Effect of Fungal Damage on Seed Composition and Quality of Soybeans

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ABSTRACT: Fungal damage caused by pathogens such as *Fusarium, Cercospora,* and *Phomopsis* can have a devastating impact on physical quality and farm price of soybeans. In some price-discount schedules, soybeans may be rejected with as low as 5% fungal damage. Although the severity of this problem varies throughout the United States, millions of bushels of fungus-damaged soybeans may be destroyed annually due to a lack of markets. The effect of fungal damage on seed composition was evaluated to assess potential utility of highly damaged soybeans. Graded samples of the cv. Centennial soybean were dried to 10% moisture and blended on a proportional weight basis to derive a series of treatments from 0 to 80% fungal damage. A positive correlation was found between fungal damage and both protein and oil concentrations. This condition was attributed to loss of residual seed mass. As a result, the protein concentration of defatted meal increased from *ca.* 54 to 66% over the range of 0 to 80% fungal damage. Mycotoxin contamination appeared to be insignificant in these high-protein meals. Fixed colors in bleached, alkali-refined oils were intensified by heat treatment prior to extraction. No significant differences, however, were noted in total polar lipid content, phospholipid, or tocopherol composition among treatments of up to 20% fungal damage. Oils from treatments of more than 40% fungal damage were more severely oxidized and could not be degummed effectively. These data suggest that fungus-damaged soybeans may be blended with high-quality soybeans to alleviate the chemical symptoms associated with unacceptable product quality. Thus, through various blend ratios, processors may consider using fungus-damaged soybeans to gain economic advantage, especially when high-quality soybeans have lower protein concentration.

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KEY WORDS: Fixed colors, fungal damage, *Glycine max,* meal, nonhydratable phosphatides, oil quality, protein concentration, soybean, test weight, tocopherol composition.

Seed discoloration, attributed to disease caused by fungal pathogens, is related to seed quality in the practice of the U.S. Official Standards for soybeans. Although severe problems with off colors are infrequent, seed lots with more than 10% discoloration may be rated as sample grade, the lowest quality recognized in the standard (1). Thus appearance of a seed lot becomes a critical factor to the sale and market price of soybeans. In that regard, the association of seed discoloration and seed quality usually is made without concern for the type of fungal pathogen present. Although many diseases may attack soybeans throughout the southern U.S.-producing region, most have limited effects on yielding ability (2), and some may cause only superficial seed discoloration (3). Yet all such visible symptoms arbitrarily are assumed to have the same impact on seed quality. Justified or not, such a liberal interpretation probably is conditioned by the associated effects of extreme fungal damage on chemical properties of soybean seed. Soybeans highly infected with *Fusarium, Cercospora, Phomopsis,* or *Alternaria* are known to produce oils with elevated free fatty acid (FFA) concentrations, which contributes to refining losses. Finished oils from such seed usually have darker colors, lower oxidative stabilities, and poor flavors (4). Obviously, these conditions limit the marketability of oils from fungus-damaged soybeans. Fungus-damaged soybeans, however, also may exhibit higher protein concentrations, lower carbohydrates, and either no change or increased oil concentrations (5,6). Although these effects tend to reduce the test weight of a bushel of soybeans, the resultant protein concentration in defatted meal suggests a useful application of fungus-damaged soybeans, assuming that other chemical quality factors are tolerable.

It would seem that fungus-damaged seed could be blended with high-quality soybeans to capitalize on the apparent benefit of higher protein concentration in fungus-damaged soybeans. Such an approach would ameliorate undesirable characteristics of the oil and theoretically could extend the utility of lower-grade or sample-grade seed. Given due consideration, the tolerable threshold of fungal damage should be determined. According to the U.S. Official Standards for soybeans, the threshold for off colors in a seed lot of No. 1 soybeans is $\leq 1\%$, between 1 and 2% for No. 2 grade, between 2 and 5% for No. 3 grade, and between 5 and 10% for No. 4 grade. Seed lots falling below these grades may be rejected under current discount schedules for contract sales and hence may be destroyed due to lack of a market. These criteria apparently discourage blending for export purposes. Should the same restrictions, however, apply to soybeans destined for

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crushing plants? Unfortunately, there seems to be little published information on the impact of blends with different levels of fungus-damaged seed on the chemical constituents and characteristics of soybeans. This investigation was conducted with a series of blended samples with known levels of fungal damage to explore the feasibility of this approach.

MATERIALS AND METHODS

Graded samples of soybean, *Glycine max* L. Merr. cv. Centennial, seed with 0 or 80% (w/w) fungal damage, were obtained from the Cooperative Inspection Service, North Carolina Department of Agriculture (Raleigh, NC). In accordance with U.S. Official Standards for soybeans, no attempt was made to identify fungal or viral organisms resident in these seed lots. Each lot was cleaned and dried at 25, 40, or 80°C to achieve 10% moisture. Three replicate sample series, consisting of 0, 5, 10, 20, 40, and 80% fungal damage, were created within each of the three heat treatments by blending the original seed lots on a proportional weight basis. Test weight of the seed in each treatment was derived on a weight/volume basis. Oil concentration was determined by wide-line nuclear magnetic resonance (NMR) (7). Protein concentration was determined by Kjeldahl (7). Residual carbohydrates and fiber were determined by weight differences. Mycotoxin assays were conducted by W.M. Hagler, Jr., Poultry Science Department, North Carolina State University (Raleigh, NC). Mean values were reported for test weight, oil concentration, protein concentration of whole seed or defatted meal, and mycotoxin levels for comparable levels of fungal damage over each temperature treatment.

Seeds were ground to a flour and extracted in two volumes of chloroform/hexane/methanol (8:5:2, vol/vol/vol) at 50°C for 30 min. Extracts were filtered and dried under a vacuum. FFA concentration was determined by American Oil Chemists' Society (AOCS) Method Ac 5-41 (8). Data were reported only from samples dried at 80°C prior to extraction. Chlorophyll levels were determined by AOCS Method Cc 13d-55 (8). These data were reported as means over all temperature treatments. Neutral glycerolipids were separated by thin-layer chromatography (TLC) with petroleum ether/diethyl ether/acetic acid (80:20:1, vol/vol/vol). Polar glycerolipids were separated by TLC with chloroform/methanol/ammonium hydroxide (65:35:1.5, vol/vol/vol). Fatty acid methyl esters derived from triacylglycerol (TG) were determined by gas chromatography (GC) (7). These data were reported as means over all temperature treatments. GC analysis of fatty acid methyl esters also was used to determine the concentration of phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and other phospholipids in oil extracts. These data were reported only from samples dried at 80°C prior to extraction. Nonhydratable phosphatides were estimated from the same samples by the procedure of List *et al.* (9). After saponification (10), total and major tocopherols in crude oil were separated by TLC (11). Trimethylsilyl derivatives of tocopherols were analyzed by GC analyses according to Fenner and Raphiou (11). Quantities were estimated against authentic α- and γ-tocopherol standards. These data were reported from seeds dried at 80°C prior to extraction.

Oils were refined from replicate samples within each temperature treatment according to AOCS Standard Method Ca 9b-52 (8). Refined oils were bleached by AOCS Standard Method Cc 8b-52 (8). Photometric color indices for the various oil samples from each treatment were determined by AOCS Method Cc 13c-50 (8). Color analyses were conducted with a Hewlett-Packard HP8452A diode array UV-VIS spectrophotometer (Hewlett-Packard, Avondale, PA). Equations in the method were modified to accommodate a 1-cm light path. Average wholesale prices for meal and crude soybean oil in Decatur, Illinois (October 1993 through September 1994), as reported by USDA-Foreign Agricultural Service (12), were used to estimate the theoretical constituent value of the soybeans from these fungal damage treatments.

RESULTS AND DISCUSSION

Analyses revealed definable relations between the level of fungal damage and the respective concentrations of the three primary seed constituents (Table 1). Because drying temperature had no effect on these responses, data were expressed as composite treatment mean values. In direct proportion to the level of fungal damage, results showed a linear increase in oil concentration from 19.5 to 22.8% of seed mass at 10% moisture, and a linear increase in protein concentration from 43.7 to 50.8% of seed mass. In both cases, statistically significant differences from the control treatment were detected at 20% fungal damage. These findings were consistent with trends suggested in prior reports (5,6). Apparently, the simultaneous increase in both oil and protein concentrations was mediated by a linear decline in residual seed mass.

Loss of residual constituents directly correlated to a linear decline in soybean test weight from 54.0 to 48.8 lb/Bu. With compensation for the change in test weight, however, there was no practical change in oil content per bushel among these treatments. Also, no appreciable loss in protein content was detected, although the apparent amount of defatted meal per bushel was inversely associated with fungal damage. Thus, by virtue of loss in residual constituents, protein concentration of the defatted meal increased from 54.2 to 65.8% (w/w). Significant differences in this trend occurred between 10 and 20% fungal damage. Based on average annual prices for crude soybean oil and meal (12), the decline in test weight did not contribute to a loss in the approximate constituent value of the fungus-damaged soybeans. Indeed, one might speculate that the apparent constituent value of highly fungus-damaged treatments in this study could be greater than the control treatment in certain hypothetical situations.

Although intriguing, the supposition that highly fungus-damaged soybeans may have equivalent or greater intrinsic economic value than No. 1 grade soybeans should be tempered with consideration of pathogen impact on oil and pro-

TABLE 1
Effect of Fungal Damage on Soybean Seed Content, cv. Centennial

Fungal damage[a] (%)	Constituent concentration (g/100 g seed @ 10% moisture)			Test weight	Constituent content (lb/Bu @ 10% moisture)		
						Defatted meal	
	Oil[b]	Protein[c]	Residual[d]		Oil[e]		% protein
0	19.5	43.7	36.8	54.0	10.5	43.5	54.2
5	19.8	44.5	35.7	53.7	10.7	43.0	55.6
10	20.1	44.9	35.0	53.3	10.7	42.6	56.2
20	20.5	46.3	33.2	52.7	10.8	41.9	58.2
40	21.3	47.5	31.2	51.4	10.9	40.5	60.3
80	22.8	50.8	26.4	48.8	11.1	37.7	65.8
$LSD_{0.05}$[f]	0.7	1.5	2.2	1.1	0.1	1.2	2.4
Slope	0.040	0.086	−0.126	−0.065	0.006	−0.071	0.139
r^2	0.996	0.987	0.991	0.999	0.898	0.999	0.991

[a]Graded samples of 0 and 80% fungus-damaged seed were dried to 10% moisture @ 25, 40, and 80°C. Seed from each drying treatment was blended separately on a proportional weight basis to derive the respective treatment series. These data represent the composite mean values from the three drying treatments.
[b]Oil concentration was determined by wide-line nuclear magnetic resonance (Ref. 7).
[c]Protein concentration was determined by Kjeldahl method (Ref. 7).
[d]Residual concentration was determined by weight difference.
[e]Oil and defatted meal content were determined relative to test weight.
[f]LSD = Least significant difference.

tein qualities. Regarding protein quality, soybeans infected with *Fusarium*, *Cercospora*, and/or *Phomopsis* fungi could contain residual amounts of certain mycotoxins that facilitate expression of the plant disease symptoms. As a worst-case scenario, the presence and concentration of common mycotoxins were determined in samples from the 80% fungal damage treatment. These assays revealed no detectable aflatoxin, T-2 toxin, diacetoxyscirpenol (DAS), or HT_2 toxin. Low levels of vomitoxin (DON), *ca.* 0.3 ppm; zearalenone (F_2), *ca.* 0.8 ppm; and fumonisin (B_1), *ca.* <5 ppm, were found in these samples. Without discounting potential concern for these toxins, however, levels diagnosed in these samples appeared to be below Food and Drug Administration regulatory specifications.

The impact of fungal damage on oil quality is quite a different matter. Crude oil may have greater FFA concentration, lower hydratable phosphatides, severe tocopherol oxidation, and intensified refined oil color (4). Elevated FFA concentration contributes to excessive refining losses. Refining losses ranging from 1 to 1.5% (w/w) usually are considered normal. These losses may approach 4% (w/w) or greater due to high FFA. Fungus-induced disruption of cellular membranes also may cause hydrolysis of hydratable phosphatides, principally lecithins, by phospholipase D activity (9). Crude soybean oil typically contains 5 to 10% (w/w) nonhydratable phosphatides (4). Higher concentrations of the, however, nonhydratable products of phospholipase D or similar reactions may obviate ability to degum the oil. Total tocopherol levels also may decline under conditions that favor lipid oxidation, with a concomitant increase in peroxide values (13). Without protection of natural antioxidants, intense pigments with absorbance between 220 and 400 nm may be formed by lipid oxidation processes (14). These undesirable colors have been attributed to conjugated or oxygenated polyunsaturated fatty acids and tocoquinones. Thus severe oxidation may result in elevated 18:1 and lower 18:2 and 18:3 acid concentrations in the oil (4,5).

Because these conditions significantly compromise oil quality, it generally may be assumed that any sign of fungal damage is unacceptable; however, there may be a tolerable threshold level of fungal damage regarding oil composition (Table 2). Examination of the relation of fungal damage to TG composition revealed an apparent breakpoint at the 10% fungal damage treatment. At or below that treatment, 18:1, 18:2, and 18:3 concentrations in TG were not significantly different from those of the control. With compensation for loss in test weight, there was no appreciable gain in TG up to 5% fungal damage or loss in total polar lipid (TPL) content per bushel up to 20% fungal damage. Significantly greater amounts of TG and lower amounts of TPL were detected at 40% fungal damage; elevated FFA concentration also was noted. Chlorophyll concentration increased from *ca.* 85 ppb in the control treatment to *ca.* 218 ppb in the 80% fungal damage treatment.

Little change was observed in the concentration of PI or PE over the treatments dried at 80°C prior to extraction (Table 3). Thus the increase in nonhydratable phosphatide (NHP) concentration appeared exclusively to be at the expense of PC. Indeed, loss in PC concentration between 0 and 80% fungal damage treatments accounted for *ca.* 89% of the gain in NHP concentration. The presence of NHP in the oil, however, did not account for the treatment effects on the amount of PC in the same samples. The amount of PC lost over the range of these treatments was roughly tenfold greater than the amount of gain in NHP. This implied that NHP was only a symptom of phospholipid oxidation in fungus-damaged soybeans.

TABLE 2
Effect of Fungal Damage on Crude Oil Composition, cv. Centennial

Fungal damage[a] (%)	Fatty acid composition (% triacylglycerol fraction)					Constituent content (lb/Bu @ 10% moisture)			
	16:0	18:0	18:1	18:2	18:3	TG[b]	TPL	FFA[c] (%)	Chlorophyll[d] (ppb)
0	10.9	3.1	25.0	52.7	8.3	9.8	0.6	0.9	85
5	11.4	3.1	25.1	52.2	8.2	9.9	0.6	1.3	122
10	11.1	3.1	25.5	52.2	8.1	10.1	0.5	1.5	155
20	11.2	3.1	26.1	51.6	8.0	10.2	0.5	1.8	119
40	10.9	3.2	27.3	51.0	7.6	10.6	0.3	2.3	149
80	11.3	3.4	29.6	48.9	6.8	10.7	0.3	3.6	218
$LSD_{0.05}$[e]	0.1	0.1	1.0	0.8	0.3	0.2	0.1	0.6	26.0

[a]Unless indicated otherwise, these data represent the composite mean values from the three drying treatments.
[b]TG, triacylglycerol; TPL, total polar lipids; lb/Bu relative to test weight.
[c]Free fatty acids (FFA) expressed as 18:1 equivalent units, determined by AOCS Method Ac 5-41 (Ref. 8). These data are reported as mean values from replicated samples dried at 80°C prior to extraction.
[d]Chlorophyll concentration was determined according to AOCS Method Cc 13d-55 (Ref. 8).
[e]LSD = Least significant difference.

Hence problems experienced in degumming the oil primarily were a function of the total amounts of PC, PI, and PE in the samples. According to these experiments, degumming could not be accomplished when the individual amounts of PC, PI, and PE were reduced to *ca.* 50% of that in the control treatment. The possibility that exogenous lecithin might be added to highly damaged oil to achieve degumming was considered. Laboratory-scale tests of this hypothesis were unsuccessful, however.

Endogenous protection against lipid oxidation may be afforded by the type and amount of tocopherols in soybean oil. In that regard, crude soybean oil typically contains *ca.* 1,140 ppm total tocopherol (15). Relative antioxidant capacity among tocopherol species may be ranked: δ-tocopherol > γ-tocopherol > β-tocopherol > α-tocopherol (16). In this study, fungal damage had no effect on total tocopherol levels among these treatments (see Table 3). Also, there was no appreciable change in the concentration or amount of α-, δ-, or γ-tocopherol until the incidence of fungal pathogens affected *ca.* 50% depletion of TPL levels in the control treatment. At that point, a significant increase in unidentified tocopherols was detected. It was presumed that those compounds represented tocoquinones and tocol dimers (17), which could contribute to the intensified oil colors in fungus-damaged soybean oil.

Apart from the undesirable nature of fixed colors in certain soybean oil applications, these lipid oxidation products may be associated with poor flavor. Therefore, refined oil from fungus-damaged soybeans may require extended deodorization time (4). Even so, the flavor characteristics of deodorized oil from soybeans containing 85% fungal damage have been reported to be inedible. Acceptable flavor scores may be attained with finished oil from seed lots of less than 35% fungal damage. Still, removal of undesirable oil colors and associated flavors is extremely difficult, even with advances in oil-processing technology (18). Certain precautions, however, may be taken prior to refining fungus-damaged soybeans that limit lipid oxidation and color formation. List *et al.* (9) demonstrated that application of live steam for short

TABLE 3
Effect of Fungal Damage on Crude Oil Fractions, cv. Centennial

Fungal damage (%)	Phospholipid composition[a] (% total phospholipid[b])						Tocopherol concentration (% total tocopherol[b])				
	PI	PC	PE	Other	NHP[c]	TPL (% TL[b])	α	δ	γ	Other	Total (ppm[b])
0	15.2	48.2	24.1	4.2	8.3	5.6	4.9	42.9	50.4	1.8	1260.2
5	15.5	48.0	23.8	4.3	8.4	5.6	5.0	41.8	51.7	1.5	1253.7
10	16.0	47.4	24.0	3.9	8.7	4.7	4.7	42.3	50.5	2.5	1261.9
20	16.6	46.2	24.2	4.0	9.0	4.6	4.8	42.4	50.0	2.8	1259.9
40	16.4	43.5	24.2	3.8	12.1	2.7	4.0	39.5	49.4	7.1	1262.6
80	16.1	31.8	22.8	2.5	26.8	2.5	1.9	29.5	37.7	30.9	1259.0
$LSD_{0.05}$[d]	0.3	3.6	0.3	0.4	4.2	0.8	0.7	3.0	3.0	6.7	1.8

[a]PI, Phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Other, residual hydratable phosphatides; NHP, nonhydratable phosphatides; TPL, total polar lipid.
[b]These data represent mean values from replicated samples dried at 80°C prior to extraction; TL, total lipid.
[c]% NHP determined from phosphorus content of degummed/crude oil.
[d]LSD = Least significant difference.

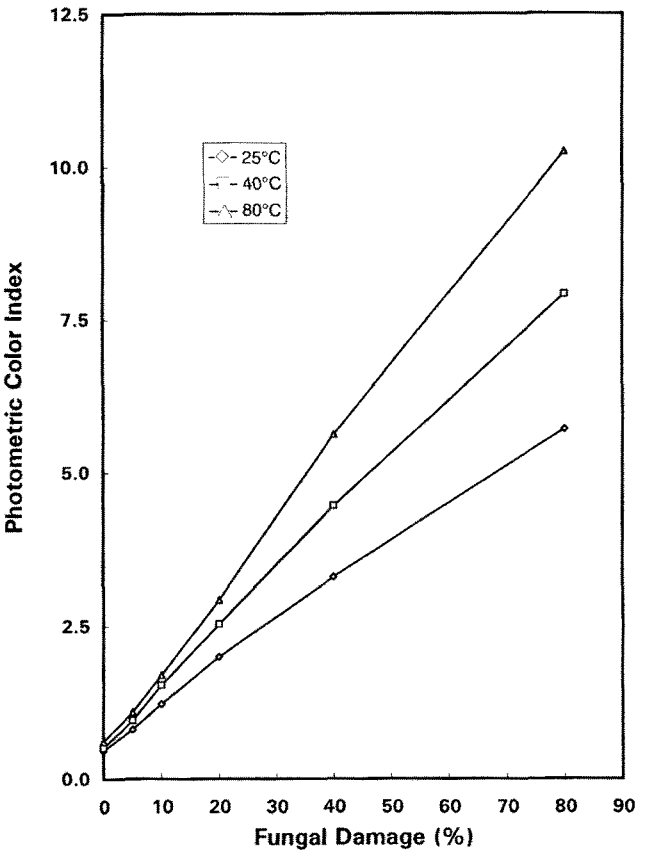

FIG. 1. Effect of drying temperature on color of bleached, alkali-refined oil from fungus-damaged soybeans. Refined oils were bleached after AOCS Standard Method Cc 8b-52 (Ref. 8). Photometric color indices were determined by AOCS Method Cc 13c-50 (Ref. 8).

periods effectively eliminated increases in NHP. Although such a treatment could denature storage proteins, other ideas were tested concerning the impact of storage temperature and moisture level on refined oil quality. As an extension of that work, we examined the effect of drying temperature prior to crushing on the refined-oil color of fungus-damaged soybeans. Replicated series of fungal damage treatments were dried to 10% moisture at 25, 40, or 80°C. In these experiments, the color of bleached alkali-refined oil increased linearly with fungal damage in all temperature treatments (Fig. 1). The rate of increase in the photometric color index, however, was twofold greater when the drying temperature prior to dehulling was increased from 25 to 80°C. These data suggested that the rate of oil-color formation also may be controlled by blending high-quality and fungus-damaged soybeans (assuming mycotoxin levels are within acceptable limits) and by attention to low drying temperatures prior to crushing.

In summary, fungal damage in soybeans usually implies poor seed quality and results in lower grade ratings at the first point of sale. Although seed quality may be interpreted in many ways, this study has shown that the threshold levels of fungal damage defined in the U.S. Official Standards for soybeans may have insignificant impact on the chemical quality of seed constituents. Indeed, one might conclude from these data that price discounts should not be imposed until fungal damage exceeds 5%. Furthermore, problems associated with seed lots of higher levels of fungal damage (assuming acceptable mycotoxin levels) may be alleviated through appropriate blends with high-quality soybeans. Therefore, with proper precautions during crushing or refining and certain marketing considerations, it may be possible to reclaim economic benefits apparent in the high-protein meals derived from fungus-damaged soybeans.

REFERENCES

1. *Official United States Standards for Grains. Subpart I: U.S. Standards for Soybeans, Grade and Grade Requirements*, U.S. Department of Agriculture, Federal Grain Inspection Service, Washington, D.C., 1990.
2. Mulrooney, R.P., *Plant Dis. 69*:92 (1985).
3. Sinclair, J.B., *J. Am. Oil Chem. Soc. 72*:1415 (1995).
4. List, G.R., in *Handbook of Soybean Oil Processing and Utilization*, American Soybean Association/American Oil Chemists' Society, Champaign, 1980, pp. 355–376.
5. Patil, K.B., S.C. Shivamurthy, and R.C. Badami, *Fette Seifen Anstrich. 88*:18 (1986).
6. Pathan, M.A., J.B. Sinclair, and R.D. McClary, *Plant Dis. 73*:720 (1989).
7. Kwanyuen, P., J.W. Burton, and R.F. Wilson, in *Proceedings of the World Conference on Edible Oils and Fats Processing*, edited by D. Erickson, AOCS Press, Champaign, 1990, pp. 351–354.
8. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D.R. Firestone, American Oil Chemists' Society, Champaign, 1989, 4th edn., Methods Cc 8b-52, CcBc-50.
9. List, G.R., T.L. Mounts, and A.C. Lanser, *J. Am. Oil Chem. Soc. 69*:443 (1992).
10. Gutfinger, T., and A. Letan, *J. Food Sci. 37*:938 (1972).
11. Fenner, G.P., and I. Raphiou, *Lipids 30*:253 (1995).
12. *Oilseeds: World Markets and Trade*, FOP 11-94, U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C., 1994.
13. Wong, M.L., R.E. Timms, and E.M. Goh, *J. Am. Oil Chem. Soc. 65*:258 (1988).
14. Chapman, D.M., E.A. Pfannkock, and R.J. Kupper, *Ibid. 71*:401 (1994).
15. Gutfinger, T., and A. Letan, *La Rivista Italiana delle Sotanze Grasse 52*:191 (1975).
16. Patterson, H.B.W., in *Bleaching and Purifying Fats and Oils*, AOCS Press, Champaign, 1992, pp. 11–147.
17. Koskas, J.P., J. Cillard, and P. Cillard, *J. Chromatogr. 287*:442 (1984).
18. Chapman, D.M., *INFORM 5*:505 (1994).

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