A Methodology Study to Evaluate Quality of Soybeans Stored at Different Moisture Levels

E.N. Frankel, A.M. Nash and **J.M. Snyder**
Northern Regional Research Center, ARS/USDA, 1815 N. University St., Peoria, IL 61604

The quality of soybeans and oil extracted from seeds stored at different moisture contents was evaluated by static headspace gas chromatography, near-infrared spectrometry, fluorescence measurements, and silicic acid chromatography. Headspace gas chromatographic analysis of both ground beans and crude oils provided a sensitive measure of oxidative deterioration based on hexanal and total volatiles. Near-infrared analyses at 2260 nm showed a correlation coefficient of 0.864 with titratable free fatty acids. Fluorescence measurements on chloroform-methanol extracts were much less sensitive and showed an increase only in the most damaged samples. Silicic acid chromatography of crude oils showed a significant decrease of polar lipids and increase of less polar lipids with storage at high moisture levels, in agreement with the decrease in phosphorus observed. Among the methods tested, headspace gas chromatography is most sensitive to evaluate oxidative deterioration, and near-infrared analysis is most suitable and rapid to evaluate hydrolytic deterioration in stored soybeans. This methodology can be used to evaluate factors affecting the food quality of soybeans for domestic and foreign markets.

Storage of soybeans under moist conditions is known to adversely affect the quality of the extracted crude oil and finished refined, bleached and deodorized oil (1-7). Previous studies of the relationship between bean damage and oil quality have relied on traditional analytical methods such as free fatty acids, phosphorus, peroxide values, metals, refining loss, fatty acid composition and flavor scores on the finished oils. These methods are generally impractical for routine analyses and often do not provide enough information to determine whether the quality damage is due to hydrolytic or oxidative deterioration. These traditional methods also require extraction of the oil before analysis and cannot be applied directly on the beans as is often necessary for screening purposes at the extraction plants and prior to shipments abroad. With the increased focus on export markets of soybeans, there has been a great need for reliable, sensitive, quantitative and fast methods to evaluate quality of the beans directly before and the oil after extraction.

This paper describes improved methods developed to evaluate the effect of known storage history on oxidative and hydrolytic deterioration by analyzing either the beans directly or the extracted crude oils. We were particularly interested in rapid instrumental methods that could be used by seed graders, shippers and processors.

EXPERIMENTAL PROCEDURES

Storage. Seed grade Century 1984 soybeans were obtained from a local distributor. The moisture contents of beans were adjusted to 13, 16 and 20% by shaking with calculated amounts of water, and monitored by the AOCS oven method Ac 2-41 (11). The beans were stored in 800-g quantities in several 0.5-gal bottles and allowed to heat endogenously in a forced-draft oven for periods of 19 to 50 days. Samples were stirred and aerated 3 times a week to prevent mold formation. During storage the beans generated their own heat from initial to final temperatures of 41 to 48 C at 13% and 16% moisture, and 47 to 49 C at 20% moisture. Samples of 400 g were withdrawn periodically for analyses and processing.

Processing. A portion of beans was ground and analyzed directly by gas chromatography (GC) for volatiles and by near-infrared spectroscopy (NIR) for free fatty acids. One portion was extracted with chloroform-methanol and analyzed for development of fluorescence. Another portion was flaked and extracted with hexane

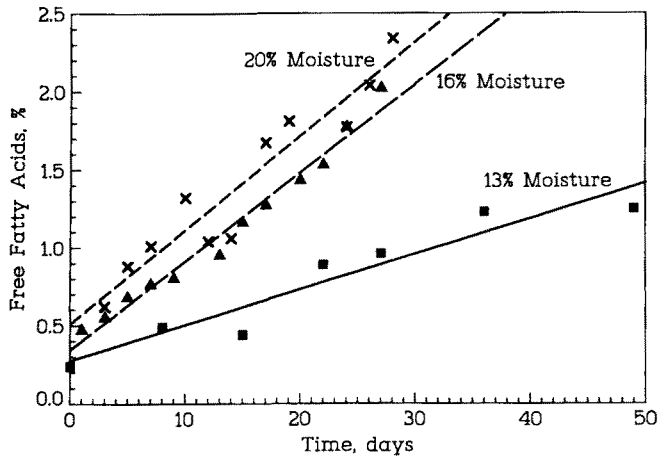

FIG. 1. Titratable free fatty acids in crude oils from soybeans stored at different moisture levels.

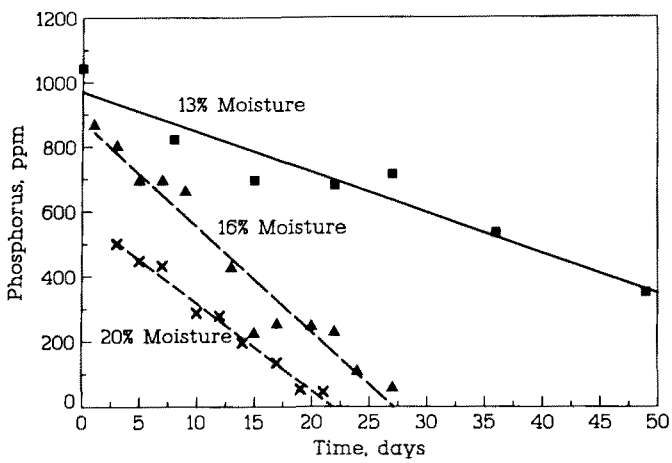

FIG. 2. Phosphorus in crude oils before degumming from soybeans stored at different moisture levels.

in a conventional Soxhlet apparatus (8). The resulting crude oil was analyzed directly for volatiles, free fatty acids, phosphorus and peroxide values, and after degumming for phosphorus.

Static headspace capillary gas chromatography. Soybean samples were ground with an electric coffee grinder, and the resulting flour was passed through a 40-mesh sieve. Samples of 0.5 g were placed in the vials of a Perkin-Elmer gas chromatograph (Model Sigma 3B, Norwalk, Connecticut) equipped with a headspace sampler (Model HS-6). The vials were sealed with a septum secured by an aluminum cap. They were then heated in the headspace GC magazine at 90 C for 20 min. The magazine was placed in the injection position and the pressure equilibrated for 0.5 min before injection. After injection, the volatiles were eluted onto a Durabond DB-5 fused silica capillary column (30 m × 0.32 mm, 1 micron film thickness, J & W Scientific, Rancho Cordova, California) in the splitless mode. The helium linear velocity was 30 cm/sec at 100 C, the injector temperature was 120 C and detector temperature 250 C. Crude oil samples (0.5 g) in vials were heated to 180 C for 10 min and pressurized for 0.5 min. These are the same capillary DB-5 column and gas chromatographic conditions as used previously for vegetable oils (9).

Near infrared reflectance spectroscopy. Soybean samples were analyzed with a computerized NIR spectrometer (Neotec Model 6350 Mark II, Pacific Scientific Co., Silver Spring, Maryland) interfaced to a computer (Nova/4), after grinding and sieving to obtain particles of uniform size. Readings were taken at 2-nm intervals in the range 1100–2500 nm. The NIR instrument was calibrated by scanning 35 samples of freshly ground beans, which had been analyzed previously for free fatty acids, volatiles, peroxide values and phosphorus contents. All spectral data were mathematically transformed to obtain first and second derivative values across the spectra. To evaluate how chemical test results correspond with specific NIR wavelengths, spectral data and analytical values were subjected to multiple regression analysis.

Fluorescence measurements. Ground bean samples (2 g) were extracted for three hr with 50 ml of a chloroform-

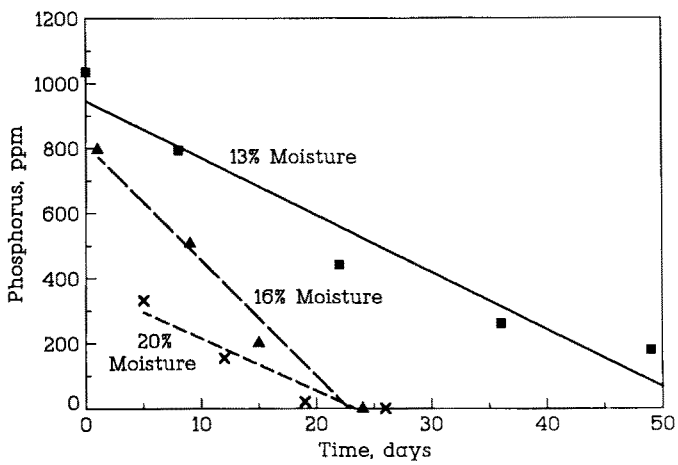

FIG. 3. Phosphorus in degummed oils from soybeans stored at different moisture levels.

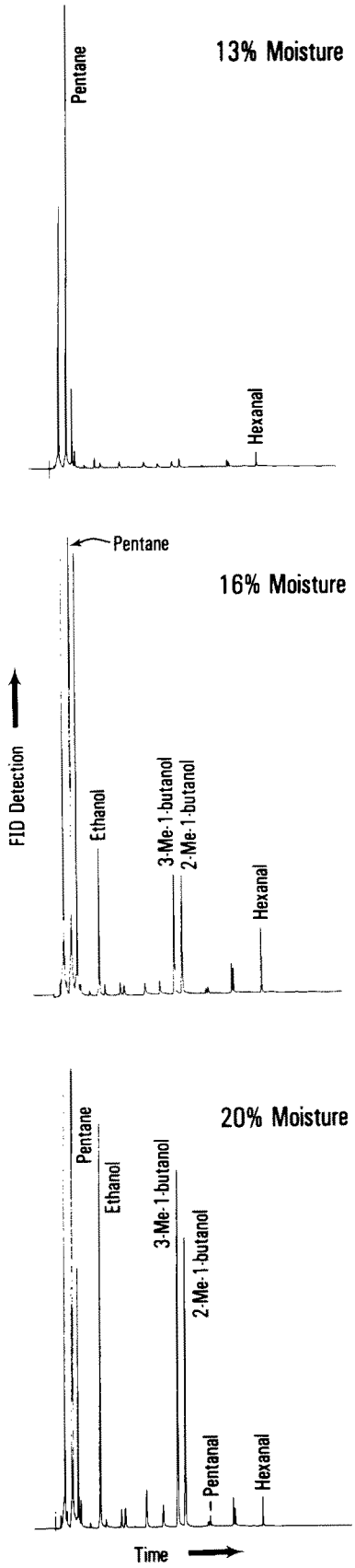

FIG. 4. Capillary headspace gas chromatograms of volatiles from ground samples of soybeans stored at 13% and 16% moisture for 27 days and at 20% moisture for 28 days.

methanol (79:21, v/v) azeotrope mixture in a butt extractor. Fluorescence was measured directly on the chloroform-methanol extracts with a Perkin-Elmer MPF-44B Fluorescence Spectrophotometer and a 1-cm cell. Instrumental conditions were standardized with a solution of 1 μg quinine sulfate/ml 0.1 N sulfuric acid, which gave a reading of 1400 scale units (excitation 364 nm and emission 437 nm) (10).

Silicic acid chromatography. Crude oil samples (1 g) were dissolved in 10 ml of a mixture of petroleum ether-diethyl ether (95:5, v/v) and chromatographed through a silicic acid cartridge column (Sep-Pak, Waters Associates, Milford, Massachusetts). The neutral lipids were eluted with an additional 10 ml of petroleum-diethyl ether mixture. Polar lipids were eluted with 20 ml of diethyl ether and 10 ml of methanol, successively. The weighing and thin layer chromatographic examination of each fraction were carried out as described previously (8).

Conventional analyses. Free fatty acids (Ca 5a-40) and peroxide values (Cd 8-53) were determined by standard AOCS methods (11). Phosphorus was determined colorimetrically (Ca 12-55) in crude oils and after degumming. A small-scale degumming procedure was used with samples of 30 g as described previously (12).

RESULTS AND DISCUSSION

Storage experiments. To develop useful methods for quality evaluation, soybeans were purposely abused by storage under adverse conditions of moisture to obtain a wide range of oxidative and hydrolytic deterioration. The effect of storing beans at three moisture levels on titratable free fatty acids in the extracted crude oil is plotted in Figure 1. Storage at 13% moisture showed the lowest increase in free fatty acids (from 0.2 to 1.25 after 49 days). Storage at 16 and 20% moisture resulted in a much greater increase in free fatty acids with time (0.5 to 2.0 after 27 days and 0.6 to 2.3 after 28 days, respectively), but no significant difference (95% confidence level) could be detected between these two levels of moisture.

The initial total phosphorus contents of the crude oils before degumming decreased with both increasing moisture levels and storage time (Fig. 2). The phosphorus loss was significantly greater at 16% than at 13% moisture. The phosphorus level decreased from 1044 to 680 ppm at 13% moisture and to 228 ppm at 16% moisture after 22 days of storage. At 20% moisture, the phosphorus content decreased to 46 ppm after 21 days of storage. The quantity of phosphorus removed by degumming represented hydratable phospholipids and was calculated as the difference between the phosphorus determined before and after degumming (Fig. 3). The crude oil from beans stored at 13% moisture initially had more hydratable phospholipids, which were lost during storage. The oil from the other beans stored at 16 and 20% moisture had less hydratable phospholipids, and after 25 days of storage all the phospholipids remained non-hydratable (Fig. 2). These results are in agreement with previous work showing a loss in total and hydratable phosphorus as well as in specific phospholipids in crude oils from field and storage damaged beans (2-4,7). These losses in phosphorus have been explained by the formation of non-hydratable phosphatides consisting of Ca and Mg salts of phosphatides (13).

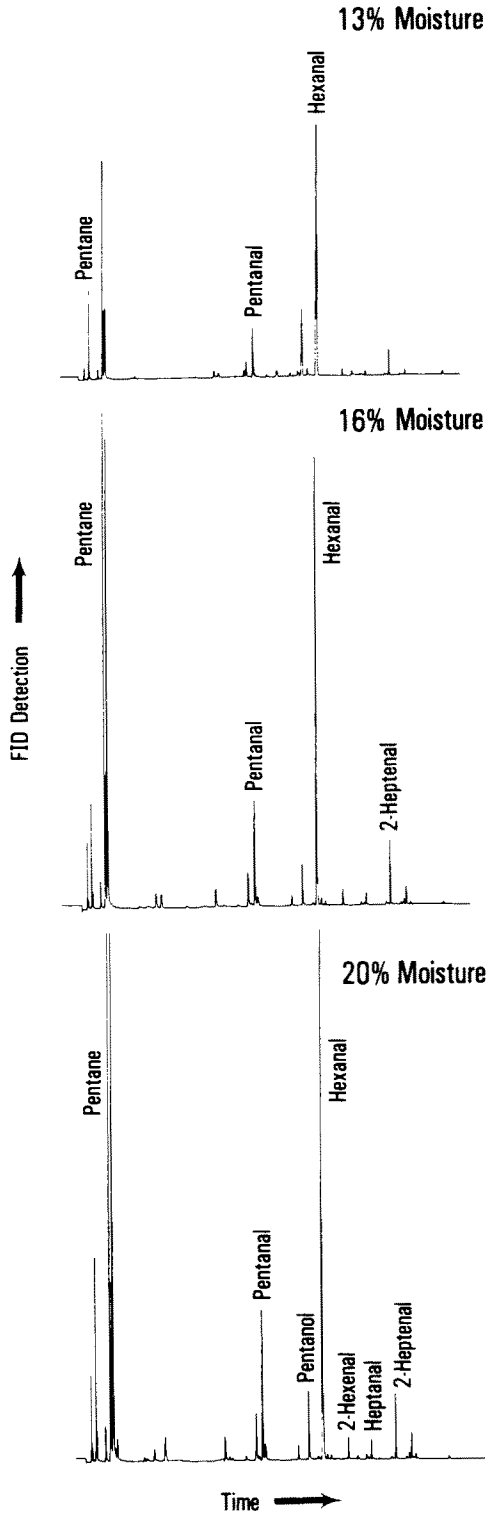

FIG. 5. Capillary headspace gas chromatograms of volatiles in crude oils from soybeans stored at 13% and 16% moisture for 22 days and at 20% moisture for 21 days.

Volatile analyses. Figure 4 shows the headspace gas chromatograms obtained with samples of beans stored at 13, 16 and 20% moisture. Except for pentane, the sample stored at 13% moisture showed very few peaks; samples stored at 16 and 20% moisture showed increased amounts of volatiles including compounds expected by

oxidation such as pentane, pentanal and hexanal. Unexpected major peaks were also found in chromatograms from beans stored at the higher moisture levels and were shown by gas chromatography-mass spectrometry (9) to include ethanol, 3-methyl-1-butanol and 2-methyl-1-butanol. These compounds apparently are degradation products resulting from fermentation (14) and oxidation.

Figure 5 shows chromatograms obtained with the crude oils extracted from the same beans stored at different moisture levels. These chromatograms are typical of those usually obtained from vegetable oils (9), and the peaks represent lipid oxidation products, including pentane, pentanal, hexanal, 2-hexenal, heptanal and 2-heptenal (15). These results show that oxidative deterioration occurs on storage and increases with the moisture level.

Quantitative GC analyses showed a marked difference in total volatiles formed in beans stored at 16 and 20% moisture compared to 13% moisture (Fig. 6). A drastic increase in total volatiles was noted at the 16–20%

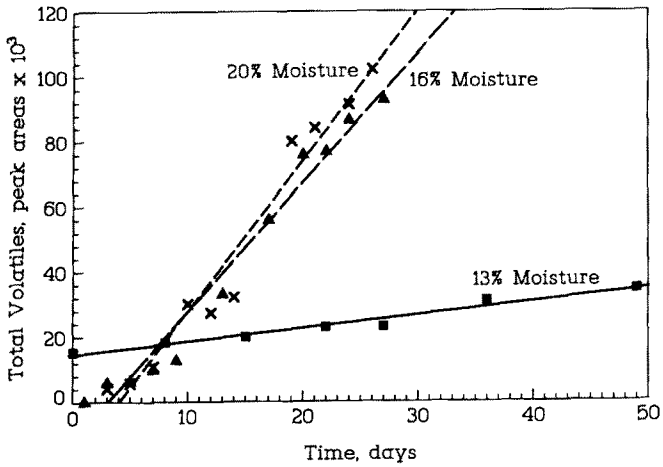

FIG. 6. Total volatiles in ground samples of soybeans stored at different moisture levels.

TABLE 1

Quality of Soybeans Stored at Different Levels of Moisture

Storage time (days)	Hexanal (pk areas)[a]		Peroxide value (me/kg)	Fluorescence (relative units)[b]	Sil. acid chromatography	
	Beans	Cr. oil			Ether (%)	Methanol (%)
			13% Moisture			
8	0.6	3.2	0.0	603	2.7	0.7
15	0.8	8.3	0.0	619	—	—
22	1.3	6.1	0.0	603	3.3	0.4
27	1.2	9.8	0.0	505	—	—
36	1.7	13.4	0.74	517	3.7	0.3
49	1.5	21.6	0.98	621	4.3	0.3
			16% Moisture			
7	1.3	20.4	0.0	321	—	—
9	1.6	23.4	0.18	280	4.2	0.6
13	2.0	20.0	0.18	288	—	—
15	2.5	27.9	0.20	321	4.1	0.4
17	2.7	25.9	0.42	314	—	—
20	4.2	26.1	0.42	326	—	—
22	3.2	32.1	0.69	337	—	—
24	2.9	28.4	0.67	371	4.9	0.3
27	2.7	32.3	0.92	418	—	—
			20% Moisture			
5	0.1	15.3	0.0	261	3.8	0.6
7	0.3	21.2	0.5	234	—	—
10	3.0	35.0	3.8	309	—	—
12	1.5	20.0	3.0	317	5.1	0.5
14	1.2	17.4	3.5	387	—	—
17	1.2	18.3	3.0	520	—	—
19	4.6	26.0	4.2	632	5.2	0.3
21	1.7	22.7	4.9	1039	—	—
24	0.6	24.8	4.3	1213	—	—
26	1.6	23.2	5.7	1451	5.3	0.2
28	1.2	29.8	3.3	1242	—	—

[a]Integrated peak areas × 10^3.

[b]Excitation 364 nm, emission 437 nm relative to quinine sulfate (1 μg/ml of 0.1N H_2SO_4 gives 1400 scale units).

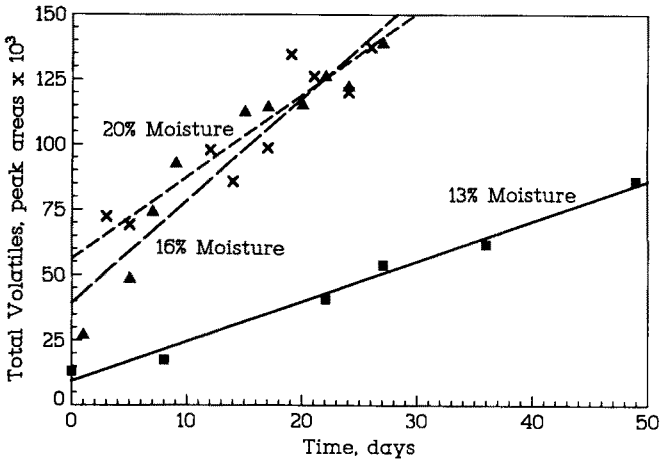

FIG. 7. Total volatiles in crude oils from soybeans stored at different moisture levels.

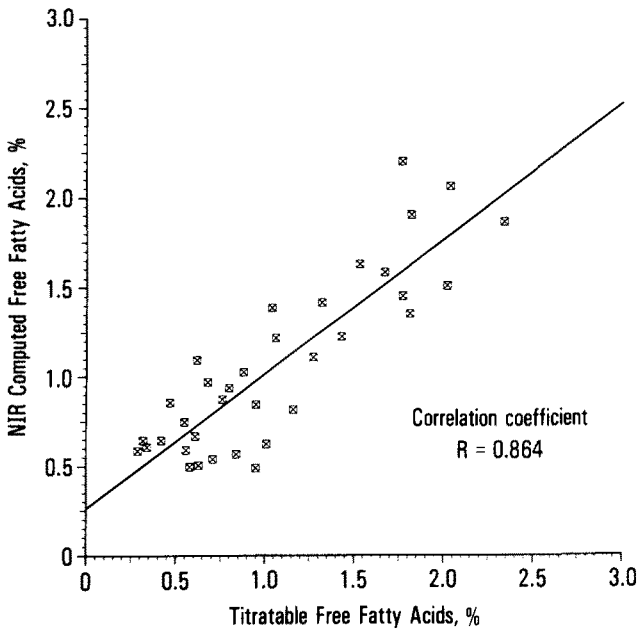

FIG. 8. Relationship between near-infrared (NIR) computed and titratable free fatty acids in soybeans stored at different moisture levels.

moisture range, but the difference between 16 and 20% moisture was not significant. Although the increase in hexanal was slight in beans stored at 13% moisture, the increase observed at 16% was unexpectedly larger than at 20% moisture (Table 1). The differences observed in the hexanal content of whole beans were not as marked as those in the total volatiles.

Similar trends were observed in the GC analyses of total volatiles in extracted crude oils. The increase in total volatiles in crude oils with storage of beans was significantly less at 13% moisture than at 16 or 20% moisture (Fig. 7). No difference was noted in the total volatiles from samples stored between 16 and 20% moisture. Measurements of hexanal in the crude oils also showed similar trends, but with greater scattering of points in the samples stored at 16 and 20% moisture (Table 1).

Thus, headspace GC analyses of volatiles provide a sensitive method to evaluate oxidative deterioration of beans directly and in extracted crude oils. Direct GC analyses of the ground beans would provide a valuable tool at the extraction plants and control laboratories for grading and evaluating the food quality of soybeans in domestic and foreign markets.

Near-infrared reflectance spectroscopy. Reflectance spectra were taken on 35 samples by averaging 50 scans for data points at each of 700 wavelengths. A mathematical transformation was carried out by computer on all spectral data to obtain the first and second derivatives of the absorption data. A plot of the second derivative of spectral data versus NIR wavelengths showed that the highest correlation with free fatty acids occurred at 2260 nm with a coefficient R = 0.864 and a standard error (S.E.) of 0.240. A high negative correlation of −0.722 was also obtained at 1810 nm, but the former wavelength of 2260 nm was chosen because of its higher and positive correlation. Additional correlations were sought for total volatiles, hexanal, phosphorus and peroxide values, but no satisfactory correlation could be obtained with these analyses.

The spectral data were subjected to multiple regression analysis. Testing of the calibration in Figure 8 shows the best correlation of 0.864 obtained between titratable free fatty acids and NIR-computed free fatty acids on all samples tested with a sample standard deviation of 0.2155. The errors associated with these NIR computations cannot, of course, be less than the errors introduced by the primary titratable free fatty acid determination. With proper calibration, this NIR method permits the analysis of a large number of samples of intact beans with rapid acquisition of data and offers a viable alternative to chemical analysis of free fatty acids for screening and routine analyses.

Fluorescence and peroxide values. Fluorescence in lipid extracts is attributed to conjugated Schiff base compounds from the interaction of oxidation products with proteins, phospholipids and nucleic acids (16). The fluorescence spectra of the chloroform-methanol extracts from storage-damaged beans showed an emission maximum at 437 nm and an excitation maximum at 364 nm. Similar fluorescent chromophores were observed during peroxidation of phosphatidylethanolamine and phosphatidylserine with emission maxima in the region of 435–440 nm and excitation maxima in the region of 365–370 nm (17). Fluorescence measurements of chloroform-methanol extracts of beans are compared with peroxide values of corresponding crude oils in Table 1. Four samples run in triplicate and averaging 343 fluorescence units gave a relative standard deviation of 4.6%. The development of fluorescence was not significant in beans stored at 13% moisture. Significant changes in fluorescence values were observed in more highly damaged samples. A smaller increase in fluorescence was noted in samples stored at 16% than at 20% moisture. The increase in fluorescence values between 7 and 24 days of storage was 50 units at 16% moisture and 989 units at 20% moisture (Table 1). No peroxide development was noted in the crude oils from beans stored at 13% moisture. Between 7 and 24 days of storage, the peroxide values increased from 0.0 to 0.67 in oils from beans stored at 16% moisture, and from 0.5 to 4.3 in oils from beans stored at 20% moisture. Therefore, analyses of fluorescence on chloroform-methanol extracts and of peroxide values on

crude oils were not sufficiently sensitive to detect the deterioration of beans stored at 13% moisture. These methods can detect significant changes only when the beans were severely abused by storage at 16 and 20% moisture.

Silicic acid chromatography. Table 1 shows an increase in the proportion of the ether eluate fraction and a small decrease in the proportion of the methanol eluate fraction with storage of soybeans. The decreases in methanol eluate fraction observed correspond to decreases in the phosphorus contents (Fig. 2). Thin layer chromatography of the ether eluate showed the presence of unsaponifiable materials, including sterols, tocopherols, pigments and other unidentified components (8). The increase in ether eluate with storage is apparently due to hydrolytic and/or oxidative components that have not been identified. The isolation and identification of these components will be the subject of another study.

Among the new methods evaluated, we conclude that the headspace gas chromatographic analysis for volatiles is most useful and sensitive to evaluate the oxidative deterioration occurring in beans during storage under moist conditions. The NIR analysis is most suitable and rapid to determine hydrolytic deterioration of beans on storage. Fluorescence and silicic acid chromatography showed a detectable effect from storage of beans only when the moisture level was above 13%. Flavor evaluation studies were carried out on the crude and refined oils from similar beans stored at 13% moisture and will be the subject of another publication.

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