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Determination of Phytic Acid in Cottonseed by Near-Infrared Reflectance Spectroscopy

Frederick W. Parrish, John P. Madacsi, Brian Q. Phillippy,* Antonia G. Wilfred, and Steven M. Buco†

Southern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124

A rapid and simple method was developed to determine phytic acid (from 1.64% to 2.99%) in dehulled cottonseed kernels. Ground samples of 14 varieties of cottonseed were directly analyzed by near-infrared reflectance and by an ion chromatography reference method. Analysis of variance revealed that the highest F value of 60.6 was obtained with a wavelength combination of 1594 and 1226 nm. A correlation, R, of 0.88 was obtained between phytic acid values obtained by near-infrared reflectance and those obtained by ion chromatography of acid extracts with a standard error of 0.118. The new method obviates the need for an extraction step, which may take several hours, that is required in all previous methods.

The market value of grains and oilseeds is based on their proximate composition. Water, protein, and lipid contents of many agricultural commodities in worldwide commerce are now determined by near-infrared analysis (Wetzel, 1983). The phytic acid content of the seed could be used as an additional factor in pricing, because phytate [myoinositol hexakis(phosphate)], which is present in all seeds, can function as an antinutrient when consumed in excess. The major products derived from cottonseed are oil, cake, and meal. In 1985 the United States produced 546 500 tons of cottonseed oil and 1 587 000 tons of cake and meal (USDA, 1987). The cake and meal are currently used in animal feeds and in the future may be used to provide protein for human consumption.

In humans, major concern is over the bioavailability of minerals such as zinc, calcium, and iron, which are not readily absorbed when insolubilized as calcium phytate complexes (Wise, 1983). Phytic acid has also been linked to the inhibition of digestive enzymes such as protease (O'Dell and De Boland, 1976), lipase (Knuckles, 1988), and α-amylase (Knuckles and Betschart, 1987). In poultry feeds, a primary concern is the utilization of phytate phosphorus (Nelson, 1967; Scheideler and Sell, 1987).

In recent years the literature has been voluminous with methods for measuring phytic acid. Originally iron precipitation methods were used (Thompson and Erdman, 1982). A step-gradient ion-exchange procedure has been adopted as the official method of the Association of Official Analytical Chemists (Harland and Oberleas, 1986). While these also measure polyphosphates other than phytate (Phillippy et al., 1988), specific methods utilizing HPLC (Phillippy and Johnston, 1985) and NMR (Mazzola et al., 1986) are also available. All of the above methods require a time-consuming acid extraction prior to analysis. We report here a rapid, direct near-infrared (near-IR) analysis method for the determination of phytic acid in ground cottonseed that is simple to perform.

EXPERIMENTAL SECTION

Materials. Nineteen samples of whole fuzzy cottonseed were obtained from the National Cotton Variety Testing Program and represent fourteen varieties from three crop years grown in four regions of the United States (Eastern, Delta, Plains, and Arizona). Three of the samples were glandless seed. Sample preparation included delinting and dehulling of the fuzzy seed and grinding the cottonseed kernels in a Krups home-style coffee mill to a fine powder. The powder was sieved through a 590-μm screen to obtain uniform particle distribution. Sodium phytate, obtained from Sigma Chemical Co., was used as a reference to characterize the absorbance of phytic acid in the cottonseed powder.

Determination of Phytic Acid by Near-Infrared Reflectance. About 8 g of the milled sample was required to fill the sample cup for presentation to the instrument. Care was taken to ensure consistency of sample density when loading each sample. A Neotec 6350 scanning monochromator (Pacific Scientific) was used to collect the spectra in the near-infrared region. Spectral data for each sample, after 50 scans, were recorded at 2-nm intervals from 1100 to 2498 nm. Absorbance from the near-IR reflectance mode is described in terms of log $(1/R)$. Second derivatives of the log $(1/R)$ data were used to determine the wavelengths and equation constants for the calibration sample set.

† Present address: Statistical Resources, Inc., 7332 Highland Rd., Baton Rouge, LA 70808.

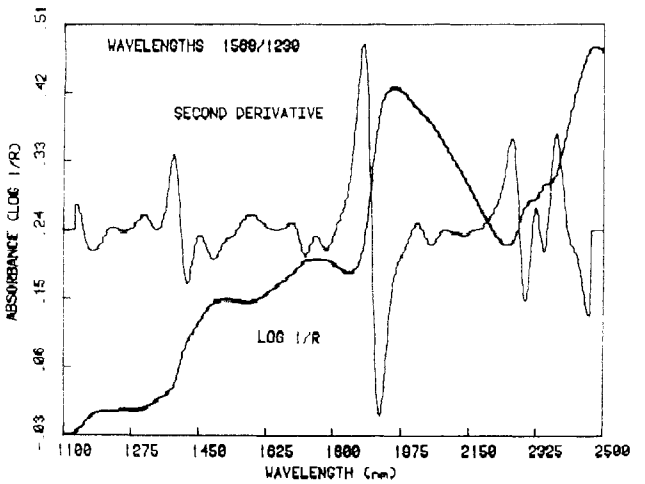

Figure 1. Spectrum of sodium phytate showing the log $(1/R)$ and second deriative of log $(1/R)$ for all 700 data points in the near-IR region.

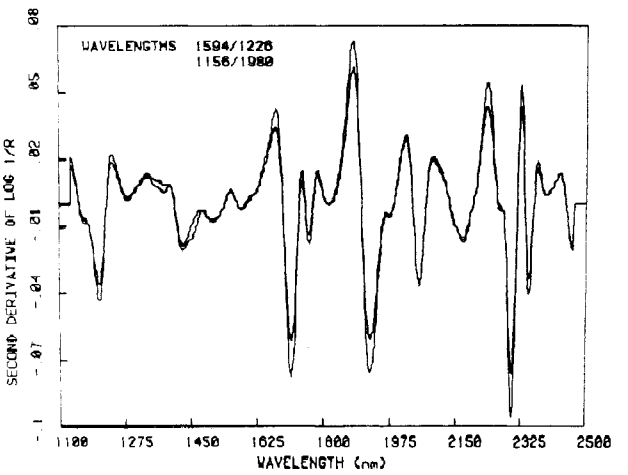

Figure 2. Comparison of the second-derivative plot for cottonseed containing high and low phytic acid contents.

Determination of Phytic Acid by Ion Chromatography or Spectrophotometry. Extracts were prepared by the method of Latta and Eskin (1980) in which cottonseed samples (300 mg) were shaken for 1 h at 25 °C with 0.65 N HCl (3 mL). The mixture was then centrifuged and the supernatant filtered through a 0.45-μm Millex filter for analysis by ion chromatography (Phillippy and Johnston, 1985) or spectrophotometry (Latta and Eskin, 1980). Duplicate test solutions of each extract were made by diluting 100 μL of extract with 400 μL of H_2O and were analyzed by ion chromatography (Phillippy and Johnston, 1985).

In the spectrophotometric method (Latta and Eskin, 1980), an aliquot (1 mL) of the 0.65 N HCl extract was applied to a column of Dowex AG1-X8 resin (Cl form, 200–400 mesh, 1 g). Inorganic phosphorus was eluted with 0.1 M NaCl (15 mL) followed by phytate phosphorus with 0.7 M NaCl (15 mL). An appropriate voluem of the 0.7 M NaCl eluant was made to 3 mL with water and mixed with modified Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in water, 1 mL). The absorbance of the mixture was read at 500 nm.

Statistical Analysis. Stepwise linear regression techniques were applied to spectra and "wet chemistry" data to determine wavelengths that correlated with phytic acid. The F value and standard error of calibration (SEC) were statistical considerations in generating the performance equation.

RESULTS AND DISCUSSION

The near-infrared spectrum of sodium phytate and its second derivative are shown in Figure 1. Absorbances at wavelengths of 1568 and 1230 nm in combination as a "best" pair are highly correlated, $R = 0.998$, with the phytic acid content by the ion chromatography method. The procedure was further substantiated when these wavelengths were identified with phytic acid as a constituent in cottonseed. The derivatives of the log $(1/R)$ of the spectra were computed for the 700-nm wavelengths in the scan. Segment size (20 nm) and distance between segments (20) were specified for this mathematical treatment.

Figure 2 is a second-derivative plot of the two samples that contained the highest (2.99%) and lowest (1.64%) phytic acid contents in the cottonseed for the sample set. The strong absorbance at 1226-nm wavelength shows significant differences between the samples. The ratio of absorbance at 1594 nm to absorbance at 1226 nm in combination with a ratio of absorbancies of 1156–1980 nm shows significant multiple correlation ($R = 0.952$) with the amount of phytic acid constituent in cottonseed whether the cottonseed be glanded or glandless.

Table I lists the wavelengths and statistical data for both the commercially prepared sodium phytate and phytic acid as a constituent in cottonseed as determined by near-IR/ion chromatography methods. The 1594/1226 nm wavelength combination indicates a significant correlation, $R = 0.884$, and a relatively low standard error of calibration, 0.118. By comparison, the standard deviation of differences between reference measurements on duplicate samples is 0.145. More than two wavelengths would overfit the data. The F statistic increased from 27.6 to 60.6 when the 1226-nm wavelength was added in combination to the 1594-nm absorption. Other wavelengths were searched to decrease standard error and increase the correlation coefficient. Equation 1 was used to calculate the phytate contents of the samples.

Table I. Regression Results

λ, nm	correln coeff	F	std error	eq const
Near-IR: Phytic Acid from Commercial Source				
1568/1230	0.998	926.9	0.007	$K(0) = 100.625$
				$K(1) = -0.657$
Ion Chromatography Method: Phytic Acid from Cottonseed				
1594	0.786	27.6	0.156	$K(0) = 2.923$
				$K(1) = 609.326$
1594/1226	0.884	60.6	0.118	$K(0) = 2.840$
				$K(1) = -0.793$
1594/1226 + 1156	0.932	10.5	0.094	$K(0) = 3.125$
				$K(1) = -0.935$
				$K(2) = 86.209$
1594/1226 + 1156/1980	0.952	6.5	0.079	$K(0) = 3.233$
				$K(1) = -1.037$
				$K(2) = -0.496$
Spectrophotometric Method: Phytic Acid from Cottonseed				
1540	0.838	40.0	0.190	$K(0) = 3.151$
				$K(1) = 699.417$
1536/2374	0.879	58.0	0.165	$K(0) = 4.354$
				$K(1) = 8.809$
1536/2374 + 1820	0.920	7.6	0.141	$K(0) = 4.194$
				$K(1) = 7.918$
				$K(2) = 314.405$
1536/2374 + 1578/1990	0.945	12.9	0.118	$K(0) = 4.382$
				$K(1) = 6.667$
				$K(2) = 0.233$

$$\% = K(0) + K(1)W_1/W_2 + K(2)W_3/W_4 \qquad (1)$$

While four wavelengths gave a higher correlation between methods, the two-wavelength equation was chosen to minimize the effect of overfitting the calibration equation to the small set of 19 samples.

Good correlation ($R = 0.879$) was obtained between near-IR and the amount of phytic acid determined with

Wade reagent by spectrophotometry (Latta and Eskin, 1980), resulting in a two-wavelength equation (1536/2374). The table shows a standard error of 0.165 and an F value of 58.0. Although different from wavelengths determined by the ion chromatography reference method, the near-IR wavelengths determined from the spectrophotometric method are considered to be within the phytic acid band. However, the 0.7 M NaCl eluant from the anion-exchange resin contains organic phosphorus compounds in addition to phytic acid. This was shown by the fact that the phytic acid content by the spectrophotometric method was consistently greater than that found in ion chromatography. In addition, the correlation between these two methods was only 0.705. Though statistically significant at the $P < 0.05$ level, this level or correlation is insufficient for precise prediction of phytic acid content. Therefore, calibration of the near-IR procedure is best done by ion chromatography.

In addition to measuring the phytic acid, near-infrared reflectance is a reliable method for measuring protein, moisture, and free fatty acids in ground cottonseed (Madacsi, unpublished results). Correlations between standard and near-infrared methods for these components were all greater than 0.97.

The near-infrared method is not specific for phytic acid, and therefore calibration curves must be made by a standard method, such as ion chromatography. A calibration is limited to one particular commodity, such as cottonseed, where matrix effects are similar. The minimum number of calibration points required can be determined statistically. Near-infrared reflectance should also prove useful for other seeds, such as soybeans, from which foods containing significant amounts of phytic acid are made for adult and infant human consumption.

Once a valid method has been established with a continuous-wavelength near-IR instrument, an inexpensive instrument, which uses fixed wavelength filters, together with a set of samples of known phytic acid could be used for rapid, routine determination of phytic acid in seeds for quality control.

INTERPRETIVE SUMMARY

Phytic acid is present in cottonseed and reduces the nutritive value of this feedstuff. Current methods for measuring the amount of phytic acid in seeds are time-consuming and laborious. A method using near-infrared spectroscopy has been developed for measuring phytic acid. The method is rapid and simple and could be used in the feed marketing system with relatively inexpensive instrumentation.

Registry No. Phytic acid, 83-86-3.

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