A New Method for Determining Gossypol in Cottonseed Oil by FTIR Spectroscopy

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ABSTRACT: A new method was developed to determine the gossypol content in cottonseed oil using FTIR spectroscopy with a NaCl transmission cell. The wavelengths used were selected by spiking clean cottonseed oil to gossypol concentrations of 0–5% and noting the regions of maximal absorbance. Transmittance values from the wavelength regions 3600–2520 and 1900–800 cm^{-1} and a partial least squares (PLS) method were used to derive FTIR spectroscopic calibration models for crude cottonseed, semirefined cottonseed, and gossypol-spiked cottonseed oils. The coefficients of determination (R^2) for the models were computed by comparing the results from the FTIR spectroscopy against those obtained by AOCS method Ba 8-78. The R^2 were 0.9511, 0.9116, and 0.9363 for crude cottonseed, semirefined cottonseed, and gossypol-spiked cottonseed oils, respectively. The SE of calibration were 0.042, 0.009, and 0.060, respectively. The calibration models were cross-validated within the same set of oil samples. The SD of the difference for repeatability and accuracy of the FTIR method were better than those for the chemical method. With its speed (*ca.* 2 min) and ease of data manipulation, FTIR spectroscopy is a useful alternative to standard wet chemical methods for rapid and routine determination of gossypol in process and/or quality control for cottonseed oil.

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The cotton plant is grown mainly for its fiber, and the seed oil produced is a by-product of the cotton industry. Although cotton breeders are mainly concerned about fiber quality, they are also trying to enhance the oil quality by reducing the gossypol content. Gossypol is a natural toxin in the cotton plant that protects it from insect damage, and it is also toxic to humans; in addition, it gives the crude oil its strong brownish-red color. It is a biologically active phenolic pigment that exists in three tautomeric forms: gossypol–aldehyde ($C_{30}H_{30}O_8$; M.W. 518.6) (Scheme 1), gossypol–lactol, and gossypol–cyclic carbonyl (1). The level of gossypol in crude solvent-extracted oil is 0.05–0.42%, and in crude screw-pressed oil 0.25–0.47% (2). Although almost all gossypol is removed during refining (3) to prevent the risk of toxicity, a sufficient amount remains to affect the keeping quality of the oil: The rate of darkening in the bleached oil is directly proportional to its gossypol content as well as to the time and temperature of storage (4). Historically,

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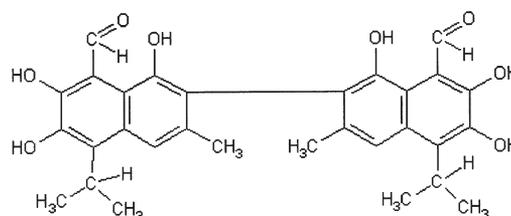
SCHEME 1

there has been little concern over gossypol in cottonseed oil, but interest in it has been spurred by its recent implication as a fertility control agent (5).

A breakthrough of sorts in the production of stable-color oil and high-quality meal was made with the introduction of glandless cotton (6). Unfortunately, without gossypol, cotton is also more susceptible to pests, and glandless cotton has not been wholly accepted for planting. Because the oil produced from glandless cottonseed may still have some glands containing gossypol, it must be analyzed for its gossypol content.

With the current high quality of refined oil, the method for determining gossypol developed by Yabe *et al.* (7) is being used at its lower limit, and gossypol often is not detectable. Other methods of analysis have been tried in a search for greater sensitivity, for example, HPLC (8). Nomeir and Abou-Donia (9) reported on the qualitative and quantitative analysis of gossypol by HPLC and its stability in various solvents. AOCS (10) has adopted methods for determining free and total gossypol that use a spectrophotometer or colorimeter equipped with a filter for maximal transmittance at 440–460 nm.

FTIR spectroscopy has been used for measurement of moisture content and iodine value in palm oil (11,12). In conjunction with chemometric techniques, FTIR data can be used to quantify, classify, and enumerate the differences between samples (13). In this work FTIR spectroscopy has been used to determine gossypol in cottonseed oil.

MATERIALS AND METHODS

Materials. Crude, refined, and semirefined cottonseed oils were obtained from Sudan through the National Oilseed Processing Research Institute. All the reagents used were of analytical grade. Gossypol was from Sigma Chemical Company (St. Louis, MO). Forty-five freshly refined, semirefined, and crude cottonseed oil sets (of 15 samples each) were prepared. The refined oil samples were spiked with gossypol over the range

0–5% (50,000 ppm) as standards for calibration. However, the crude and semirefined oil samples already contained unknown amounts of gossypol. Therefore, we randomly added known amounts of refined oil to make some sort of dilution regarding gossypol content. Chemical analysis was used to determine the gossypol content of each sample.

Chemical method. The total gossypol content in the spiked cottonseed oil was determined according to AOCS method Ba 8-78 (10).

Instrumental method. The instrument used for analysis was a PerkinElmer 1725 series FTIR spectrometer (PerkinElmer Corporation, Norwalk, CT) equipped with a room temperature deuterated triglycine sulfate detector and controlled by a PerkinElmer 7300 PC. The software used for collecting the FTIR data was the Infrared Data Manager system. The instrument was purged with dry nitrogen and maintained with two automatic dehumidifiers to minimize CO_2 and water vapor interference. Melted drops of each standard were placed in a transmission cell with sodium chloride (NaCl) windows. The transmission path of 25 µm was adjusted using a polytetrafluoroethylene spacer. The cell was then placed in the cell holder in the FTIR spectrometer and the sample was scanned. The transmission cell was rinsed three times with acetone and then dried with a soft tissue before the next sample was put in. Calibration spectra were collected by 81 scans of each of the 45 standards at a resolution of 4 cm^{-1}, gain of 1.0, and strong apodization throughout the mid-IR region of 4000–600 cm^{-1}. The spectra were ratioed against the background air spectrum. All the samples were scanned in duplicate, with the spectra recorded as absorbance and/or transmittance at each data point. The absorbance spectra were stored on a disk as Joint Committee on Atomic and Molecular Physical Data—Data Exchange (JCAMP—DX) format files for subsequent chemometric analysis (14).

Statistical analysis. All experiments and/or measurements were duplicated. The relationships between each of the parameters obtained by the FTIR spectrum and standard chemical method were determined using the software Nicolet Turbo Quant IR-Calibration and Prediction Package, Version 1.1 (Nicolet Instrument Co., Madison, WI). By using this software, a partial least squares (PLS) regression statistical approach was developed for mathematical treatment of the FTIR-predicted data. The data were transferred to a Microsoft Excel 97 spreadsheet to derive regressions between the FTIR-PLS predicted and chemical analysis data. The similarity between the chemical data and PLS predictions of gossypol content for the samples

used in the calibration indicated the adequacy of the PLS model. Accuracy of the PLS model was assessed based on the smallest SE and the highest coefficient of determination (R^2) (15).

Validation. The set of standards was used for the cross-validation method, leaving out one sample at a time. Given the set of 15 calibration spectra, the PLS calibration was performed on 14 calibration spectra and, with this calibration, the concentration of the sample left out during calibration was predicted. This process was repeated until each sample had been left out once. The concentration of each sample was then predicted and compared with the known concentration of this reference sample. The predicted residual error sum of squares (PRESS) was computed from the error in prediction from the standards by validation and plotted as a function of the number of factors used in the calibration (16). PRESS values are indications of how closely a model fits the calibration data. The accuracy was assessed by the standard error of cross-validation (SECV) and R^2. Further, cross-validation results were employed to compute the mean difference (MD) and SD of difference for repeatability and accuracy between the chemical and FTIR-predicted gossypol contents.

RESULTS AND DISCUSSION

Table 1 shows the results obtained from chemical and FTIR analyses as means, SD, and relative SD (or CV) for total gossypol contents in the calibration sets of crude, semirefined, and refined gossypol-spiked cottonseed oil samples. With the AOCS method (10), the mean gossypol contents were 0.54, 0.05, and 2.62%, respectively. The FTIR spectroscopic analysis gave the corresponding values of 0.61, 0.07, and 2.84% (Table 1). The CV was high (poor precision) for the low gossypol content by both the AOCS and FTIR methods.

Spectra. Gossypol melts at 177–182°C and decomposes in the same temperature range, which makes it difficult to scan pure gossypol in liquid form. Figure 1 shows the spectra of gossypol-spiked cottonseed oil (A), pure cottonseed oil (B), and hence the difference spectrum (C) of gossypol in the frequency range 4000–600 cm^{-1}. In spectrum C (gossypol) there is a band at 3365 cm^{-1} for –OH associated in phenol, and bands at 3130 and 3015 cm^{-1} were assigned as unsaturated and aromatic (phenyl) –CH vibrations, respectively. The band at 2725 cm^{-1} suggests the presence of aldehydic –CH, and the bands at 2518, 1881, 1642, 1551, 1492, and 1404 cm^{-1} are due to aromatic aldehydes, phenyl ring stretching, and –C–OH in-plane bending. The weak but sharp bands at 1321, 1132, 982, 918, and 838 cm^{-1} were

TABLE 1
Gossypol Contents Determined by the AOCS and FTIR Methods

Sample	AOCS method			FTIR method		
	Mean[a]	SD	CV (%)	Mean[a]	SD	CV (%)
Crude cottonseed oil	0.54	0.040	7.41	0.61	0.050	7.87
Semirefined cottonseed oil	0.05	0.006	12.00	0.07	0.009	12.86
Gossypol-spiked cottonseed oil[b]	2.42	0.095	3.63	2.84	0.058	2.04

[a]Mean is in percentage.
[b]Refined oil.
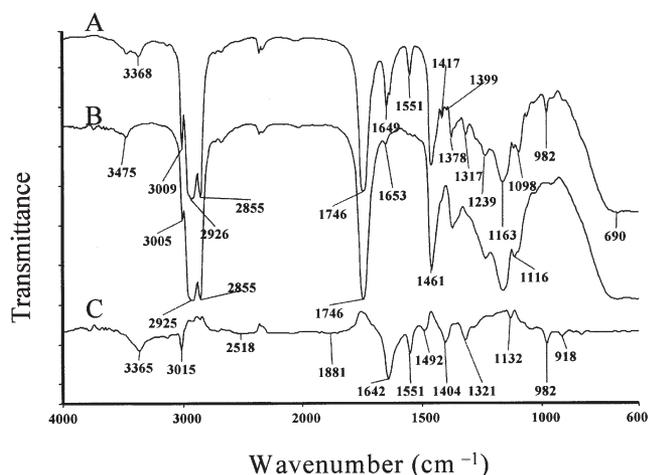
FIG. 1. IR spectra of (A) gossypol-spiked cottonseed oil, (B) pure cottonseed oil, and (C) the difference between A and B, representing gossypol only.

assigned to the in-plane –CH bending of phenyl, phenolic –OH, and two adjacent phenyls, and the band at 752 cm^{-1} to –CH out-of-plane bending (17–19). The bands in the spectrum of solid gossypol (Fig. 2) are at 1620 and 1551 cm^{-1} for aromatic C=C, 1443 and 1383 cm^{-1} for methyl bending vibrations, 1338 and 1303 cm^{-1} for the in-plane –CH bending of phenyl, 1124 and 1055 cm^{-1} for the in-plane –CH of 1,2-substituted phenyl, 967 and 915 cm^{-1} for the two adjacent phenyls, 876 and 844 cm^{-1} for the –CH of p-disubstituted phenyl, and 772 and 700 cm^{-1} for out-of-plane –CH and ring bending (17–19).

Development of calibration models. The correlation and variance spectra were used to select the best spectral regions for analysis. Only the data from the regions with features of interest were abstracted by the PLS software in order for the calibration standard(s) to be especially representative of the samples to be analyzed (20). The spectral regions with the highest correlation between the gossypol content and spectral response were set (based on the correlations) to include all the data from 3600 to 2520 cm^{-1} and from 1900 to 800 cm^{-1} for determining the gossypol content in the oil samples as shown in Table 2. The data from these regions produced the highest R^2 and low-

TABLE 2
Calibration and Cross-validation Using PLS of Wavenumber Regions 3600 to 2520 and 1900 to 800 cm^{-1} for Gossypol Content in Cottonseed Oil^a

Oil	Calibration		Validation	
	R^2	SEC	R^2	SECV
Crude cottonseed oil	0.9511	0.042	0.8954	0.063
Semirefined cottonseed oil	0.9116	0.009	0.9008	0.007
Gossypol-spiked cottonseed oil	0.9363	0.060	0.9272	0.066

^{a}PLS, partial least squares; R^2, coefficient of determination; SEC, standard error of calibration; SECV, standard error of cross-validation.

est SE of calibration and SECV for calibration and cross-validation, respectively.

A regression was derived by plotting the gossypol contents in the spiked cottonseed oil as determined by the AOCS method against the calibration set (Fig. 3). The equation of $y = 1.0075x + 0.1768$ ($R^2 = 0.9363$) had an intercept and slope not significantly different ($P > 0.05$) from 0.0 and 1.0, respectively. The calibration was evaluated by cross-validation, and Figure 4
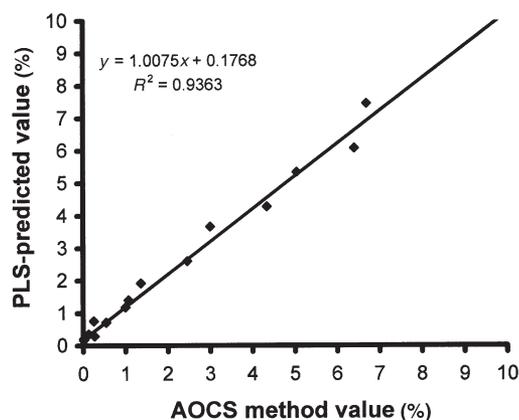
FIG. 3. Plot of gossypol content in gossypol-spiked cottonseed oil by AOCS chemical method (10) vs. partial least squares (PLS)-predicted values for calibration.
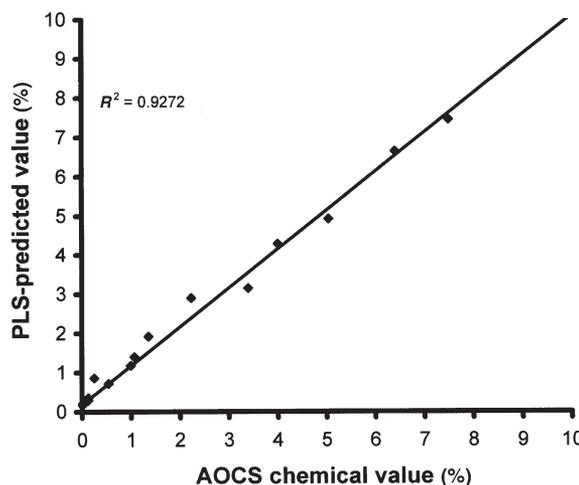
FIG. 4. Plot of actual vs. FTIR-predicted gossypol content in gossypol-spiked cottonseed oil for validation. For abbreviation see Figure 3.

FIG. 2. IR spectrum of solid gossypol.

TABLE 3
Calibration Statistics for Gossypol Content in Gossypol-Spiked Cottonseed Oil Using Data from Chemical Analysis and FTIR Spectroscopy[a]

Statistic	AOCS method	FTIR method
MDr	0.126	0.043
SDDr	0.025	−0.007
Min. value	0.00	0.015
Max. value	5.84	6.28
MDa	−0.220	
SDDa	0.033	

[a]MD, mean difference; SDD, SD of difference; r, repeatability; a, accuracy.
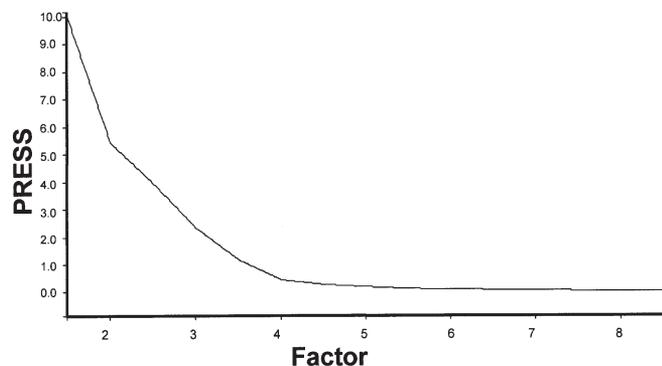
FIG. 5. Plot of predicted residual error sum of squares (PRESS) values vs. the different number of factors included in the cross-validation of the PLS model for gossypol content in gossypol-spiked cottonseed oil. For abbreviation see Figure 3.

shows the validation of the predictive model with the actual gossypol contents compared with the data obtained by the FTIR spectroscopy. The plot was linear with a slope of 0.965 and R^2 of 0.9272. The SECV was 0.066, which indicated the accuracy of the method was good, reflecting that the statistically predicted gossypol contents were very close to the actual values. Table 3 shows the calibration statistics for gossypol content in gossypol-spiked cottonseed oil obtained by the AOCS reference method and the new FTIR spectroscopic method. The FTIR method was comparable to the AOCS method, as it could detect down to 0.015%. Further, the MD for repeatability was lower for the FTIR method (0.043) than for the AOCS method (0.126). The MD for accuracy also showed the FTIR spectroscopy method obtained a higher mean (2.84) than the AOCS method (2.42) (Table 1). The maximum number of factors used to calculate the optimum PRESS was selected as 8 (half the number of standards + 1). The number of factors of 4 was selected as the optimum that resulted in the minimum PRESS value, as shown in Figure 5. In conclusion, FTIR spectroscopy was found to be a potential analytical tool for simple and rapid quantitative determination of gossypol in cottonseed oil.

ACKNOWLEDGMENTS

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