Spectrophotometric determination of total gossypol in cottonseeds and cottonseed meals

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 1984

Impact Factor: 5.64 · DOI: 10.1021/ac00265a010 · Source: PubMed

2 AUTHORS, INCLUDING:

B. S. Chandravanshi
Addis Ababa University
126 PUBLICATIONS **577** CITATIONS

SEE PROFILE

Spectrophotometric Determination of Total Gossypol in Cottonseeds and Cottonseed Meals

Atnafseged Admasu

Ethiopian Nutrition Institute, P.O. Box 5654, Addis Ababa, Ethiopia

B. S. Chandravanshi*

Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

A new spectrophotometric method for the determination of total gossypol content in cottonseeds and cottonseed meals has been developed. The method is based on the reaction of gossypol with 3-amino-1-propanol and its subsequent complexation with iron(III). The green colored iron(III)-bis(aminopropanol)-gossypol complex has a characteristic absorption maximum at 620 nm. The colored system obeys Beer's law in the concentration range of 4-80 ppm of gossypol. The effects of several experimental variables on the determination of gossypol have been studied and the stoichiometric composition of the complex has been determined. The new method has been found to be simple, rapid, and precise and to yield results comparable with the standard AOCS method. The method has been applied for the determination of total gossypol in several cottonseed and cottonseed meal samples.

Cottonseed products in the forms of oil and meal are widely used for human and animal food. However, the utilization of cottonseed products in human nutrition is limited by the presence of a phenolic compound, gossypol. This undesirable constituent is toxic to different animal species (*1-3*). The toxicity of gossypol also limits its own application in many areas (*4-9*).

Gossypol and its analogues are found primarily in the pigment glands of cottonseed. The structures of the three tautomeric forms of gossypol itself are given in Figure 1. Analogues of gossypol have a similar structure and can either occur naturally in cottonseeds or form as a result of storage and processing of cottonseed. The actual pigment composition of cottonseed pigment glands varies with the genetic type of the cotton plant and with environmental conditions during the development of cottonseed.

Several methods have been reported for the determination of gossypol in a variety of samples (*10*). The results of the analysis are reported in terms of percent of free gossypol, percent of bound gossypol, i.e., gossypol that is bound to protein, and percent of total gossypol by weight. Methods used for the analysis of gossypol include spectrophotometric methods (*11-16*), an NMR method (*17*), a chemiluminescent method (*18*), a gas-liquid chromatographic method (*19*), polarography, thin-layer chromatography, and paper chromatography (*20*). Most of these methods are tedious, time-consuming, and suffer from interferences due to the presence of the other pigments. Therefore, it is necessary to look for the development of a simple and precise method for the routine analysis of gossypol in cottonseed materials.

The reactions of gossypol with several metal ions including iron(III) have been reported in the literature (*21-23*). However, no fundamental investigation has been made on the application of such reactions for the quantitative determination of gossypol. Hence, it is worthwhile to study the complexation of gossypol with iron(III) in detail and, if possible, to develop a new method for the determination of gossypol in cottonseed samples.

The present investigation deals with the development of a new spectrophotometric method for the determination of total gossypol in cottonseeds and cottonseed meals. The method is based on the reaction of gossypol with 3-amino-1-propanol and its subsequent complexation with iron(III). The composition of the complex has been determined and the method has been applied successfully for the analysis of total gossypol in several cottonseeds and cottonseed meals. The results obtained by the new method have been compared with the AOCS method.

EXPERIMENTAL SECTION

Apparatus and Equipment. A Beckman Model 26 UV-VIS spectrophotometer equipped with a pair of 1-cm path length quartz cuvettes was used for absorbance measurements. A Beckman Expandometric SS-2 pH meter was used for pH measurements.

Reagents and Standards. All the chemicals used were of analytical reagent grade. A 1.79×10^{-2} M iron(III) solution was prepared by dissolving hydrated ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$, BDH AnalaR) in 40:60 (v/v) hexane–isopropyl alcohol containing a few drops of concentrated hydrochloric acid. The complexing agent solution was prepared by mixing 2 mL of 3-amino-1-propanol (E. Merck) with 10 mL of glacial acetic acid. The solution was cooled to room temperature and diluted to 100 mL with dimethylformamide in a volumetric flask.

A 4.821×10^{-3} M standard solution of gossypol was prepared by dissolving gossypol acetic acid (Sigma Chemical Co.) in complexing agent solution. A working solution of gossypol was prepared by diluting a 10-mL aliquot of standard solution to 100 mL with 40:60 (v/v) hexane–isopropyl alcohol in a volumetric flask.

Procedure for Extraction of Gossypol from Cottonseeds and Cottonseed Presscakes. Samples of seven varieties of cultivated cottonseeds, commonly grown under different conditions in Ethiopia, belonging to the *hirsutum* species of the genus *Gossypium* were collected from the Institute of Agricultural Research, Addis Ababa. Samples of cottonseed presscakes were collected from Addis Ababa Oil Mills. The dehulled cottonseeds and cottonseed presscakes were ground in a laboratory mill (Max Luscher AG Seon, Type 12E8) to pass through a 2-mm screen.

A weighed quantity of the sample containing 2–20 mg of total gossypol was transferred into a 100-mL Erlenmayer flask and 10 mL of complexing agent solution was added to it. The mixture was heated in a boiling water bath for 30 min, cooled, and diluted to about 30 mL with 40:60 (v/v) hexane–isopropyl alcohol. The solution was filtered and diluted to 50 mL with hexane–isopropyl alcohol in a volumetric flask.

Procedure for Determination of Gossypol. An aliquot of the solution containing 0.2–2.0 mg of gossypol was transferred into a 25-mL volumetric flask and 2 to 4 drops of 5 M hydrochloric acid were added to it. A 5-mL aliquot of iron(III) solution was added to the flask, the solutions were mixed well and then allowed to stand for 5 min. One milliliter of distilled water was added to the flask, and the solution was immediately diluted to volume

0003-2700/84/0356-0030$01.50/0

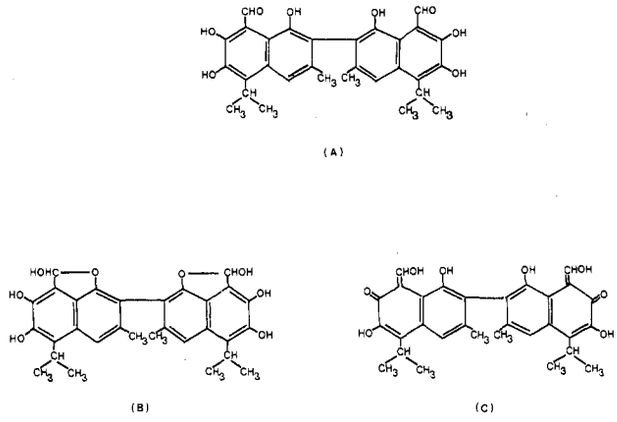

Figure 1. The three tautomeric forms of gossypol: (A) hydroxy aldehyde; (B) lactol; (C) cyclic carbonyl.

with 40:60 (v/v) hexane–isopropyl alcohol. The absorbance of the colored solution was measured at 620 nm against the hexane–isopropyl alcohol mixture as a reference. Calibration curves were prepared by measuring the absorbance of the solutions containing known amounts of gossypol by the same procedure.

RESULTS AND DISCUSSION

Colorimetric Reaction. Gossypol reacts with 3-amino-1-propanol to form bis(aminopropanol)gossypol. The bis(aminopropanol)gossypol reacts with iron(III) in the acidic medium to form a stable, green colored complex, freely soluble in hexane–isopropyl alcohol mixture. This sensitive color reaction forms a basis for the development of a new spectrophotometric method for the determination of gossypol.

Absorption Spectra. The green bis(aminopropanol)–gossypol–iron(III) complex showed an absorption maximum around 620 nm while iron(III) and 3-amino-1-propanol showed negligible absorption in the region of 450–700 nm. Thus, excess quantities of iron(III) and 3-amino-1-propanol did not interfere with the determination of gossypol. The absorption spectra are given in Figure 2.

Effect of pH. To obtain a complete complexation reaction with constant and maximum absorbance, the pH of the final solution should be in the range of 1.0–1.5. At higher and lower pH values the solutions exhibit no fixed absorption maxima in the visible region.

Effect of Concentration of Iron(III). A 1:10 molar ratio of gossypol to iron(III) was found to be necessary for the maximum color development. A large excess of iron(III) up to 500-fold molar excess has no adverse effect on the determination of gossypol.

Effect of Amount of Water. It has been found that a stable color intensity is developed only in the presence of a small amount of water. The amount of water can vary from 0.8 to 1.5 mL in the reaction mixture without any change in the color intensity for a total volume of 25 mL. The role of water may be to satisfy the coordination number of iron(III) in the complex.

Effect of Time and Temperature. It was found that the optimum color intensity is obtained within 5 min of reaction time and the absorbance of the colored solution remains constant for at least 3 h. Variation in the temperature of the colored solution from 20 to 30 °C did not produce any measurable change in the absorbance of the solution. At higher temperature, absorbance of the colored solution decreases slowly.

Beer's Law, Optimum Concentration Range, Sensitivity, and Molar Absorptivity. The colored system obeys Beer's law in the concentration range of 4–80 ppm of gossypol at 620 nm. The optimum concentration range for the determination as evaluated from the Ringbom plot (*24*) was found to be 8–64 ppm of gossypol. The effective molar absorptivity in terms of gossypol was found to be 6480 L mol^{-1} cm^{-1} and the photometric sensitivity (*25*) of the color reaction was found to be 0.08 μg of gossypol cm^{-2} at 620 nm.

Table I. Determination of Total Gossypol by Weight in Cottonseed Samples

type of cottonseed variety	% total gossypol by wt: new method	% total gossypol by wt: AOCS tentative method
Acala 1517/70	0.489	0.483
Albar 637	0.613	0.616
AMS 1.74	0.331	0.329
Acala 1517c	1.069	1.063
Acala 63-64 glandless	0.029	0.032
Frago glandless	0.415	0.411
Frago Bract glandless	0.168	0.166

Table II. Determination of Total Gossypol by Weight in Cottonseed Presscakes

type of sample	% total gossypol by wt: new method	% total gossypol by wt: AOCS tentative method
Nazrawi Oil Mills	0.689	0.685
Arsina Mertu Oil Mills	0.518	0.519
Teramaj Oil Mills	0.962	0.952
Akaki Oil Mills	0.850	0.845
United Oil Mills	0.758	0.757

Composition of Complex. The composition of the complex was determined by the continuous variations (*26*) and molar ratio (*27*) methods. In the continuous variations method the mole fractions of bis(aminopropanol)gossypol (BAPGP) and iron(III) were varied with a constant total molarity. The absorbances were plotted against the mole fractions of BAPGP. Maximum absorption occurred at a mole fraction of 0.33 (Figure 3). This suggests that the combining ratio of BAPGP to iron(III) in the complex is 1:2. This result was also supported by the molar ratio method.

These results are similar to the result obtained by Haas and Shirley (*23*) in which they have reported that the aldehyde groups and the adjacent 7-hydroxyl groups of the gossypol are the site of complexation. In the present investigation the bis(aminopropanol)gossypol was used for the complexation with iron(III); hence, the structure of the complex may be as given in Figure 4.

Precision. The precision of the newly developed method has been evaluated in terms of relative standard deviation. Ten independent analyses on samples each containing 36 ppm of gossypol gave a mean absorbance value of 0.450. The standard and relative standard deviations were found to be ±0.0030 and 0.67%, respectively. These results indicate that the method is precise and gives reproducible results.

Application of the Method. The validity of the newly developed method has been tested by determining the total gossypol content of cottonseed and cottonseed presscake samples. These results have been compared with the results obtained by the AOCS tentative method which is based on the extraction of gossypol with 3-amino-1-propanol in dimethylformamide, its subsequent reaction with aniline to form dianilinogossypol, and measurement of the colored reaction product at 440 nm (*11*). The data given in Tables I and II clearly indicate that the two methods give almost identical results.

To test the effect of interferences, known concentrations of pure gossypol, treated with 3-amino-1-propanol, were added to the gossypol extracts from cottonseeds and cottonseed presscakes. The total gossypol was then determined by the

Table III. Recovery of Gossypol Added to Cottonseed and Cottonseed Presscake Extracts

type of sample extract	amt of gossypol, g				% recovery of gossypol
	in extract	added	total	found	
cottonseed variety	0.513	0.302	0.815	0.809	99.3
cottonseed presscake	0.497	0.160	0.657	0.654	99.5

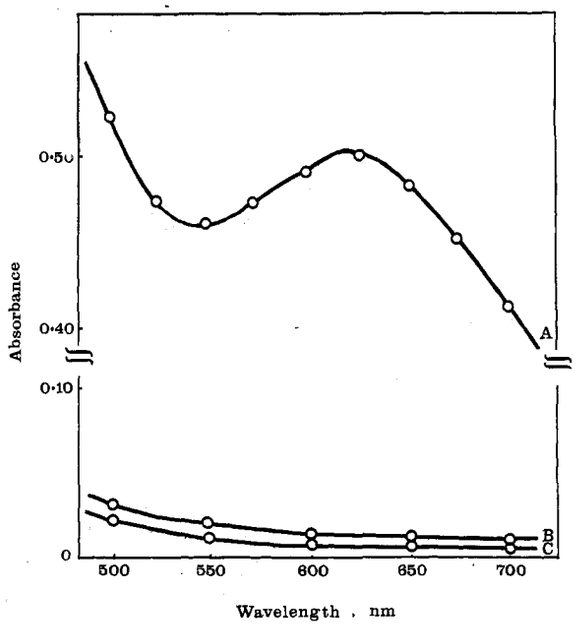

Figure 2. Absorption spectra of: (A) bis(aminopropanol)–gossypol–iron(III) complex (6.94 × 10^{-5} M gossypol); (B) iron(III) solution (1.79 × 10^{-2} M); (C) reagent blank in 40:60 (v/v) hexane–isopropyl alcohol.

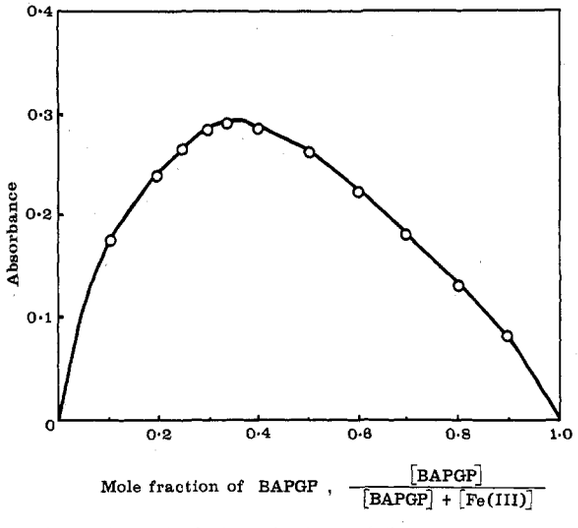

Figure 3. Job's method of continuous variations.

procedure described earlier. The results of the analysis of two samples are given in Table III. These results show satisfactory recovery of gossypol and establish the precision of the method. It has also been found that there is no shift in the absorption maximum of the iron(III) complex by the presence of other consituents in the gossypol extracts from the samples.

The precision of the method was also tested on the cottonseed meal extract containing a mean total gossypol content of 0.705%. Ten independent analyses gave a standard deviation of ±0.005 with a relative standard deviation of 0.71%.

Thus, the proposed method is sensitive, selective, and free from the interferences of other constituents present in cottonseeds. The method is simple, rapid, precise, and accurate. The reagents involved are cheap and readily available and the analysis can be completed within a short period of time. The

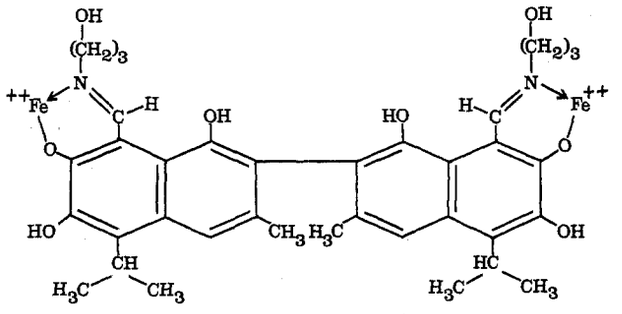

Figure 4. Structure of the bis(aminopropanol)–gossypol–iron(III) complex.

method can be applied for the determination of total gossypol in a variety of cottonseed and cottonseed product samples.

LITERATURE CITED

(1) Abou-Donia, M. B. *Residue Rev.* **1976**, *16*, 125–160.
(2) Liener, I. E. "Toxic Constituents of Plant Food-Stuffs"; Academic Press: New York, 1969; p 251.
(3) Qian, Shao-Zhan; Xu, Ye; Jing, Quang Wol *Yao Hsueh Hsueh Pao* **1979**, *14*, 116–119. *Chem. Abstr.* **1980**, *92*, 33909*f*.
(4) Askarov, M. A.; Dzhalilov, A. T.; Fatkhullaev, E.; Sukhinina, L. A. *Otkrytiya, Izobret., Prom. Obraztsy, Tovarnye Znaki* **1980**, *8*, 99–100. *Chem. Abstr.* **1980**, *92*, 216236*d*.
(5) Bickford, W. G.; Pack, F. C.; Castillon, L. E.; Mack, C. H. *J. Am. Oil Chem. Soc.* **1954**, *31*, 91–93.
(6) Bailey, A. "Cottonseed and Cottonseed Products"; Interscience: New York, 1948; p 148.
(7) Markman, A. L.; Rzhekhin, V. P. "Gossypol and its Derivatives"; Israel Program for Scientific Translations: Jerusalem, 1968; p 148.
(8) Wang, Nai-Gong; Lei, Hai-Peng *Chung-hua I Hsueh Tsa Chih (Peking)* **1979**, *59*, 402–405. *Chem. Abstr.* **1980**, *92*, 174961*d*.
(9) Liener, I. E. "Toxic Constituents of Plant Food-Stuffs"; Academic Press: New York, 1969; p 255.
(10) Pons, W. A. *J. Assoc. Off. Anal. Chem.* **1977**, *60*, 252–259.
(11) American Oil Chemists Society "Official and Tentative Methods"; American Oil Chemists Society: Champaign, IL, 1978; Tentative Method Ba 8-58.
(12) Crouch, Floyd W.; Bryant, Melton F. *Anal. Chem.* **1982**, *54*, 242–246.
(13) Pons, W. A.; Guthrie, J. D. *J. Am. Oil Chem. Soc.* **1949**, *26*, 671–676.
(14) Pons, W. A.; Hoffpauir, C. L.; O'Connor, R. T. *J. Am. Oil Chem. Soc.* **1951**, *28*, 8–12.
(15) Storherr, R. W.; Holley, K. T. *J. Agric. Food Chem.* **1954**, *2*, 745–747.
(16) Mathur, J. M. S.; Sharma, N. D.; Singh, M. *J. Food Sci. Technol.* **1972**, *9*, 138–140.
(17) Waiss, A. C.; Chan, B. G.; Benson, M.; Lukefahr, M. J. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 146–149.
(18) Aver'yanov, A. A.; Merzlyak, M. N.; Rubin, B. A. *Biokhimiya (Moscow)* **1978**, *43*, 1594–1601. *Chem. Abstr.* **1978**, *89*, 211079*h*.
(19) Raju, P. K.; Cater, C. *J. Am. Oil Chem. Soc.* **1967**, *44*, 465–466.
(20) Markman, A. L.; Rzhekhin, V. P. "Gossypol and Its Derivatives"; Israel Program for Scientific Translations: Jerusalem, 1968; Chapter 8.
(21) Ramaswamy, H. N.; O'Connor, R. T. *J. Am. Oil Chem. Soc.* **1968**, *45*, 841–844.
(22) Jonassen, H. B.; Demint, R. J. *J. Am. Oil Chem. Soc.* **1955**, *32*, 424–426.
(23) Haas, R. H.; Shirley, D. A. *J. Org. Chem.* **1965**, *30*, 4111–4113.
(24) Ringbom, A. *Z. Anal. Chem.* **1938**, *115*, 332–343.
(25) Sandell, E. B. "Colorimetric Determination of Traces of Metals", 3rd ed.; Interscience: New York, 1959; p 83.
(26) Job, P. *Ann. Chim. (Paris)* **1928**, *9*, 113–203.
(27) Yoe, J. H.; Jones, A. L. *Ind. Eng. Chem., Anal. Ed.* **1944**, *16*, 111–115.

RECEIVED for review April 19, 1983. Accepted September 1, 1983. The authors are thankful to the Chairman, Department of Chemistry, Addis Ababa University, Addis Ababa, Ethiopia, and Director of Ethiopian Nutrition Institute for providing the facilities. They are also thankful to the Swedish Agency for Research Cooperation with Developing Countries for the financial assistance.