Application of the Antimony Trichloride-Spectrophotometric Method to the Determination of Gossypol in Cottonseed and Cottonseed Products[1]

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Introduction

SEVERAL years ago Boatner *et al.* (1) described a spectrophotometric method for the quantitative determination of gossypol in cottonseed meats and meals which was based upon the absorption spectrum of the stable reaction product which antimony trichloride forms with gossypol in chloroform. The red-colored gossypol-antimony trichloride complex formed in this reaction exhibits a characteristic absorption spectrum in the visible wave length region having broad maxima at 520 and 380 mμ, and a minimum at 430 mμ, when measured with a Coleman monochromator spectrophotometer, which permits selection of a 10-mμ band. The existence of two maxima in the absorption spectrum of the gossypol-antimony trichloride reaction product permits mathematical characterization of the absorption in terms of the ratios of the extinction coefficients at the maxima and minimum. It is possible therefore to determine the specificity of the antimony trichloride reaction for gossypol in any mixture in which the pigment occurs by comparing the ratios of the maxima and minimum exhibited by the reaction product with those of the reaction product of pure gossypol.

The use of a mixture of aqueous ethanol and diethyl ether has recently been reported (2) to extract all of the gossypol from cottonseed and cottonseed meal within 10 minutes when a Waring Blendor is used, in contrast to 72 hours which is required for extraction of gossypol with diethyl ether in a soxhlet type extractor (3, 4), or 24 hours which is required for extraction of gossypol with chloroform by simple equilibration (1, 5). The use of a Waring Blendor eliminates the necessity for preliminary grinding or flaking of the seed. However, if finely ground or flaked seed is available, simple equilibration of these materials with aqueous ethanol effects complete extraction of gossypol within 10 minutes without the use of a Waring Blendor or the addition of ether (6, 7). It has also been shown that aqueous ethanol extracts gossypol quantitatively from defatted cottonseed meals (8) and from separated pigment glands prepared by the flotation method (9, 10).

A method was recently developed (5) for quantitatively extracting gossypol from extracts and solutions in which it occurs. In applying this method, gossypol is extracted with dilute aqueous alkali containing sodium dithionite ($Na_2S_2O_4$) to prevent oxidation. The alkaline extract is then acidified, and the precipitated gossypol is dissolved in chloroform prior to the application of the antimony trichloride test. This method has now been applied to isolation of gossypol from cottonseed oils and to extracts of cottonseed meats, meals, and pigment glands.

Procedure

Antimony Trichloride Test. The gossypol-antimony trichloride reaction with pure gossypol is carried out as follows: to 1 ml. of a chloroform solution of pure gossypol in a glass-stoppered absorption cell, there are added 1 drop of acetic anhydride and 5 ml. of a saturated solution of antimony trichloride in chloroform. The acetic anhydride is added to prevent the development of the hazes produced by the presence of moisture in the extracts. For the same reason it is desirable to mix the reagents directly in the absorption cells and thus avoid unnecessary exposure to atmospheric moisture. The volumes of reagents indicated were chosen to suit the capacity of the cells designed for the Coleman double monochromator spectrophotometer. Ten minutes after the reagents are mixed, the transmission at 380, 430, and 520 mμ is read against a chloroform blank.

For pure gossypol, the specific extinction coefficient ($E^{1\%}_{1.27\,cm.}$) at 520 mμ is 65.5 ± 1.9 in terms of the concentration of gossypol in the solution to which the antimony trichloride solution is added. R_a, the ratio of the extinction at 520 mμ to that at 430 mμ is 2.68 ± 0.23; and R_b, the ratio of the extinction at 520 mμ to that at 380 mμ, is 1.22 ± 0.07.

For determination of the concentration of gossypol in a given chloroform solution or extract the same procedure is followed except that one ml. of the solution or extract[3] is substituted for the gossypol solution. For determination of the specificity of the reaction, the transmission is read at 380, 430, and 520 mμ, in the order listed, as rapidly as possible, starting 10 minutes after the reagents are mixed. The values of R_a and R_b are calculated and compared with the values of R_a and R_b for the reaction product of antimony trichloride with pure gossypol.

Preparation of Samples. For extraction of gossypol from cottonseed meats and meals the samples should be ground to pass a U. S. No. 50 sieve or flaked to a thickness of 0.008 to 0.010 inch. Grinding or flaking to this degree is necessary in order to assure contact of the solvent with the glands in which all of the gossypol of cottonseed is segregated.

Chloroform Extraction. Weighed samples are equilibrated with measured volumes of chloroform for 24 hours at approximately 3°C.; the extracts are then filtered cold and the antimony trichloride test is applied to a 1-ml. aliquot of the filtrate within 48 hours after filtering. The size of sample employed for preparation of the extract depends upon the gossypol content of the sample. In the case of cottonseed meats,

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[3] The original solution or extract should be of such gossypol content that the transmission of the antimony trichloride reaction product at 380 and 520 mμ is 20 to 60%.

the gossypol content of which varies from about 1 to 3%, samples varying from 0.25 to 1.0 g. are equilibrated with 25 ml. of chloroform. When extracting gossypol from pigment glands, the gossypol content of which varies from 30 to 50%, 0.050-g. samples are equilibrated with 25 ml. of chloroform.

When using the Coleman spectrophotometer,[4] the gossypol content is calculated by means of Formula I.

$$\text{I. Per cent gossypol} = \frac{\frac{V_1}{G} \times \frac{V_2}{V_3} \times \log \frac{I_o}{I}}{65.5}$$

where V_1 is the volume of chloroform used for extraction; G is the weight of the sample extracted; V_2/V_3 is the dilution of the extract which may be used in order to avoid transmission values at 380 and 520 mμ of less than 20%; V_3 is the volume of the aliquot of the extract, and V_2 is the volume to which it is diluted; log I_o/I is the extinction at 520 mμ; and 65.5 is the value of $E^{1\%}_{1.27\,cm.}$ at 520 mμ of the reaction product of antimony trichloride with pure gossypol.

Aqueous Ethanol Extraction. For extraction of gossypol with 60% aqueous ethanol, samples of meals (ground to pass through a U. S. No. 50 sieve) are weighed into 200-ml. centrifuge bottles. To each bottle there is added 100 ml. of 60% ethanol (by volume). The mixture of sample and solvent is shaken vigorously and allowed to stand for 30 minutes.

After centrifugation, a 25-ml. aliquot of the extract is removed by pipette and placed in a centrifuge tube containing 25 ml. of chloroform and the two liquids are thoroughly mixed. For the purpose of transferring the gossypol dissolved in the ethanol solution to the chloroform, 100 ml. of distilled water is added, the mixture is shaken vigorously, and then centrifuged. A one-milliliter aliquot of the chloroform solution can be transferred directly from the centrifuge tube to the absorption cell if care is taken to avoid the presence of water in the pipette. The antimony trichloride test can then be applied as described above. Samples of pigment glands or meats (ground to pass through a U. S. No. 50 sieve) are weighed into 200-ml. centrifuge bottles. To each sample there is added 30 ml. of 30% aqueous ethanol (by volume). The mixture of sample and solvent is shaken vigorously and allowed to stand for a period of 10 minutes. In order to dissolve the resulting suspension of gossypol, 70 ml. of 72% aqueous ethanol (by volume) is added, thus producing a mixture which contains 60% of ethanol by volume. Gossypol is transferred from the aqueous ethanol extract to the chloroform by the procedure described above.

The size of the sample employed for preparation of the extract depends upon the amount of gossypol present in the sample. Relatively small samples (0.25 to 1.0 g.) are suitable for raw cottonseed meats, cooked meats, hexane- and other petroleum naphtha-extracted meals, the gossypol content of which varies from 0.5% to 2%. When analyzing samples of diethyl ether-extracted meals, hydraulic- or screw-pressed meals, or meals obtained by the gland flotation process, the gossypol content of which is usually very small (0.0006% to 0.08%), much larger samples (2.0 to 10.0 g.) must be used. When pigment glands are being analyzed, very small samples (0.025 to 0.10 g) are used. Occasionally, it is necessary to dilute the final chloroform solution before applying the antimony trichloride test.

The gossypol content of the original sample is calculated by means of Formula II.

$$\text{II. Per cent gossypol} = \frac{\frac{V_1}{G} \times \frac{C_1}{A} \times \frac{V_2}{V_3} \times \log \frac{I_o}{I}}{65.5}$$

where V_1 is the volume of aqueous ethanol used for extraction; G is the weight of sample extracted; C_1 is the volume of chloroform; A is the volume of aliquot of ethanol extract; V_2/V_3 is used only when it is necessary to dilute the chloroform solution; V_3 is the volume of the aliquot of the extract; V_2 is the volume to which the aliquot is diluted; log I_o/I is the extinction at 520 mμ; and 65.5 is the value of $E^{1\%}_{1.27\,cm.}$ at 520 mμ of the reaction product of antimony trichloride with pure gossypol.

Alkaline Extraction. If the antimony trichloride reaction is found not be specific for gossypol, the alkaline extraction method for gossypol must be applied to the chloroform extract, and the concentration of isolated gossypol then determined.

The method may be applied to chloroform extracts of meats, meals, or pigment glands, chloroform solutions of aqueous ethanol extracts, or chloroform solutions of crude cottonseed oils, either expressed or solvent-extracted. The method is applied as follows:

To a 5-ml. aliquot of the chloroform solution there is added 25 ml. of a 0.05 molar aqueous solution of NaOH, which contains 1% sodium dithionite ($Na_2S_2O_4$) by weight to prevent oxidative decomposition of gossypol in alkaline solution. The mixture is shaken vigorously at least 50 times and is then centrifuged. The acidic gossypol is extracted by the alkali whereas nonacidic components remain in the chloroform. Sodium gossypolate is stable in the solution for a period of about one-half hour. Quantitative recovery of gossypol from the sodium gossypolate solution is accomplished by mixing a 10-ml. aliquot of the alkaline extract with a measured volume of chloroform (2, 5, or 10 ml., depending upon the amount of gossypol present in the original extract), and acidifying the mixture with 6 to 8 drops of concentrated hydrochloric acid. After thorough agitation, this mixture is centrifuged, and an antimony trichloride test is applied to a one-ml. aliquot of the chloroform layer.

The gossypol content of the original sample is calculated by means of Formula III.

$$\text{III. Per cent gossypol} = \frac{\frac{V_1}{G} \times \frac{25}{5} \times \frac{C_2}{N} \times \log \frac{I_o}{I}}{65.5}$$

where V_1 is the volume of solvent (chloroform or aqueous ethanol) used for preparation of the original

[4] When instruments are used which are equipped with cuvettes of different capacity, the values of Ra, Rb, and E 1% at 520 mμ for pure gossypol should be determined with the appropriate volumes of antimony trichloride and gossypol solutions. For example when the test is carried out with Beckman spectrophotometers which are equipped with glass-stoppered cuvettes of 3-ml. capacity and 1-cm. optical path, 1 drop of acetic anhydride and 2 ml. of antimony trichloride solution are added to 1 ml. of gossypol solution.

TABLE I

Determination of Gossypol in Cottonseed Pigment Glands

No.	Solvent used for extraction[1]	Treatment of chloroform solution	Antimony trichloride reaction product			
			R_a	R_b	$E^{1\%}_{1.27\,cm.}$ at 520 mμ	Gossypol content[2]
						%
1	Chloroform	None	2.48	1.18	24.5	35.8
1	Chloroform	Acidified with conc. HCl	2.75	1.26	22.0	33.4
1	30-72% ethanol	None	2.15	1.09	32.3	47.4
1	30-72% ethanol	Extracted with NaOH	2.04	0.94	30.6	46.8
2	Chloroform	None	2.82	1.39	21.4	32.9
2	30-72% ethanol	None	2.40	1.15	24.6	37.6
3	Chloroform	None	2.43	1.48	21.6	32.9
3	30-72% ethanol	None	2.20	1.18	24.5	37.2
3	30-72% ethanol	Extracted with NaOH	2.24	0.96	24.4	37.3
4	Chloroform	None	2.51	1.22	25.4	38.8
4	Chloroform	HCl	2.45	1.30	25.1	38.2
5	Chloroform	None	2.33	1.30	30.7	46.9
5	Chloroform	HCl	2.77	1.44	29.9	45.6
6	Chloroform	None	2.34	1.31	22.5	34.3
6	30-72% ethanol	HCl	2.42	1.37	29.1	33.8
6	Chloroform	None	2.36	1.12	23.1	35.3
7[3]	Chloroform	None	2.40	1.27	6.14	9.4
7[3]	30-72% ethanol	None	2.66	1.27	30.8	46.9

[1]Extractions with chloroform were accomplished by equilibration of pigment glands with chloroform for 24 hours at 38°F. Extractions with ethanol were carried out by mild agitation for 10 minutes at room temperature of a suspension of pigment glands in 30% aqueous ethanol, followed by addition of 72% aqueous ethanol.

[2]Average values obtained from antimony trichloride test applied to two to five independently prepared extracts.

[3]Pigment glands prepared from defatted meal. Note incomplete extraction of gossypol with $CHCl_3$. Castillon, Hall, and Boatner, J. Am. Oil Chem. Soc., *25*, 233-236 (1948).

TABLE II

Determination of Gossypol in Uncooked Cottonseed Meats

No.	Preparation of meats	Solvent for extraction[1]	Treatment of chloroform solution	Antimony trichloride reaction product				
				R_a	R_b	$E^{1\%}_{1.27\,cm.}$ at 520 mμ	Gossypol content %	Selectivity of test
1	Ground	Chloroform	None	2.32	1.35	1.65	2.52	Good
1	Ground[3]	Chloroform	None	2.34	1.05	2.45	3.73	Good
2	Ground	Chloroform	None	2.39	1.29	1.27	1.94	Good
2	Ground[3]	Chloroform	None	2.12	1.04	1.30	2.09	Good
3	Ground	Chloroform	None	2.34	1.29	1.27	1.94	Good
3	Ground[3]	Chloroform	None	1.75	0.82	1.33	2.03	Poor
4	Ground	Chloroform	None	2.15	1.14	1.11	1.70	Good
4	Ground[3]	Chloroform	None	1.46	0.85	1.22	1.86	Poor
5	Flaked	Chloroform	None	2.10	1.26	1.30	1.99	Good
5	Flaked	30-72% ethanol	Aq. NaOH	2.15	1.12	1.38	2.11	Good
6	Ground	Chloroform	None	1.99	1.02	1.24	1.90	Fair
6	Ground	Chloroform	Aq. NaOH	2.35	1.01	1.01	1.54	Good
7	Ground	Chloroform	None	.....	.....	0.98	1.50	Good
7	Ground	30-72% ethanol	None	.....	.....	0.99	1.51	Good

[1]Extractions with chloroform were accomplished by equilibration of meal and solvent for 24 hours at 38°F. Extractions with ethanol were carried out by mild agitation for 10 minutes at room temperature of a suspension of meal in 30% aqueous ethanol, followed by addition of 72% aqueous ethanol.

[2]Average values obtained from antimony trichloride test applied to two independently prepared extracts.

[3]Meats from seed stored for 6 months; note variation of gossypol content during storage. Boatner, Castillon, Hall, and Neely, J. Am. Oil Chem. Soc., *25*, still in press (1948).

TABLE III

Determination of Gossypol in Cooked Cottonseed Meats

No.	Cooking conditions	Solvent for extraction[1]	Treatment of chloroform solution	Antimony trichloride reaction product			
				R_a	R_b	$E^{1\%}_{1.27\,cm.}$ at 520 mμ	Gossypol content %
1	Dry, 104°C. for 1 hour	Chloroform	None	1.21	0.91	0.17	0.26
1	Dry, 104°C. for 1 hour	Chloroform	Acidified with conc. HCl	1.23	0.94	0.18	0.27
1	Dry, 104°C. for 1 hour	30-72% ethanol	Extracted with NaOH	1.79	1.29	0.94	1.43
2	10% added H_2O for 1 hour at 104°C.	Chloroform	None	1.38	0.88	0.17	0.27
2	10% added H_2O for 1 hour at 104°C.	30-72% ethanol	Extracted with NaOH	2.03	1.00	0.97	1.48
3	5% added H_2O at 104°C. for 1½ hours	Chloroform	None	1.48	0.78	0.14	0.215
3	5% added H_2O at 104°C. for 1½ hours	30-72% ethanol	Extracted with NaOH	2.13	1.11	0.86	1.31
4	10% added H_2O at 118°C. for 1½ hours	30-72% ethanol	Extracted with NaOH	2.52	0.98	0.58	0.889

[1]Extractions with chloroform were accomplished by equilibration of meal with the solvent for 24 hours at 38°F. Extractions with ethanol were carried out by mild agitation for 10 minutes at room temperature of a suspension of meal in 30% aqueous ethanol, followed by addition of 72% aqueous ethanol.

extract; G is the weight of sample; 25 is the *invariable volume* of NaOH; 5 is the *invariable volume* of chloroform solution extracted with NaOH; C_2 is the volume of chloroform to which the alkali-extractable material is transferred; N is the volume of the aliquot of alkaline extract used to transfer the alkali-extractable material to chloroform; log I_o/I is the extinction at 520 mμ; and 65.5 is the value of $E^{1\%}_{1.27\,cm.}$ at 520 mμ of the reaction product of antimony trichloride with pure gossypol.

When aqueous ethanol is used for extraction, the term, $\frac{C_1}{A}$, in Formula II should be included in Formula III. In the case of alkaline extraction of oils ½ is the volume of chloroform in which the oil was dissolved and G is the weight of oil dissolved.

Results

Pigment Glands. As may be seen by reference to Table I, complete extraction of gossypol from pig-

TABLE IV

Determination of Gossypol in Solvent-Extracted Meals

No.	Description of raw material	Extraction conditions		Treatment of chloroform solution	Antimony trichloride reaction product				
		Solvent for extraction	Time		R_a	R_b	$E^{1\%}_{1.2\,cm.}$ at 520 mμ	Gossypol %	Selectivity of test
1	Hexane-extracted flakes	Chloroform	24 hours	None	1.55	0.87	0.815	1.40	Poor
1	Hexane-extracted flakes	Chloroform	24 hours	Acidified with HCl	.....	.....	1.02	1.57	Poor
1	Hexane-extracted flakes	60% ethanol	10 minutes	None	.....	.....	1.25	1.92	Poor
2	Diethyl-ether-extracted flakes	Chloroform	24 hours	None	0.67	0.56	0.009	0.01	Poor
2	Diethyl-ether-extracted flakes	Chloroform	24 hours	Extracted with alkali	.....	.....	0.00	None	.....
3	Diethyl-ether-extracted flakes	Chloroform	24 hours	None	0.60	0.65	0.015	0.025	Poor
3	Diethyl-ether-extracted flakes	60% ethanol	30 minutes	None	0.71	0.75	0.023	0.035	Poor
4	Light petroleum naphtha-extracted flakes	Chloroform	24 hours	None	2.34	1.30	0.94	1.45	Good
4	Light petroleum naphtha-extracted flakes	30-72% ethanol	10 minutes	None	2.12	1.16	1.18	1.81	Good
5	Pigment glands removed by flotation process	Chloroform	24 hours	None	0.61	0.57	0.015	0.023	Poor
5	Pigment glands removed by flotation process	60% ethanol	30 minutes	None	0.63	0.51	0.022	0.035	Poor
6	Pigment glands removed by flotation process	60% ethanol	30 minutes	None	0.725	0.725	0.041	0.063	Poor
6	Pigment glands removed by flotation process	60% ethanol	30 minutes	Extracted with alkali	0.822	0.559	0.026	0.039	Poor
7	Pigment glands removed by flotation process	60% ethanol	30 minutes	Extracted with alkali	.....	.....	0.00	None	.....

ment glands is not obtained by contact for 24 hours with chloroform but is obtained in 10 minutes with aqueous ethanol. Alkaline extraction of extracts of pigment glands is not necessary as specific antimony trichloride tests are obtained with the original extracts.

Cottonseed Meats. It has also been found, as shown in Table II, that complete extraction of gossypol from cottonseed meats can be obtained in 24 hours with chloroform and in 10 minutes with aqueous ethanol. Specific antimony trichloride tests are obtained with extracts of fresh seeds. However, in some cases the antimony trichloride tests on extracts of stored seed are not specific, as may also be seen by reference to Table II, and alkaline extraction is necessary for accurate determination of gossypol.

Cooked Cottonseed Meats. Equilibration of cooked cottonseed meats with chloroform for 24 hours yields incomplete extraction of gossypol, and the antimony trichloride tests obtained with such extracts are not specific for gossypol. Gossypol is completely extracted from cooked cottonseed meats by contact with aqueous ethanol for 10 minutes, as may be seen from the data in Table III. Application of the antimony trichloride method to chloroform solutions of the ethanol extracts of cooked cottonseed meats yields unreliable results for gossypol as may be seen from the ratios R_a and R_b shown in Table III. However, the method is applicable when used in conjunction with the alkaline extraction procedure as is evident from the ratios also shown in Table III.

Solvent-Extracted Meals. As may be seen from Table IV, complete extraction of the gossypol which is present in solvent-extracted meals is usually not obtained in 24 hours at 38°F. by equilibration with chloroform. Contact with aqueous ethanol for 10 minutes is usually adequate for complete extraction of gossypol from solvent-extracted meals of relatively high gossypol content, that is, for meals which have been defatted with light petroleum naphtha (pentane-hexane).

The antimony trichloride tests obtained with chloroform solutions of the ethanol extracts are usually specific for gossypol in the case of meals which have been defatted at moderate temperatures with light petroleum naphtha whereas alkaline extraction must be used for determination of gossypol in meals which have been defatted at relatively high temperatures with petroleum naphthas of high boiling point range (hexane).

In the case of meals which have been exhaustively extracted with diethyl ether and meals which have been freed of pigment glands by the flotation process (9, 10), the antimony trichloride test applied to alkali-extractable components of ethanol extracts gives either negative reactions or reactions which are not specific for gossypol.

Hydraulic and Screw-Pressed Meals. Application of the antimony trichloride test to the alkali-extractable components of ethanol or chloroform extracts of expressed cottonseed meals gives reactions which are not characteristic of gossypol. The values for the contents of gossypol shown in Table V have been calculated on the assumption that all of the absorption at 520 mμ is attributable to the reaction product of gossypol with antimony trichloride. In view of the low values obtained and the non-specificity of the ratios R_a and R_b it is probable that there is little, if any, native gossypol in such meals.

Solvent-Extracted Oils. As shown by the typical example given in Table VI, direct application of the antimony trichloride test to chloroform solutions of oils obtained by solvent-extraction of cottonseed gives reactions which are not specific for gossypol. Application of the test to the alkali-extractable material gives reactions which are specific for gossypol only

TABLE V

Determination of Gossypol in Expressed Cottonseed Meals

No.	Conditions of extraction		Treatment of chloroform solution	Antimony trichloride reaction product			
	Solvent	Time		R_a	R_b	$E^{1\%}_{1.2\,cm.}$ at 520 mμ	Gossypol content %
1 HP[a]	Chloroform	48 hours	None	0.574	0.542	0.052	0.08
1 HP	Chloroform	48 hours	Extracted with NaOH	1.03	0.716	0.017	0.03
2 HP	Chloroform	24 hours	None	0.885	0.675	0.061	0.093
3 HP	60% ethanol	30 minutes	None	0.745	0.721	0.024	0.037
3 HP	60% ethanol	30 minutes	Extracted with NaOH	1.12	0.691	0.014	0.021
4 SP[b]	Chloroform	24 hours	None	0.542	0.517	0.006	0.01
4 SP	60% ethanol	30 minutes	None	0.596	0.511	0.010	0.016
4 SP	60% ethanol	30 minutes	Extracted with NaOH	0.491	0.390	0.004	0.006
5 SP	Chloroform	24 hours	None	0.61	0.293	0.002	0.003
5 SP	60% ethanol	30 minutes	Extracted with NaOH	0.398	0.243	0.0026	0.004

[a] Hydraulic-pressed meal.
[b] Screw-pressed meal.

TABLE VI

Determination of Gossypol in Solvent-Extracted Cottonseed Oils[1]

No.	Description of raw material	Antimony trichloride reaction product				
		R_a	R_b	$E^{1\%}_{1.2\text{ cm.}}$ at 520 mμ	Gossypol content %	Selectivity of test
1	*Light petroleum naphtha extract*[2] of ground meats	0.621	0.421	0.047	0.0712	very poor
2	Light petroleum naphtha extract of ground meats from dried seed	1.61	0.82	0.230	0.286	poor
3	Hexane extract of flaked meats	1.07	0.700	0.0544	0.066	poor
4	Diethyl-ether extract of flaked meats	2.69	1.10	1.42	2.16	good
5	Light petroleum naphtha extract of flaked meats	2.17	1.28	0.22	0.336	good
6	Hexane extract of flaked meats	1.68	0.862	0.0533	0.081	poor

[1] AAntimony trichloride test applied to alkaline extracts chloroform solutions of the oils.
[1] Atimony trichloride test applied to alkaline extracts chloroform solutions of the oils.

in the case of oils obtained by extraction of seed with diethyl ether or relatively moist, light petroleum naphtha.

Expressed Oils. As shown in Table VII, tests for gossypol in expressed oils are usually not specific for gossypol even when they are applied to the alkali-extractable components of the oils.

Summary

A method is described which permits application of the antimony trichloride spectrophotometric method to the determination of gossypol in a variety of cottonseed products.

TABLE VII

Determination of Gossypol in Crude Expressed Cottonseed Oils[1]

Sample No.[2]	Antimony trichloride reaction product				
	R_a	R_b	$E^{1\%}_{1.2\text{ cm.}}$ 520 mμ	Gossypol content %	Selectivity of test
1	.....	.....	no reaction	none	.....
2	.....	.....	no reaction	none	.....
3	1.75	1.19	0.0695	0.106	poor
4	1.54	1.22	0.0524	0.0799	poor
5	1.59	1.02	0.1564	0.239	poor
6	1.63	1.02	0.1553	0.236	poor
7	1.38	0.819	0.0227	0.0347	bad
8	1.51	1.22	0.0935	0.143	poor
9	1.75	1.05	0.1504	0.22	poor
10	1.26	0.858	0.0553	0.085	bad
11	1.77	1.004	0.093	0.0447	poor
12	1.77	0.972	0.0280	0.0426	poor

[1] Antimony trichloride test applied to alkaline extracts of chloroform solutions of the oils.
[2] Samples Nos. 1 and 2 were hydraulic-pressed, all others were screw-pressed oils.

Gossypol is determined by means of the following series of operations: 1. extraction of gossypol from cottonseed or cottonseed products by use of chloroform or aqueous ethanol; 2. isolation of gossypol from the extracts by use of aqueous alkali; and 3. application of the antimony trichloride-spectrophotometric test.

Data are presented to show the results obtained by application of this procedure to the determination of gossypol in pigment glands, raw cottonseed meats, cooked cottonseed meats, hydraulic- and screw-pressed meals, solvent-extracted meals, gland-free meals, and oils, both expressed and solvent-extracted.

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