cated. The determination of the solubility of the protein in different solvents, analysis of the isolated protein to determine its nitrogen and ash contents and its color when dispersed in sodium hydroxide solutions, determination of the viscosity characteristics of concentrated peanut protein solutions, and specific product testing are used to evaluate peanut protein for industrial utilization.

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Determination of Free Gossypol in Cottonseed Materials

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SEVERAL spectrophotometric methods (1, 2, 3) have been proposed for the determination of free gossypol in cottonseed products. They require extensive manipulation and employ either lengthy or tedious extraction procedures or reagents lacking in specificity. Difficulties have been experienced with them in the hands of independent investigators (4).

A method is proposed in which the gossypol is extracted with 70% aqueous acetone and determined by the color developed with *p*-anisidine. In preparing pure gossypol for another investigation (5), it was observed that gossypol is quite stable in acetone. This stability appears to be due to the formation of a "loose" acetone-gossypol compound which is easily dissociated and in which the aldehyde groups of gossypol are stabilized. This compound was mentioned briefly by Carruth (6) and is being investigated further.

Aniline is usually employed for the colorimetric determination of gossypol because it reacts with gossypol to form dianilino gossypol (1, 2). In order to avoid the repeated distillation of aniline, several solid aromatic amines were investigated, namely, *o*-dianisidine, *p*-aminobenzoic acid, α-napthylamine, β-napthylamine, benzidine, *o*-tolidine, 2,4-diaminophenol, and *p*-anisidine. All of these compounds were found to give yellow-colored solutions when reacted with gossypol. In all cases the presence of a trace of acetic acid led to the development of a more intense color. *p*-Anisidine was selected because it is a white crystalline solid (m.p. 57°C.), easily purified, and stable in the solid state.

Reagents

1. *70% Aqueous Acetone (by Volume).* 700 ml. A.C.S. grade acetone plus 300 ml. distilled water.

2. *95% Ethyl Alcohol, Aldehyde Free.* Reflux U.S.P. 95% ethyl alcohol over potassium hydroxide and aluminum (10 grams KOH plus 5 grams aluminum per liter) for one hour and distill.

3. *Glacial Acetic Acid.* A.C.S. reagent.

4. *p-Anisidine.* Prepare a saturated solution of technical grade *p*-anisidine in hot water and filter through paper. Upon cooling in a water bath with stirring at room temperature the black oxidation products settle out on the sides of the beaker. Decant the slightly yellow supernatant into a clean beaker and keep overnight in a refrigerator. The crystalline product is usually pure. If slightly yellow, recrystallize. Dry in a desiccator over phosphorus pentoxide and store in a brown bottle. *p*-Anisidine in the solid state has proven to be stable for at least several months.

5. *p-Anisidine-Acetic Acid-Ethyl Alcohol Reagent.* Dissolve 0.500 gram recrystallized *p*-anisidine in purified ethyl alcohol. Add 1 ml. of glacial acetic acid and make to 50-ml. volume with the ethyl alcohol. Use 3 ml. of this reagent for each determination. Since *p*-anisidine is not stable in solution, this reagent must be made fresh each day.

6. *Ethyl Alcohol-Acetic Acid Reagent.* Dilute 1 ml. glacial acetic acid to 50-ml. volume with purified ethyl alcohol. Use 3 ml. of this reagent for each gossypol blank.

7. *Standard Gossypol Solutions.* Weigh accurately 25 mg. of pure gossypol, dissolve in 70% aqueous acetone and make to 200-ml. volume with 70% aqueous acetone. This stock solution of gossypol contains 0.125 mg. gossypol per ml. Dilute 2, 5, 10, 15, 20, 25, 30, 35, and 40 ml. of this stock solution to 50 ml. with 70% aqueous acetone. Two-milliliter aliquots of each solution are used for developing the standard curve as outlined below.

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Sample Preparation

a) Raw Cottonseed: Dehull cottonseed in a Bauer mill and separate the meats from hulls and lint. Grind the meats through the 20-mesh screen in a Wiley mill. Do not preheat the cottonseed before dehulling and avoid overheating during grinding.

b) Hydraulic- and Screw-Pressed Cottonseed Meals or Cake: Grind through the 20-mesh screen in a Wiley mill, avoiding overheating of the sample.

c) Solvent-Extracted Cottonseed Meals: No preparation is required unless sample is lumpy, in which case grind through the 20-mesh screen in a Wiley mill.

Analytical Procedure

Weigh sufficient sample material to contain about 2.5 milligrams of free gossypol into a glass-stoppered 250-ml. Erlenmeyer flask. This will require 0.25-0.30 g. for raw cottonseed meats and hexane-extracted meals, and 1.0-1.5 g. for hydraulic-, screw-pressed meals, and solvent-extracted meals containing very little gossypol. Cover the bottom of the flask with 6-mm. solid glass beads, add 50 ml. of 70% aqueous acetone by pipette. Stopper the flask and shake on a mechanical shaker for one hour at room temperature at such a rate that the sample material which collects around the top of the flask will be constantly washed back into the solution. Filter through a dry 11-cm. paper of medium retentivity into a small glass-stoppered flask, discarding the first portion of the filtrate. Place a watch glass over the funnel to reduce evaporation. If necessary, gossypol may be determined in the filtrate the next day since gossypol is stable in aqueous acetone.

Pipette duplicate 2-ml. aliquots of the filtrate into 25-ml. volumetric flasks. To one of the aliquots add 3 ml. of ethyl alcohol-acetic acid reagent and make to volume with ethyl alcohol. This is the gossypol blank. To the other aliquot add 3 ml. of the *p*-anisidine-acetic acid-ethyl alcohol reagent and place in a water bath (with the flask loosely stoppered) at 60°C. for one-half hour. At the same time run a reagent blank containing 2 ml. of 70% aqueous acetone and 3 ml. of *p*-anisidine-acetic acid-ethyl alcohol reagent parallel with the sample. Remove from the bath, cool to room temperature, and make to volume with 95% ethyl alcohol. Also prepare a solvent blank consisting of 2 ml. of 70% aqueous acetone and 3 ml. of acetic acid-ethyl alcohol reagent made to 25-ml. volume with ethyl alcohol.

Determine the per cent transmittance of the sample, designated as T_1, with an Evelyn colorimeter (No. 470 filter), or equivalent, setting the instrument at 100% transmittance with the reagent blank. If a spectrophotometer is used, make the measurements at 447 mμ. Determine the per cent transmittance of the gossypol blank, designated as T_2, using the solvent blank to set the instrument. Obtain the ratio T_1/T_2 and use the logarithm of this ratio ($\log T_1/T_2$) to find the concentration of gossypol in the sample aliquot from the standard curve. If desired, the measurements can be made in terms of density rather than transmittance provided of course that the standard curve is plotted also on a density basis. As a 2/50 aliquot is used for analysis, multiply the milligrams of gossypol found in the sample aliquot by 25 to obtain the milligrams of gossypol in the original sample.

Preparation of Standard Curve: Pipette duplicate 2-ml. aliquots of the standard gossypol solutions in 70% aqueous acetone, prepared as outlined above, into 25-ml. volumetric flasks. To one aliquot add 3 ml. of ethyl alcohol-acetic acid reagent and make to volume with ethyl alcohol. To the other aliquot add 3 ml. of *p*-anisidine-acetic acid-ethyl alcohol reagent and proceed as outlined above. Plot logarithms of T_1/T_2 values against milligrams gossypol to obtain the standard curve.

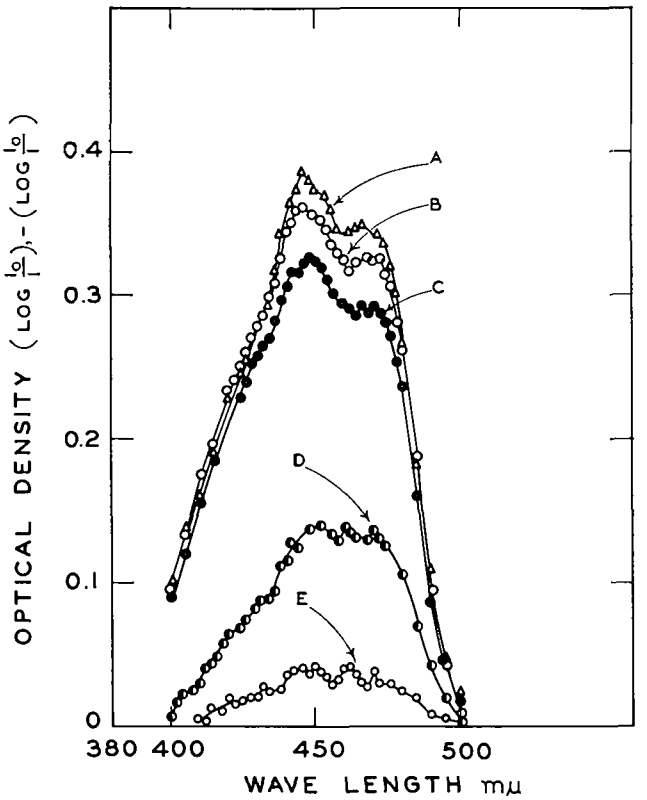

FIG. 1. Absorption spectra of 70% aqueous acetone extracts of various cottonseed materials reacted with *p*-anisidine as described under Analytical Procedure. Optical density values are corrected for the absorption of the gossypol blanks.

A. Hexane-extracted meal; 0.2390 g. (1.20% gossypol), 2/50 aliquot.
B. Raw meats; 0.3276 g. (0.837% gossypol), 2/50 aliquot.
C. Pure gossypol; 0.0991 milligram in 25-ml. volume.
D. Hydraulic-pressed meal; 2.000 grams (0.060% gossypol), 2/50 aliquot.
E. Screw-pressed meal; 2.000 g. (0.022% gossypol), 2/50 aliquot.

Investigation of the Reaction of Gossypol with p-Anisidine

The concentration of *p*-anisidine in the reaction is not very critical. Three ml. of a 1% solution in ethyl alcohol gave optimum results and was selected. Increasing the concentration above this does not increase the color intensity. With a given concentration of gossypol and *p*-anisidine a greater color intensity is developed in the presence of acetic acid than without acetic acid. The amount of acetic acid may vary from 0.01 to 0.10 ml. glacial acetic acid in the reaction volume without any change in the color intensity. Since traces of acids may be found in cottonseed extracts, a serious error might be introduced if the standard curve was run without the added acid.

As reported by Smith (2) for the reaction of gossypol and aniline, heating is necessary for complete color development in a reasonable time. Variation in the heating time from 15 to 120 minutes at 60°C. did not affect the color intensity. A 30-minute heating

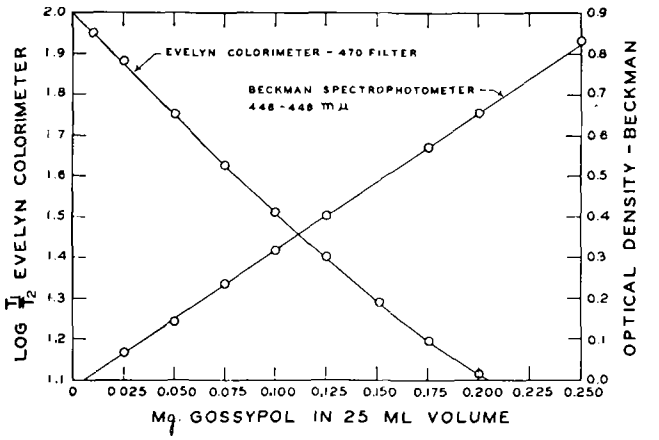

FIG. 2. Standard curves—gossypol and *p*-anisidine prepared as described under Analytical Procedure.

period was chosen. When the reaction between gossypol and *p*-anisidine in the presence of acetic acid was allowed to proceed at room temperature, optimum color intensity was reached only after 3 to 4 hours. The reaction volume is not critical. Experiments were conducted in which the reaction volume was kept at 5 ml. and at 20 ml. For high concentrations of gossypol the color intensity remained constant. For low concentrations of gossypol the color intensity was slightly greater when the reaction volume was kept at 5 ml. Consequently a 5-ml. reaction volume was chosen.

The color is stable for at least four hours after development. There is a slight reaction between acetone and *p*-anisidine in the presence of acetic acid. The reagent blank has about 97-98% transmittance when measured against the solvent alone (Evelyn colorimeter No. 470 filter). In 20 hours the reagent blank shifts to about 90% transmittance. The same change takes place in the samples however, and the transmittance values of standards remain unchanged for at least four hours when read against the reagent blank. For this reason the sample solutions are read against the reagent blank which insures cancellation of this effect and also insures against contamination of the reagents. The gossypol blanks are read against the solvent and division of the transmittance values of the samples (T_1) by that of the gossypol blanks (T_2) cancels out the extraneous color which is present in cottonseed extracts.

Evaluation of the p-Anisidine-Gossypol Color

Curve C in Figure 1 is a spectrophotometric curve of the reaction product of gossypol with *p*-anisidine, measured with a Beckman spectrophotometer using a 1-cm. cell. The optical density value of the gossypol blank was subtracted from the optical density value of the reaction product of gossypol and *p*-anisidine at each wave length. The curve shows two distinct maxima at 447 mμ and at 468 mμ. As the maximum at 447 mμ is much sharper than that at 468 mμ, measurements should be made at 447 mμ for greatest sensitivity.

The standard curve determined with the Evelyn colorimeter, using the No. 470 filter, is shown in Figure 2. The No. 470 filter with a peak transmittance at 464 mμ and a half-band width of 30 mμ has been found to be the most satisfactory filter for this instrument. The values of log T_1/T_2, obtained as outlined above, were plotted against concentrations of gossypol in 25-ml. volume. For concentrations from 0 to 0.10 mg. of gossypol a straight line was obtained. For concentrations from 0.10 to 0.20 mg. of gossypol a definite curvature was found. The standard curve has been repeatedly checked and is reproducible throughout the concentration range of 0 to 0.20 mg. gossypol in 25-ml. volume.

All gossypol determinations reported in this paper were obtained with the Evelyn colorimeter, using the No. 470 filter. Any colorimeter equipped with a filter isolating a band with the range 447-468 mμ can be used.

The standard curve was also determined with a Beckman spectrophotometer at 447 mμ and is shown in Figure 2. A plot of optical density, corrected for the optical density of the gossypol blanks, against concentration of gossypol is a straight line from 0 to 0.20 mg. of gossypol in 25-ml. volume. Several gossypol determinations obtained with the Evelyn colorimeter were checked with the Beckman spectrophotometer and the values were in good agreement.

Specificity of the Method

Curves A, B, D, and E in Figure 1 are spectrophotometric curves of the reaction products of various cottonseed extracts with *p*-anisidine, measured with a Beckman spectrophotometer, using a 1-cm. cell. The curves were obtained by subtracting the optical density values of the gossypol blanks at each wave length from the optical density values of the same extracts reacted with *p*-anisidine. In this manner extraneous color due to pigments other than gossypol are cancelled out unless these pigments react with *p*-anisidine, in which case the curves would be substantially different from that for pure gossypol and *p*-anisidine (Curve C). The curves for extracts of raw meats (Curve B) and hexane-extracted meal (Curve A) are identical in shape with the curve for pure gossypol (Curve C). All three show the double maxima at 447 and 468 mμ, typical of the reaction product of gossypol and *p*-anisidine. The curve for the extract of hydraulic-pressed meal (Curve D) has the double maxima at 447 and 468 mμ although the maxima are not well defined. The extract for screw-pressed meal (Curve E) shows a rather broad absorption over the entire range 447-468 mμ. The curves for hydraulic- and screw-pressed meals indicate that *p*-anisidine reacts with other gossypol-like pigments to produce absorption bands in the region 447-468 mμ. For the extract of screw-pressed meal the absorption of these pigments seems to be superimposed on the absorption of the gossypol-*p*-anisidine reaction product since close inspection discloses maxima at 446 and 468 mμ for the reaction product of gossypol and *p*-anisidine in addition to a maximum at 460 mμ. It should be emphasized therefore that for hydraulic- and screw-pressed meals this method will measure gossypol and all other related compounds which react with *p*-anisidine in the region 447-468 mμ.

Gossypurpurin, a gossypol-like pigment, reacts with *p*-anisidine to give a reaction product with maxima at 447 and 468 mμ identical with the reaction product of gossypol and *p*-anisidine. Gossypurpurin is soluble in 70% acetone which indicates that this method will measure any gossypurpurin present in cottonseed as gossypol.

Investigation of Extraction Procedure

The conditions required for complete extraction of gossypol from cottonseed materials by aqueous acetone were determined by extraction on a mechanical shaker at room temperature for periods of time from 1 to 4 hours as shown in Table I. It was found that covering the bottom of the flask with 6-mm. glass beads has a grinding effect on coarse materials such as raw meats. Low results on raw meats were obtained when the beads were omitted. While the beads were not necessary for finely divided samples of solvent-extracted meals, they were used for all samples to insure uniform extraction. Static overnight extractions (16 hours) at room temperature were also made.

TABLE I

Effect of Time on Shaker Extraction of Gossypol From Cottonseed Products With 70% Aqueous Acetone

Sample Material	Free Gossypol[1]			
	Shaker Extraction[2]			Static Extraction, 16 hr.
	1 hr.	2 hr.	4 hr.	
	%	%	%	%
Raw Meats	0.802	0.790	0.806	0.785
Raw Meats	1.33	1.33	1.33	1.29
Hexane-Extracted Meal	1.20	1.22	1.23	1.20
Acetone-Extracted Meal	0.025	0.028	0.032	0.027
Hydraulic-Pressed Meal	0.037	0.042	0.042	0.061
Hydraulic-Pressed Meal	0.078	0.082	0.086	0.087

[1]Average of duplicate determinations.
[2]Bottom of flask covered with 6-mm. glass beads.

From the data in Table I it seems that extraction of gossypol from raw meats and hexane-extracted meals is complete in 1 hour on the shaker and that the values agree with overnight static (16 hours) extraction. Commercial hydraulic-pressed meals however show a small increase in gossypol after 2- and 4-hour shaker extractions and a still greater increase after 16-hour static extraction. Presence of considerable bound gossypol in these meals makes possible the slow hydrolysis of bound gossypol on increased extraction time in the presence of the aqueous solvent. In order to demonstrate this effect the various cottonseed materials as shown in Table II were allowed to stand in contact with 50 ml. of 70% aqueous acetone

TABLE II

Effect of Time on Static Extraction of Gossypol From Cottonseed Products With 70% Aqueous Acetone

Sample Material	Free Gossypol[1]					
	Shaker Extraction, 1 hr.	Extended Static Extraction[2]				
		16 hr.	24 hr.	48 hr.	72 hr.	
	%	%	%	%	%	
Unheated flakes of meats	0.947	0.939	0.918	0.906	0.889	.....
Raw meats	0.778	0.749	0.742	.....	0.717	0.614[3]
Hexane extracted meal	1.20	1.20	1.21	1.20	1.18	.....
Acetone-extracted meal	0.025	0.027	0.027	0.027	0.028	.....
De-pigmented; de-fatted meal	0.065	0.072	0.075	0.081	0.088	0.108[4]
Hydraulic-pressed meal	0.060	0.070	0.074	0.088	0.100	0.208[4]

[1]Average of duplicate determinations.
[2]Samples in stoppered flasks in dark cabinet.
[3]After 336 hours.
[4]After 400 hours.

in a dark cabinet for long periods of time at room temperature, after which they were filtered and analyzed. Raw cottonseed meats showed a decrease in gossypol on extended time of extraction, which indicated a degradation of gossypol. The reason for this effect is not known with certainty. It may be pointed out that when raw meats are extracted for 1 hour on the shaker and filtered, the gossypol in the filtered extracts is very stable (Table III). Hexane- and acetone-extracted cottonseed meals gave essentially constant values up to 72 hours' extraction, which indicated that complete extraction was attained on the shaker in one hour. Depigmented cottonseed meal and hydraulic-pressed meals showed a slow progressive increase in gossypol content on extended extraction up to 400 hours. The results indicate a progressive hydrolysis of bound gossypol in these meals. The 1-hour shaker extraction should lead to a minimum of hydrolysis for meals which contain bound gossypol.

TABLE III

Stability of Pure Gossypol and Extracts of Various Cottonseed Products in 70% Aqueous Acetone

Gossypol Solutions and Products Extracted	Mg. Gossypol Found in Filtered Extracts[1]		
	0 hr.	21.5 hr.	96 hr.
0.235 mg. pure gossypol	0.235	0.225	0.225
2.503 mg. pure gossypol	2.503	2.463	2.463
4.990 mg. pure gossypol	4.990	4.990	4.990[2]
Raw meats	3.973	3.973	3.975
Pigment glands	4.035	4.035	4.035
Hexane-extracted meal	2.865	2.865	2.843
Hydraulic-pressed meal	0.800	0.800	0.805

[1]Flasks stored in dark cabinet.
[2]After 121 hours.

From these results the 1-hour shaker extraction with 70% aqueous acetone was chosen for the extraction of free gossypol in order that the extraction conditions should be consistent for all types of cottonseed samples.

Stability of Gossypol in 70 Per Cent Aqueous Acetone

Investigations showed that the stability of pure gossypol, dissolved in 70% aqueous acetone, is not affected by mechanical shaking for a period of 1 hour at room temperature.

Three concentrations of pure gossypol were prepared in 70% aqueous acetone and four typical cottonseed materials were extracted on the shaker for 1 hour with 50 ml. of 70% aqueous acetone and filtered. All of the extracts were analyzed for gossypol and then placed in a dark cabinet at room temperature. After various times 2-ml. aliquots of each extract were analyzed for gossypol. The results are shown in Table III. All of the extracts gave essentially unchanged values for gossypol on standing for periods of time up to 96 hours. The fact that the filtered extract of raw cottonseed meats was stable for 96 hours in aqueous acetone is of interest. The results in Tables I and II indicated a lower value for raw meats on extended static extraction where the solvent stood in contact with the meats. The data given in Table III show that filtered extracts can be reserved and analyzed for gossypol several days later if necessary.

Recovery of Gossypol Added to Cottonseed Materials

To test the effect of interferences in various extracts, 1.0-gram samples of the various materials (Table IV) were weighed, and known concentrations

TABLE IV

Recovery of Gossypol Added to Various Cottonseed Materials

Sample Material	Gossypol in Sample	Gossypol Added	Total Gossypol Present	Gossypol Found	Recovery
	mg.[1]	*mg.*[1]	*mg.*[1]	*mg.*[1]	%
De-pigmented; de-fatted meal	0.717	1.004	1.721	1.700	98.8
De-pigmented; de-fatted meal	0.717	2.008	2.725	2.659	97.6
Hydraulic pressed meal	0.838	1.007	1.845	1.850	100.3
Hydraulic pressed meal	0.838	2.013	2.851	2.873	100.8
Hexane-extracted peanut meal	None	1.004	1.004	0.975	97.1
Hexane-extracted peanut meal	None	2.008	2.008	1.973	98.3

[1]Average of duplicate determinations.

of pure gossypol in 70% aqueous acetone were added, with aqueous acetone, to make the total volume to 50 ml. Controls, omitting the added gossypol, were also run. The samples were extracted either on a shaker for 1 hour or by overnight static (16 hours) extraction. A sample of hexane-extracted peanut meal was also used to determine the effect of protein on the recovery of gossypol. The results (Table IV) show satisfactory recovery of gossypol from these samples, and demonstrate that the presence of considerable protein does not interfere with the extraction procedure.

General Applicability of the Method

Comparison of the method with the procedure of Smith (2) is shown in Table V. For raw meats and hexane-extracted meals the two methods are in good agreement. The shaker extraction (1 hour) shows a trend toward slightly higher values although the differences are not marked. The Smith method gives higher results than the proposed method for kettle meats, hydraulic- and screw-pressed cottonseed meals, and depigmented meals. As these samples all contain bound gossypol and have been shown (Tables I and II) to give increased gossypol values on extended extraction, it is possible that the heating of the sample in the blendor operations of the Smith method leads to hydrolysis of some of the bound gossypol. The values obtained with the Smith method vary in the same direction as those obtained by the proposed method.

Alternative Procedures

In the event that 95% ethyl alcohol is not available, 80% aqueous isopropyl alcohol can be substituted for it throughout the procedure. The standard curve is the same for both alcohols. A number of cottonseed samples were analyzed with the substitution of 80% isopropyl alcohol for ethyl alcohol. The results were identical with previous analyses in which 95% ethyl alcohol was used. Both 90 and 98% isopropyl alcohol cause turbidity with some extracts, but 80% isopropyl alcohol is applicable to all types of cottonseed extracts.

For purposes such as plant control operations, where a rapid analysis of a sample is required, the following extraction procedure was found to be adequate:

The analytical sample is weighed into a small-sized Waring Blendor jar, 100 ml. of 70% aqueous acetone is added by pipette, and the sample is blended for 5 minutes. The aqueous acetone is a foaming solvent and keeps the sides of the jar washed down continuously. The slurry is immediately filtered through a paper of medium retentivity and a portion of the filtrate collected as soon as it comes through clear. Duplicate 2-ml. aliquots are pipetted into 25-ml. volumetric flasks and analyzed for gossypol exactly as outlined above.

An analysis can be completed within 1 hour, using the rapid extraction procedure. Filtration of the warm slurry without making to volume after blending does not seem to materially affect the results. Table VI shows that the 5-minute blendor and 1-hour shaker

TABLE V

Comparison of Proposed Method With Smith (2) Blendor Method for Gossypol in Cottonseed Products

Type of Sample	Free Gossypol[1]	
	Proposed Method[2]	Smith Blendor Method
	%	%
Raw meats	0.837	0.812
Raw meats from seed stored at room temperature	1.26	1.19
Raw meats from seed stored at -18°C.	1.32	1.28
Commercial shaker meats	1.18	1.17
Commercial second kettle meats	0.049	0.081
Hexane-extracted meal	0.571	0.598
Hexane-extracted meal	1.21	1.17
De-pigmented; de-fatted meal	0.065	0.080
Acetone-extracted meal	0.025	0.034
Hydraulic-pressed meal	0.047	0.062
Hydraulic-pressed meal	0.110	0.119
Screw-pressed meal	0.015	0.033
Screw-pressed meal	0.031	0.048
Pigment glands	38.77	38.71

[1]Average of duplicate determinations.
[2]1 hr. shaker extraction.

extraction methods give essentially the same values. In view of the possible errors in blendor operations the 5-minute extraction is not recommended for the most precise work. When a number of samples are to be analyzed, the shaker extraction will materially reduce the time required per sample. The rapid 5-minute blendor procedure should prove to be desirable in cases where a single sample must be analyzed in as short a time as possible.

Precision of the Method

Samples of raw meats, hexane-extracted meal, and hydraulic-pressed meal were analyzed repeatedly over a period of several months. The number of determinations made on each of these samples were 23, 24, and 17, respectively; the average free gossypol contents were 1.31, 1.20, and 0.064%, respectively; and the standard deviations of the determinations were 0.021, 0.020, and 0.0046, respectively.

TABLE VI

Comparison of 5-Minute Blendor Extraction With 1-Hour Shaker Extraction for Gossypol in Cottonseed Products

Type of Sample	Free Gossypol[1]	
	1-Hour Shaker Extraction	5-Minute Blendor Extraction
Raw meats	0.947	0.938
Raw meats	1.32	1.33
Hexane-extracted meal	1.20	1.18
De-pigmented; de-fatted meal	0.065	0.061
Hydraulic-pressed meal	0.060	0.056
Hydraulic-pressed meal	0.109	0.091
Screw-pressed meal	0.015	0.016
Screw-pressed meal	0.031	0.029

[1]Average of duplicate determinations.

Summary

A method for the determination of free gossypol in cottonseed materials is described. The method consists of extraction of gossypol with a measured volume of 70% aqueous acetone on a shaker for one hour, filtration, and colorimetric analysis for gossypol in an aliquot of the filtrate by means of the reaction between gossypol and *p*-anisidine.

The conditions for complete extraction of gossypol from various types of cottonseed materials have been investigated, the stability of gossypol in aqueous acetone has been demonstrated, and data are presented on recovery of gossypol added to cottonseed materials. The method has been compared with the method of Smith (2).

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☆ ☆ ☆ ABSTRACTS ☆ ☆ ☆

Oils and Fats

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BETTER WAY TO BLEACH VEGETABLE OILS. J. V. Hightower. *Chem. Eng. 56,* 102-4(1949). Continuous vacuum process equipment is described. Tables compare amount of absorbent needed, removal of soap, oil stability during bleaching, and change in fatty acid content during bleaching with atmospheric and vacuum bleaching.

ANOTHER OLEO? FARM BLOC EYES FALLING FAT AND OIL PRICES, PROBES SYNTHETIC DETERGENTS GROWTH. *Chem. & Inds. 65,* 342-3(1949). There is a 400-million-lb. oversupply of tallow and grease.

SOLUBILITY AND SPECIFIC ROTATION OF *l*-ASCORBYL PALMITATE AND *l*-ASCORBYL LAURATE. D. Swern. *J. Am. Chem. Soc. 71,* 3256(1949).

FAT HYDROLYSIS. V. Mills and H. K. McClain. *Ind. Eng. Chem. 41,* 1982-85(1949). Data are presented on the reaction of tallow and coconut oil with water, in the temperature range used in continuous fat splitting. The maximum amount of splitting which can occur is determined by the glycerol concentration in the aqueous phase. This value is not affected by changes in temperature. In the range 90-100% completeness, the unsplit fat contains more glycerol than the original fat, an indication of the presence of a large amount of monoglyceride. The percentage of glycerol in the unsplit fat is constant, which is strong evidence that the reaction proceeds stepwise.

REPORT ON VITAMIN A. FURTHER COMPARISON OF THE SPECTROPHOTOMETRIC AND ANTIMONY TRICHLORIDE METHODS FOR VITAMIN A IN MARGARINE. J. B. Wilkie. *J. Assoc. Official Agr. Chemists 32,* 455-9(1949).

ANALYSIS OF MIXTURES OF ORGANIC ACIDS BY EXTRACTION. K. R. Tsai (National Amoy Univ., Amoy, China), and Y. Fu. *Analytical Chem. 21,* 818-21 (1949). The extraction method for the analysis of fatty acid mixtures has been critically studied. The effects of dissociation and association of the acids and of the ratios of the volume of extractant to that of the aqueous solution on the accuracy of analysis have been expressed in the form of equations. In the case of ternary mixtures better results are obtained by the back-extraction of the organic layer with water or dilute aqueous solution than by the extraction of the aqueous solution repeatedly with organic solvent. The method has been successfully applied to the analysis of binary mixtures of acetic, propionic, and butyric acids, and to the ternary mixtures of the above acids either in the absence or in the presence of formic acid.

ACROLEIN FORMATION FROM FATS. K. Taufel and U. Freimuth. *Z. Lebensm.-Untersuch. u. -Forsch 89,* 121-51(1949). When fat was heated with $KHSO_4$ the acrolein evolved from both free and bound glycerol. With elaeostearic acid and tung oil which has been altered in light (oxidized) the glycerol radical is unimportant as the source of acrolein. That is during oxidation, unsatd. fatty acids are affected in some unknown manner so that they yield acrolein on heating. A parallel between this mechanism and that of conjugation does seem possible.

HEME-CATALYZED REACTIONS OF ORGANIC PEROXIDES. J. Glavind and S. Hartmann. *Acta Physiol. Scandinavica 16,* Supplement 53, 26-7(1948). If a few drops of peroxidized oils are added to a solution of benzidene in alcohol containing heme a weak bluish color, which soon fades, is seen. However, if the water is substituted by an organic solvent (acetone) the addition of organic peroxides gives a very strong reaction in the course of a few minutes.

A HISTOCHEMICAL METHOD FOR THE DEMONSTRATION OF PEROXIDES. H. Grandos, J. Glavind, S. Hartmann, and H. Dam. *Acta. Physiol. Scandinavica 16,* Supplement 53, 28-9(1948). This is a staining technic for adipose tissue such as bacon. The section is stained with a solution made from 20 mg. hemin, 5 cc. pyridine, and 10 cc. glacial acetic acid, mixed with a solution of 500 mg. leuco-base of 2,6-dichlorophenolindophenol dissolved in 50 cc. absolute alcohol and 120 cc. of distilled water.

THE ANTI-OXIDANT PROPERTIES OF NORDIHYDROGUAIARETIC ACID IN CREAM. V. N. Krukovsky, D. A. Theokas, and F. A. Whiting. *J. Dairy Sci. 32,* 695-M2 (1949). Abstracts of papers presented at the 44th annual meeting of the American Dairy Science Association. NDGA was added at the rate of 0.005% of the bulk fat to milk prior to pasteurization at 82.2°C. for 30 minutes and separation. The stability of fat