Estimation of Free Gossypol in Cottonseed Meal and Cottonseed Meats: Modified Method

An earlier modification (3) of a spectrophotometric method (2) for the determination of free gossypol, as the dianilino derivative, in cottonseed meal and meats has been simplified. Ethanol, water and ethyl ether are combined into a single solvent mixture for the extraction of the gossypol in a homogenizer which is cooled by immersion in a water bath. The modified method has the precision and accuracy of the original.

Reagents are as follows: (a) Solvent mixture: 715 ml 95% ethanol, 285 ml of distilled water, 200 ml ethyl ether (peroxide free) and 0.2 ml glacial acetic acid. Ether is tested as follows: a mixture of 0.1 g of vanadic oxide and 2 ml concentrated sulfuric acid is heated on a steam bath for 15 min, cooled and diluted to 50 ml with water (1). A pink color is obtained when 2 ml of reagent is shaken with 10 ml of ether containing peroxides. (b) Aniline: Freshly distilled from approximately 1 g of 30 mesh granular zinc. The distillate should be colorless. (c) Acid washed Hyflo Super-Cel: Prepared as previously described (3,4).

The procedure is as follows: Add 60 ml of the solvent mixture to either 1 g of finely ground cottonseed meal or 0.250 g of cottonseed meats placed in a 200 ml chamber of a Sorvall omni-mixer or similar comminution apparatus. Homogenize for 5 min after surrounding the mixing chamber with water at 27–30 C. Filter the homogenate under vacuum through a filter tube (Corning 9480) previously prepared by inserting a porcelain plate over which is formed a layer of asbestos followed by a ⅛ in. mat of Hyflo Super-Cel. The filtrate and washings are received in a 100 ml volumetric flask containing 5 ml of ether, placed under a bell jar. Wash the equipment and residue with the ethanolic solvent mixture dispensed from a fine-tipped wash bottle. Dilute the extract to 100 ml, mix and transfer 5 ml aliquots to two 25 ml volumetric flasks. Dilute one of the aliquots to 25 ml with the solvent mixture, mix and reserve as the reference solution. Add 0.5 ml of freshly distilled aniline to the other aliquot and heat gently either on a steam bath or in a water bath at 70–75 C for 40 min. After cooling dilute the sample to 25 ml with the ethanol-water-ether solvent mixture, mix and determine the absorbance at 445 mμ using the aliquot without aniline as the reference.

TABLE I

Free Gossypol Values for Cottonseed Meals by Modified Methods[a]

Number		Modification	
Sample	Determination	Present %	Earlier %
1	a	0.328	0.320
	b	0.334	0.328
	c	0.324	0.325
	d	0.320	0.321
	Average	0.327	0.324
2	a	0.026	0.027
	b	0.029	0.027
	c	0.029	0.029
	Average	0.028	0.028

[a] The present modification is compared with an earlier one (3).

The gossypol content of the samples may be calculated from the extinction coefficient or determined graphically from the absorbance-concentration curve, as previously described (4,5).

Typical results for two samples of cottonseed meal by the present and the earlier modifications are shown in Table I and are in good agreement.

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[Received July 8, 1968]

Identification of Arachidic Acid in Porcine Glycerides

Literature reports indicate that porcine glycerides contain about 1% linolenic acid, but there is little support for the presence of arachidic acid in such amounts. Magidman et al. (1) reported that lard contains 0.5% linolenic acid and 0.3–0.4% arachidic acid. Gas-liquid chromatography of the methyl esters of porcine glycerides, using an F & M Model 720 dual column gas chromatograph with thermal conductivity detectors and 10 ft × ¼ in. OD stainless steel columns packed with 20% diethylene glycol succinate on 60/80 mesh firebrick, shows a peak with a retention time between methyl linoleate and methyl eicosanoate (Table I), which could represent the methyl ester of either linolenic acid or arachidic acid or both. Chromatographic conditions were as follows: column temperature, 230 C; injection temperature, 275 C; detector temperature, 240 C; and flow rate of helium gas, 50 ml/min.

Standard samples of methyl linolenate and methyl eicosanoate, when chromatographed under the above conditions revealed the same retention time as the unknown peak. Semilogarithmic plots of relative

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TABLE I

Relative Retention Times of Methyl Esters of Porcine Milk and Body Fat on a Diethylene Glycol Succinate Column

Methyl ester	Relative retention time
14:0	0.35
16:0	0.59
16:1	0.68
18:0	1.00
18:1	1.17
18:2	1.40
unknown	1.72
20:1	1.86

TABLE II

Peak Area Ratios Obtained From the GLC Analysis of Porcine Methyl Esters on Polar (DEGS) and Nonpolar (SE 30) Columns

Fat sample	Peak area ratio 3:2[a] on DEGS	Peak area ratio 4:1[b] on DEGS	Peak area ratio $C_{18}:C_{20}$ on SE30
sow milk fat	26:1	102:1	28:1
sow body fat	24:1	51:1	25:1
piglet body fat	36:1	72:1	36:1

[a] Combined peak areas of methyl stearate, oleate and linoleate: combined peak areas of unknown and methyl eicosenoate.
[b] Combined peak areas of methyl stearate, oleate, linoleate and unknown: peak area of methyl eicosenaoate.

TABLE III

Fatty Acid Composition (wt %) of Porcine Milk and Body Fat, With and Without the Addition of Standard Methyl Esters of 20:0 and 20:1, Before and After Hydrogenation

Methyl ester	Milk Fat				Body Fat			
	Without 20:0 and 20:1		With 20:0 and 20:1		Without 20:0 and 20:1		With 20:0 and 20:1	
	Not hydro-genated	Hydro-genated	Not hydro-genated	Hydro-genated	Not hydro-genated	Hydro-genated	Not hydro-genated	Hydro-genated
14:0	2.9	3.1	2.6	3.3	1.4	1.1	1.1	1.2
16:0	30.7	37.3	27.5	34.3	22.0	23.2	19.5	22.3
16:1	8.5	......	7.8	......	4.1	......	3.5	......
18:0	4.6	57.3	3.9	51.8	8.7	73.4	8.0	64.7
18:1	37.4	......	33.7	......	55.5	......	50.6	......
18:2	13.3	......	13.6	......	6.1	......	5.5	......
unknown	1.9	2.2	6.1	10.6	1.0	2.3	5.7	11.8
20:1	0.5	......	4.9	......	1.2	......	6.0	......

retention times of the porcine methyl esters vs. number of carbon atoms, and relative retention time vs. number of double bonds supported the possibility that the unknown peak could represent the methyl ester of either linolenic acid or arachidic acid.

Negative results were obtained when the presence of linolenic acid was tested by the methods of Mehlenbacher (2) and White and Brown (3). Chromatography of the porcine methyl esters on a nonpolar silicone column (5% SE 30 on 60/80 mesh Chromosorb W) and subsequent comparison of peak area ratios on the polar and nonpolar columns (Table II) indicated that the unknown peak was primarily arachidic acid, not linolenic acid.

Hydrogenation of porcine milk and body fat, with and without the addition of standard methyl eicosanoate and eicosenoate (Allied Laboratories, Inc.), was accomplished using a Brown Hydro-analyzer (Delmar Instruments, Inc.). Fat samples before and after hydrogenation were methylated according to the method of Metcalfe, et al. (4) and chromatographed on the DEGS column. Results (Table III) verify that the unknown peak represents primarily the methyl ester of arachidic acid. Trace amounts of linolenic acid could be present, especially in porcine milk fat, but these could not be detected by the methods reported here.

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ACKNOWLEDGMENT

This work was supported by a grant from the National Research Council of Canada.

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[Received September 16, 1968]