Determination of Oil and Lipids in Cottonseed Meal

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ABSTRACT

Two simple methods for determining total oil equivalent in cottonseed meal are described. The meal is extracted with methanol, and the crude extract is either (a) saponified and subsequently acidified to find the weight of acids set free, or (b) subjected to methanolysis to determine the weight of esters formed. When applied to a meal previously extracted with petroleum ether, these methods determine lipids. Results obtained on three commercial cottonseed meals were in excellent agreement with those obtained by the more cumbersome method requiring direct saponification of the ground meal. The methods described should also be applicable to soybean and sunflower seed meals and to other systems containing lipids, such as flours, doughs, and leaf proteins. One of the methods is particularly useful as a preliminary step in the determination of cyclopropenoid acids in cottonseed meal because much larger samples can conveniently be used, and the oil is isolated as methyl esters.

INTRODUCTION

The official AOCS method for determining the oil content of either cottonseed (1) or cottonseed meal (2) consists of extracting with petroleum ether, evaporating the solvent, and weighing the residue. When this procedure is used to determine the materials balance in the production of oil from cottonseed, the amount of oil produced plus that left in the meal is always slightly less than that originally present in the seed. In a panel discussion (3) of this unexplained hidden loss of oil, evidence was presented which indicated that during processing a small portion of the oil is converted to lipids and is thus rendered insoluble in petroleum ether. The oil content of cottonseed meals as determined by the official method, therefore, will not include the lipids.

Two methods were described (3) for determining the glyceride equivalent of the lipids remaining in the meal after removal of the oil by petroleum ether extraction. Fowler used a method suggested by W.J. Miller that involved saponification of the ground meal followed by acidulation and recovery of the fatty acids. Norris extracted the meal with methanol, evaporated the alcohol, and then extracted the residue with petroleum ether. Neither of these methods was published. In 1965, Szutowicz (4) reported the determination of the lipids in a number of cottonseed meals from which the oil had been extracted with petroleum ether. He used a modification of the Miller method that required direct saponification of the ground meal. The saponification procedure applied to the unextracted cottonseed meal determines the free oil plus the glyceride equivalent of the lipids, the sum of which will be referred to as the total oil equivalent in the meal. The purpose of the present investigation was to develop and evaluate simpler methods for determining total oil equivalent and lipids in cottonseed meals.

MATERIALS AND PROCEDURES

Three commercial cottonseed meals were used. Meals 1 and 2 were screw press meals, and Meal 3 was a prepress solvent extracted meal. Moisture content was calculated from the loss in weight when heated at 103 C for 2 hr in a forced draft oven.

Petroleum ether, b.p., 35-38 C, was used throughout. Sodium methoxide solution was made by weighing 1.5 g of metallic sodium into a dry flask and cautiously adding 1000 ml of absolute methanol. The solution was stored in a stoppered bottle. Alcoholic potassium hydroxide solution was prepared with 95% ethanol. Litmus paper was used in adjusting acidity.

All determinations were made in duplicate and are reported on a moisture-free basis.

EXPERIMENTAL PROCEDURES

Two simple methods for determining total oil equivalent were developed. The meal is first extracted with methanol. The crude methanol extract is then either (a) saponified and subsequently acidified to determine the weight of acids set free, or (b) subjected to methanolysis to determine the weight of esters formed. When applied to a meal previously extracted with petroleum ether, these methods determine lipids. These methods were evaluated by comparing results obtained for three commercial cottonseed meals with those obtained with a modification of the method of Szutowicz (4). The procedures were as follows.

Method A: Direct Saponification of the Meal

This is a modification of Szutowicz's method. Ground cottonseed meal (5 g) was saponified by heating for 1 hr on a steam bath with 35 ml of a 7.5% alcoholic solution of potassium hydroxide in a 250-ml beaker covered with a watch glass. The resulting mixture was acidified with a 5-ml excess of 30% acetic acid and filtered through S&S White Ribbon filter paper in a Buchner funnel, with suction. The paper and residue were dried at room temperature, wrapped in the form of a thimble as described in the official AOCS method for residual oil in cottonseed meals (2), and extracted with petroleum ether in a Soxhlet extractor for 3 hr. The solvent was removed under reduced pressure at 60 C on a rotary evaporator. The acids were weighed and calculated as glycerides, using the factor 1.045, based on an average molecular weight of 273 for cottonseed fatty acids.

Method B: Saponification of the Methanol Extract

Ground cottonseed meal (5 g) was Soxhlet extracted for 4 hr with 50 ml of methanol. A 5-ml portion of 50% alcoholic potassium hydroxide was added to the methanol extract and heated for 1 hr on a steam bath. A funnel was placed in the neck of the extraction flask to prevent evaporation. The contents were cooled, made acid to litmus with 30% acetic acid, and 25 ml of distilled water was added. The solution was transferred to a 250-ml separatory funnel and extracted with six 25-ml portions of petroleum ether. The combined petroleum ether layers were washed with three 25-ml portions of 10% ethyl alcohol and freed of solvent on a rotary evaporator. The fatty acids were weighed and calculated as glycerides.

Method C: Methanolysis of Methanol Extract

Ground cottonseed meal (5 g) was Soxhlet extracted for 4 hr with methanol. Sodium methoxide solution (50 ml) was added to the methanol extract and refluxed for 0.5 hr. After cooling, 30% acetic acid was added to destroy the remaining methoxide. Distilled water (25 ml) was added, and the methyl esters were extracted by the same proce-

TABLE I

Analyses of Three Commercial Cottonseed Meals by Different Procedures

Meal	Oil		Total oil equivalent			
	Petroleum ether extract[a] (%)	Lipids[b] Method A[c,d] (%)	Column 2 plus column 3 (%)	Method A[c] (%)	Method B (%)	Method C (%)
Meal 1	4.40	1.75		6.22	6.16	6.19
	4.35	1.69		6.17	6.27	6.07
Screw press	Av. 4.38	Av. 1.72	6.10	Av. 6.20	Av. 6.21	Av. 6.13
Meal 2	4.72	1.52			6.27	5.98
	4.76	1.46			6.16	6.10
Screw press	Av. 4.74	Av. 1.49	6.23		Av. 6.21	Av. 6.04
Meal 3	1.44	1.66			3.10	3.19
	1.43	1.72			3.15	3.01
Solvent prepress	Av. 1.44	Av. 1.69	3.13		Av. 3.13	Av. 3.10

[a]Official AOCS method (2).
[b]As glycerides.
[c]Szutowicz method (4) modified.
[d]Applied to meal after extraction with petroleum ether.

dure as that used for the fatty acids in Method B, weighed, and reported as glycerides.

RESULTS AND DISCUSSION

The total oil equivalent of the three meals as determined by Methods B and C was compared to the sum of (a) the value obtained by the official AOCS method (2), i.e., by petroleum ether extraction, and (b) the value for the lipids left in the extracted meal as determined by Method A. Also included was the determination of total oil equivalent in Meal 1 by Method A. The results, summarized in Table I, are in excellent agreement, proving that the four methods are equally reliable, and confirming the validity of the implicit assumption that all of the oil is extracted by the methanol.

Methods A, B, and C determine total oil equivalent when applied to cottonseed meal, and lipids when applied to a meal previously extracted with petroleum ether. When these methods are used instead of the official AOCS methods, a suitable materials balance can be expected in the processing of cottonseed and there will be no unexplained hidden loss of oil. These methods should also be applicable to soybean, rapeseed, and sunflower seed meals and to other systems in which the oil is bound by the protein, e.g., flours and doughs (5), and leaf protein (6).

Method B is the most convenient and best suited for general use. In addition to being simple and precise, Method C affords the best procedure for determining the cyclopropenoid acid content of cottonseed meals, since the oil in the meal is isolated as the methyl esters instead of the acids. This is important because the acids would have to be converted to esters to remove the interfering substances before HBr titration (7).

Another advantage of Methods B and C is that they can be conveniently applied to much larger samples, resulting in higher yields of isolated acids or esters, such as are needed, for example, to determine cyclopropenoid acid content.

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