

Conductivity Analysis for Free Fatty Acids In Oil from Glandless Cottonseed

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Abstract

Attempts to determine free fatty acids in petroleum ether extracts of cottonseed and crude cottonseed oil by conductivity measurements were unsuccessful due to interference of gossypol. With new strains of glandless cottonseed, which do not contain gossypol, analysis of free fatty acids by conductivity was successful on both the petroleum ether extracts and on the crude oil. An important advantage of conductivity over titration is that sample preparation is eliminated, since conductivity measurements are made on the oil extracts from the total oil determination.

Introduction

THIS LABORATORY is investigating the possible use of conductivity measurements for the determination of free fatty acids in crude cottonseed oils. Vegetable oils have a low dielectric value and so are poor conductors of electrical current in solution. The resistance of a crude vegetable oil in n-butyl alcohol is about the same as that of a refined oil; equal amounts of crude and refined oil in n-butyl alcohol are indistinguishable by electrical conductivity.

If, however, ammonium hydroxide is added to n-butyl alcohol (ammoniated-butanol reagent) the free fatty acids present in the crude oil will react with ammonium hydroxide to form soluble ammonium salts. These salts increase the conductance of the solution above the level of ammoniated-butanol solution. The increase in conductance of the crude oil solution is proportional to the concentration of the salts formed. The precision of free fatty acid measurement by conductivity, at the 1% level, is within $\pm 3\%$.

We have been able to form and measure the conductance of ammonium salts of linoleic, oleic, stearic, and palmitic acid by dissolving the acids in orthodichlorobenzene (ODB) and neutralizing them with ammoniated-butanol reagent. The reaction is instantaneous and proceeds practically to completion. Ammonium salts of palmitic, stearic, oleic, and other organic acids have been prepared and their crystals analyzed by McMasters (1), Lehman (2), and Malkin

(3). Standard curves of the predominant free fatty acids in crude cottonseed oils are shown in Figure 1.

Experimental

Apparatus

Components of the conductivity instrument are: 1) Conductivity cell immersed in a constant-temperature bath. 2) Wheatstone bridge. 3) Variable oscillator supplying alternating current at 400 or 1,000 cycles per second. 4) A vacuum tube voltmeter.

The conductivity cell has a capacity of 10 ml and a cell constant of $K = 0.1$. A bath temperature higher than ambient simplifies control of the bath and increases the electrical conductance of solution. From the available commercial thermoregulators, we selected 37°C for the temperature of the bath.

The fixed legs of the Wheatstone bridge are 1,000- and 10,000-ohm resistors. The cell leg is balanced by a variable digital resistor with a range from 0 to 10,000 ohms in steps of 100 ohms. A multiple-range voltmeter with ranges from 0.01 to 10 volts is used to detect the balance point of the bridge.

Preparation of Ammoniated-Butanol Reagent

One volume of reagent-grade ammonium hydroxide is added to 18 volumes of n-butyl alcohol (reagent grade). A slow stream of air is bubbled through the solution, by mouth, until the resistance decreases to approximately 13,000 ohms, due to the formation of ammonium carbonate salts. Five gallons of this solution require about 2 min of blowing.

Preparation of Standard Oleic Acid Curve

Reagents: 1) Oleic acid, ACS purified. 2) Orthodichlorobenzene (ODB), 99%. 3) Neutral vegetable oil: refined cottonseed oil which had been chromatographed according to AOCS Method Ca 9f-57.

Standard Solution: 1) Solution containing 2 mg oleic acid per ml ODB.

Plot a standard oleic acid curve (as shown in Fig. 1) in the following manner: Weigh 1 g of neutral oil (± 0.01 g) into each of eight 50-ml glass-stoppered erlenmeyer flasks. Add 1-8 ml of standard solution to flasks. Adjust the volume in each flask to contain a total volume of 11 ml by adding ODB from a pipette or burette (assume the volume of 1 g oil to be 1 ml). Add 30 ml ammoniated-butanol reagent with a pipette and shake well. The volume of this solution should provide for two or three full rinses of the cell plus a final volume for the recorded measurement. The standard curve can be extended for samples with higher acid concentration.

Analysis of Sample

Add 10 ml of ODB to each flask containing the weighed petroleum ether extract used in the determination of the total oil content of the cottonseed by AOCS method (these extracts normally range in weight from 0.9 to 1.1 g). Add 30 ml of ammoniated-butanol reagent to the flask; stopper, and shake well. Rinse the conductivity cell two or three times with this solution. Add a final volume to the cell and

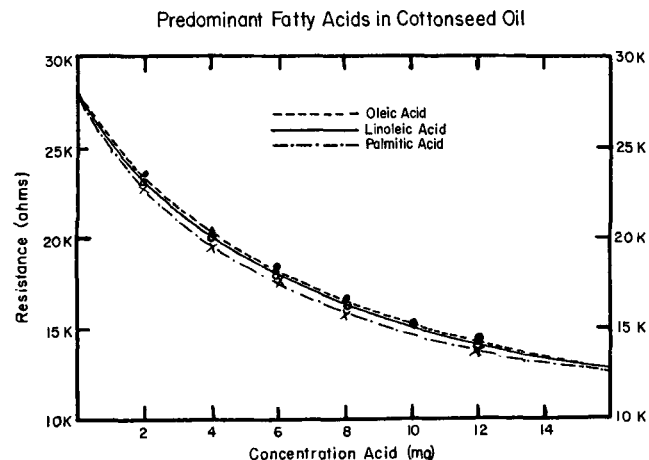


FIG. 1. Predominant fatty acids in cottonseed oil.

measure the resistance, 2 min after addition to allow for temperature equilibration. Read free fatty acid content of the extract from the prepared standard oleic acid curve and calculate the percent oleic acid in oil from the weight of extract. Note: If the weight of the extract is less than 0.9 g, an additional volume of ODB must be added to increase the resistance to the level of 1 g extract. A volume of 0.2 ml ODB gives an approximate resistance of 0.1 g extract. Similarly, if the weight of extract is over 1.1 g, the volume of ODB must be reduced in the above ratio to bring extract to 1 g level. This correction will occur in less than 5% of the extracts (4).

Results and Discussion

Early investigation of crude cottonseed oils revealed that in the crude oil :: ammoniated-butanol solution, conductance was due mainly to the formation of ammonium salts of free fatty acids, gossypol, and phospholipids.

The initial effect of gossypol ammonium salts on conductivity is 1.5 to 2 times as great as that of oleic acid salts; the gossypol salts, however, are unstable and their breakdown can be observed by the steady decrease in conductance of the solution.

The ammonium salts of phospholipids, on the other hand, were found to conduct only about 5% as much as the oleic acid salts. The error introduced in free fatty acids calculation by phospholipids at the 3% level would amount to 0.15%. This error is in line with the error introduced by phospholipids in the titration of free fatty acids. The acid value of vegetable phospholipids was found to be about 6% that of oleic acid.

In order to determine free fatty acids present in crude cottonseed oils by the proposed conductivity method elimination of gossypol is necessary. A way of accomplishing this was thought possible by utilizing the oil samples from the total oil determination of cottonseed. These oil samples are extracted from cottonseed with petroleum ether (bp 30–60C) which according to literature (5) will not dissolve gossypol. In this manner we would have an oil sample free of gossypol and one for which time of preparation had been eliminated.

Preliminary conductivity investigation of free fatty acids in petroleum ether extracts was very encouraging. However, as a greater number of extracts were analyzed, serious variation in free fatty acid results began to occur between the titration and conductivity methods. Investigation of this variation revealed that some of the petroleum ether extracts contained gossypol.

New strains of cottonseed have been bred to eliminate the undesirable glands of gossypol. Crude oil

TABLE I
Determination of Free Fatty Acids in Extracts of Glandless Cottonseed by Titration and Conductivity

Sample	Petroleum ether extraction	
	Room temp	Reflux temp
	FFA	FFA
	Titration ^a	Conductivity ^b
Crude oil	(%)	(%)
C	0.5	0.4
U	0.4	0.4
Cottonseed	(Commercially extracted with hexane)	
N. Carolina (M)	0.2	0.2
Mississippi (D)	2.8	2.7
California (S)	0.3	0.3
N. Carolina (S)	0.2	0.2
Mississippi (S)	0.4	0.5

^a AOCS Official Method Aa 6-38.

^b Extracts on hand from AOCS Official Method Aa 4-38 Oil Content.

extracted from glandless cottonseed presented an excellent opportunity to determine the reliability of the conductivity method on oil free from gossypol. Two crude oil lots were analyzed with good results (Table I). These oils had been commercially extracted with hexane.

Recently six new varieties of glandless cottonseed were made available to us through the cooperation of Mr. Garlon Harper of the National Cottonseed Products Association. These new varieties were developed by cottonseed breeders from the states of North and South Carolina, Georgia, Mississippi, Oklahoma, and California. Analyses of free fatty acids, determined by the titration and conductivity methods, in the petroleum ether extracts of these seeds were found to be in close agreement (Table I). The petroleum ether extracts used in the conductivity measurements were found to be free of gossypol and phospholipids as determined by the official colorimetric methods of the AOCS.

Results of these tests indicate that free fatty acid content of oil from glandless cottonseed can be determined as accurately by conductivity as by titration. The advantage of conductivity over the titration method is that sample preparation is eliminated, thereby increasing the efficiency of analyzing oils from cottonseed.

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Several oil mills and cottonseed breeders, as mentioned in the text, submitted samples for this study.

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