Modified Indirect Conductivity Method for Determining Water in Cottonseed Meal

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Indirect conductivities and moisture were determined on 11 cottonseed meal samples (3.1 to 14.5% water). The following values were obtained from a statistical analysis of the data: equation of regression line, % H_2O = (73.4)(millimhos) — 22.1; correlation coefficient, 0.997; standard deviation from regression line, 0.27% water. Replicate determinations were made on each of two samples (4.2 and 9.6% water), for both samples the standard deviation was 0.20% water. These results show that the accuracy and precision of the indirect conductivity method are satisfactory for practical applications. The time required per determination was about 9 minutes; it could be reduced to about 5 minutes by using duplicate sets of stirring apparatus.

An indirect conductivity method for the determination of water in soils (*1*) involves the use of alcohol-acetone-water–sodium chloride systems, the fundamental basis of the method being that the conductivity of the system will be proportional to the sodium chloride concentration, which, in turn, will be proportional to the water concentration. The use of a large excess of sodium chloride tends to mask the effect of other electrolytes that may be present in the material being tested.

First attempts to apply this method to the determination of water in cottonseed meal were unsuccessful, largely because of ineffective stirring. This fault was corrected by use of a homogenizer-type stirring apparatus. Other modifications of the original procedure were made, in order to keep the method as simple and as rapid as possible. The present article describes the modified procedure and presents encouraging results from the determination of moisture in 11 cottonseed meal samples.

Apparatus

A portable Model RC-1B conductivity bridge (Industrial Instruments, Inc., Cedar Grove, N. J.) was used. This bridge operates on 115-volt, 50- to 60-cycle alternating current circuits. The Wheatstone bridge is supplied with 1000-cycle alternating current from an integral vacuum tube oscillator, balance being indicated by a cathode ray tube null indicator.

A glass, dip-type conductivity cell with cell constant of 1.020 $cm.^{-1}$, determined at 35.0° C. with 0.01*N* potassium chloride (*2*), was used.

Satisfactory agitation of mixtures was obtained by use of a Model 10 Oster mixer (John Oster Mfg. Co., Racine, Wis.).

Materials

Absolute ethyl alcohol and acetone were the same as those used in previous work (*1*). A 5-gallon bottle of 70% ethyl alcohol–30% acetone was equipped with a long thermometer, a siphon tube connected to a 100-ml. automatic pipet, and appropriate pinch clamps and drying tubes. The pipet was calibrated and graduated to deliver 100.00 ml. of mixed solvent (corrected to 35.0° C.) over the range of 28° to 35° C.

Common table salt (Morton Salt Co., Chicago, Ill.) was used directly from a 100-pound bag. A 10.000-gram, air-exposed sample of this salt was weighed 18 times during a 29-day period and the maximum variation in weight was found to be only 0.002 gram; consequently, it was considered unnecessary to dry the salt before use. This practice may not be permissible in extremely humid climates or weather; however, at several weighing times during the 29-day period, although the relative humidity was near 100%, the 10.000-gram sample of salt showed no significant gain in weight. The approximate sieve analysis of this salt is shown in Table I.

Cottonseed meal samples from several sources were combined, and mixed thoroughly, and the mixture was divided into two portions. Enough water was added to one of the portions to bring the moisture content to about 12%. Varying amounts of the wetted and unwetted portions were combined and mixed to obtain 10 samples with a representative range of moisture contents. A sample from the wetted portion was wetted further to ensure having one sample with more than 12% water. The resulting 11 well-mixed samples were sealed in 1-quart Mason jars and stored for 6 days to obtain uniform distribution of the moisture.

All glassware was washed with soap solution, rinsed with tap water, drained, and air-dried. Comparative tests showed that rinsing with distilled water or drying in an oven was of no advantage.

Table I. Approximate Sieve Analysis of Morton's Evaporated, Granulated Salt

Sieve No.	% Passing	Sieve No.	% Passing
10	100	60	21
20	99.7	80	10
40	38	100	4

Procedure

A 50.0-gram portion of Morton's salt, plus 10.00 grams of cottonseed meal plus 100.00 ml. (corrected to 35.0° C.) of 70% ethyl alcohol–30% acetone mixture, was placed in a 1-pint Mason jar and agitated for 5 minutes in an Oster mixer. The temperature rose to about 55° C., resulting in a pressure increase in the jar. Consequently, safety goggles and gloves were used in handling the warm jar, and this precaution was followed rigorously, even though no jars exploded. The sealed mixture was cooled for 1 minute in running tap water in a pneumatic trough. The agitator assembly was replaced with a regular screw cap (the agitator assembly was cleaned and used immediately in the next determination) and the mixture was allowed to settle for 4 minutes while being cooled in the running tap water bath. The supernatant extract was decanted into a 6-inch test tube (inside diameter of 28 mm.). The dip-type conductivity cell was lowered and raised several times in the extract and then clamped so that the top of the extract was about 0.5 inch above the ports in the cell.

Table II. Water Contents and Indirect Specific Conductances of Cottonseed Meal Samples

% Water by Oven-Dry Method	Indirect Specific Conductance, Millimho/Cm.
7.3	0.396
5.7	0.376
3.1	0.351
4.2	0.360
14.5	0.502
6.6	0.392
7.9	0.407
5.6	0.378
9.6	0.432
9.0	0.422
5.2	0.369

Four measurements of the resistance were made. As the deviation among these four readings was never significant, the average was used. The average of closely agreeing duplicate determinations on each sample was taken. When this procedure was used, at the time of the resistance measurements, the temperatures of the various decanted extracts all fell within the range of 29.4° to 30.2° C. The average resistance in ohms was converted to specific conductance in millimhos per centimeter in the usual manner by using the cell constant of 1.020 $cm.^{-1}$ After this research was completed, an examination of all the resistance readings showed, in all cases, that the average of the first two values did not deviate significantly from the average of the four readings; consequently, it appears that two readings are sufficient to give a reliable average.

Portions of the 11 cottonseed meal samples were used for duplicate determinations of moisture by the oven-dry method (5-gram sample dried for 2 hours at 101° C.). The average of the closely agreeing duplicate determinations was used.

Table III. Precision of Resistance Measurements at 29.4° to 30.0° C.

	Water, Weight %	
	4.2	9.6
Run No.	Resistance, Ohms	
1	2830	2365
2	2830	2354
3	2785	2364
4	2835	2325
5	2785	2352
6	2825	2362
7	2795	2355
8	2792	2323
9	2838	2372
10	2810	2344
11	..	2348
12	..	2362
13	..	2350
Av., ohms	2812.5	2352
Av. dev., ohms	19.10	10.77
Stand. dev., ohms	21.53	14.66
Stand. dev., % water	0.20	0.20

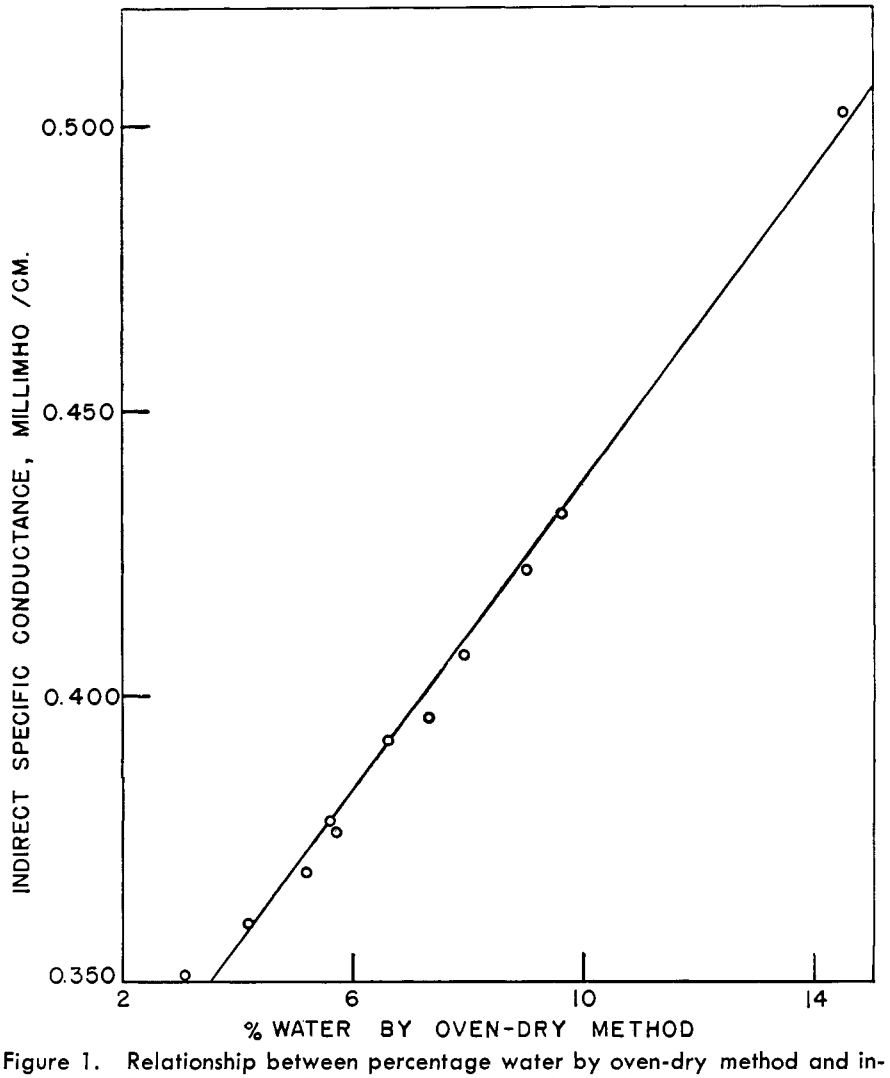

Figure 1. Relationship between percentage water by oven-dry method and indirect specific conductance for 11 cottonseed meal samples

Results

For the 11 cottonseed meal samples, the indirect specific conductances and the percentages of water by the oven-dry method are listed in Table II and plotted in Figure 1. Statistical treatment (*3*) of the data of Table II yields the following results: (1) equation of the regression line, per cent water = (73.4) (specific conductance) −22.1; (2) correlation coefficient, 0.997; (3) standard deviation from the regression line, 0.27% water. The regression line is shown in Figure 1.

In order to evaluate the precision of the method, replicate resistance measurements were made on each of two of the cottonseed meal samples (4.2 and 9.6% water). The results and statistical data (*3*) resulting therefrom are given in Table III.

For the series of determinations, the average time required per determination was about 9 minutes. This time could be reduced to about 5 minutes by using two Oster mixers.

Discussion

The modified indirect conductivity method offers much promise as a simple, rapid laboratory control procedure for the determination of water in cottonseed meal.

Several measurements of the temperature coefficient of conductance of alcohol-acetone-water–sodium chloride systems were made, the average value being about 0.6% per ° C. This value is considerably smaller than the coefficient of 2% per ° C. that is generally accepted for aqueous systems; consequently, temperature control is less critical for the indirect conductivity method. The temperatures of the decanted extracts, at the time of the resistance measurements, were between 29.4° and 30.2° C. In view of this narrow temperature range and the relatively low temperature coefficient, no temperature corrections were applied to the data given in Tables II and III.

If temperature corrections had been applied to these data, statistical treatment of the corrected data might have yielded slightly more accurate and precise values. It is believed that temperature

corrections in this study were less significant than the errors introduced by using percentages of water determined by the oven-dry method as criteria of accuracy. In any event, the final practical answer to this problem is the incorporation of a temperature-compensation feature in the Wheatstone bridge.

The intimate mixing and temperature rise resulting from agitation with an Oster mixer are both beneficial, in that both help toward transferring water from the cottonseed meal sample to the mixed solvent and subsequently saturating the alcohol-acetone-water system with salt. As shown by the results, very nearly reproducible temperatures can be obtained by cooling the warm mixture for 5 minutes in a running tap water bath.

Close inspection of Figure 1 indicates the possibility of some curvature in the relationship at lower percentages of water. This was disregarded in the statistical treatment of the data, the analysis being made by linear regression (*3*). This appears justifiable in view of the degree of accuracy and precision found. If the indicated curvature at lower percentages of water is real, the curved portion could be eliminated by adding an appropriate amount of water to the stock supply of alcohol-acetone mixed solvent.

Further research on this method is in progress. The bridge of a Model RB-26 Solu-Bridge (Drawing A230E, Industrial Instruments, Inc.) has been modified (by changing four of the bridge resistances to the following values in ohms: *R*1, 1230; *R*2, 1000; *R*3, 874; *R*4, 560), so that the full scale represents 0.2 to 1.0 millimho. The Model RB-26 is a small, portable, battery-operated, 1000-cycle instrument with an adjustable temperature compensator. By suitable calibration, the scale has been modified to read percentage of water directly and the temperature compensator modified to apply to alcohol-acetone-water–sodium chloride systems. A pipet-type conductivity cell is being used with this modified bridge.

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EGG PROTEINS

Separation of Egg White Proteins by Paper Electrophoresis

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The need for a rapid method for the quantitative determination of the individual egg white proteins led to a study of their separation by paper electrophoresis. A procedure was developed whereby the proteins in whole egg white were separated by the ridgepole technique of Durrum, using a pH 8.6 diethylbarbiturate buffer of 0.05 ionic strength. The separated proteins were dyed with bromophenol blue, the color was eluted with dilute sodium hydroxide solution, and absorbance was determined at 590 mμ in the spectrophotometer. Fresh egg white protein contained 65.2% ovalbumin (48.4% A_1, 12.6% A_2, and 4.2% A_3), 11.2% ovomucoid plus ovoglobulin, 17.0% conalbumin, 2.1% nonmobile protein, and 4.5% lysozyme. The method is ideal for studying possible changes in egg white proteins during storage of shell eggs, because of its speed and the reproducibility of results.

PAPER ELECTROPHORESIS offers a new technique for the study of proteins. It is less expensive and more rapid than the conventional moving-boundary electrophoresis method and has been successfully used, especially for the separation and study of blood proteins. Tiselius and Flodin (*22*), Block, Durrum, and Zweig (*3*), and McDonald (*15*) have reviewed the literature on the general subject of paper electrophoresis.

Longsworth, Cannan, and MacInnes (*14*) were the first to make an extensive study of the protein of egg white by means of moving-boundary electrophoresis. Bain and Deutsch (*2*), Forsythe and Foster (*10*), and Csonka and Jones (*6*) have also used the Tiselius moving-boundary electrophoresis method for the study of egg white proteins.

Nothing has been published on the use of paper electrophoresis for the separation of egg white proteins. This method, however, should be useful in the quantitative study of the proteins of egg white, if these proteins can be satisfactorily separated by this means. The purposes of the experiments reported herein were to study the behavior of egg white proteins when subjected to