Effect of Relative Humidity On the Determination of Oil In Soybeans

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It is well known that the quantity of oil extractable from soybean meal with petroleum ether varies with the moisture content of the meal. Within reasonable limits, the higher the moisture level the greater the quantity of oil extracted (3, 6).[3] However, experience has shown that duplicate samples at the same original moisture level may give variable results even in the hands of experienced operators when the analyses are made on different days or under different conditions. There are wide discrepancies among the results obtained by collaborators making a study of oil extraction so that the routine determination of oil by solvent extraction merits thorough investigation. In reporting such a study, Taylor (9) states that "one of the factors contributing to these discrepancies is probably a lack of uniform procedure with respect to moisture under the A. O. C. S. method, particularly in the case of soybean meal." The need for an increase of precision in the determination of oil in soybeans is evident. Since in collaborative tests humidity conditions during analysis were not specified and were only a matter of chance, this factor may account for much of the disagreement among results obtained.

It has been noted at the U. S. Regional Soybean Laboratory that analyses made under conditions of high relative humidity give from .6 to 1.2 percent higher results than are obtained from the same sample having the same original moisture content under dry atmospheric conditions. Analysis of filter paper showed that under conditions of high humidity, the filter paper may contain a greater quantity of water than the meal wrapped in it and under conditions of low humidity contains much less. There could easily be an exchange of moisture between meal and paper so that the moisture content of the meal is altered. Also at the time of regrinding, the amount of water in the meal is increased or decreased according to the humidity of the air and the amount of water in the sample. Frost often forms on the sample and paper at this time due to the rapid evaporation of the petroleum ether causing cooling and condensation of water from the atmosphere on the paper and meal. It is evident from these observations that the moisture level at which the sample is actually run is determined only partially by the original moisture level and might vary widely from it according to the atmospheric conditions.

It is the purpose of this study to determine the effect of variations in relative humidity in the laboratory on the moisture content of the meal during extraction and its effect on the precision with which the percent of oil in the meal can be determined by the usual method of analysis (1), but using a two-hour extraction after regrind.[4]

In order to determine the actual moisture content of the meal sample under extraction conditions, a modification of Fischer reagent (5) was used which gave very satisfactory results both on the original samples and on samples wet with petroleum ether. The results checked those obtained by the regular oven analysis (10) within one-tenth of one percent for samples of moisture content between 1.5 and 16.8 percent.

Methods

Dunfield, Illini, Lincoln, and Peking varieties of soybeans were ground with a Wiley mill through a 1 mm. screen. Moisture levels were then adjusted at room temperature by drying in a desiccator or placing in a desiccator over water to give the desired moisture content. Three moisture levels were prepared from each variety as follows: 4.35-5.00 percent, 6.40-6.90 percent, and 8.00-8.65 percent.

After conditioning, each meal sample was thoroughly mixed and a moisture analysis made by the official forced draft oven method (10). The meal was then kept in tightly sealed jars to prevent loss or gain in moisture content. Samples were weighed from these jars and wrapped in filter paper for oil extraction. Since the normal time required to weigh samples and prepare to extract the oil is approximately thirty minutes, these samples were allowed to condition for thirty minutes at the relative humidity level at which they were to be analyzed. The extraction room, which was also used for conditioning and regrinding the samples, was equipped to keep the air in circulation in addition to an exhaust for ventilation. These fans were placed so that strong air currents did not strike the samples. Relative humidity was determined and recorded by a Friez Hygro-Thermograph which had been checked for accuracy by comparing it with the United States Weather Bureau's instrument located in the building housing our laboratory. In the case of analyses at 20 percent relative humidity, the filter papers were oven dried for one hour at 130° centigrade and kept in a desiccator until used. Since it was not possible to obtain conditions of 20 percent relative humidity in the laboratory, these samples were weighed out, wrapped, and extraction started in the shortest possible time. After the two-hour extraction, the samples were reground in a room maintained at the desired relative humidity.

Three relative humidity levels representing the range of humidity encountered in this laboratory were used: 20-25 percent, 50-55 percent, and 75-80 percent. For the 20 percent humidity level, the samples were reground in a seed storage room maintained

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[3] Figures in parenthesis refer to "Literature Cited."

[4] Experience in this laboratory has shown that this method is equivalent to the A. O. C. S. method in results obtained.

at 20 percent and 70° Fahrenheit (4). For the 50-55 percent relative humidity level, the room temperature ranged from 70-87° Fahrenheit and for 75-80 percent, from 80-97° Fahrenheit. The temperature for any one extraction varied not more than 5° Fahrenheit throughout the period and much less at regrind time. The condensers used during the extraction were capped with lead foil to prevent any possible condensation of water inside the condenser from the atmosphere. Two-gram samples were analyzed according to the regular A. O. C. S. method using Butt apparatus, extracting for two hours with petroleum ether, regrinding for one minute, and then extracting for two more hours. The petroleum ether extract was then heated on a steam bath for 45 minutes and the remaining oil cooled and weighed. The percent oil was calculated to a moisture-free basis. In order to approximate normal operating conditions, the wrapped samples were exposed to the atmospheric conditions about 30 minutes at regrind time. A sufficient number of identical samples were started extracting simultaneously to allow the removal of samples for moisture and oil determination for the following time intervals of extraction: 30 minutes, 60 minutes, 120 minutes, after regrind (for moisture determination only), 150 minutes, 180 minutes, and 240 minutes. Data obtained in this study seem to indicate that a shortened extraction period could be used when the meal is at a high moisture level and the relative humidity is 75-80 percent. In order to check the accuracy of this method, a random selection of samples was analyzed in the following manner: extraction with petroleum ether in Butt extraction apparatus for one hour, regrind one minute, and then extraction for one hour with petroleum ether. The moisture content of the samples ranged from 4.73 to 16.8 percent and the relative humidity was maintained at 75-80 percent during the regrind period. A duplicate of each of the above samples was analyzed by the regular four-hour extraction method and very good agreement was shown between results from the two methods for moisture levels from 8.0 to 16.8 percent as shown in Table I. It was not necessary to maintain the extraction room humidity at 75-80 percent during the entire extraction but only during that time when the samples were being reground. The samples might well be reground in a hood equipped to maintain a high humidity and circulate the air to remove the petroleum ether fumes.

For moisture determination, the meal while still wet with petroleum ether was transferred as rapidly as possible from the filter paper to an oven-dried flask. Fifty milliliters of dry methyl alcohol was then added and the mixture shaken, allowed to stand one hour, and then shaken for fifteen minutes on a mechanical shaker. The samples were then titrated with a modified form of Karl Fischer reagent which was prepared as follows: add 280 grams of I_2 crystals to a mixture of 1170 ml. dry synthetic methyl alcohol and 450 ml. pyridine and mix thoroughly. Cool this mixture by placing in a refrigerator or in an ice bath and add 220 grams of dry refrigeration grade SO_2 slowly, shaking the mixture occasionally during the addition of the gas.

Since the reagent is less stable after the SO_2 is added (8), the mixture of pyridine and methyl alcohol may be prepared in quantity and SO_2 and I_2 added to only as much as will be used within a week. The reagent was standardized with a standard water solution containing about ten grams of H_2O per liter in dry CH_3OH. One hundred and fifty milliliters of the freshly prepared reagent will titrate approximately one gram of H_2O, but gradually loses its strength so that it should be standardized daily. The reagent was

TABLE I

Percentage Oil [1] Extracted from Soybeans by Two-hour Extraction and Four-hour Extraction at Different Moisture Contents and Humidity Levels

Variety	Original Analysis [2]		Analysis Under Controlled Humidities and Adjusted Moisture Levels				
			Adjusted Moisture Level % Moisture	50% Relative Humidity		80% Relative Humidity	
	% Moisture	% Oil		Short Extraction [3] % Oil	Long Extraction [4] % Oil	Short Extraction [3] % Oil	Long Extraction [4] % Oil
Dunfield			4.73	22.9	23.6	23.3	24.0
Dunfield	6.67	23.7	6.67	23.5	23.7	24.1	23.9
Dunfield			8.40	24.0	23.9	23.9	23.9
Dunfield			16.80	24.1	24.1	24.0	24.1
Illini	6.40	22.2	4.73	21.5	22.2	22.2	22.7
Illini			6.40	22.0	22.2	22.1	22.8
Peking			5.03	16.1	17.7	17.3	18.0
Peking	6.85	17.7	6.85	17.3	17.7	17.7	18.0
Peking			8.65	17.7	17.9	17.9	18.0
Lincoln	6.85	22.5	8.03	22.3	22.5	22.5	22.8
Mandarin	5.10	20.1	13.55	20.1	20.2	20.3	20.3
Richland	5.40	22.1	13.40	21.4	22.0	22.1	22.1
Mukden	5.30	21.3	13.60	21.4	21.4	21.5	21.5
Illini	5.60	20.4	13.78	20.5	20.5	20.8	20.8
Illini	5.25	22.4	14.09	22.7	22.9	23.0	23.0
Boone	5.55	22.9	12.77	23.3	23.4	23.4	23.4
Boone	5.55	23.3	12.72	23.3	23.5	23.5	23.5
Dunfield	6.05	21.8	13.43	22.1	22.3	22.2	22.3
Illini	6.05	20.5	13.53	20.9	21.0	21.0	21.1
Illini	5.50	20.7	12.90	20.9	20.8	21.0	21.0
Sioux	6.05	13.1	13.50	13.4	13.5	13.7	13.5
Sioux	6.20	12.3	13.90	12.5	12.5	12.5	12.3
Richland	5.45	21.0	12.25	20.9	21.0	21.2	21.2
Sioux	6.30	14.1	13.75	14.6	14.5	14.7	14.7
Manchu Kota	5.70	20.6	13.20	21.0	21.0	21.1	21.2
Herman	5.40	21.7	12.46	21.5	21.9	21.9	22.0
Mean		20.50		20.46	20.69	20.73	20.85

[1] Calculated to a moisture-free basis. [2] Data taken from laboratory records of original analyses. [3] One-hour extraction, one-minute regrind, and one-hour extraction. [4] The long extraction was the usual method consisting of a two-hour extraction a one-minute regrind, and a two-hour extraction.

added to the sample in dry methanol and the sample was shaken occasionally during the titration. A brown iodine color which persists for at least fifteen minutes is taken as the end point. The concentration of the reagent given above is much higher than that of the original Fischer reagent, and the proportions are altered somewhat; but after some experimentation, the above proportions proved most satisfactory. The reagent was stored in a glass-stoppered bottle and dispensed from a 50 ml. automatic burette protected from atmospheric moisture by means of Drierite.

In order to check the accuracy of the method, samples of the meal were analyzed for moisture content by the official method (10) and with the reagent and in each case results checked within one-tenth of one percent. The accuracy of the determination of moisture during extraction was also checked by determining the amount of moisture in sample and paper before extraction of the oil and by accounting for the moisture after two hours of extraction while the sample and paper were still saturated with petroleum ether. The moisture in the sample and paper was also determined by drying duplicates of the wrapped sample for five hours in a forced draft oven at 130° centigrade. The dried wrapped sample was transferred while still in the oven to a tared weighing bottle, cooled, and weighed. Table II is a tabulation of these results.

TABLE II

Total Moisture Before and After Extraction

	H_2O in Meal and Wrappings	H_2O from Reflux Condenser	H_2O from Extracted Oil + Solvent	Total Moisture
Before Extraction:				
Oven analysis	290 mg.	.....	....	290 mg.
Titration Meal	114 mg.			
Wrappings	177 mg.	.....	....	291 mg.
After Extraction:				
Sample I	212 mg.	81 mg.	0	293 mg.
Sample II	203 mg.	88 mg.	3	294 mg.
Sample III	211 mg.	82 mg.	0	293 mg.

It is interesting to note that in first attempting to account for all water before and after extraction as shown in Table II total moisture after extraction always exceeded the initial moisture by five to fifteen milligrams when the regular Butt extraction apparatus was used. However, when the cork stopper connections were replaced by ground glass connections, these results were obtained indicating that the cork stoppers were a source of a variable amount of moisture. In order to check this conclusion, the regular Butt apparatus with cork connections was operated without sample or wrappings. The condenser was capped with lead foil to prevent the entrance of any atmospheric moisture. Visible amounts of water accumulated in the reflux condensers which when washed out with alcohol and titrated gave results accounting for the additional moisture found. The amount of moisture involved would be of little importance so far as its effect on the oil determination is concerned but this experiment does demonstrate the accuracy of the method of moisture determination. It is rather surprising that the iodine of the reagent does not seem to react with the unsaturated oil in the meal, but the iodine color often persists in titrated samples overnight indicating that the iodine is not absorbed to any significant extent. Fischer in his original work (5) and Smith, Bryant, and Mitchell (8) in their later investigations with Fischer reagent found that the iodine shows practically no tendency to react with ethylenic bondings. McKinney and Hall (7) allowed an excess of the reagent to stand with pine oil for fourteen minutes and then back titrated with a standard water solution. They found that no iodine had been absorbed by the oil.

The determination of moisture by use of the Fischer reagent seems to merit consideration for regular use especially where it is necessary to obtain results of reasonable accuracy where conditions make the regular oven method impractical.

Discussion

It is obvious from graphs such as Figure I that relative humidity of the atmosphere in which the de-

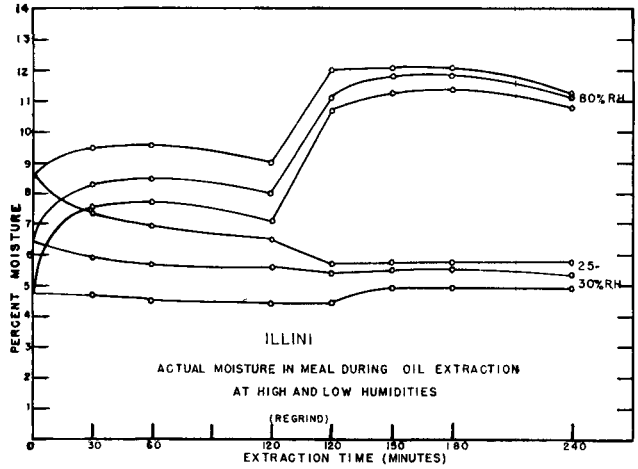

FIG. I. Effect of high and low relative humidity on moisture content of soybean meal during oil extraction.

termination is made is a very important factor in determining the actual moisture level at which the sample is extracted. Since the quantity of oil which can be extracted from the beans is known to be related to the percent of moisture in the sample, any change in the moisture content of the meal during the extraction period or at the regrind period may cause a decrease or increase in the rate of oil extraction.

In the extraction of oil from the Illini variety at an original moisture level of 4.73 percent at 25 percent, 50 percent, and 80 percent relative humidity the amount of oil which is extracted is 1.2 percent greater for the 80 percent humidity than it is for the 20 percent relative humidity as shown in Figure II. At 80 percent humidity, the amount of oil at any period of time during the extraction is always greater than that which is extracted in a like period at lower humidities. The corresponding study of the moisture in the meal is in close agreement with these results as shown in Figures I and II. In relatively low moisture content meals, the percent of oil extracted at 80 percent, 50 percent, and 20 percent humidity is greatly affected by the amount of moisture in the meal as shown by moisture analysis of the same sample of meal from which the oil was extracted. Samples beginning with the same moisture contents may actually be extracted at much different moisture levels if they are analyzed at different humidity levels. By the end of the extraction period, the moisture level is determined partially by the original moisture level but largely by the relative humidity during the regrind

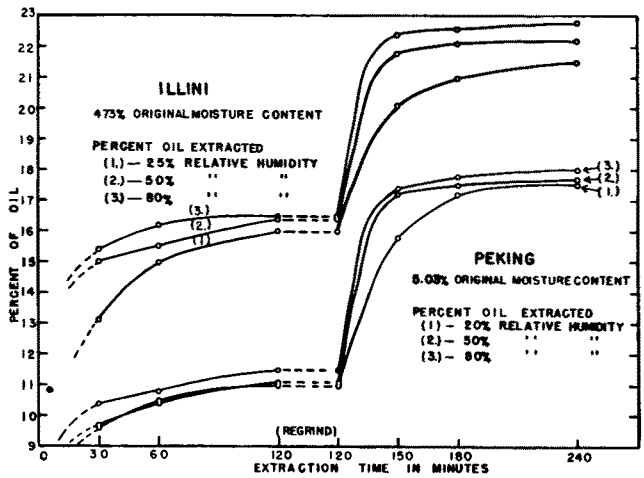

FIG. II. Showing different rates of oil extraction at three relative humidities for one original moisture level of soybean meal.

period. It may be noted in Figure I that under conditions of 25-30 percent relative humidity that a meal at about 5.5-6.0 percent H_2O remains at practically the same moisture level throughout the extraction. Similarly in Figure III under conditions of 20-25 percent relative humidity meal at about 4.7 percent remains at that moisture level. This indicates that under these conditions the moisture in the meal is in equilibrium with that in the atmosphere. These results are in close agreement with the results obtained by Beckel and Cartter (2) in another study. Figure III very strikingly shows the fact that especially after the regrind, the differences in moisture level are greatly reduced. The original samples had a range in moisture

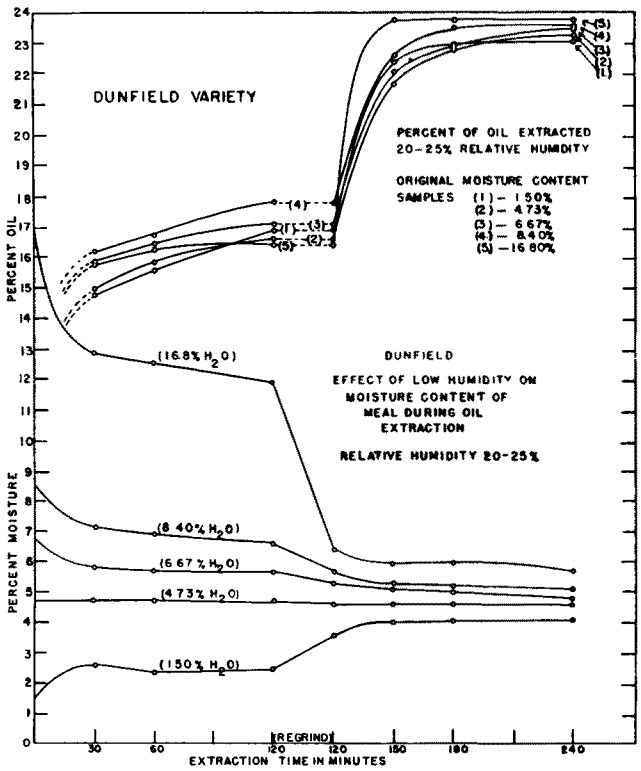

FIG. III. Showing effect of low humidity on moisture content and rate of oil extraction during the determination of oil in soybean meal.

content of over 15 percent, but during the last half of the extraction this was reduced to 2 percent or less. A very good example of the effect of relative humidity is shown in Figure I. Within less than 30 minutes after the beginning of the extraction, the sample having an original moisture content of 4.73 percent under conditions of 80 percent relative humidity actually was analyzed at a slightly higher moisture level than the 8.57 percent sample under low humidity conditions. Figure III shows that although the wide differences in moisture content of samples are greatly reduced at regrind time as indicated by the lower moisture curve, original moisture in the meal does have a very noticeable effect on the amount of oil extracted at low humidity levels as shown in the upper curves of the same figure.

In the corresponding study under high relative humidity conditions shown in Figure IV, original mois-

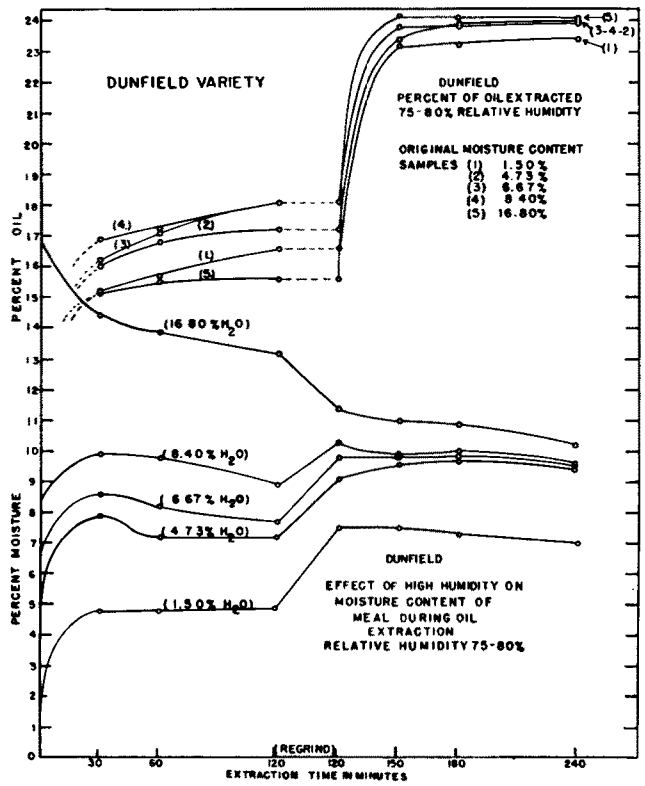

FIG. IV. Showing effect of high humidity on moisture content and oil extraction during the determination of oil in soybean meal.

ture level has much less effect. For moisture contents between 4.73 and 8.40 percent differences between results obtained are practically negligible. There is very good agreement between moisture curves and oil curves both indicating that extremely low original moisture of 1.5 percent does have an effect and that very high moisture of 16.8 percent gives higher results. It is interesting to note the low rate of oil extraction at 16.8 percent moisture until after the regrind when probably due to loss of water at regrind the oil is extracted very rapidly.

Similarly in Figure V at high relative humidities, original moisture levels between 4.73 and 8.65 percent had practically no effect on the total amount of material extracted under conditions of high humidity. The total amount of oil extracted was practically the same

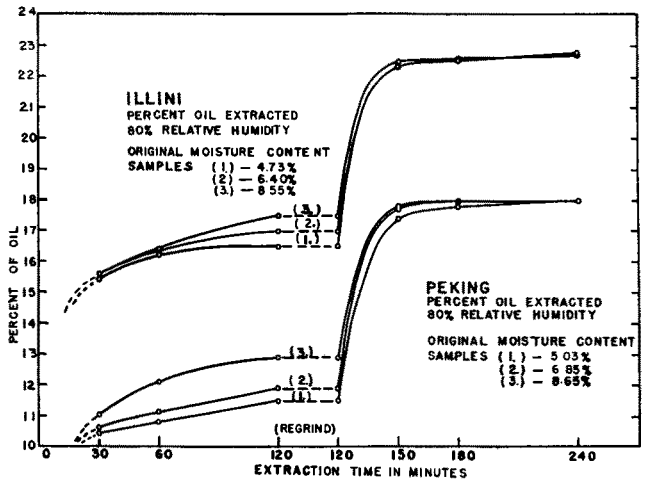

FIG. V. Showing effect of high humidity on rate of oil extraction at three moisture levels.

in either case. The data suggest the possibility of shortening the time of extraction when analyzing soybean meals of relatively high moisture content if the atmospheric conditions are such that a humidity of 75-80 percent exists. Table I shows that the extraction of oil at 75-80 percent relative humidity and high moisture levels can be accomplished without serious loss of accuracy in a two-hour period when these results are compared with those obtained from a four-hour extraction under like conditions. However, according to our laboratory records of analyses, the percent of oil extracted under these conditions was about .4 percent higher than was obtained when these same meals were analyzed at lower moisture levels and lower humidities by the usual four-hour method. We have no record of the relative humidity at the time these samples were analyzed originally but estimate that it must have been from 25 to 50 percent. When the short extraction period was used under conditions of 50 percent relative humidity, less consistent results were obtained particularly at lower moisture levels. At higher moisture levels, difficulty is experienced in grinding the samples for analysis and it was necessary to grind the samples at lower moisture content and then adjust the moisture in the meal by exposing it in open dishes under high humidity conditions.

In order to study the effect of moisture content, four Dunfield samples from the same lot of meal were adjusted to various moisture levels for analysis as shown at the top of Table I. Two Illini samples and three Peking samples were prepared in the same way. The remainder of the samples listed in the table are a random selection of samples of a number of varieties from various locations. It has been reported that at high moisture levels, a greater proportion of the phosphatides are extracted with the oil than when the moisture content of the meal is lower (6). This altered composition of the oil may not be objectionable if only total extractable material is the object of the analysis.

Conclusions

From the results shown it may be concluded that the percent of extractable material which is obtained is highly dependent upon the atmospheric conditions under which the sample is analyzed. When soybean meals with moisture content from 4.35 to 16.8 percent are analyzed for oil content at 75-80 percent relative humidity, the amount of extractable material is not dependent upon the original moisture level. However, at lower relative humidities or lower moisture levels this is not true. Under conditions of relatively high humidity with meals of high moisture content, the short two-hour extraction gives results which check satisfactorily with the results obtained by the official four-hour method under like conditions. The data tend to emphasize the fact that the determination of oil in soybeans is empirical and that any analysis does not necessarily represent the total amount of lipids present in the sample. The data shows the necessity of control of moisture conditions under which seed is stored and under which it is analyzed if reproducible results are to be obtained.

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