Methods for Cottonseed Analysis

Preliminary Report Presented at Twentieth Annual Convention American Oil Chemists' Society

By G. S. JAMIESON and R. S. MCKINNEY

AT the request of the industry, methods for the sampling and the analysis of cottonseed are being studied by a committee which has been appointed for this purpose at the Department of Agriculture. Briefly, the object of this Committee's work is to study different methods of sampling and analyzing cotton seed in order to determine those most efficient and accurate, so that they may be used in all analyses made on the authority and responsibility of the Department of Agriculture. The present report will be confined to methods of analysis, as another report covering sampling, together with certain recommendations, will be given by Mr. Meloy this week at the convention of the Interstate Cotton Seed Crushers Association, which will be published by that association.

Before commenting on the several methods studied in connection with the results which we have obtained to date and those obtained by others who have made a special study of these methods, attention is directed to the importance of getting portions for analysis from the sample of seeds which will be representative in so far as that is possible. The difficulty of drawing representative portions naturally increases with the quantity of bald, damaged, aborted seeds in the sample. Experience has shown that the sample, preferably on a smooth stout sheet of paper, must be very thoroughly mixed in a manner so as not to segregate the lighter and heavier portions that will result with any stirring or rotary motion, such as frequently witnessed by the Committee. In Mr. Meloy's report the committee has recommended that samples of approximately 1,000 grams should be sent to the laboratory in sealed airtight containers. It is further recommended that on the receipt of the sample, the chemist should carefully divide it into two equal portions; one portion being returned to the container as a referee sample, the other portion to be used for his analyses.

In reducing samples it is recommended that the mixing and quartering be done by passing the hands upward through a cone-shaped pile, flattening this and quartering by cutting through to the table. The pile of seeds is thus divided as nearly as possible into quarters of equal size; each of these quarters so obtained may be used for each of the determinations to be made, which include those for moisture, ammonia, free fatty acids and oil. Each quarter should be again thoroughly mixed (unless the entire quarter is to be used as a portion for analysis) and flattened into the form of a shallow pile. From this, a few seeds are to be taken at a time, from as many parts of the pile as possible, until a portion of the desired weight has been obtained. The mixing of the sample and drawing the portions for analysis should be done, of course, as rapidly as possible to avoid loss of moisture. It is assumed that the samples submitted for analyses are representative of the shipment of seed, but in actual practice, unfortunately, this assumption heretofore in many cases has not been true.

Determination of Moisture

FOR the sake of comparison, the moisture was determined by the Official Method, in which the weighed portion of cracked seeds was dried at 102-3°, for five hours, and the method based upon drying the seeds for one hour at 130°. All of the moisture determinations were made in ventilated De Khotinsky drying ovens. The results by both procedures checked each other in a satisfactory manner. Only in a few cases did the results by these two methods differ as much as .2 per cent. In so far as we have been able to ascertain the Official Method for moisture gives good results, when the directions are actually followed using the above mentioned ovens.

Determination of Ammonia

THE accuracy of the Official Method for the determination of nitrogen (which is usually stated in terms of ammonia) in seed is well known, provided a suitable sample is used; consequently, no particular study of the method was required. However, a number of analyses were made principally by way of comparison between the results that might be obtained by this method and a modification of it that was suggested in connection with making the entire analysis of a sample of seeds on a single 10 gram portion. In connection with this suggestion the other deter-

minations will be discussed later. After the removal of the oil, the 10 gram portion of seed was digested etc., in the usual manner and finally the solution was diluted to a definite volume in a graduated flask, and the ammonia was determined, using accurate aliquot portions of this solution. The results obtained by these two procedures in no case differed by more than .1 per cent; this was as expected. The results, however, would indicate that in so far as the determination of ammonia is concerned, the smaller portion of seeds taken according to the Official Method are sufficient to yield satisfactory results.

Determination of Free Fatty Acids

SOME attention was given to the determination of the free fatty acids in the oil extracted from the seeds by cold percolation with petroleum ether according to the Tentative Method given in the book of "Rules." In order that more concordant results may be obtained by different laboratories, it is believed that a detailed description of the procedure to be followed in arriving at the end point of the titration should be given. In other words, the end point should be the same in all cases. A visit to various laboratories showed that this was not the case, besides it was observed that several different indicators were being used; comparatively few were found using Alkali Blue, whereas the majority of those visited used phenolphthalein as indicator, which is the one we prefer to all others that have been proposed for this titration. Furthermore, we believe that the titation should be conducted with vigorous shaking until a pink color is obtained that remains permanent for a minute, as this constitutes a perfectly definite end point. To date, we have not made any comparisons of the results given with phenolphthalein and Alkali Blue as indicators.

Determination of Oil in Seed

FOUR methods for the determination of oil were studied. They include the so-called Copes Bag Method, the Cut and Pick Method, the Modified Cut and Pick Method, and the Official (fuming) Method. At the present time, this investigation has not progressed to a stage at which it is possible for us to make any definite recommendation in regard to the procedure best adapted for the determination of oil in cottonseed. This will require some months of further intensive study before such a decision can be made. On the basis of the work that we have done, taken in consideration with that of others who have extensively studied one or more of these methods, it is proposed to discuss certain features of these methods. The criticism that is frequently made of the Official Method is that it gives too high a result for oil in seed, and the principal criticism of the other methods appears to be in regard to the smallness of the portions taken for analyses. Of course it is obvious that if the portions taken are so small that they are not representative of the sample, the results are of little or no value (assuming that the sample itself is representative of the shipment of seed).

It was found that the Cut and Pick Method and the Copes Bag Method gave identical results when the portion taken for analysis was reground and extracted until only negligible quantities of substances were obtained after a further 2 hour extraction. This was found to require from 5 to 7 2-hour extractions on 5 grams of meats. In practice the extractions are not carried to this limit. The last few extractions gave, upon evaporation of the solvent, a semi-solid resinous deposit. In the case of the modified Cut and Pick Method, in which the separated "meats" are dried before extraction, it was observed an appreciable quantity of the resins are not obtained upon further extraction. Consequently this method will give lower results than the original Cut and Pick Method, or the Copes Bag Method, provided the extractions in each case are taken to completion. The smaller quantity of extractable matter (oil, etc.) which is obtained from dried meats (Modified Cut and Pick Method) is in all probability due to the partial polymerization of the resins that takes place during the drying of the meats. Several years ago a study was made at the Department in which it was found that these resins readily polymerize and as the polymerization progresses, the solubility of the resulting product in petroleum, ether, and other solvents diminishes. This alone would account for the somewhat lower results obtained by this method. On account of the fact that we were unable to extract the oil completely from the undried meats without at the same time extracting an excessive quantity of the resins, etc., the original Cut and Pick Method was soon discarded, and attention was then given to the more recent modification of this procedure. In addition to this, it was found through experimentation that different proportions of moisture were lost during the separation of the hulls from meats, but this does not affect the results obtained by the modified method in which the completely dried meats are extracted.

In the case of the Official Method more or less trouble has been experienced in grinding the sample after treatment with hydrochloric acid so as to get the seed in the form of a fine meal that, according to the directions, should pass through a 30-mesh sieve. When

the Wiley mill was used, a notable quantity of the sample that was only partially ground remained behind the blades of the mill. This trouble was largely overcome by passing the entire sample two or three times through the mill but even then not all of the ground sample would pass through a 30-mesh sieve. After extraction of some of these samples, for 4 hours as directed, the meal was further ground in a mortar and again extracted for several hours with the result that almost half a per cent more of extractable material was obtained. This seems to indicate that in the Official Method all of the ether soluble constituents are not extracted, but the results obtained may check very closely the amount of oil in the seed provided the sample has been ground to a certain degree of fineness, prior to extraction. If on the other hand, the sample is more coarsely ground, and to compensate for this defect, the meal is reground after the first extraction, then the results obtained will be higher than the amount of oil in the seed. We believe that it is essential for the seed to be reduced in the beginning to a certain fineness in order to obtain reasonably correct results, and the failure to accomplish this in all probability accounts to no small extent in many instances for the variation in the results obtained by different laboratories. However, it should also be observed that failure to get agreeing results for the same sample of seed is not infrequently due either to the portions of the sample submitted to different laboratories not being representative of the entire sample of the seed or through the failure of the analyst himself in taking portions for his analyses that are not representative of the sample received by him. Several laboratories now use the Official Method for their routine work and in so far as can be ascertained they obtain satisfactory results. It is apparent that they not only employ the same method for grinding the seed, but actually succeed in duplicating each other in this respect to such an extent that their results for oil in seed not only check each other by making one extraction, but they appear to agree very well with the actual quantity of the oil present in the seed. With few exceptions it is believed that much more attention should be paid to getting representative portions of the seed and to the grinding of the seed or meats from which the oil is to be extracted. The writers are acquainted with the much discussed subject regarding what is oil and what is not oil. The quantity of the latter depends to some degree upon the method, but to a larger extent upon the actual procedure used in extracting the seed. It is the presence or absence of more or less of these non-oil constituents in the extracted oil that accounts for the different results obtained by the different methods in the hands of different analysts, as has been previously indicated. However, we are not in a position to discuss the subject further in this report.

A method has been proposed that is based on taking a 10-gram portion of the sample and using it for the determinations of moisture, oil, ammonia and oil instead of the more customary practice of taking a separate portion of the sample for each determination. Directions were sent along with a request that we should study this method. In view of some obvious advantages such a method might possess over the others being investigated, considerable attention was given to it. After the moisture has been determined on the 10-gram portion of seed which previously had been cracked, the next step is to grind it in an iron mortar with the addition of some ground glass to facilitate the grinding; the next step is to extract the oil. Incidentally, it was found that the sample could be ground alone as readily as with the glass, the absence of which later on in the analysis was particularly desirable. The results obtained for oil in seeds were always lower than those obtained by either the Official or the Cut and Pick Methods, and the variations ranged from .5 to 1.9 per cent. Duplicate analyses by this method differed up to .7 per cent, even though every precaution possible was taken to check results. It should be observed that the samples which were selected for this investigation contained a considerable quantity of damaged seeds. It was not definitely known why the results for oil showed such wide variations. Although some of this difference may have been due to the non-uniformity of the portions of seeds taken for analysis, it is believed that the chief difficulty was on account of not being able to grind the seeds so that each portion would be in approximately the same condition of fineness.

The next step, according to the directions, was to titrate the free fatty acids in an aliquot portion of the petroleum ether extract and use the remainder of the solution for the determination of oil. Owing to the difficulty of taking an accurate aliquot portion and at the same time avoiding the introduction of an error in the estimation of the oil in the remainder of the solution, this was not done. Following the extraction of the oil, the meal is digested and the ammonia determined as previously described.

It is our intention to continue the investigation as rapidly as possible. Any suggestions from the members of this Society will be welcomed.