Analytical Methods for Cottonseed

Report Covering the Methods Developed in Bureau of Chemistry and Soils for Sampling and Analysis

By GEORGE S. JAMIESON *and* ROBERT S. MCKINNEY

ABOUT a year ago a preliminary report was given by us (*Oil and Fat Industry*, 1929, VI, No. 7, p. 11) at the annual meeting of American Oil Chemists' Society. At that time, we gave the results of a detailed study of the various methods in use for the analysis of cottonseed for the purpose of determining its quality. Further study convinced us that all these methods contained one or more objectionable features. When the sample contained a large percentage of either slick or damaged seed, it was found that the methods in which a 10 gram portion was taken for analysis, seldom gave duplicate results and frequently the results showed a wide variation. Moreover, it is recognized that such small samples cannot represent aborted or immature seed, and the present practice of trying to evaluate such seed on a percentage basis is one that cannot be defended. Also, it is extremely doubtful whether samples of this size can be considered as representative when there are wide variations in the percentage of hull.

It is inconceivable that 90 or 100 seeds, more or less consciously picked from a specimen of seed of doubtful authenticity, may represent, in all of its probable variables, the two hundred millions of cottonseed contained in a carlot of 25 tons. On the other hand a series of very carefully conducted tests has demonstrated the fact that the limit of reduction of a sample of cottonseed in its natural form is between 50 and 60 grams. The gram portion of seed used in the Official Method is, therefore, sufficiently large to be representative of the sample provided it is properly obtained. Mr. Meloy has devised a machine for the purpose of accurately subdividing the laboratory sample into representative portions and thereby has largely removed the personal element from this phase of cottonseed analysis.

It was found that the quantity of oil extracted in the usual manner varied considerably, owing to the fact that different laboratories grind the seed to different degrees of fineness. However, it was repeatedly observed that the laboratories which grind their samples to approximately the same degree of fineness consistently checked each other's results with the same samples of seed. By using a properly adjusted Bauer mill and following in detail the directions given in our method, this source of error can be avoided.

After giving careful consideration to all the various procedures that have been proposed and to the criticisms and suggestions of various members of the American Oil Chemists' Society, all of which were much appreciated, we finally chose the Official Method as a working basis both on account of the size of the portions of seed taken for analysis and the possible elimination, insofar as that is possible, of the personal element in the analysis. You will find the method to be quite similar to your Official Procedure, but it differs in the following respects: The 60 gram portion of seed is dried for two hours at 130° previous to the treatment with hydrochloric acid. Instead of directing that the seed be ground fine enough to pass through a 30 mesh sieve, which has been a source of considerable criticism on account of the difficulty of actually meeting this requirement, it is recommended that the seed be ground as fine as possible in a Bauer Laboratory mill, so that after mixing the ground sample has a homogeneous appearance. Although the ground sample may appear to be homogeneous, it may not actually be so. Consequently, it is necessary to follow the directions for getting representative portions of the ground sample for the several determinations. Furthermore, as it is not practical to direct that the seed be ground to a definite degree of fineness, in the determination of oil, after the first extraction, the material is to be reground in a mortar and extracted a second time.

Our method has been applied to the analysis of many regular daily mill samples, which

were kindly sent from a mill at which the yield of crude oil checked very closely with that estimated by the chemist from his analyses. Being familiar with the method employed, we know that it would give correct average figures. As expected, the results which we obtained by our method closely checked the average results reported to us. For example, we obtained an average of 21.89 per cent of oil on the dry basis, whereas the mill reported 21.81 per cent, for a set of 20 samples of seed.

Samples of cottonseed were carefully subdivided by quartering, and representative portions sent to a commercial chemist who uses the present Official Method in his laboratory. His average results checked ours very closely. For example, on a set of 13 samples he obtained an average of 21.83 per cent of oil, and we obtained 21.90 per cent of oil.

The advantages of our method are: A large sample is taken to be representative, even if the sample contains damaged, slick, aborted or immature seed. The moisture of the seed does not influence the results. The fuming of the seed does not result in a predicted oil content of the cottonseed higher than can be obtained in plant practice. The grinding of the sample does not affect the results of the analyses.

Our methods include those for the determination of moisture, free fatty acids, oil and ammonia. It is believed that when the sampling and the analyses of cottonseed are actually made according to the methods recommended by Mr. Meloy and ourselves, results will be obtained which will indicate accurately the quality of the seed. It is expected that upon further study some additional details may be added to the methods presented here today.

The committee appointed at the request of your industry has spent about a year and a half studying methods for the sampling and analysis of cottonseed, and it is now recommended that the selected methods be considered with a view to their adoption by the National Cottonseed Products Association.

The Methods in Detail

Laboratory Sample

THE sample received at the laboratory shall consist of approximately 1,000 grams of cleaned seed. It shall be sealed in an air-tight container and shall be accompanied with a statement as to the original weight and that of the separated foreign matter.

Quartering of Samp'e

THE sample shall be examined by the chemist, who, if it is not thoroughly cleaned, shall correct the weights reported to him by the sampler, for such additional foreign matter as he may find in the sample. The cleaned sample should be quartered and one half of it returned to the original can and retained as a referee sample. The second half shall be preserved in an air-tight container and used by the chemist for his analysis. The sample to be used for the analysis shall be quartered down until the combining of opposite quadrants yields two samples of about 120 grams. One of these shall be used for the free fatty acid determination. The second half is requartered, yielding two samples of 60 g. One of these is used for the moisture determination and the other for oil and ammonia determinations.

Quartering shall be done as follows: Place the sample on a large piece of paper and mix thoroughly, passing the hands upward through the pile and separating any masses of seed with the fingers. Particularly, if there is a large percentage of damaged seed present, the greatest care should be exercised to get the seed well mixed. If a large amount of bald seed is present they tend to segregate to the bottom of the pile. In such cases, it is preferable to remove the bald seeds, and later distribute them uniformly over the thoroughly mixed flattened pile of fuzzy seed. Divide into four equal parts by cutting twice through the pile to the bottom in the usual manner through the center at right angles to each other. This can well be accomplished with a large spatula. Quadrants No. 1 and No. 3 are discarded, while quadrants No. 2 and No. 4 are combined, mixed and requartered. Or the sample shall be mixed and quartered by such mechanical means as is approved by the Chemists' Committee.

Original Moisture

DETERMINATION.—Carefully crack each seed coat by means of an approved laboratory crimper. Weigh duplicate samples of about 5 grams into official aluminum dishes (2 inches diameter and ¾ inch high, fitted with cover). Dry for 5 hours at 101° C. in A. O. C. S. Official Jacketed Oven, or Dr. Khotinsky Constant Temperature Oven. (Any other make of electric oven that can be shown to give as uniform a temperature throughout as the ovens specified, may also be used.) The cover is then placed on the dish and the dish placed in an efficient desiccator until cool (one-half hour). The sample is reweighed and loss in weight calculated as moisture.

DETERMINATION.—Optional Method. The sample of about 60 grams resulting from quartering is weighed into an aluminum dish with cover (of about 9 cubic inches capacity) cap-

able of holding sample. The sample in the moisture dish with lid removed, is placed in the drying oven at 101° C. to 102° C. for 10-14 hours or over night. The cover is then placed on the dish and the dish placed in an efficient desiccator until cool (one-half hour). The sample is reweighed and loss in weight calculated as moisture.

DETERMINATION.—Optional Method. Weigh into official moisture dish, as rapidly as possible to avoid moisture change, between 5 and 10 grams of the whole seed, the exact weight being recorded. The uncovered dish containing the sample is placed in the oven at 102° for from 10 to 14 hours, or most conveniently over night. The dish when removed from the oven is covered, cooled in an efficient desiccator (one-half hour) and weighed, the loss in weight being calculated as moisture.

Fuming and Grinding

APPARATUS.—1. *De Khotinsky Constant Temperature Oven.*

2. *Fuming Oven.*—A well ventilated oven capable of reaching and maintaining a temperature of 125° C., the variations from this temperature being not more than 5° C. in any part of the oven.

3. *Fuming Pots.*—A porous earthenware vessel, such as a 3 inch flower pot.

4. *Grinding Mill.*—Bauer Bros. No. 148 Laboratory Mill, using No. 6912 plate.

PROCESS.—Dry the sample of about 60 grams for two hours at 130° C. ‡1°. Absorb into the inner walls and bottom of the flower pot 1.5 cc. of concentrated hydrochloric acid. The acid is distributed all over the side of the pot and when absorbed, the inside of the pot must appear dry, otherwise a new pot must be substituted. Place the dried seed in the pot, cover with a watch glass and place in the fuming oven at 125° C. for 1 hour. The lint should be loose and brittle, not scorched. Grind the sample in Bauer Mill which has been adjusted to produce a fine meal. After grinding, open up the mill and carefully brush out all remaining ground seed onto a sizable smooth sheet of paper. It is important that the top of the hopper of the Bauer mill be fitted with a cover to prevent loss of seed during grinding. There should be practically no loss of material in grinding and if more than 1 gram of material is lost the whole process should be repeated as the lost material is not necessarily representative of the whole.

Mix ground sample thoroughly. It is recommended that this be done by placing in a one-half gallon Mason fruit jar, containing a large rubber stopper. Replace the cover and shake violently for three minutes, then transfer to a well stoppered bottle or container.

Second Moisture

DETERMINATION.—Weigh 5 grams of ground sample into moisture dish and dry at 101° C. for 3 hours. Place cover on dish, cool in desiccator (one-half hour) and reweigh. Calculate loss in weight as per cent of moisture of fumed sample.

Oil

APPARATUS.—Extraction apparatus of Butt or other continuous percolation type. The use of Allihn condensers with 12 inch jackets, fitted with cork connections is recommended.

DETERMINATION.—Weigh accurately duplicate samples of 4 to 5 grams, wrap in a 125 or 150 mm. filter paper (S & S No. 597, or equivalent grade) and rewrap in a second paper or papers in such manner as to prevent escape of the meal, leaving the top of the second paper open like a thimble. Place a piece of absorbent cotton in the top of the thimble to distribute the dropping ether. Place 25 cc. of petrolic ether (see Specifications Rule 272, Sec. 3) in a tared flask of 50 cc. or of 120 cc. capacity and extract sample for 2 hours. The ether should drop on the center of the thimble at a rate of at least 150 drops per minute. The volume of the solvent should be kept approximately constant. Regrind the sample in a mortar, rewrap in the filter papers as before and re-extract for one hour. The solvent is evaporated off until no trace remains, the sample cooled to room temperature, and weighed. The last traces of ether are sometimes difficult to detect by odor and in case of doubt evaporate for an hour or longer until constant weight is obtained.

Example of Calculation

Petrolic Ether Extract ..... 1.0255 grams
Original Moisture ..... 12.22 per cent
Second Moisture ..... 2.54 per cent

$$\text{Per cent oil} = \frac{1.0255}{5} \times \frac{87.78}{97.46} = 18.45$$

Nitrogen

Nitrogen to be made as per Rule 272, Sec. 5.

Free Fatty Acid

DETERMINATION.—Heat about 120 g. of seed, obtained by quartering, for 30-40 minutes at a temperature of 100-5° and cool. Separate the meats by any laboratory huller or mill that will approximate factory conditions and grind sufficiently to pass a 1.5 mm. sieve. At least 20 g. of meats should be obtained. Extract the thoroughly mixed meats by cold percolation with petrolic ether. The following procedure is recommended. Place the lower disc from a Knorr Extraction Apparatus in a Butt

(*Turn to page* 315)

Cottonseed Analysis

(*From Page 292*)

tube and place a layer of absorbent cotton on the plate. Place the meats in the prepared tube and pour sufficient portions of petrolic ether on the meats to extract at least 5 grams of oil. Receive the extract in a tared flask. Evaporate the ether from the oil on a steam bath. Care must be taken to see that all the ether is removed from the oil. Weigh the oil, add 30 cc. neutralized alcohol (Formula 30) and titrate the free fatty acid of the oil with standard alkali, using phenolphthalein as the indicator. (0.1 N alkali is used if f. f. a. if low, but 0.25 N is used for oils with f. f. a. above 3 per cent). The addition of a small amount of petrolic ether before titration makes the end point sharper. The titration is performed in a flask which is shaken vigorously during the titration, the end point being taken when a permanent pink is obtained which persists for at least one minute.

$$\text{Per cent F. F. A.} = \frac{28.2 \times \text{Normality of alkali} \times \text{cc. used}}{\text{weight of oil}}$$

California Olive Oil

(*From Page 296*)

that California oils be further studied along these lines, with the idea of calling attention to cases of considerable variation from the average; second, that the average figures of 80 yellow—5 red—2 blue for color, 15-16 for relative viscosity, and 1.0 per cent. for free fatty acid be considered as tentative standards for the purpose of such investigation; and third, that a slight but distinct positive Kreis test be considered tentatively as maximum permissible rancidity for the purpose of such investigation.

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Soap Chemists' Report

(*From Page 301*)

The committee and cooperating laboratories follow:

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The Institute for Commercial Expansion of the Ministry of Agriculture, Industry and Commerce of Brazil has issued a very complete discussion entitled "The Babassu Nut," published in Portuguese and English, which gives extensive information on the occurrence in Brazil of the Babassu palm, the products which may be developed from the palm and the conditions surrounding its exploitation. The booklet is well prepared and attractively printed. It is profusely illustrated with half-tones and contains maps of the babassu-producing areas, together with many beautifully designed colored charts setting forth exports of babassu products in recent years and imports of various commodities which may be affected by the development of the babassu industry.

Referee Applicant

Mr. Clinton Morris, of Morris-Flinn Company, Macon, Georgia, has applied to the American Oil Chemists' Society for Referee Chemist certification on all products covered by the Rules of National Cottonseed Products Association. (*First Publication*)