Report of Seed Analysis Committee

C. H. COX, Chairman

IN a collaborative study of the analysis of cottonseed, it was observed that frequently there was a wide variation in the results for free fatty acids in cottonseed reported by the different laboratories. On account of the importance of the free fatty acid determination in connection with the evaluation of cottonseed, this laboratory undertook a further study of the present method (*Oil and Fat Industries, VII, No.* 8, *pp.* 291-294, 1930) and of other methods which appeared promising.

It occurred to us that, in the present method, the varying quantity of meats separated by the different types of laboratory hullers might account in part for the wide range of results. To study this factor a laboratory huller was used that has two adjustable corrugated steel rolls which rotate in opposite directions. This huller operates as do the "breaks" (rolls) of a flour mill. When the sample was first sent through, the rolls were set so as to slightly break the seed. The broken meats were separated by a 6-mesh screen. The rolls then were set closer together, and the unbroken seed and the hulls were passed between them again. The broken meats then were screened out. This operation can be repeated again and again, the rolls being closer together each time until all the meats are separated.

Two samples of 110 g. of seed each were selected from well mixed samples of cottonseed of high free fatty acid content. The first sample was sent through the huller twice, yielding 27.5 g. of meats. From the second sample, which was sent through the huller five times, 58 g. of meats were obtained. The two samples of meats were ground fine, and the oil was extracted with petroleum ether by cold percolation. The oil extracted from the first sample contained 25.2 per cent of free fatty acids, whereas the oil from the second sample contained 20.7 per cent of free fatty acids. This experiment indicated that the cottonseed meats which separate first in a gradual reduction of the seed are damaged the most. This accounts for the greater acidity of the oil from the first sample.

To obtain further information on this phase of the problem the following experiment was conducted: Four samples of cottonseed were selected with a free fatty acid content of 20.9 per cent, 7.3 per cent, 3.1 per cent and 1.8 per cent, respectively. After each sample was thoroughly mixed, 200 g. were taken for the test. Each sample was heated for 40 minutes at 102° C. and cooled, then put through the huller and screened in the manner previously described. The first 25 g. of meats separated in the gradual reduction of the sample constituted portion No. 1; the second 25 g., portion No. 2; the third 25 g., portion No. 3, and the remaining meats, usually slightly more than 25 g., portion No. 4. The four portions of meats obtained from each sample of seed were ground and extracted in the usual manner with petroleum ether. The oils were freed of solvent, and the free fatty acids were titrated, a weighed portion of the residual oil being used. The following results for free fatty acids were obtained:

Sample	*Portion* No. 1 Per Cent	*Portion* No. 2 Per Cent	*Portion* No. 3 Per Cent	*Portion* No. 4 Per Cent
A	27.1	20.8	19.1	16.5
B	9.7	7.3	6.0	6.4
C	5.0	2.8	2.3	2.4
D	2.8	1.8	1.4	1.1

These results definitely show that the meats which separate first contain oil having the highest acidity. Furthermore, these experiments demonstrate the importance of separating all the meats present in the portion of the sample of seed taken for this determination.

It appeared at first that the simplest procedure for obtaining the oil for the free fatty acid determination from all the meats present, would be to grind the seed, then extract the oil, but it was found very difficult to extract the oil by cold percolation. Experiments showed that the oil obtained in this way contained considerably less free fatty acids than that which still remained in the ground seed. Then an experiment was made in which a 60-g. portion of

ground seed was extracted hot in a large *Smalley* extraction tube. In this case, the extracted oil gave a high result which was believed to be due to some action of the lipase in the seed upon the oil. When, however, the seed were previously heated for two hours at 130° C., then ground and extracted as described, satisfactory results for free fatty acids were obtained, provided all the oil in the seed was recovered. No further experimental work with this procedure was attempted because it was found at this time that a complete separation of the meats could be made with the expenditure of much less time and effort.

In connection with our investigation of the determination of free fatty acids, the recently proposed Albert method (*Albertschrift* No. 15, p. 55; *Chem. Abstracts* 25, 332 (1931) for the titration of free fatty acids was given some attention as it was claimed to give very satisfactory results. The method consists in dissolving an accurately weighed two-gram portion of the oil or other substance to be tested in 25 cc. of a mixture consisting of two parts benzene (C_6H_6) and one part of neutralized alcohol, adding 25 cc. of a neutral saturated salt solution along with 3 to 4 g. of salt and phenolphthalein indicator solution. This mixture is titrated with standard alkali solution, using a slight excess over that required to neutralize the free fatty acids. The excess of alkali used is titrated then with 0.1 N acid solution. The method gives a very sharp end-point, even in the case of very dark-colored oils, but, unfortunately, it was found that the results ranged from 0.1 to 0.5 per cent below those obtained by the method now in use for the analysis of cottonseed. Another laboratory studying the Albert method obtained similar results.

We made a few titrations, also, for free fatty acids with portions of the same sample of crude cottonseed oil, in order to compare the results obtained by using cold and hot neutralized alcohol, and a cold mixture of neutralized alcohol with 10 cc. of petroleum ether. These three procedures gave results which were practically identical.

It has been suggested from time to time that some further attempts should be made to express the oil from cottonseed for the determination of free fatty acids. Such a method, if practical, would have much advantage over those based upon extracting the oil in which the removal of the solvent was necessary before the free fatty acids can be determined, because the quantity of oil dissolved in the solvent is not known. At first, several experiments were made by cold pressing the separated meats in the Carver laboratory press and titrating the acids present in the expressed oil. With samples of seed containing small quantities of acids, the results obtained were similar to those by the present method, but when the free fatty acids amounted to two per cent or more, the cold pressed oil gave low results, the average being about 0.7 per cent below those obtained by the cold percolation method now in use.

As cold pressing the seeds proved unsatisfactory, attention was given to hot pressing. One set of experiments was made in which the meats were pressed at a temperature of about 105° C. (about 220° F.) for a ten-minute period a pressure of 5,000 pounds per square inch being used. Another series was run, which differed only in that the pressing period was thirty minutes. The oil obtained by pressing for ten minutes was found to give results that averaged about 0.5 per cent below those by the cold solvent extraction method, whereas the oil from the meats pressed for thirty minutes gave results which averaged about 0.3 per cent low. One result was 0.6 per cent low for which we have no explanation. Whether or not the hot pressing method can be made to give satisfactory results remains to be determined.

The problem of mixing the sample of seed sent to the laboratory so that it will be homogeneous, which is required in order to obtain representative portions of it by the quartering process, for the determination of free fatty acids as well as other constituents, has been constantly kept in mind. It was believed that in certain cases the wide range of the results reported for free fatty acids was due either to the imperfect mixing of the sample or to the failure to subdivide it into representative portions. Although previous tests with the Maclellan mixer indicated that it functioned properly, some later tests showed that this was not always the case. Several months ago, the occasion arose to repeat the determination of free fatty acids on one of the collaborative samples

of cottonseed. Much to our surprise, the results did not check with those previously obtained. After mixing sample Number 15 of the collaborative series of seed in the Maclellan mixer, then quartering according to the official direction, four portions were obtained with which the following results were obtained: (1) 2.36 per cent; (2) 2.07 per cent; (3) 1.78 per cent, and (4) 1.42 per cent. A duplicate sample, Number 15, was put through the Meloy sampler. Upon analysis, two portions of the seed gave 1.55 and 1.64 per cent of free fatty acids, respectively. These results indicated that a satisfactory subdivision of the sample had been made. Another experiment was made on a sample which contained 25 per cent of seed with 20 per cent of free fatty acids, and 75 per cent of seed with 0.62 per cent of free fatty acids. This sample was mixed in the Maclellan mixer, quartered, and analyzed in the usual manner. Two portions of the seed gave 5.03 and 6.12 per cent, respectively, of free fatty acids. The experiment was repeated, but this time the Meloy sampler was used. One portion of seed gave 5.32 per cent, and a second portion 5.73 per cent of free fatty acids. Although these results are much too far apart, still they agree more closely than those given by the Maclellan mixer. From this time on, free fatty acid determinations were all made in duplicate at this laboratory, separate portions of seed being used for each test, and when duplicate results failed to check use was made of the Meloy sampler. The results obtained are as follows:

Sample	With Maclellan Mixer Per Cent of F. F. A.		With Meloy Sampler Per Cent of F. F. A.	
	(1)	(2)	(1)	(2)
16	0.83	0.71		
17	1.01	0.59	0.83	0.96
18	0.38	0.34		
19	0.65	0.67		
20	0.19	0.23		
21	0.52	0.53		
22	4.23	4.69	4.14	4.32
23	0.37	0.46		
24	1.18	1.27		
25	8.10	8.35		
26	4.89	3.88	4.37	4.67

From the results given in this table, it is apparent that in our hands at least, the Maclellan mixer, with certain samples of seed, failed to give a homogeneous mixture from which it would be possible to get representative portions for the determination of the free fatty acids, and that this was not confined to those samples which were high in free fatty acids. Undoubtedly this mixer is decidedly superior to the older method of hand mixing of the sample previous to the quartering process, but as it has been shown that some samples cannot be properly mixed by means of the Maclellan apparatus, we believe that the Meloy sampler is to be preferred. Further study may show that the best results can be obtained by mixing the sample first in the Maclellan mixer, then putting it through the Meloy sampler.

From the present investigation it is obvious that in addition to getting for analysis portions that are representative of the sample, it is necessary to make a complete separation of the kernels or meats from the hulls, and to extract practically all the oil from the meats in order to get satisfactory results for the free fatty acid content.

Agash Refining Corp. have just completed a 10,000 square foot addition to their vegetable oil refinery in Bush Terminal, Brooklyn, New York, and now occupy buildings No. 81 and No. 82. They have moved their New York City offices from 99 Hudson St. to their new, enlarged quarters in Brooklyn.