SOME ADDITIONAL FACTORS AFFECTING THE ACCURACY OF THE FREE FATTY ACID DETERMINATION AS APPLIED TO COTTONSEED ANALYSIS

By

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For some years our laboratory, along with several others, has been engaged in studying methods for the analysis of cottonseed. During this time special attention has been paid to ascertaining the causes of variations in the results obtained by those taking part in the annual collaborative analysis of specified samples of cottonseed. As a result of these studies helpful information has been obtained, the use of which has caused a marked improvement in the quality of the collaborative work, except in the determination of free fatty acids. Certain factors were found which influence the accuracy of this determination. Some of the important factors are as follows: The difficulty in obtaining a suitable sample, the rapid deterioration of some seed, and the failure to completely separate all the kernels or "meats" from the portion of the seed taken for this determination. In spite of the suggestions based upon these findings and the sincere efforts of the collaborators to follow them, the improvement during the past three years in the results for free fatty acids has been surprisingly little.

Complete Separation

Until quite recently it was believed that the chief difficulty in many cases was the failure to get a complete separation of all the meats from the hulls. Consequently, considerable time was spent in attempting to perfect a method which would eliminate their factor. Experiments were made in which the whole seed was ground without any treatment, but this was found to be impractical, owing to the resistance of the lint in grinding, the effect of lipase on the oil, and the difficulty in extraction. Drying the seed for two hours at 130° C. before extraction, eliminated the lipase action, but the oil could be obtained from the ground seed only by use of a large Smalley extractor. However, such a procedure is not adapted to general laboratory use on account of the time and special apparatus required. An attempt was then made to remove the lint before grinding the seed, with the expectation that it would be possible to extract the oil in the usual manner by percolation with cold solvent. After the seed had been dried for two hours at 130°, it was fumed with hydrochloric acid in the usual manner and as much as possible of the lint was rubbed off by hand. Even then, however, sufficient lint remained to seriously interfere with the extraction of the oil with cold solvent unless suction was applied to the extractor tube containing the ground seed. Although this procedure is not recommended for general use, it may be of some interest to give the results obtained with five samples of seed which were in the series analyzed this year by the collaborators. Comparative results obtained with the official method are also given, steel rolls being used for hulling the seed. These results are given in table 1.

TABLE 1

Sample No.	Official Method Percent as Oleic Acid	Delinted Seed Percent as Oleic Acid	Accepted Averages Percent as Oleic Acid
21	3.3	3.3	3.4
22	2.0	2.4	2.5
23	1.1	1.1	1.2
24	3.4	3.8	3.9
25	2.2	2.6	2.6

The close agreement of the results for the delinted ground seed and the results of the collaborators are noteworthy.

Low Fifteen Times

For several years it has been observed that some of the collaborators showed a marked tendency to obtain results either above or below the accepted average for the free fatty acid content of the seed, although in each case these collaborators were able to closely check their own results by subsequent analyses. For example, during the 1932-1933 season, our results covering the analysis of twenty-eight samples of seed showed that they were low fifteen times, high seven times and in agreement six times with the accepted averages for free fatty acids. Based upon the entire season our results averaged 0.15 percent below the accepted values for free fatty acids. As the example given is typical of others, it is evident that there was some essential difference in the manner in which the analysis was conducted. As it was impossible for either of us to visit each laboratory in order to discover any differences in the technic used for this determination, it was suggested

that such information might be obtained by correspondence. From the replies received to our inquiries, we were much impressed with the efforts of each collaborator in attempting to follow the method as described. However, these replies brought to our attention the fact that both the type of huller and grinder for the meats was left to the choice of the analyst. Consequently, it was decided to look into the effect of the several hullers and grinders used on the determination of free fatty acids.

Hulling

For hulling the seed, some use the Bauer mills and others a plumber's crimper, which is somewhat similar to the rolls used by us. Several types of grinders for the meats were used, including the Wiley mill, coffee mills, and a household food grinder or chopper.

The effect of hulling the seed and grinding the meats on the determination of free fatty acids was investigated in the following manner: Each sample of seed was thoroughly mixed and carefully divided into several portions. One portion was hulled between rolls, and the meats were ground in a Wiley mill. A second portion was hulled with the Bauer mill, and the meats were ground in the Wiley mill. A third portion of the seed was hulled with the Bauer mill, and the meats were ground in a coffee mill. The percentage of free fatty acids was determined in the usual manner except that in the case of the meats ground in the coffee mill it was necessary to use suction in order to draw the solvent through the ground meats in the extractor tube. The results obtained are given in table 2.

These results show definitely that the method of hulling the seed and grinding the meats affects the free fatty acid results. The seed hulled by the rolls with few exceptions gave lower results than the seed hulled with the Bauer mill. Attributing this difference in the results to the action of lipase on the oil when the seed was hulled with the Bauer mill, a fourth portion of the seed was heated for two hours at 130° C. to check enzyme action before being hulled. The results for free fatty acids obtained after heating the seed were 2.8 percent for sample 26 and 5.0 percent for sample 27.

TABLE 2

Sample No.	Rolls and Wiley mill free fatty acid. Percent	Bauer and Wiley mill free fatty acid. Percent	Bauer and Coffee mill free fatty acid. Percent
26	2.3	2.8	—
27	4.5	5.6	—
28	6.7	8.4	8.7
29	1.2	0.6	0.6
1	4.1	4.4	3.8
3	—	13.1	9.1
4	2.7	2.1	3.0
5	18.3	18.8	18.7
11	15.6	18.2	17.3
14	6.3	7.1	7.2
45	2.7	3.9	4.4

Apparently the higher results obtained for the seed hulled with the Bauer mill cannot be attributed entirely to lipase action.

Much Less

It was found that hulling the seed with rolls or by the Bauer mill gave practically the same percentage of meats, but the quantity of oil extracted from the meats obtained with the Bauer mill was always much less than that from the meats obtained by the use of the rolls. In the case of sample 27 it was found that the meats separated by the rolls and extracted in the usual manner retained 2.5 percent of oil, whereas those obtained with the Bauer mill retained 9.0 percent of oil after extraction.

Repeats Experiments

In view of these results it was decided to repeat the experiment on a larger scale in order to determine whether the oil remaining in the meats from the Bauer mill had the same acidity as the oil previously removed by percolation with cold solvent. For these experiments cottonseed sample No. 28 was selected. The seed hulled with rolls gave 6.7 percent of free fatty acids and those with the Bauer mill gave 8.4 percent. The extracted meats (33 grams) of the Bauer mill experiment were reground and extracted in a large Smalley tube. Three grams of oil were recovered which contained 5.9 percent of free fatty acids. In the same manner cottonseed sample No. 11 was examined. It gave 15.6 percent of free fatty acids when hulled by rolls and 18.2 percent when hulled with the Bauer mill. The extracted meats (32 grams) in the latter case were reground and extracted, using the Smalley tube. The oil, which amounted to 3.2 grams, contained 9.4 percent of free fatty acids. These results show that the oil not removed from the meats by extraction with cold solvent differs greatly in acidity from the portion first removed. A possible explanation is that the oil in the radicle differs in composition from the oil in the cotyledons, which make up the bulk of the seed, as has actually been found to be the case with peanuts and some other seeds. Doubtlessly the relative proportions of the oil extracted by percolation with cold solvent from these two sources is dependent on the type of hullers and grinders used in the separation and grinding of the meats.

Table 3 gives some additional results for free fatty acids which were determined as indicated on six samples of seed.

Well Adapted

It should be observed that samples 26, 27 and 30, which were previously dried at 130° C. for two hours, hulled with the Bauer mill, and the meats ground in the Wiley mill gave respectively 2.8, 5.0 and 4.5 percent of free fatty acids. These results are identical with the accepted averages for these samples.

The present investigation indicates that the method based upon drying the seed for 2 hours at 130° C., hulling with the Bauer mill adjusted so as to just crack all of the seed, and grinding the separated meats in the Wiley mill, is well adapted for general use for the determination of free fatty acids. This procedure not only removes the possibility of enzyme action on the oil, but also permits the more complete removal of the oil from the ground meats, thereby minimizing errors caused by the differential action of the solvent as previously discussed.

TABLE 3

Sample No.	Rolls and Wiley mill free fatty acid Percent	Bauer and Wiley mills free fatty acid Percent	Bauer and Coffee mills free fatty acid Percent	Heated seed Bauer and Wiley mills free fatty acid Percent
30	4.4	4.3	—	4.5
48	4.7	5.0	5.4	4.8
50	3.9	3.3	3.8	3.4
59	7.8	7.4	7.4	7.4
61	6.1	6.9	6.1	5.9
79	2.7	2.5	2.4	2.8