Report of Committee on Determination of Free Fatty Acid of Oil in Seed

R. K. BRODIE, *Chairman*

THE present report divides itself into two parts, corresponding to the double task assigned to the committee; namely, the development of a method for determining free fatty acid of oil in seed and investigation of the relation of free fatty acid to quality of oil.

I. Method for Free Fatty Acid

The development of a suitable method has accounted for the main effort of the committee. The aim has been to propose a method which will be simple as to manipulation, give uniform results in different laboratories, and give a result corresponding closely to the fatty acid of oil produced from the same seed by the customary milling processes. The proposed method. which had best be stated in advance of the discussion of the committee's work, is as follows.

"At least 100 grams of the well mixed sample of seed are heated 30-45 minutes at 100-105 Deg. C. and cooled. The meats are then separated by any laboratory huller or mill that will approximate factory conditions and ground sufficiently to pass 1½ m.m. sieve. Not less than 10 grams of the thoroughly mixed meats are extracted by cold percolation with gasoline boiling below 70 Deg. and the gasoline evaporated off and the oil weighed. 30 c. c. of neutralized denatured alcohol are added and the free fatty acid of the oil is titrated with a standard caustic using alkali blue as an indicator. The free fatty acid is calculated by the formula:

$$\% \text{ F. F. A. equals } \frac{28.2 \times \text{normality of alkali} \times \text{c.c. used}}{\text{Weight of Oil}}$$

Notes: The gasoline percolation should be continued sufficient time to give at least 2 grams of oil.

The addition of a small amount of gasoline to the flask after the alcohol has been added before titrating makes the end point sharper.

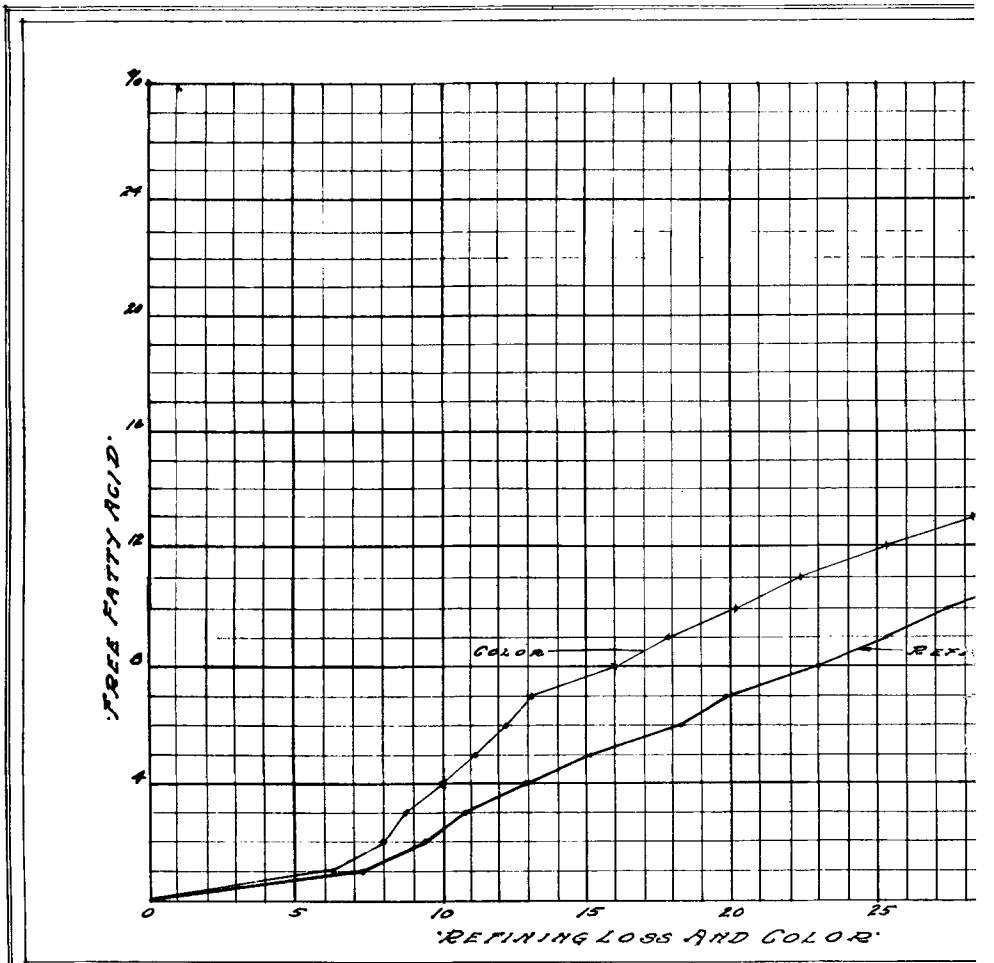
Tenth normal caustic soda is preferable for low fatty acid oils but for oils above 5 per cent, quarter or fifth normal is preferable.

In case it is desired to make the determination where analytical balance is not available, extract a larger quantity of meats and after evaporating all the gasoline from the oil, pipette 7.05 grams and titrate with quarter normal caustic. The reading in this case is percentage directly."

Sampling: The problem of uniform sampling of large bodies of cottonseed is in no way peculiar to the fatty acid determination. Consequently the committee has not concerned itself with the broader aspects of this difficult problem, but study was made of the minimum size of seed sample required for a uniform free fatty acid determination. When prime seed of low fatty acid and off seed of high fatty acid were well mixed in equal quantity, no distinction in the uniformity of observed free fatty acid was noted in samples of 70 grams as compared with larger size, while samples of 35 grams and smaller size gave progressively more erratic results. Also experiments on the mixing of seed dyed with different colors confirm the

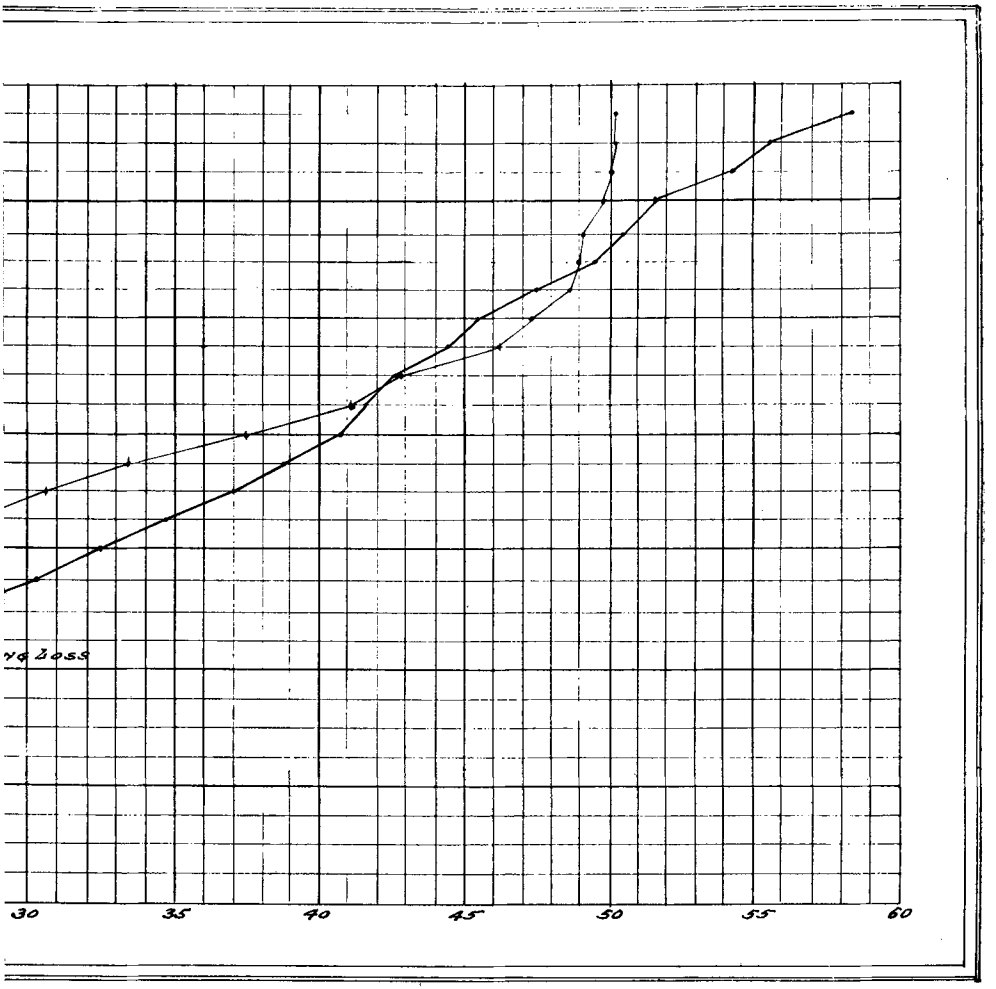
idea that there is a roughly defined minimum size of sample below which increasing lack of uniformity is noted and above which no additional advantage is obtained. The committee believes that a larger sample can be reduced to a representative sample of 100 grams for purpose of determining free fatty acid, provided the larger sample is itself truly representative of the seed in the car or other commercial unit specifically under consideration.

Preliminary Heating of Seed: Under all normal conditions of storage, increase of free fatty acid of oil in whole seed is relatively slow. After hulling, fatty acid in meats increases more rapidly, but still shows no appreciable change within the time ordinarily devoted to an analytical procedure. After rolling or grinding, free fatty acid in meats increases so rapidly that there is danger of results being too high on account of this source of error, especially in seed of high



moisture content. Preliminary heating of seed checks the tendency towards subsequent increase in fatty acid and reduces the chance of error from this source. Although in the committee's own results with the procedure described above no appreciable difference was noted when heating was omitted, the general experience of the committee has been in favor of including this step as an extra precaution.

Crushing the Sample. At the outset it was hoped to devise a very simple procedure involving the crushing of whole seed. After a series of disappointments, the committee reluctantly abandoned even the hope of suggesting an alternative method based on crushing whole seed without separating hulls and meats. It is unnecessary to review this phase of the work, since results were negative, but a few points may be of value in case anyone wishes to undertake further work along this line. Oil ex-



tracted from hulls is higher in free fatty acid than oil from meats, but this factor alone is not very serious. Grinding of whole seed is mechanically difficult and tends to produce a serious rise in free fatty acid which is only partially overcome by preliminary heating. The Copes bag method of crushing, i.e., hammering seed in a canvas bag, does not ordinarily produce increase in free fatty acid, the contrary result being obtained, strangely enough, in some cases. However, this method seems tedious when applied to samples of sufficient size for reliable results; and cannot be recommended.

All factors considered, it seems preferable to separate hulls and meats by any laboratory method which gives a completeness of separation approximating that obtained in ordinary plant practice. The meats can then be ground in almost any type of laboratory mill or household food grinder.

Equipment for Hulling: A number of laboratory mills and even some coffee grinders can be coarsely adjusted so as to serve as hullers. The committee particularly recommends the Bauer Laboratory Grinder, made by The Bauer Bros. Company, Springfield, Ohio. Screening is easily done by hand. A special laboratory machine for hulling and separating, originally made for John Malowan and subsequently modified by The Fort Worth Laboratories, has recently been still further improved and used with considerable success.

Extracting the Oil: This has not been found to be a critical operation. Completeness of extraction and time of extraction are not factors which appreciably affect the results. Simple percolation in the cold is preferred to reflux extraction on account of convenience.

Titration: The committee's experience with the brine method of titrating for free fatty acid was unfavorable. On the average, lower results were obtained by brine titration than by the alcohol method.

Reproducibility: The final results of the committee are frankly disappointing as regards reproducibility. The trouble is believed to be due to the inherent difficulty of sampling. However, reproducibility is good as compared with the methods heretofore employed in seed grading and hence the committee has no apologies to make on this score. Table I gives values for free fatty acid as reported by three different laboratories, working independently on samples from the same lot of prime and off seed respectively and instructed to follow directions given above.

TABLE 1. CHECK DETERMINATIONS OF FREE FATTY ACID OF OIL IN SEED BY THE METHOD RECOMMENDED

Prime Seed		
Lab. 1	Lab. 2	Lab. 3
0.51	0.82	0.9
0.53	0.77	0.7
0.85	0.95	..
0.72	...	..
0.66	...	..
0.82	...	..
0.79	...	..
1.19	...	..

Off Seed		
Lab. 1	Lab. 2	Lab. 3
13.14	12.85	11.3
13.46	13.00	11.9
12.77	12.25	...
13.43	....	...
12.14	....	...
11.08	....	...
12.28	....	...
14.15	....	...

Fatty Acid in Seed vs. Fatty Acid in Expressed Oil

At the outset of the committee's work, there was already available experimental evidence showing good agreement between fatty acid of oil produced by regular milling and oil extracted in the laboratory from the meats used in the mill, provided samples were properly taken and handled thereafter. Subsequent mill experience has confirmed this conclusion. Also in a carefully controlled experiment, a lot of seed which showed 1.5 per cent free fatty acid by the above method of analysis was put through a small experimental oil mill and gave oil of 1.4 per cent free fatty acid. In the case of oil produced on a commercial scale, due to difficulty of sampling, the average agreement is not so good, but is fairly satisfactory. Table II shows the observations made at one mill during thirty-one consecutive days.

TABLE II. RELATION OF FREE FATTY ACID OF OIL IN SEED TO FREE FATTY ACID OF OIL PRODUCED

Day	Per Cent Free Fatty Acid: Oil in Seed	Per Cent Free Fatty Acid: Oil Produced
1	2.5	2.5
2	2.8	3.5
3	3.5	3.9
4	3.3	3.6
5	2.4	2.8
6	2.6	3.3
7	1.4	1.4
8	3.9	5.4
9	2.5	1.4
10	4.4	4.4
11	3.8	3.5
12	5.3	5.9
13	5.4	5.0
14	5.7	7.0
15	4.8	3.9
16	6.5	3.9
17	7.3	6.5
18	3.6	4.3
19	4.8	3.6
20	4.1	3.5
21	4.6	4.1
22	6.4	4.0
23	5.0	3.8
24	5.1	4.5
25	4.8	5.3
26	5.1	5.1
27	8.0	6.1
28	5.3	4.9
29	5.5	4.9
30	2.7	2.3
31	2.1	2.1

II. Relation of Free Fatty Acid to Quality of Oil

About 15,000 analyses of crude cottonseed oil from seven independent sources, including both refinery and commercial laboratories, were grouped according to free fatty acid in steps of one per cent (0.5 to 1.5 per cent, 1.6 to 2.5 per cent, etc.) The average free fatty acid, refining loss, and Lovibond red color of the refined oil of the groups are given in Table III. The results are also plotted in the accompanying figure.

TABLE III. RELATION OF FREE FATTY ACID TO QUALITY OF OIL

% F.F.A.	No. Samples	Loss	Color
1	3623	7.25	6.4
2	2662	9.34	8.0
3	1747	10.95	8.8
4	1196	13.00	10.0
5	818	15.06	11.1
6	595	17.21	12.2
7	402	19.96	14.1
8	327	23.00	16.0
9	325	25.36	17.9
10	359	27.50	20.1
11	331	30.35	22.4
12	313	32.48	25.3
13	356	34.70	28.2
14	331	37.00	30.7
15	357	38.80	33.4
16	334	40.80	37.3
17	244	41.72	41.1
18	157	42.82	42.9
19	136	44.52	46.2
20	129	45.42	47.4
21	105	47.45	48.7
22	88	48.62	49.0
23	95	50.40	49.1
24	36	51.60	49.8
25	42	54.20	50.0
26	48	55.50	50.0+
27	49	58.15	50.0+

(*Continued on page* 190)