cluded from his studies that only compounds containing an allyl group or a substituted allyl group are capable of giving positive (red) Kreis tests. Since the ferric chloride test given by malonic dialdehyde shows it to be partially in the enol form, it would satisfy Kobert's observation. In a general sense any unsaturated fatty acid would conform to this requirement. According to Holm and Greenbank (4), autoxidized oleic, ricinoleic, linoleic, and linolenic acids give positive Kreis tests.

Patton and Kurtz (10) have shown that oxidized milk fat when heated with thiobarbituric acid gives a red color. The absorption spectrum of this color (maximum at 532 mμ) is very similar to the Kreis color (maximum at 543 mμ) of oxidized milk fat. In a similar manner these workers demonstrated that malonic dialdehyde gives a red color with thiobarbituric acid reagents and that this color is spectrally identical with that obtained from oxidized milk fat. Further it may be noted that the enol form of 2-thiobarbituric acid bears a close structural relationship to phloroglucinol. With respect to measurement of milk fat oxidation the thiobarbituric acid test appears more sensitive than the Kreis test. The chemical mechanisms of the two seem similar.

The present paper constitutes a preliminary report. The question concerning the roles of malonic dialdehyde and epihydrin aldehyde in fat oxidation would be considerably clarified by conclusive demonstration of their presence or absence in oxidized fat. Research efforts toward this end are under way in this laboratory.

Summary

Evidence is presented which suggests that epihydrin aldehyde and its derivatives are not necessarily solely responsible for the Kreis color reaction of oxidized fats. Malonic dialdehyde has been shown to give a positive reaction in the Kreis test and the resulting color demonstrated to be spectrally similar to the Kreis colors obtained with epihydrin aldehyde diethyl acetal, acrolein treated with H_2O_2 rancid lard, and oxidized milk fat. Characteristics of a water-soluble, low molecular weight, Kreis positive, carbonyl compound from oxidized milk fat were observed to be very similar to those reported for malonic dialdehyde, i.e., strongly acidic, enolic as indicated by the ferric chloride test, and relatively stable to heating with dilute mineral acids. These properties would not be expected of epihydrin aldehyde. Three highly sensitive colorimetric reactions, involving reactions with ferric chloride, 2-thiobarbituric acid or the Kreis reagents, might well serve as the basis for quantitative measurement of malonic dialdehyde.

Acknowledgment

The helpful counsel of L. H. Sommer is gratefully acknowledged. The authors are also indebted to Union Carbide and Carbon Corporation for a generous supply of certain research chemicals employed in this study.

The paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 326 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the Department of the Army.

REFERENCES

1. Beilsteins Handbuch der organischen Chemie, Berlin, 4th edition, *1*, 765 (1918).
2. Claisen, L., Ber. Chem. Ges., *36*, 3664 (1903).
3. Goto, R., Bull. Chem. Soc., Japan, *15*, 103 (1940). Cited in Chem. Abstracts, *34*, 5827 (1940).
4. Holm, G. E., and Greenbank, G. R., Ind. Eng. Chem., *16*, 518 (1924).
5. Keeney, M., and Doan, F. J., J. Dairy Sci. (in press).
6. Kobert, K., Ztshr. Analyt. Chem., *46*, 711 (1904).
7. Kreis, H., Chem.-Ztg. *26*, 897 (1902).
8. Lea, C. H., Rancidity in Edible Fats, New York, N. Y. Chemical Publishing Co. Inc., 1939, p. 100.
9. Nef, J. U., Ann. Chem. *335*, 191 (1904).
10. Patton, S., and Kurtz, G. W., J. Dairy Sci. (in press).
11. Owen, J., and Somade, H. M. B., J. Chem. Soc., 1947, p. 1030-1034.
12. Powick, W. C., J. Agr. Res., *26*, 323 (1923).
13. Rehm, R. H., Union Carbide and Carbon Corp., Philadelphia, Pa. Personal communication (1950).
14. Täufel, K., and Russow, F. K., Z. Untersuch. Lebensm., *65*, 540 (1933). Cited in Chem. Abstracts, *27*, 4702 (1933).
15. Wohl, A., Ber. Chem. Ges., *31*, 1796 (1898).
16. Wohl, A., and Momber, F., Ber. Chem. Ges., *47*, 3346 (1914).

[Received March 8, 1951]

The Centrifugal Acetone Foots Test Applied to Crude Soybean Oil. I. Rapid Estimation of Phosphatide Content[1]

EGBERT FREYER and VICTOR SHELBURNE, Spencer Kellogg and Sons Inc., Buffalo, New York

ALTHOUGH most crude edible vegetable oils are graded according to the refining loss as determined by the official A.O.C.S. cup method, there are many occasions when it is desired to know the content of the phosphatides and related constituents, for example, as estimated by the acetone insoluble content. The HCl heat break (Gardner break) content of soybean oil indeed continues to be used to some extent despite its displacement by refining loss methods as the primary means of evaluating crude soybean oil for refining purposes.

In the early days of the rapid growth of the American soybean industry, a committee (1) conducting cooperative analyses intended to lead to the adoption of a suitable method for grading soybean oil in trade, investigated the acetone foots test which had been in use for some time in grading raw linseed oil. This committee found that test to be far less reproducible when applied to soybean oil as compared with its performance with raw linseed oil (on which its application has been the subject of much criticism). Considering this test therefore altogether unsuitable for the purpose, the committee investigated the HCl heat break content and found the method to

---

[1] Presented at 42nd Annual Meeting, American Oil Chemists' Society, May 1-3, 1951, New Orleans, La.

be reasonably reproducible and to correlate in a general way with phosphatides content. Nevertheless the acetone foots test is still designated as the official method for grading crude soybean oil in A.S.T.M. specifications though presumably the oil is not traded under these specifications to an appreciable extent with respect to this factor of phosphatides content.

Upon the development of the centrifugal foots method (2) for raw linseed oil as an improvement on the gravity separation method which has been in regular use for many years and in the realization of the value of the results obtained by it when applied to laboratory studies of raw linseed oil properties (3), it was hoped that the method might be applied with equal success to crude soybean oil as a quick and easy means of evaluating that oil for gum content. It was hoped the test might correlate well enough with HCl heat break content to justify substituting it for that determination in the routine examination of oils for various purposes.

Experiments were first performed to determine how the test as devised for raw linseed oil could be modified to yield the best results on crude soybean oil. At the outset it was recognized that since the phosphatidic constituents of soybean oil were partially soluble in the acetone-oil solution, probably in proportion to the percentage of oil present, a greater acetone-to-oil ratio than that used in the linseed oil test would be required to obtain anything approaching complete precipitation of the phosphatides. Since the volume of the acetone-oil solution was limited by the size of the centrifuge tube to 60 ml. at most, the problem became one of finding the optimum size sample of oil which, when diluted to volume with acetone, would yield the largest volume of insoluble material.

A series of oils was selected and tested with the result that a 10-ml. sample of oil was found to give the largest volume of foots when diluted with 40 or 50 ml. of acetone. A series of 55 determinations was then made, using HCl heat break content as a reference, in which 40 ml. acetone was used in 29 tests, and 50 ml. in 26. An analysis of the data showed that identical results were obtained on the average in relation to break content and that there was no choice from the standpoint of the degree of correlation with break content. Forty ml. was chosen as the amount of acetone for the test since it afforded easier mixing. The ratio of break content to centrifugal foots percentage by volume averaged 0.123 and varied through the range of 0.095 to 0.163. The average of the individual ratio deviations from 0.123 was .012, or 10%. Continued accumulation of comparative data between break content and centrifugal foots on more than 100 samples of oil has verified this average ratio.

Variations in the gravity settling time before centrifuging had a slight but unmistakable effect on the results. Longer times, up to one hour, gave results about 0.5% lower than obtained using the 5 to 8-minute period adopted.

Tests were run on a series of samples to determine if the heating and cooling cycle required in the testing of linseed oil was necessary when applying the test to soybean oil. Results tabulated below showed that no significant difference was obtained in the quantity of foots separated whether the sample was or was not subjected to this temperature cycle. The first four crudes were also chilled overnight in a refrigerator at 43°F., then brought to room temperature, and tested. Again no significant difference in quantity of foots separated was obtained. It is conceivable however that an oil which had been subjected to abnormally low temperatures for an extended period might give different results, and for this reason there is included in the method a provision that oils which have been chilled abnormally should be heated and again cooled to room temperature before testing.

Centrifugal Acetone Foots, %		
Heated and Cooled	Run as Received	Chilled
2.9	3.3	3.0
3.2	3.4	3.2
3.2	3.4	3.3
3.1	2.9	2.9
2.3	2.3	
1.8	2.1	
3.3	2.9	
3.0	2.9	
3.9	3.8	
1.8	1.7	
3.1	3.3	

By this simple test the equivalent break content, accurate to ± 10% on the average, can be obtained on a crude soybean oil sample in about 25 minutes with very little manipulation. Extensive experience in inter-laboratory checking shows that this accuracy corresponds to about the same reproducibility as obtained in the HCl heat break test itself. However having achieved the original and main objective of this work, subsequent interest was focussed less on HCl heat break content and more on the centrifugal foots value itself when it was realized, as will be shown in a forthcoming paper, that this quantity has more significance from the standpoint of indicating refining quality than has Gardner break.

Table I shows the compartive accuracy with which the acetone insoluble content of an oil may be predicted by the HCl break content and by the centrifugal foots. It must be recognized that the acetone insoluble determination itself when applied to crude vegetable oils, and especially when it may be quite low, is subject to such inaccuracies as to make us believe that departures from more exact correlation between centrifugal foots or HCl break content and gravimetric acetone insoluble content are not entirely

TABLE I

Correlation of Break, Centrifugal Foots, and Acetone Insoluble Contents in Crude Soybean Oil of Varying Origins

(A) Acetone Insoluble, %	(B) HCl Heat Break, %	(C) Centrifugal Foots, %	Ratio A/B	Ratio A/C	Ratio B/C
1.61	0.43	3.30	3.74	0.488	0.133
1.93	0.41	3.20	4.71	0.603	0.128
2.27	0.49	4.30	4.64	0.528	0.114
1.46	0.34	3.00	4.29	0.486	0.113
2.09	0.46	4.00	4.54	0.522	0.115
1.45	0.34	2.40	4.26	0.604	0.142
1.53	0.38	2.50	4.05	0.611	0.152
2.01[a-b]	0.47	5.80	4.28	0.347	0.081
1.82	0.45	3.10	4.04	0.586	0.145
1.94	0.46	3.80	4.22	0.510	0.121
3.75[a]	0.78	9.90	4.81	0.379	0.079
1.64[a]	0.38	4.00	4.31	0.410	0.095
2.46[a]	0.50	6.40	4.92	0.384	0.078
Average, extracted oils			......	0.548	0.129
Average, expeller oils			......	0.380	0.083
Average, all			4.37	0.497	0.115
Average deviation from average			±0.27	±0.075	±0.020

[a] These are expeller oils.

[b] A low temperature processed oil of rare occurrence. All others were extracted oils.

due to the empirical nature of the first two tests as a measure of phosphatides content. Table I also shows the ratio of break content to centrifugal foots and illustrates the close adherence of even limited data to the average ratio of 0.123.

It appears from the lower values of the ratios A/C and B/C for expeller oils that better prediction of acetone insoluble or heat break content from centrifugal foots values would obtain through using different conversion factors for expeller and extracted type oils. Making the approximation that centrifugal foots values represent weight percentage (instead of volume percentage), the different ratios shown then represent fractional compositions of the foots stratum emulsions separated in the test. That is, the emulsions from extracted oils range from 48 to 61% acetone insoluble content; those from expeller oils average 38% AI content. The differences are considered to be measures of differences in chemical and emulsifying properties of the phosphatides and as such make it appear that the centrifugal foots values measure something about crude oils and their refining responses that refining loss tests measure; i.e., to some extent they distinguish emulsifying capacities of the gums, which do not appear in straightforward chemical analyses, whether composite analyses like chromatographic separation and the Wesson loss or analysis of separate loss components.

The following table shows the reproducibility of the method when applied in routine testing of samples by different laboratories.

Centrifugal Acetone Foots, %			
Central Control	Lab. No. 1	Central Control	Lab. No. 2
2.8	2.8	1.6	1.8
2.6	2.6	1.5	1.7
3.5	3.5	2.5	2.2
2.9	3.1	2.0	2.3
3.0	3.1	2.2	2.1
3.0	3.0	1.8	2.1
3.0	3.2	2.5	2.4
3.0	3.1		
3.0	3.0		

It must be pointed out that the centrifugal foots method has dubious applicability to degummed soybean oils, especially on those which come within the authors' experience, i.e., generally having HCl heat break contents below .10%. The method however is hardly more defective in that respect than is the conventional gravimetric acetone insoluble determination which gives results on degummed oils that seem to lack significance. This is primarily due to the fact that the phosphatides and other material commonly considered to be acetone insoluble actually are soluble in some degree, and this solubility amounts proportionately to a considerable value on oils like degummed in which the amount of phosphatides is very small. It is believed however that further development may show that the centrifugal foots method, or a variation, may yet have some value in connection with degummed soybean oils, for example, possibly as an easy rapid means of distinguishing between degummed and non-degummed oils.

Summary

The centrifugal acetone foots test developed for use on linseed oil was successfully adapted for estimating the acetone insoluble or HCl heat break content of soybean oil. Application of the method makes it possible to obtain results in a half hour with an accuracy comparable to that of the HCl heat break test; it requires only a few minutes of the analyst's time and involves no exacting technique. Data are given showing good reproducibility of the centrifugal foots values and their degree of correlation with acetone insoluble and HCl heat break contents.

Centrifugal foots test results are being studied in relation to those obtained by other methods of predicting the refining quality of crude soybean oil, and an account of this work will be reported in a paper now in preparation.

Acknowledgment

Grateful acknowledgment is accorded to Mahlon D. Smith and Robert N. Varnum for making the acetone insoluble determinations in our Bellevue, Ohio, plant laboratory.

The Centrifugal Foots Method for Crude Soybean Oil

Apparatus:

Centrifugal foots test tube and apparatus as previously described (2) and a 25-ml. graduate with 0.2-ml. graduations.

Reagents:

1. Acid calcium chloride solution. Dissolve 800 g. of reagent grade $CaCl_2 \cdot 2H_2O$ in a mixture of 80 ml. of HCl (Sp. Gr. 1.18) and 500 ml. of water. Dilute to 1 liter and mix. Filter or allow to settle undisturbed and decant the clear supernatant liquid.
2. Acetone. Acetone shall conform to the standard specifications for acetone (A.S.T.M. designation: D329 of the American Society for Testing Materials).

Procedure:

(NOTE: Crude samples which may have been subjected to extremes of cold for extended periods of time should be first heated to at least 65°C. for about 10 minutes and then cooled to ca. 25°C. before being tested.) With sample and all reagents at ca. 25°C., measure 10 ml. of the crude soy in the graduate and transfer to the centrifuge tube. Measure 40 ml. of acetone in the same graduate, using the acetone in two 20-ml. portions as a rinse to transfer all of the oil to the tube, and add to the centrifuge tube. Add 10 ± 0.2 ml. of acid calcium chloride solution to the centrifuge tube, stopper, and mix the contents thoroughly for one minute by shaking vigorously as the tube is alternately inverted to allow the air bubble to pass back and forth through the constricted waist. Place the tube in the supporting cup and allow to stand for five to eight minutes, during which the tubes and cups may be balanced for centrifuging. Centrifuge for 15 minutes at a speed determined by the dimensions of the centrifuge as follows:

For radius (R) center of rotation to bottom of cup in cm.	Use the following speed (N) ±50 r.p.m.
20	1850
23	1730
25	1660
28	1570
30.5	1500

Or, for a centrifuge of different dimensions, use the formula: $N = 8273\sqrt{1/R}$. After centrifuging, remove the tubes and read the volume of the separated material lying between the acid calcium chloride solution and the acetone-oil solution, estimating both interfaces to the nearest 0.01 ml. The interfaces will be sharp and distinct though the separated material may or may not be solid and opaque. Disregard any hazy or cloudy and indistinct layer appearing in the acetone-oil solution. Multiply the volume by 10 to obtain the percentage of centrifugal foots.

REFERENCES

1. Unpublished committee correspondence, National Soybean Processors' Association, Finished Materials' Standards Committee 1935-36.
2. Freyer, E. B., and Shelburne, V. B., J. Am. Oil Chem. Soc., 27, 545 (1950).
3. Freyer, E. B., and Shelburne, V. B., J. Am. Oil Chem. Soc., 27, 551 (1950).

[Received March 12, 1951]