of this method however the refining loss grade of crude soybean oils may be estimated in as little as one hour of elapsed time, and it requires only 5 to 10 minutes of the operator's time.

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Notes on the Centrifugal Foots Test Applied to Crude Cottonseed Oil

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AFTER modifying the centrifugal foots method originally developed for raw linseed oil (1) so that it gave satisfactory results on crude soybean oil (2) (and indeed proved to be in the second case a more useful test for control purposes than when applied to raw linseed oil), we considered that it might have some usefulness for the approximate evaluation of quality in crude cottonseed oil, if applicable.

To determine this, a group of crude samples of different origins and varying quality was requested of John J. Thoede of the South Texas Cotton Oil Company, who also supplied the f.f.a. and refining loss values on the oils. Centrifugal foots tests were run on a few of these under different conditions. The results which follow are decidedly preliminary and are presented merely as a point of departure for any other investigators who may be interested in working on the development of a quick easy test for estimating the phosphatides content of crude cottonseed oil since it is unlikely that we will do any further work on this particular application of the method.

In tests run to determine effect of oil to acetone ratio, it was found that the use of 10 ml. of oil to 40 ml. of acetone (ratio originally developed for soybean oil) resulted in a higher percentage of separated foots than obtained with any other ratio. In a test to determine the minimum time of centrifuging, four oils were run for 15 minutes and 30 minutes. Only very slightly lower results were obtained after 30 minutes. The reduction ranged 0.1 to 0.3% on values of 3.3 to 5.1% foots, due to the extra 15 minutes of centrifuging.

Briefly, the method discussed here involves the precipitation of the phosphatides, etc., in a 10-ml. portion of the oil with 40 ml. of C. P. acetone and in the presence of 10 ml. of a saturated and acidified solution of calcium chloride. (See A.S.T.M. Method D555-47.) The reagents and oil are mixed by violent shaking for one minute in a specially designed centrifuge tube and, after settling 5 minutes, are centrifuged for 15 minutes under a given force, determined by a specified r.p.m. for the radius used. Then the volume of the separated foots stratum is read to the nearest 0.01 ml. For a detailed statement of the method (bearing in mind the qualifications discussed here), reference is made to a previous paper (2) by the authors.

Results and Discussion

Referring to Table I, the heating cycle may not always be necessary, but as the amount of data here is quite limited, this point should be investigated further. On high foots oil it would be advisable to spin for a longer time as complete compaction was probably not attained in 15 minutes of centrifuging; or, alternately, the crude could be diluted with 100 to 300% of its volume of refined oil before subjecting the oil to the test, in which case appropriate factors would have to be used to convert the volume separated to percentage by volume of the original oil.

TABLE I

Relation of Centrifugal Foots Results to Refining Loss. Effect of Preheating and Cooling

Type	F.F.A.	Centrifugal Foots			Calculated Loss[b]	A.O.C.S. Loss
		Not Heated	Heated to 65°C. and Cooled			
		Average	Checks	Average		
	%	%	%	%	%	%
Hyd.	0.7	0.3	0.3, 0.3	0.3	2.3	2.6
Exp.	0.7	3.3	3.3, 3.2	3.3	4.1	4.8
Hyd.	0.8	4.5	4.2, 5.2, 5.1	4.8	5.3	4.9
Exp.	0.9	2.3	2.2, 2.2	2.2	4.0	5.1
Hyd.	1.4	2.9	3.2, 3.2	3.2	6.1	5.4
Exp.	1.2	3.6	3.7, 3.8, 3.9, 3.7	3.8	5.9	6.5
Hyd.	0.8	3.7	5.1,[a] 3.9, 3.2, 3.5	3.5	4.5	6.9
Hyd.	1.4	4.9	4.2, 4.4	4.3	6.8	7.7
Hyd.	1.0	17.0	17.5, 17.0	17.3	13.4	11.4
Hyd.	2.0	>20	17.8, 18.5	18.2	16.9	14.2

[a] Value not in average.
[b] = 3 × f.f.a. % + 0.6 × centrifugal foots % (heated).

We know that the principal reason for some lack of precision in these results was the presence of quantities of meal in some of the oils. Much, but not all, of this meal separated from the foots layer and settled to the bottom of the centrifuge tubes. When a large quantity of meal was present or when centrifuging

was extended to 30 minutes, the bottom interface of the foots layer became indistinct (or torn), probably because meal particles were dragged from the layer by the centrifugal force. Otherwise they are held within the foots by the forces of interfacial tension. These observations suggest therefore that the method could be improved by removing the meal by centrifuging or filtering after the oil has been heated to 65°C. to disperse or dissolve any petroleum naphtha-soluble gums which may have been separated with the meal; i.e., redisperse the material which it is the intent of the method to determine.

If there is meal present which has to be removed before applying the test, it is apparent that the usefulness of centrifugal foots results on such oil would depend upon the result being used in connection with the measured meal content, provided the main object is to obtain an estimate of probable refining loss (i.e., by combining those results with f.f.a. content). This requirement undoubtedly detracts from the potential value of the test as applied to cottonseed oil for this purpose, although the authors have found it especially useful when so applied to soybean oil, all of which in our experience has been substantially meal-free.

However, aside from this complicating effect of meal content in cottonseed oil, it seems likely that wider variations in the chemical nature of the acetone insoluble material in crude cottonseed oil and in the pigments, and especially variations in the gossypol content, may be such that it is beyond reasonable expectation to find anything approaching the same degree of correlation of refining loss and centrifugal foots results (combined with f.f.a.), as we have found in the instance of crude soybean oil (3). That conclusion indeed seems indicated by the poor agreement between "calculated loss" and the official cup loss results on the present series of samples even when the factors used in the equation for "calculated loss" have been chosen to give agreement on the average on most oils.

Still, where there may be a need for an empirical measure of the phosphatide content of crude cottonseed oil, the centrifugal foots value determined on the sample from which meal had been separated should have value as a simple rapid test. Or, as a rapid measure of "foots," as the term is used in reference to raw linseed oil, embracing both phosphatides and meal, this test may find application in programs involving the evaluation of crude cottonseed oil with respect to these components.

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Photometric Determination of Esterase in Animal or Plant Tissues and in Microorganisms

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THE method described here is similar in principle to the colorimetric method for phosphatase (1) and β-glucuronidase (2). Phenolphthalein dibutyrate is hydrolyzed to phenolphthalein by animal or plant esterase without any compensating activation, the esterase inactivated by trichloroacetic acid, proteins removed, and the phenolphthalein estimated at pH 10 by measuring the extinction with a Pulfrich photometer with filter S 53, cell depth 49.96 mm., and comparing with this value a calibration curve established for phenolphthalein solutions at pH 10.

Preparation of the Substrate

Phenolphthalein dibutyrate is synthesized by adding butyryl chloride (13 gm.) drop by drop to phenolphthalein (10 gm.) dissolved in water-free benzene (80 ml.) in the presence of pyridine as a catalyst at higher temperatures (under reflux). The pyridine (6 ml.) is added to the reacting mixture also drop by drop after the main portion of the butyryl chloride has already reacted. The major part of the solvent is evaporated *in vacuo* at room temperature. The residue is diluted with not too much water (100 ml.). Then 3 to 4 times as much ethyl ether (as water) is added. To remove the pyridine the solution is shaken 8 to 10 times with about 200 ml. of water. The water is removed and the solution evaporated *in vacuo* at room temperature. The residue is taken up with a little methanol and the phenolphthalein dibutyrate crystallized from it at −8 to −10°C. By recrystallizing the initial product (8 gm.), to which traces of unchanged phenolphthalein still adhere, from the solution in methanol, 6.5 gm. of chemically pure phenolphthalein dibutyrate are obtained in colorless long thin flakes (see Figure 1) with m.p. 91.5°C. (uncorrected), constituting a 65% yield. The crystalline substance is not soluble in water, somewhat in methanol and ethanol at room temperatures, easily soluble at higher temperatures and also easily soluble in ethyl ether, benzene, and chloroform.

Analysis of the crystallized phenolphthalein dibutyrate. The carbon and hydrogen content of the refined crystals correspond closely to their theoretical values.

$C_{28}H_{26}O_6$ C calculated 73.77%, found 73.70%.
H calculated 5.72%, found 5.76%.

Esterase Estimation Procedure

Solutions required.

1. *N trichloroacetic acid.* Check titer with N sodium hydroxide, using phenolphthalein as indicator.

2. *0.20 M glycine buffer solution* (Sørensen) for pH 8.0 (with esterase in pancreas, saliva, milk, and blood plasma, respectively) and for pH 8.3 (with es-