3. The method can be employed for the semi-micro estimation of unsaturated acids.
4. There is no chance of substitution and hence prolonged reaction times are without any influence.
5. The results are affected by the presence of ketone bodies, but beta-hydroxy acids cause no interference with the estimation.

The method has been successfully extended for the estimation of total unsaturation of oils and fats, which will form the subject matter of a future communication.

Acknowledgment

The author is indebted to Prof. M. N. Goswami for his keen interest in the work and useful suggestions.

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A Comparison of Several Methods for Determination of Non-Oil Constituents of Raw Linseed Oil

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DURING the year of 1950 a total of 1 billion, 87 million pounds of linseed oil was produced (1). A large portion of this oil was sold and consumed as raw linseed oil. In establishing quality, a grading factor such as refining loss is not applicable. Raw linseed oil must be evaluated by tests showing its quality as a raw oil.

Both the American Society for Testing Materials and the Federal Government have set up similar specifications for the evaluation of raw linseed oil. With one exception the tests such as specific gravity, acid value, iodine value, saponification value, etc., are common to the grading of all vegetable oils. The exception is the "Foots" test. This test, the Walker-Wertz Method of Foots Determination, has been in use for approximately 22 years, and during this period the only modification has been the inclusion of a heated oil and chilled oil section, a change made within five years of the initial adoption of the test.

TABLE I

A.S.T.M. and Federal Specifications for Raw Linseed Oil

Test	Specification
Specific Gravity at 15.5/15.5°C.	0.931 to 0.936
Acid Value, Maximum	4.0
Saponification Value	189.0 to 195.0
Unsaponifiable, Maximum, %	1.50
Iodine Value, Wijs, Minimum	177.0
Loss on Heating at 105 to 110°C., Maximum, %	0.3
Appearance	Clear and transparent at 65°C.
Color	Not darker than a freshly prepared solution of 1.0 g. of $K_2Cr_2O_7$ in 100 ml. of pure H_2SO_4 (Sp. Gr. 1.84)
Foots, Maximum, %	
Heated Oil	1.0
Chilled Oil	4.0

In 1923 and 1924 the ASTM Subcommittee V of D-1 spent considerable time on collaborative work on the Foots Determination (4, 5). Nine samples of oil from various sources and of different ages were examined by seven laboratories during each year. Various modifications were considered, including preheating of the oil in a water bath at 65.5°F. for one hour or pre-cooling of the oil in an ice bath for 12 hours before carrying out the Foots Determination. A centrifuge modification was also considered.

After collecting all of the data and examining it very critically, the subcommittee reached this conclusion in 1923: "There is but one conclusion that can be drawn from the data . . . and that is that in all modifications and upon all types of oil the (Foots) test is very inaccurate. The average error varies from 55% to 130% when the results of the determinations by the several modifications of the methods are considered . . . therefore the subcommittee concludes that the ASTM and the Sutherland modifications (centrifuge procedure) of the ASTM Foots test are both incapable of yielding accurate results in the hands of skilled operators. Consequently it is recommended that this test not be advanced to standard at this time." And in 1924 the subcommittee came to this conclusion: "The variables in the method seem to be too great to allow for satisfactory results. The figures speak for themselves. The subcommittee, therefore, must reluctantly reaffirm the position taken in its last year's report, namely, that neither this test, nor any modification of it proposed to date, is sufficiently accurate for use in the standard specifications."

However, in spite of the above conclusion, the Foots test was made a part of the tentative specifications for linseed oil in 1926 and shortly thereafter became a standard specification, which has remained essentially unchanged to this date.

Original Foots Test. American Society Materials, D51-18T (6)

I. PERCENTAGE OF FOOTS

1. The amount of Foots in properly clarified pure raw linseed oil from North American seed, as determined by the test specified below, shall not exceed 2% by volume.

II. METHOD OF DETERMINATION

2. The following reagents are required:
   a. Acetone that will pass United States Pharmacopoeia specifications.

b. Acid $CaCl_2$ solution, made by saturating with $CaCl_2$ a mixture of 90 parts water and 10 parts concentrated HCl, sp. gr. 1.2, at room temperature.

3. With all materials at a temperature between 70 and 80°F., mix by shaking in a stoppered flask for exactly one minute 25 cc. of the well-shaken sample of oil, 25 cc. of acetone, and 10 cc. of the acid $CaCl_2$ solution. Transfer the mixture to a burette where settling can take place for 24 hours. The temperature during this period should be between 70 and 80°F.

The volume of the strata lying between the clear calcium-chloride solution and the clear acetone and oil mixture is read in tenths of a cubic centimeter, or a fraction thereof. This reading multiplied by four expresses the amount of Foots present as percentage by volume of the oil taken.

The present ASTM Method, D-555-47, for Foots is essentially the same as the test initially considered by the original ASTM committee with some refinement in the description of the method of mixing and more exact specification of heating and cooling procedures for use in the "heated oil test" and "chilled oil test."

According to the ASTM proceedings of 1924, "the Foots portion of linseed oil is made up primarily of the following three constituents (5).

1. Moisture.
2. High melting point saturated fats which solidify at or slightly below normal temperature (causing the higher values experienced in the Chilled Foots Procedure).
3. A mucilaginous material which is held largely in suspension and colloidal dispersion in the oil." It was stated that "this albuminous material appeared to be identical with the substance forming the break when a linseed oil is heated." It is apparent that the processor would be most concerned with the latter of the three constituents since the moisture and high melting point saturated fats can be controlled in the processing.

Because of dissatisfaction with the Foots test as it now exists, a committee of the ASTM has been studying possible improvements in the method for the past two years, and several independent laboratories also have devoted time to a study of this test method. This paper is a summary of the work done in the Archer-Daniels-Midland Laboratories.

Experimental Results

Experience with the Foots test has demonstrated two rather disturbing factors. First, it is quite possible by careful manipulation to obtain fair check results within a single laboratory. However check results between different laboratories are difficult to obtain, especially when the oil samples are of different ages at the time of the test, or when the samples have been subjected to varying conditions before testing. Secondly, samples of certain types of oil, when subjected to the Foots test, will sometimes give results showing 15 to 20% Foots content. Obviously the oil does not contain 15 to 20% of foreign or non-oil material.

In order to demonstrate the difference between the true content of non-oil material and the values obtained by the Foots test, oils containing varying levels of Foots were passed through an activated alumina adsorption column using ether as a solvent and eluant. The chromatographic adsorption procedure used is essentially similar to that described by Linteris and Handschumaker for removal of loss constituents from crude oils (8). Polar materials such as fatty acids, moisture, and phosphatides are adsorbed whereas the neutral oil almost quantitatively passes through the column. The non-oil portion of the starting oil is determined by subtracting the percentage of free acids from the percentage of material adsorbed. These non-oil constituents are referred to in this paper as *Non-Neutral Oil—%. Free Fatty Acids.*

Using a larger column than the analytical unit previously described, sufficient oil was passed through the activated alumina to give a sample for running the Foots test. Table II illustrates the Foots values obtained in such a series of oils before and after passage through the adsorption column. It is noteworthy that the removal of relatively small levels of non-acidic constituents results in a large reduction in Foots content. This is strikingly shown in samples 7 and 8 which represent a second passage of samples 5 and 6 through the column. In sample 7 the second adsorption resulted in removal of an additional 0.39% of material. However, corresponding to this reduction of only 0.39% by the column, there was a reduction of 4.36% of Foots. Similarly in sample 8, the removal of an additional 0.09% of material resulted in a change of 2.0% in Foots. It is obvious that the Foots test cannot measure the amount of non-oil constituents in raw linseed oil.

TABLE II

The Relationship of the Foots Content of an Oil Sample Before and After Passage Through an Adsorption Column of Activated Alumina

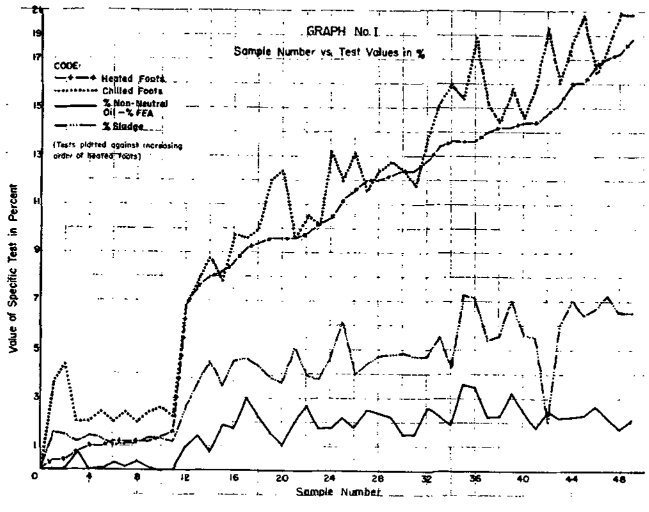
Sample	% Foots Before Adsorption	% Foots After Adsorption	Difference in Foots Content,%	% Non-Neutral Oil—% F.F.A.
1	17.19	7.80	9.39	2.63
2	7.39	3.36	4.03	1.56
3	6.81	2.16	4.65	1.80
4	2.67	0.40	2.27	1.78
5	22.84	7.80	15.04	2.83
6	7.00	2.84	4.16	1.51
7*	22.84	3.44	19.40	3.22
8*	7.00	0.84	6.16	1.60

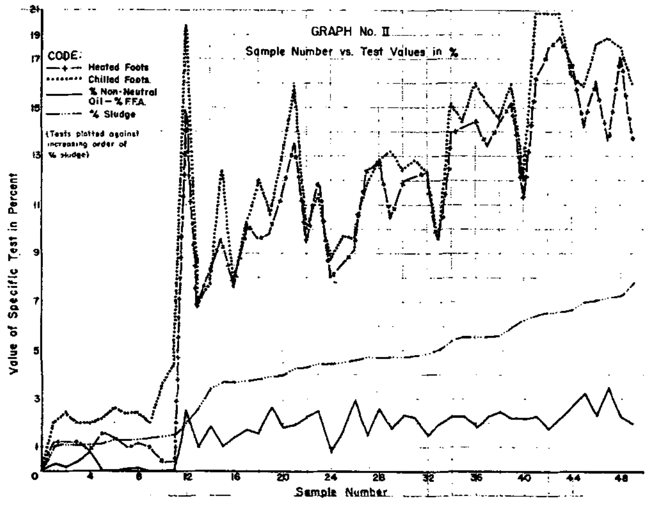
* Samples 7 and 8 are samples 5 and 6 respectively which have been passed through two adsorption columns.

In order to establish a basis for further work on the development of a suitable new test to measure the non-fatty portion of linseed oil, correlation was made of a number of tests known to measure, in part at least, the non-oil portion of linseed oil.

Forty-nine samples of raw linseed oil of varying Foots content were analyzed by four methods, including the previously described heated Foots, chilled Foots, and Lever Brothers' chromatographic adsorption. In addition, an arbitrary sludge test was used. A sample of oil in a centrifuge cup was subjected to alternate cooling and warming by storage for one day in a refrigerator, one day at room temperature, one day in a refrigerator again, and finally at room temperature until warm. The sample was centrifuged to separate the sludge, and the tube was inverted for five minutes to drain off the bulk of the supernatant oil.

The data were arranged in increasing order of heated Foots and increasing order of percentage of sludge and plotted in Graphs I and II. The graphs show that while there is a gradual increase in the percentage of non-neutral oil and percentage of sludge to follow the increase in the percentage of Foots value, it is by no means a consistent function.





Since a graph is somewhat misleading in the correlation of one test with another, it is desirable to obtain one simple figure—having known limits—so that it may be readily interpreted. The "Coefficient of Correlation" is such a measure. This figure serves as an index of the degree of linear relationship between the particular tests and the Foots test.

This coefficient has been calculated by the standard product-moment method giving the correlations between tests shown in Table III for the 49 samples of linseed oil analyzed.

If the relationship between the data can be represented by a straight line, the correlation is unity. If, on the other hand, there is no relation at all between the variables, the coefficient is zero.

The chilled Foots, as one would expect, has the best correlation with the heated Foots. We may roughly say that 97% of the non-oil portion measured by the heated Foots is measured by the cold Foots.

TABLE III

The Correlation of % Non-Neutral Oil and % Sludge With the Foots Test

Test Correlation	Coefficient of Correlation
Heated Foots versus Chilled Foots	0.970
Heated Foots versus % Non-Neutral Oil—% FFA	0.824
Heated Foots versus % Sludge	0.825
Chilled Foots versus % Non-Neutral Oil—% FFA	0.789
Chilled Foots versus % Sludge	0.795
% Sludge versus % Non-Neutral Oil—% FFA	0.898

On the other hand, the non-oil constituents measured by both the Lever Brothers' Adsorption and the Sludge Test have only fair correlation with the heated Foots and somewhat poorer correlation with the chilled Foots. However it should be noted that the non-oil constituents measured by the Lever Brothers' Adsorption test correlate fairly well with the Sludge Test which measures the settlings in linseed oil.

With the somewhat encouraging results of the first series in mind, several modifications were made in the analysis of a second group of samples by these procedures with the hope that better correlations would be attained. In addition, two more procedures were examined for possibilities as a substitute for the Foots procedure.

The Lever Brothers' Adsorption procedure was modified by introducing the activated alumina as a slurry, using a separatory funnel in an attempt to avoid entrapped air bubbles which greatly impair the efficiency of the column. In addition, the sample and ether rinses were added through a separatory funnel using the "Dropping Funnel" technique. In this way the height of the liquid over the bed of alumina in the column could be regulated to an absolute minimum to keep a constant flow through the column.

The percentage of sludge by the alternate cooling and warming procedure was not modified. However the period of time of drainage of the oil was closely adhered to, and the sides of the centrifuge tube were wiped to remove as much oil as possible.

The two procedures added were the percentage break by the modified Gardner method (9) and a centrifuge procedure for Foots developed in the ADM laboratories (10).

The Break procedure involves heating of a sample of oil to a high temperature in the presence of a small amount of hydrochloric acid. Mucilaginous material and other non-oil constituents are coagulated and weighed after filtering and washing with carbon tetrachloride.

The second added procedure was a centrifuge modification of the Foots test. A 15-ml. centrifuge tube was connected by a ground glass joint to a 1-cm. internal diameter tube to give an over-all capacity of 70 ml.

TABLE IV

The Use of 5 ml. Versus the Standard 10 ml. of Acid Chloride Solution

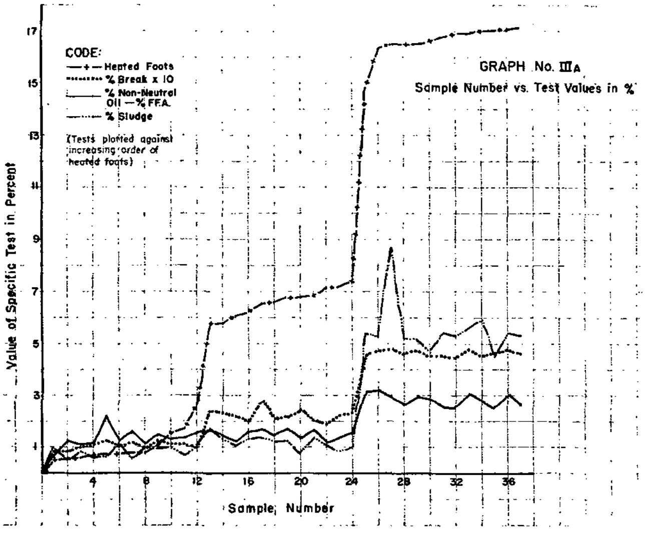
Procedure	Sample Number			
	A	B	C	D
Standard ASTM Method				
(a) Heated Foots	1.63	5.20	0.52	Trace
(b) Chilled Foots	3.01	5.51	0.53	0.80
Revised Method using 5 ml. of acid $CaCl_2$ solution				
(a) Heated Foots	1.60	5.20	0.54	Trace
(b) Chilled Foots	2.80	5.20	0.60	......

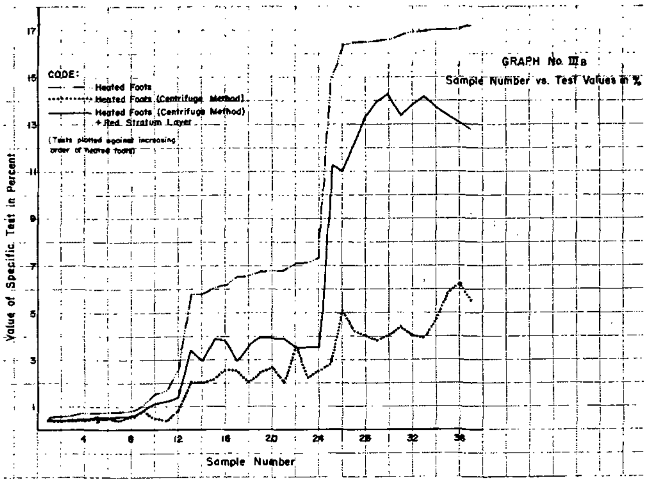
A change in the procedure involving the amount of saturated acid $CaCl_2$ solution added was made to facilitate the reading of the Foots layer in the 15-ml. centrifuge tube. Only 5 ml. of the acid chloride solution was used, and the following table shows that there seemingly is no adverse result.

The procedure for determining the centrifuge Foots involves a settling period of at least three hours for oils having a Foots by the present method of 8.0 or less. (For oils having a Foots content of 2.0 or less a half-hour settling period is entirely adequate.) After the settling period the clear acetone-oil layer was removed by suction to a point below the ground glass joint and the tube disconnected. The remaining mixture was then centrifuged for 15 minutes at 2,000 r.p.m., giving an even compact stratum of Foots centered between the acid $CaCl_2$ solution and the remaining acetone-oil layer.

In the determination of the Foots by the centrifuge procedure, a characteristic red layer was observed between the solidly packed Foots stratum and the acid $CaCl_2$ solution. As this was translucent, but not transparent, it was recorded, if only for the sake of completeness, in all data. This red layer gradually disappeared into the Foots stratum on very prolonged centrifuging.

It has been a great problem in all collaborative study of the Foots test to get repeatability and reproducibility of results. On this basis all analyses were run in triplicate on all 37 samples in this second group. No individual test was run in duplicate on any one day to eliminate, in part at least, the possibility that check determinations could be attributed to the particular atmospheric conditions or induced personal errors during the analysis. The only test that was found to be consistently repeatable was the percentage break by the modified Gardner method. All of the other tests were quite poor in this respect. Graphs IIIa and IIIb represent this data averaged and arranged according to the increasing order of heated Foots. It is seen that the break content, although of a much lower percentage than the heated Foots, follows a similar pattern. The other results show much greater variation throughout although they do show a gradual increase with an increase in the heated Foots.





Since the original adoption of the Foots test by ASTM centered around the specification of the amount of Foots allowable in a raw linseed oil, it was decided to classify the oils in this second set of samples into three separate groups of low, medium, and high Foots content based on analysis by the present ASTM Method D-555-47. In this way it was hoped that some other test would show its ability to classify particular oils into particular groupings.

The high, low, and mean values of the averages of the triplicate determinations for each specific test are tabulated in Table V.

TABLE V

The Classification of Linseed Oils by Their Foots Content

	High Value	Low Value	Mean Value
Group I (13 Samples)			
Foots, Heated	17.19	14.72	16.63
% Non-Neutral Oil—% FFA	3.14	2.51	2.83
% Break	0.480	0.448	0.466
% Sludge	8.62	4.53	5.52
Foots (Centrifuge)	6.24	2.83	4.53
Foots and Red Stratum Layer (Centrifuge)	14.31	11.28	13.37
Group II (12 Samples)			
Foots, Heated	7.39	5.88	6.59
% Non-Neutral Oil—% FFA	1.72	1.21	1.51
% Break	0.281	0.192	0.226
% Sludge	1.89	0.77	1.29
Foots (Centrifuge)	3.57	2.00	2.41
Foots and Red Stratum Layer (Centrifuge)	3.97	2.93	3.60
Group III (12 Samples)			
Foots, Heated	2.67	0.57	1.10
% Non-Neutral Oil—% FFA	2.27	0.72	1.40
% Break	0.125	0.085	0.107
% Sludge	1.16	0.53	0.85
Foots (Centrifuge)	0.88	0.39	0.55
Foots and Red Stratum Layer (Centrifuge)	1.41	0.39	0.72

The following conclusions may be drawn from examination of Table V.

1. The Lever Brothers' Adsorption procedure for the determination of non-neutral oil does not correlate well with the Foots test. It will be noted that the tabulated mean value for the % non-neutral oil—% FFA is practically identical for both Group II and III and also that the high value observed in Group III is approaching the low value experienced for the oils comprising Group I. However since the Lever Brothers' Adsorption procedure has been shown to

measure—and indeed does measure the non-oil portion of linseed oil—we cannot preclude the possibility that this test is the best one for the job at hand. The basis of this work was not to find a particular test which would correlate 100% with the Foots test, but rather one which could be considered as giving a more exact and precise method for the determination of non-oil constituents in raw linseed oil.

2. The percentage of break, sludge, and Foots by the centrifuge method all show a tendency to classify the oils into exactly the same groupings as does the Foots by the present method.

3. The Foots plus Red Stratum layer by the centrifuge method also is capable of classifying the oils by particular grouping. However in the case of Group I it will be noticed that the values observed are nearly as great as those experienced using the present method for determination of Foots, values which are unrealistic as measures of non-oil constituents.

As before, the coefficient of correlation was determined to relate the values by the different analytical procedures.

TABLE VI

The Correlation of the Particular Tests Under Consideration With the Foots Test

Test Correlation	Coefficient of Correlation
Heated Foots versus % Non-Neutral Oil—% FFA	0.881
Heated Foots versus % Break	0.990
Heated Foots versus % Sludge	0.921
Heated Foots versus Heated Foots (Centrifuge)	0.941
Heated Foots versus Heated Foots and Red Stratum Layer by Centrifuge Method	0.987

By examining Table VI, one will note immediately the very excellent correlation experienced between the break test and the present ASTM Foots test on heated oil. This excellent correlation has been observed previously; in fact, it was emphasized in the report of the subcommittee on linseed oil in the 1924 ASTM proceedings.

The correlation of the % non-neutral oil and % sludge with the heated Foots test has shown a very decided improvement over what was obtained in the first series of tests. However the repeatability of these determinations is only fair.

Summary and Conclusion

1. The present ASTM method D555-47 can give fair checks in any one laboratory, but in the main checks between different laboratories have been poor.

2. It has been shown that the Gardner Heat Break procedure correlates excellently with the present Foots test, gives good repeatability, and is a relatively rapid analysis. Work is now being carried out to determine the effect of increasing the sample size, the effect of reagents other than concentrated HCl, and the advisability of substituting a sintered glass funnel for the collection of the break in place of the asbestos mat now used in a Gooch type crucible. It is the feeling of the authors that this procedure merits further study as a replacement for the present Foots procedure.

3. The centrifuge procedure developed in our laboratories follows the current ASTM Method D-555-47 rather closely as to the preparation of the sample for the test, type of Foots tube employed, and the use of common reagents. It has the advantages of giving a more acceptable figure for the actual "Foots Content" of the oil being tested and being less time consuming. However even with centrifuging, which produces a more readable, firmly packed stratum of Foots, all of the oil portion is not removed from the Foots. Furthermore a volumetric determination is not capable of the precision and accuracy enjoyed by a gravimetric procedure. It should be noted also that the repeatability is only fair. Further refinements in procedure could possibly remedy this complaint.

4. The sludge determination involves too long a time to be employed in control work, and also the repeatability is very poor. At best this test must be classed as a highly qualitative measure.

5. Further work is now being carried out on the Lever Brothers' Adsorption procedure for the determination of neutral oil in raw linseed oil. However it is felt that refinements in technique and procedure are required in order to insure the attainment of better reproducibility. Investigations now under way cover use of gentle suction during the development of the column and examination of the effectiveness of adsorption with change in column length.

In conclusion, we believe that the present Foots test is unrealistic and inadequate and that a test based on chromatographic adsorption may offer a more rational basis for comparison of the quality of samples of raw linseed oil.

Acknowledgment

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