examined in three groups of 1,000, 1,000, and 700, and the results obtained from a statistical analysis of each of the three groups are compared. The important differences in chromaticity existing among glasses of identical nominal grade are illustrated and it is found that there is a slight improvement among the glasses of the third group. The degree to which the Lovibond scale, as embodied in these 2,700 red glasses combined with 35Y, fails to be additive is shown. The second and third groups confirm not only the existence but also the magnitude of the departures from additivity found in the first group. Both regular and erratic departures are described.

It is demonstrated that the same two types of regular departures from additivity exist in each of the three groups. This is shown by means of linear equations which express the relation between the N″ and N scales. Certain small differences from group to group in the slopes of the equations defining the glasses above $N = 1.0$ appear significant in that they are greater than the uncertainties involved in their determination, but no adequate explanation of these differences is apparent.

Of even greater importance than the regular departures from additivity in the N scale are the erratic departures, of which there are also two types. It is shown that important discrepancies could still occur in grading oil both because of the variations among glasses of identical nominal grade and because of the variations in size of the average intervals between successive Lovibond tenth and unit glasses. Furthermore, these intervals show the same irregularities throughout all three of the groups. It may be concluded, therefore, that the need for uniformly graded red glasses still exists in the vegetable oil trade.

APPARATUS FOR CONTINUOUS EXTRACTION: ITS APPLICATION TO THE DETERMINATION OF UNSAPONIFIABLE MATTER IN FATS AND OF TOTAL FATTY ACIDS IN SOAPSTOCK*

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Abstract

The apparatus consists of a pipette-like glass tube (solvent distributor) into the side-wall and inside of which is sealed a somewhat smaller tube for carrying solvent vapor. This device is supported in a test tube, the sidearm of which is connected to a distillation flask containing the solvent. The solvent distills from the flask through the tube, is condensed in a condenser, runs down the inside walls of the distributor and accumulates in the test tube until its level reaches the sidearm, where a portion overflows back into the solvent reservoir.

Data are given comparing results obtained by official methods with those obtained using the apparatus described.

THE present official method for the determination of unsaponifiable matter, as described in the methods of the Fat Analysis Committee of the American Chemical Society and the American Oil Chemists' Society,[1] has been in use for many years and has yielded very satisfactory results.

The official method consists briefly, of (1) saponification of a small sample of the fat with an alcoholic potassium hydroxide solution, (2) quantitative transfer of the resulting solution to an extraction cylinder and dilution with alcohol and water to a specified concentration, and (3) subsequent extraction with seven successive portions of petroleum ether (AOCS). The individual extracts are separated from the soap solution by means of a glass syphon, and the unsaponifiable matter recovered by evaporation of the solvent.

This method is, obviously, somewhat long and tedious. Many analysts have been disturbed by the large number of extractions and the length of time and close attention required, it being necessary to shake the seven extractions vigorously and long to be certain that the extraction of the unsaponifiable matter is complete.

The apparatus described in this paper was developed with the thought of reducing the time required for this determination as much as possible and at the same time improving the extraction efficiency and general manipulative ease of the method. The extraction unit is identical in principle with that described by I. E. Knapp[2], who proposed his apparatus for use in the ethyl ether extraction of unsaponifiable matter from rosin. It differs only in the manner in which the solvent vapor is carried to the condenser and in the manner of delivery of the extracting solvent to the bottom of the extraction tube. Since the completion of this work Ashley and Murray[3] have also proposed a similar apparatus for the removal of ferric chloride from solution.

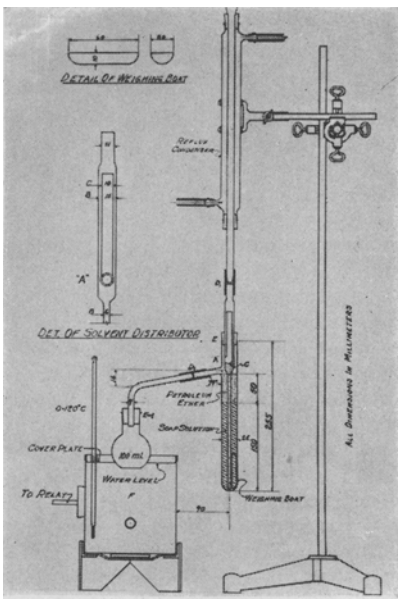


FIGURE I

Apparatus:

Figure I illustrates the principle of the unit. The solvent vapor is carried from the 100 ml. flask through the side arm of the extraction tube and the glass tube (C), sealed into the solvent distributor (B), to the condenser. The condensed solvent runs down the inside wall of (B) and accumulates in (B) until the pressure head of the liquid solvent is sufficient to force the

*Presented at the Twelfth Fall Meeting of the American Oil Chemists' Society, Chicago, October 6-7, 1938.

solvent out at the bottom opening of (B). The solvent passes upward through the soap solution and accumulates above this solution until the height of the column is such that a portion of the solvent overflows through the side arm and back into the 100 ml. flask of boiling solvent. The soap solution is thus continuously extracted.

The distributor tube (A) is suspended from the condenser by a short rubber connection (D_1). The extraction tube is suspended from the distributor tube by a cork or Duprene stopper (E) and connected to the solvent flask by a short rubber connection (D_2). The solvent flask stopper (E-1) is of cork. These rubber connections impart flexibility to the apparatus, making it possible to swing the extraction tube sideways and foreward so that its lower end can be placed in the boiling water bath (F) (see photograph A). With this arrangement, the sample, contained in a small glass boat, can be slipped into the extraction tube and the two operations, saponification and extraction, performed in a single tube. This eliminates the necessity of washing the saponified solution into an extraction cylinder as required by the present official method.

The water bath used for these determinations is a rectangular vessel 6⅜ inches deep, 6 inches wide and 48 inches long built of copper. The top or cover plate contains twelve holes, centered laterally, 2⅛ inches in diameter. This plate is rigidly fastened ⅜ of an inch from the upper edge of the bath. Since the water level is maintained at the level of the top-plate, the ⅜ inch flange produced by depression of the top plate prevents water from spilling over during filling or boiling. The steam heater, running the full length of the bath, is of the direct or open type consisting simply of a pipe capped at one end with ⅛ inch holes opening downward and spaced 2 inches apart. The electric heater is an 825 watt strip heater placed tightly against the bottom of the water bath. Opposite the end of the steam inlet is placed the cold water inlet and a water leveling device. Insulation of the back of the bath with transite, celotex, or similar material decreases the tendency of the solvent to boil in the extraction tubes during operation. During the saponification period the bath temperature is maintained at the boiling point (97° C.-100° C.) by manual control of the steam inlet valve. During the extraction period a temperature of 76° C.-78° C. is maintained by a bimetallic thermostat used in conjunction with a relay.

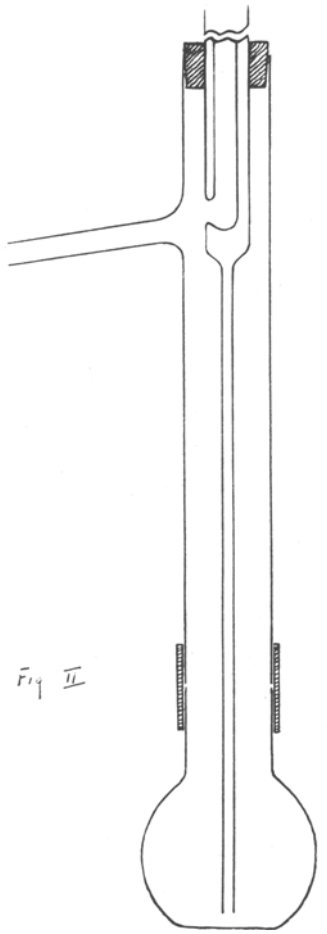


Fig II

Method:

The following procedure has yielded results which are in satisfactory agreement with those obtained by the official method:

Weigh 3 grams (± .001 gm.) of the thoroughly mixed fat into each of two weighing boats and place the boats in the bottom of the extraction tube being careful that *none* of the sample is spilled or wiped onto the upper inside walls of the tube. This is easily accomplished by holding the tube in a horizontal position and pushing the boat into the tube with a glass stirring rod. From a burette or pipette, add 12 cc. of alcohol (formula No. 30) and then 4 cc. of a 50% aqueous solution of C.P. potassium hydroxide.

Attach the extraction tube, containing the sample and boat along with the saponifying reagents to the solvent distributor (B) at the point E. Keep the solvent distributor (B) permanently attached (except for occasional washing) to the reflux condenser at D_1. Adjust the extraction tube so that the lower end of the solvent distributor (B) is not more than 3/16 of an inch from the bottom of the extraction tube. The tube opening should be as near the bottom as possible so as to insure extraction of the entire column of soap solution. Place a small cork stopper in the side arm at D_2, then swing the extraction tube sidewise and forward so that its base is immersed in the water of the bath to a depth of about one half inch. Bring the water in the bath to boiling. The alcoholic solution under these conditions will boil gently. If it does not, adjust the depth of immersion in the bath to a point where gentle boiling of the solution is obtained. Boil for one hour.

At the end of this time close the steam inlet valve, remove the extraction tubes from the water bath, resting the bottom of the tubes on the water bath top-plate. Remove the stoppers from the side arms. Add, through the side arms, sufficient 10% alcohol solution to bring the total volume of the hot saponified mixture to 45 ml.* When hard fats are being extracted add a few ml. of the 10% alcohol solution through the top of the condenser to prevent solidification of the hard soap in the narrow part of the distributor tube. Swing the tubes back to a vertical position, suspended from their respective condensers, and allow the solutions to cool to room temperature (maximum 37° C.).

Cool the water bath to a temperature of about 77° C. and turn on the electric heater unit, regulating the temperature at 76° C. to 78° C. Fill a 100 ml. Soxhlet flask to the neck with petroleum ether (AOCS) and connect the flask to the extraction tube through D_2 as indicated in Fig. 1 (see also photograph B). With the above temperature regulation a distillation rate of approximately 9 ml. per minute is obtained. If the rate varies from this significantly, adjust the temperature of the bath to produce a rate of 9 ml. per minute. Extract the soap solution for two and one-half hours.

At the end of this time, disconnect the Soxhlet flask and wash the petroleum ether extract into a separatory funnel with at least 50 ml. of petroleum ether. Wash the extract

*This point can be marked on the side of the tube. The dilution will bring the level of liquid in the tube to about 2" below the side arm. Reservation of some space above the liquid is necessary since, in some cases, considerable petroleum ether is retained in the soap layer during subsequent extraction. Furthermore, space must be reserved for adequate separation of the liquids at the interface, to prevent soap solution from carrying over into the 100 c.c. Soxhlet extraction flask.

with three successive portions of 25-30 ml. of 10% alcohol, twirling the funnel gently on the first wash and shaking vigorously on the two succeeding washes. Transfer the ether extract to a 250 ml. beaker and evaporate the petroleum ether on a steam bath in an air current. Dry to constant weight. Dissolve the residue in about 50 ml. of petroleum ether. Filter into a tared vessel, thoroughly washing the filter and insoluble residue with petroleum ether. Evaporate and dry in the same manner as before and record weight of extracted material.

Take up the final residue in 50 ml. of warm ethyl alcohol (No. 30) neutralized to phenolphthalein and titrate to the same color as the original neutral alcohol with 0.1 normal sodium hydroxide solution. Calculate to oleic acid and deduct this figure from the gross weight previously found reporting the net weight as unsaponifiable matter.

Results:

Table I gives a comparison of results between the method proposed and the official method. It will be noted that the agreement between the two methods is within experimental limits, except in two instances where the difference (+0.34 and +0.38) are somewhat wider than might reasonably be expected.† An interesting feature of the method is that it appears to reduce substantially the average correction for titratable acids, the average falling from 0.41 to 0.26.

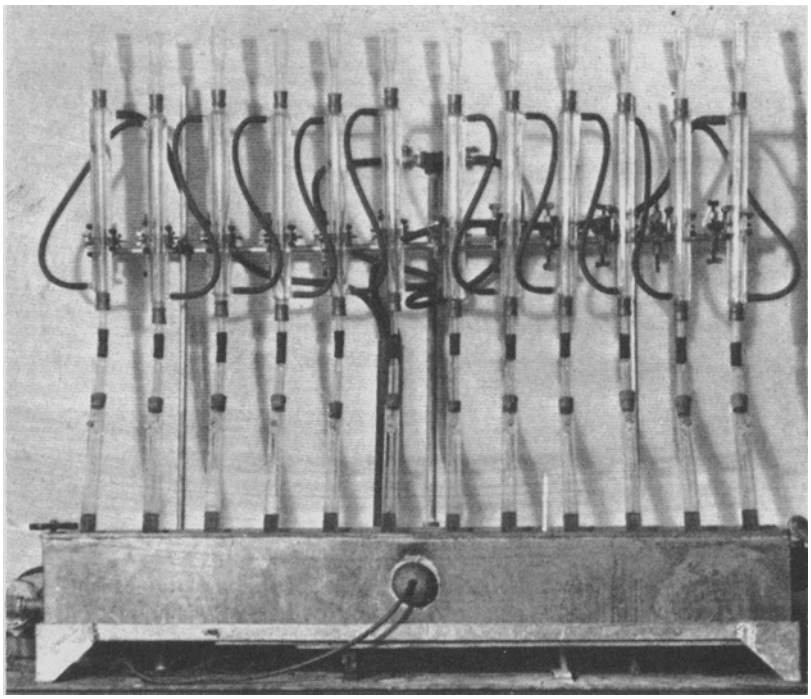
It is believed that this method substantially reduces the active time required by an analyst to perform an unsaponifiable determination and that it materially improves the ease of manipulation of the determination.

Application to Soapstocks:

The principle of extraction described above has also been applied to the determination of total fatty acids in cottonseed and other soapstocks.

The official method for this determination,[4] while not particularly tedious, is none the less quite time-consuming, since it requires chilling the fatty acids until solid and washing with cold water. This step is most conveniently and satisfactorily accomplished by storing the fatty acid in an ice-box overnight, with resultant loss of time. An alternate official method requires extraction of an acidified soap solution in a manner similar to the extraction of unsaponifiable matter discussed in the forepart of this paper. Each extraction is separated from the solution individually by means of a syphon. It, therefore, requires considerable manipulation and handling of apparatus and in this regard is open to the same objections as the official method for unsaponifiable matter.

The apparatus proposed for this test is identical with that used for extraction of unsaponifiable matter, with one exception. The bottom of



PHOTOGRAPH A

TABLE I—UNSAPONIFIABLE MATTER

Sample	Official Method: Titratable Acids in Extract (as oleic)	Official Method: Net Unsaponifiable Per cent	Proposed Method: Titratable Acids in Extract (as oleic)	Proposed Method: Net Unsaponifiable Per cent	Titratable Acids (Differences)	Unsaponifiable (Differences)
Grease	0.22	1.44	....	1.39	...	—.05
	0.33	1.66	0.27	1.69	—.06	+.03
	0.49	2.58	0.31	2.46	—.18	—.12
	0.24	1.68	0.31	1.51	+.07	—.17
	0.22	1.51	0.31	1.65	+.09	+.14
	0.40	1.93	0.15	1.76	—.25	—.17
	0.40	2.59	0.13	2.50	—.27	—.09
	0.20	2.00	0.51	1.99	+.31	—.01
	0.38	1.78	0.29	1.92	—.09	+.14
	0.35	2.50	0.35	2.73	.00	+.23
	0.35	1.43	0.09	1.50	—.26	+.07
	0.65	1.77	0.51	2.11	—.14	+.34
	0.38	1.41	0.18	1.21	—.20	—.20
	0.24	1.62	0.20	1.64	—.04	+.02
	0.60	1.02	0.31	0.92	—.29	—.10
	0.80	1.08	0.63	1.05	—.17	—.03
	0.36	0.89	0.33	1.02	—.03	+.13
	0.36	1.37	0.81	1.43	+.45	+.06
	0.56	2.38	0.22	2.33	+.44	—.05
	0.85	2.21	0.20	2.07	—.65	—.14
	0.33	2.24	0.08	2.62	—.25	+.38
	0.33	5.10	0.16	5.02	—.17	—.08
Tallow	0.22	0.89	0.80	0.93	+.58	+.04
	0.42	0.91	0.33	0.98	—.09	+.07
	0.54	0.94	0.36	0.88	—.18	—.06
	0.44	1.11	0.40	1.25	—.04	+.14
	0.20	0.85	0.22	1.05	+.02	+.20
	0.20	1.67	0.19	1.77	—.01	+.10
	0.22	1.90	0.18	1.81	—.04	—.09
	0.26	1.57	0.18	1.61	—.08	+.04
	0.26	1.07	0.33	1.23	+.07	+.16
	0.24	1.67	0.17	1.77	—.07	+.10
	0.22	0.60	0.33	0.54	+.11	—.06
	0.29	0.50	0.27	0.50	—.02	.00
	0.30	0.49	0.15	0.59	—.15	+.10
Red Oil	0.45	5.34	0.26	5.42	—.19	+.08
	1.20	4.80	0.36	4.76	—.84	—.04
	0.47	4.67	0.53	4.65	+.05	—.02
Sheanut Oil	1.00	5.20	0.04	5.16	—.96	—.04
Average	0.41	....	0.26	....	.....	.....

†It was later found that occasional results of this nature are caused by incomplete extraction when the official method is used.

the extraction tube is cut off and for it is substituted a small, 50 ml. flask similar to a Soxhlet flask but having no flange. This flask is fixed tightly to the extraction tube by means of a rubber sleeve. The apparatus is shown in Figure II. The overall length of the apparatus from the base of the flask to the top of the extraction tube is the same as the length of tube in Figure I, the removable flask being added simply to facilitate saponification and drying of the resulting soap, and to provide somewhat more room for liquid. Other dimensions are also identical with those shown in Figure I.

Method:

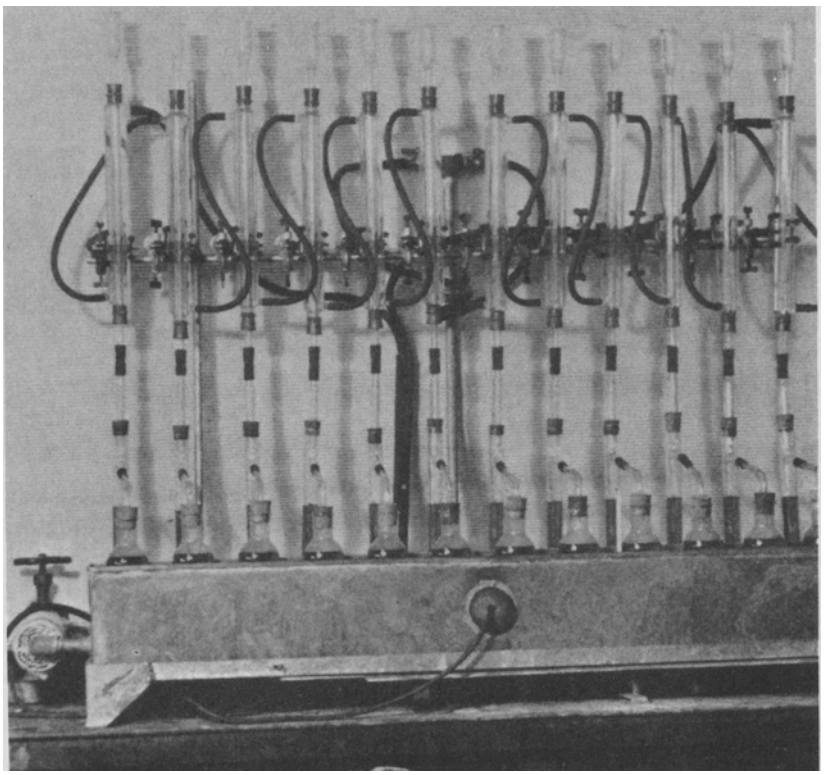
Weigh 3 grams (± 0.001 gram) of the thoroughly mixed soapstock into each of two 50 ml. Soxhlet flasks and add 30 ml. of an alcoholic potassium hydroxide solution containing 42.2 grams of C. P. KOH per liter. Place the flasks on a steam bath to saponify. At the end of one hour, or when saponification is complete, direct an air jet into the flask and completely evaporate the alcohol. Now add 25 c.c. of hot water and when solution is complete add just enough 1:1 hydrochloric acid to split out the fatty acids. Avoid a large excess. Remove from the steam bath and allow to cool. While still warm enough to keep the fatty acids melted, connect the Soxhlet flask to the extraction tube and place in position for extraction. (See Figure I.) Through the side arm add enough cold water to fill the 50 ml. flask; then add 20 ml. of formula No. 30 alcohol and follow with more water, bringing the level of the liquid up to about two inches below the side arm of the extraction tube.‡

Regulate the temperature of the water bath to 77° C. Fill a 100 c.c. Soxhlet extraction flask to the neck with petroleum ether (AOCS) and connect the flask to the extraction tube through (D_2) as indicated in the diagram. With the above temperature regulation a distillation rate of approximately 9 ml. per minute is obtained. If the rate varies from this significantly, adjust the temperature of the bath to produce a rate of 9 ml. per minute. Extract the soap solution for 2 hours.

At the end of this time filter the contents of the Soxhlet flask containing the petroleum ether extract, through a good quality filter paper into a suitable tared vessel, being sure that the filter paper is washed free from fatty acid. Evaporate the petroleum ether on a steam bath in an air current, dry to constant weight, and calculate the result to percent total fatty acids.

The same extraction procedure can be used for cocoanut soapstocks except that a four hour extraction period is required.¶ The recovered fatty acids, due to volatility, must, however, be neutralized with standard sodium hydroxide solution, evaporated, and weighed as soda soap. In place of evaporating the petroleum ether and recovering the final residue as fatty acids, the following method can be applied:

Filter the petroleum ether extract through a good quality filter paper into a 400 ml. beaker tared with a stirring rod, washing the filter thoroughly. Add 25 to 30 ml. of 95% alcohol containing a few drops of phenolphthalein indicator, titrate the solution with 0.5 normal caustic soda, and record the amount used. Evaporate the petroleum ether and



PHOTOGRAPH B

TABLE II—TOTAL FATTY ACIDS

Type of Soapstock	Total Fatty Acids—Per Cent: Official Method	Proposed Method	Difference	
Cottonseed	45.5	45.3	—0.2	
	39.5	39.8	+0.3	
	39.2	39.2	0.0	
	44.5	44.5	0.0	
	43.8	43.8	0.0	
	42.0	42.2-42.2	+0.2	
	45.5	45.2	—0.3	
	43.1	43.2	+0.1	
	43.4-43.4	43.5-43.4	+0.1	0.0
	43.7	43.9	+0.2	
	42.6	42.6	0.0	
	45.8	46.1	+0.3	
Soyabean	34.5	34.7-34.5	+0.2	0.0
	48.5	48.2	—0.3	
Mixed	43.6	43.5	—0.1	
	43.6	43.7	+0.1	
Tallow	43.6	43.4	—0.2	
Cocoanut	39.0	39.1	+0.1	
	40.2	40.0	—0.2	
	40.2	40.4	+0.2	

‡Alcohol is added here since it appears to have a dispersing effect on the solvent as it passes through the acid solution, resulting in a more efficient extraction. It is best to add the alcohol after considerable dilution with water to prevent formation of ethyl esters of the fatty acid, which would lead to high results.

¶Results obtained with a two hour interval were low, four hours being required for complete extraction of the fatty acids from the acid solution. The added time required for this material may possibly be associated with the greater solubility of the fatty acids in alcohol-water solutions.

alcohol and dry the residue in an oven at 105 to 110° C. to constant weight. In order to calculate the soda soap to fatty acids, a correction must first be made for the neutral salts in the caustic solution. Neutralize 20 c.c. of 0.5 N caustic soda with 0.5 N HCl, using a small amount of phenolphthalein as indicator. Evaporate to dryness and heat to constant weight at 105°-110° C. From the weight of the residue found, subtract the weight expected if the reagents had been 100 per cent pure. The difference divided by 20 gives the correction per c.c. for neutral salts. From the weight of the soda soap, subtract the product of the titration of fatty acids times the factor (0.011) plus correction for neutral salts. Divide the result by the weight of sample used, and multiply by 100, which gives the results in per cent.

Results:

The results of a number of determinations comparing these procedures with the official method are given in Table II. The maximum divergence between the two methods in the 20 samples reported is 0.3%. The method appears, therefore, to give results agreeing with those obtained by the official method within experimental limits.

It is felt that the procedure offers the possibility of determining total fatty acids in substantially less time than is required by the official method, with considerably less active attention required by the operator.

REFERENCES

(1) Published by the American Oil Chemists' Society, 509 Tchoupitoulas Street, New Orleans, La. (Pages 30 and 30-a.)

(2) Ind. & Eng. Chem., An. Ed. 9, page 315 (1937).

(3) Ind. & Eng. Chem. An. Ed. 10, page 367 (1938).

(4) See Reference (1), AOCS Methods, pages 18-19.

VARIATION IN THE F.F.A. CONTENT OF COTTONSEED WITHIN A SAMPLE

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Abstract

Collaborators on F.F.A. in cottonseed vary more than they do on F.F.A. in cottonseed oil. It was suspected that this variance was due to the variance in F.F.A. in the seed in a sample. This work reports on the variance in the seed in two cottonseed oil samples as determined by Edeler's micro-method. It is concluded that the collaborators' discrepancies are not all explainable on the basis of variance in seed.

THE work described in the following report was done at the end of the 1936-37 cottonseed crushing season. It was planned to extend this to the A.O.C.S. samples sent out during the 1937-38 season but the press of work would not permit. It is hoped that time will permit further work to be done during the coming season, but the variation found in the seed is so unexpected that the author feels that the values may be of interest even though they were found on only two samples.

The F.F.A. reported on cottonseed samples, such as the A.O.C.S. samples, varies much more than the F.F.A. reported on cottonseed oil samples. The tolerance allowed on Prime seed is 0.2% and is frequently exceeded whereas the tolerance allowed on Prime oil is 0.1% and is seldom exceeded.

In considering the scatteration of F.F.A.'s on cottonseed, the first question that had to be answered was: "How much do the various seed in a sample vary in their individual F.F.A. content?" It did not seem feasible to determine F.F.A. on individual cottonseed, but a method for determination on groups of 10 seed was worked out by Mr. A. Edeler, who used it in making the determinations reported.

Through the courtesy of Mr. Thomas Law we secured portions of A.O.C.S. cottonseed samples Nos. 6 and 8 of the 1936-7 series.

The results of the collaborators in the regular A.O.C.S. work on this is shown on Figure 1. The results may be summarized as having a mean value of 1.87 and a standard deviation (root — mean — square deviation) of 0.178.

When groups of ten seed were run by Mr. Edeler's micro-technique, the results were as shown in Figure II. These may be summarized by a mean of 2.04 and a standard deviation of 0.29.

Values for A.O.C.S. sample No. 8 are shown in Figures III and IV. The collaborators' results showed a mean of 1.7 and a standard deviation of 0.175, the micro method a mean of 1.65 and a standard deviation of 0.302.

We see that the seed in a sample have a rather large variation in F.F.A. content. What would the variation have been if we had been able to analyze individual seed? It is a rule of statistics that variance of the mean value is inversely proportioned to the square root of the number of units taken in a sample. If the standard deviation of units of 10 is 0.30, that of units of 1 is $\sqrt{10} \times 0.30 = 0.95$. In other words, the individual seed in samples 6 and 8 have a standard deviation of almost 1% of F.F.A. about the mean value. This is probably a skewed distribution, a few high F.F.A. seed offsetting seed of more nearly normal acid content.

To answer the question, how much of a variation shown by the collaborators is due to the variation in seed, we have to apply our square root rule again. At 10 seed per gram we can consider the whole 200 gram sample to consist of 2000 seed; then our expected variation would be

$$.95 \times \frac{1}{\sqrt{2000}} = .02$$

Even if we assume that the process of hulling and grinding are not such as to thoroughly mix the sample and we calculate our variation on the 40 grams, we would have

$$.95 \times \frac{1}{\sqrt{400}} = .05$$

We can say then, that if the sample drawn is truly representative, the variations in F.F.A.'s introduced by the difference in seed is small. However, the variation in seed makes it imperative that effort be made to draw a sample that really represents the seed submitted.

Finally, the method of determining F.F.A. in cottonseed needs investigation as the variation between chemists is greater than can be accounted for by differences in the F.F.A. of seed analyzed.

"EDELER MICRO-METHOD FOR F.F.A. IN COTTONSEEDS"

Butyl alcohol was found to be the