Report of the Committee on Analysis of Commercial Fats and Oils

Fall Meeting, October 1947

THE report of the Fat and Oil Analysis Committee comprises the work of the various subcommittees. A suggestion was made that the sampling method, C 1-41, Section D, subsections a, b, and c, be revised for clarification. This change has been agreed to by the committee and is submitted for approval. The revised procedure follows:

D. Procedure:

a) *Petcock Method for Sampling Tanks or Tank Cars During Loading or Unloading*

1. If the conditions are suitable, this is a very satisfactory method of sampling. This method is applicable only if the product is completely liquid, free-flowing and does not contain any material that may plug the petcock.
2. A petcock ⅜ inch minimum inside diameter and bleeder line is located in a vertical section of the pumping line through which the product is continuously flowing.
3. Adjust the petcock so that a continuous stream of sample flows freely without dripping during the entire pumping period.
4. Collect the sample in a clean and dry container and protect from dirt, water, or other contamination. Mix the entire sample thoroughly so as to obtain uniform distribution of moisture, meal, and any other impurities and then fill 3 clean and dry 1-gal. containers to an outage of about 2 inches.

b) *Grab Method for Sampling Tanks or Tank Cars During Loading or Unloading*

1. Use a dipper or any other convenient container and withdraw ca 1 lb. from the discharge end of the pipe at regular intervals as the product is entering the tank.
2. Collect, mix, and distribute as directed in (a), 4.

c) *Loaded Tanks or Tank Cars—Liquid Contents (Official Method of the National Cottonseed Products Association.)*

1. Lower the official oil trier vertically through the oil at a uniform rate with the bottom valve completely open so that 10 to 15 seconds will be required to reach the bottom of the car (See Note 1). Close the bottom valve and withdraw the tube.
2. Take several portions in this manner and then proceed as directed in (a), 4 above.

SUBCOMMITTEE ON F.A.C. COLOR METHOD:

(E. W. Blank, chairman)

A questionnaire has been drawn up to be submitted to various users of the F.A.C. Color Standards. The wording of this questionnaire has been approved to date by a majority of the members of the subcommittee. By the time of the fall meeting it is hoped to have unanimous approval. Various members of the subcommittee have kindly agreed to be responsible for distribution of copies of the questionnaire. On the basis of replies received the subcommittee will then attempt to formulate a program.

SUBCOMMITTEE ON DETERMINATION OF SCN VALUES:

(R. T. Milner, chairman)

Collaborative thiocyanogen analyses have been made by the full committee on the five glyceride oils, studied also by the Spectroscopy and Solid Acids Committees. In addition, five members of this committee have prepared the mixed fatty acids of three of these oils and determined thiocyanogen values for these mixed acids. Results for the five laboratories are summarized below:

Thiocyanogen Values

	Linseed		Soybean		Cottonseed	
	Oil	Acids	Oil	Acids	Oil	Acids
Average	120.9	124.1	85.3	88.5	70.8	70.2
Maximum	122.5	126.9	86.1	90.6	73.9	70.9
Minimum	120.1	121.0	84.2	86.8	69.0	69.2
Standard Deviation	0.9	2.6	0.3	1.5	1.9	0.3

For two of the three samples results are in better agreement for the oils than the acids, and this probably is caused by difficulties in preparing the acids. The reports by the individual laboratories showed more variations for each laboratory and between laboratories than are desirable, and this is reflected in the table above. In the work carried out so far, lead thiocyanate, prepared by Dollear's method, has been used and components of the reagent were tested for impurities before use. Slight modifications were made in the present official A.O.C.S. procedure, such as the use of 25% CCl_4 in the reagent. A little evidence has been obtained that unknown factors in the preparation of the lead thiocyanate are responsible for some of the difficulties encountered.

For future work, fresh samples of three oils (linseed, soybean, and cottonseed) are being obtained. A common source of lead thiocyanate and a new, detailed set of directions will be used. Determinations on the oils will be followed by measurements on the mixed acids which will come from a common source. It is hoped that rapid progress and a final report may soon result.

SUBCOMMITTEE ON DETERMINATION OF PEROXIDE VALUES:

(A. R. Baldwin, chairman)

Details of the Wheeler method (copy following) were distributed to the members of the committee along with a tentative procedure for the preparation of peroxide-containing fat samples. Comments were requested. At the suggestion of W. O. Lundberg the method of sample preparation was modified to be done as follows:

1. Oxidize the fat with air at 100°C.
2. Cool to room temperature and purge all oxygen from the oil by blowing with purified nitrogen.
3. Fill amuples which had previously been purged of oxygen.
4. Displace headspace gas in ampules several times with nitrogen.
5. Seal the ampules.

In order to test the stability of the peroxides during shipment to the collaborating laboratories, ampules were filled with oxidized corn oil in the above

manner and stored. Peroxides were determined by the Wheeler method after various storage periods in the dark at —20°F. and at room temperature (75-95°F.). Results are shown below:

Storage Time (days)	Peroxide Values (Me/Kg)*	
	—20°F.	75-90°F.
0	41.4 41.7 42.3 Avg. 41.8	
5	42.3 42.2 41.2 Avg. 41.9	
7	41.2 41.7 39.9 Avg. 40.9	41.4 41.3 41.4 Avg. 41.4
10	41.4 41.4 40.9 Avg. 41.2	41.1 41.1 41.0 Avg. 41.1
14	41.3 42.3 40.2 Avg. 41.3	41.3 40.2 39.9 Avg. 40.5

*Analyses were made in triplicate as shown.

Indications are that ampules of peroxide-containing fat prepared in this way may be distributed to the collaborators without significant changes even when shipped without dry ice packing.

Samples of oxidized lard and corn oil are in preparation for collaborative testing in the very near future.

Wheeler Method for Peroxide Determination*

1. Weigh 3 to 10 grams of fat into a 250-ml. Erlenmeyer flask.
2. Add 50 ml. of solvent (60 parts glacial acetic acid and 40 parts of anhydrous C. P. chloroform).
3. Add 1.0 ml. of saturated aqueous potassium iodide solution.
4. Stir vigorously by using a rotating motion.
5. After exactly 1 minute from the time of addition of the iodine, add 100 ml. of distilled water.
6. Titrate immediately with standard (0.10 or 0.01 N) sodium thiosulfate solution. Use starch as an internal indicator. Calculate the results as milliequivalents of peroxide per 1000 g. of oil.

$$\text{Me/Kg} = \frac{\text{ml.} \times \text{N}}{\text{grams}} \times 1000.$$

SUBCOMMITTEE ON SEPARATION OF LIQUID AND SOLID FATTY ACIDS:

(F. G. Dollear, chairman)

During the past year the Subcommittee on Separation of Liquid and Solid Acids has continued its investigation of the separation of solid acids and determination of the saturated acid content of five oils, namely, beef fat, lard, cottonseed, soybean, and linseed, which oils have also been investigated by the Thiocyanogen Committee and Spectroscopy Committee. Three methods have been compared, the official A.O.C.S. lead salt-alcohol method, Cd 6-38; a modified Bertram Oxidation method; and a solvent crystallization method (see Earle and Milner, Oil and Soap, *17*, 106 (1940). The results obtained by the four laboratories carrying out these determinations are summarized in the following table:

Comparative Results of Determination of Saturated Acids by Various Methods

	Oil	Per Cent Saturated Acids in Mixed Acids Determined by Various Methods		
		Lead-Salt-Alcohol	Bertram Oxidation	Crystallization
High	Beef Fat	47.9	52.0	54.3
Low		40.7	46.3	43.2
Average		45.3	50.0	49.2
High	Lard	35.0	39.6	36.6
Low		27.2	32.2	34.0
Average		33.2	36.5	35.7
High	Cottonseed	24.6	28.2	26.4
Low		20.5	21.4	23.6
Average		23.3	26.6	25.3
High	Soybean	13.5	15.0	14.5
Low		8.7	12.0	13.4
Average		11.7	14.2	14.1
High	Linseed	8.0	10.2	9.4
Low		5.0	8.5	7.8
Average		7.0	9.4	8.9

From the high, low, and average values for per cent saturated acids reported in this table, it may be seen that there was a rather wide spread in these values as determined in the different laboratories using the same method on the same oil. This may be due in part to the analyst's lack of familiarity with two of the methods investigated. The need for further work to improve the reproducibility of determinations by different laboratories is indicated by the results obtained thus far. When average results are considered, it is apparent that the modified Bertram oxidation and crystallization methods give results which are in fairly good agreement and which are definitely higher than the results for saturated acids determined by the lead salt-alcohol method. This indicates the desirability of carrying out further work, particularly on the crystallization method since it is the simplest of the three methods to apply.

SUBCOMMITTEE ON UNSAPONIFIABLE MATTER:

(C. P. Long, chairman)

The committee did not engage in any active organized work during the past year. However, a collaborative program is being formulated for the coming year.

SUBCOMMITTEE ON ANALYSIS OF DRYING OILS:

(J. C. Konen, chairman)

The committee is submitting the following methods for the analysis of drying oils:

Free Fatty Acids
Color
Refractive Index
Specific Gravity

Since these methods are in common use in the industry, they have not been subjected to collaborative study.

Acid Value

Definition: The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free fatty or rosin acids in 1 gram of sample.

Scope: Applicable to all natural and synthetic drying oils and their fatty acids.

A. Apparatus:

1. Erlenmeyer flasks, 250 or 300 ml.

B. Reagents

1. Sodium hydroxide, approximately 0.1 *N*, accurately standardized.

* Taken from "Peroxide Formation as a Measure of Autoxidative Rancidity"—D. H. Wheeler, in Oil and Soap, *9*, 89-97 (1932).

2. Benzene-alcohol mixture consisting of equal parts by volume of 95% alcohol (U.S.S.D. Formulas 30 or 3A) and benzene (A.C.S. grade). The mixture must give a definite, distinct, and sharp end-point with phenolphthalein and must be neutralized with alkali to a faint but permanent pink color just before using.
3. Phenolphthalein indicator solution, 1.0% in 95% alcohol. (See Note 1.)

C. Procedure:

1. Determine the size of sample from the following table:

Acid Value	Approx. Wt. of Sample, Grams	Accuracy of Weighing
0 to 5	20	±0.05 g.
5 to 15	10	±0.05
15 to 30	5	±0.05
30 to 100	2.5	±0.001
100 and over	1.0	±0.001

2. Weigh the designated size of sample into an Erlenmeyer flask.
3. Add approximately 100 ml. of neutralized solvent and 2 ml. of indicator.
4. Shake until sample is completely dissolved. Warming may be necessary for bodied oils.
5. Titrate with standard alkali solution, shaking vigorously to the appearance of the first permanent pink color of the same intensity as that of the neutralized solvent before addition. The color must persist for 30 seconds. In case of off-color materials, the color should be observed in the alcohol layer above the sample after the layer has been allowed to settle. The sample will usually settle sufficiently in a minute or less.

D. Calculation:

The acid value, mg. KOH per g. of sample =

$$\frac{\text{Ml. of alkali} \times N \times 56.1}{\text{Weight of sample}}$$

Notes:

1. A "masked phenolphthalein indicator" may be used with off-color materials. Prepare by dissolving 1.6 grams of phenolphthalein and 2.7 grams of methylene blue in 500 ml. of alcohol (U.S.S.D. Formula 30 or 3A). Adjust the pH with alkali solution so that the greenish blue color is faintly tinged with purple. Color change is from green to purple when going from acid to alkali.

Color

Gardner (1933) Standard Colors

Definition: This method determines the color by comparison with standards of definite color composition.

Scope: Applicable to natural and synthetic drying oils which do not differ from the standards in hue.

A. Apparatus:

1. Color tubes of clear glass 10.75 mm. ± 0.03 mm. inside diameter, 12.3 mm. outside diameter, 112 mm. long. Standard Gardner-Holdt viscosity tubes meet these specifications. (See Note 1.)
2. Comparator block, constructed of wood and painted dull black, dimensions as shown on the illustration.
3. Light source, north sky background, avoiding reflections from buildings, or a daylight type fluorescent bulb.
4. Gardner, 1933, Color Standards. This set consists of 18 color standards numbered from 1 to 18. (See Note 2.)

B. Procedure:

1. Pour the sample into a color tube. Place this tube and 2 of the standards in the comparator block with the sample in the center.
2. Hold the block containing the standards and sample up to the light in a vertical position and in such a way that the standards and sample are viewed simultaneously.
3. The sample is reported as not darker than the standard which it matches or not darker than the darker of the 2 standards between which it falls. Any sample darker than 18 is reported as darker than 18.

C. Notes:

1. Color tubes may be obtained from:
   Henry A. Gardner Lab., Inc., Apparatus Division,
   4723 Elm Street, Bethesda, Maryland.
2. The color standards are made from solutions of ferric chloride, cobalt chloride, and hydrochloric acid. The composition of each is given by Gardner in *Physical and Chemical Examination of Paints, Varnishes, Lacquers, and Colors,* 10th Edition, p. 94.

Refractive Index

Definition: The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. The index of refraction of oils is characteristic with certain limits of each kind of oil. It is related to the degree of saturation, but it is affected by other factors such as free fatty acid content, oxidation and heat treatment.

Scope: Applicable to natural and synthetic drying oils.

A. Apparatus:

1. Refractometer with Abbe scale or dipping type refractometer. The temperature of the refractometer prisms must be controlled to within ± 0.1°C., and for this purpose it is preferably provided with a thermostatically controlled water bath and motor driven pump to circulate water through the instrument. The instrument is standardized following the manufacturer's instructions, with a liquid of known purity and refractive index or with a glass prism of known refractive index. Distilled water, which has a refractive index of 1.33251 at 25°C. is satisfactory in some cases.
2. Light source, a tungsten or daylight bulb. An electric sodium vapor lamp gives much sharper readings and is preferred.

B. Reagents:

1. Toluene or some other fat solvent satisfactory for cleaning the prisms. Lens tissue or cotton is recommended for cleaning the prisms in order to avoid injury to them.

C. Procedure:

1. Adjust the temperature of the refractometer to 25.0°C.
2. Be sure that the prisms are clean and completely dry and then place several drops of the sample on the lower prism of the Abbe refractometer, or if the dipping refractometer is used immerse the prism in the material to be examined at 25°C. Allow to stand for three minutes so that the sample and prisms will both be at 25°C. before taking a reading.
3. Adjust the instrument and light to obtain the most distinct reading possible and determine the refractive index. Take several readings and calculate the average of all.

D. Notes:

1. Approximate temperature corrections may be made by the following calculations:
   $R = R' + K(T' - T)$
   R = the reading reduced to temperature T
   R' = the reading at T' °C.
   T = the standard temperature
   T' = the temperature at which the Reading R' is made
   K = 0.000365 for fats and 0.000385 for oils.

Specific Gravity of Oils and Liquid Fats

Definition: This method determines the specific gravity of the sample in liquid form as compared with water at 25°C.

Scope: Applicable to natural and synthetic drying oils.

A. Apparatus:

1. Specific gravity bottles with well fitting ground glass joints having at least 25 ml. capacity.
   a. Leach type pycnometer for oils with viscosities up to 500 Stokes.

b. Wide mouth sp. gr. bottle such as Hubbard (A.S.T.M. Specification D70) for oils with viscosities above 500 Stokes.

Calibrate these bottles as follows:

Clean and dry thoroughly and then fill with recently boiled and cooled distilled water at 20° to 23°C. Fill the bottle to overflowing by holding the bottle on its side in such a manner as to prevent the entrapment of air bubbles. Insert the stopper and immerse in water bath at 25°C. ±0.2°C. Keep the entire bulb completely covered with water and hold at specified temperature for 30 minutes.

Carefully remove any water which has exuded from the side opening and tightly cover with the cap. (See Note 1.) Remove the bottle from the bath and wipe completely dry. Weigh the bottle and contents. Calculate the weight of water in the flask by subtracting the weight of empty bottle from the weight of bottle plus water.

2. Water bath maintained at 25°C. ± 0.2°C.
3. Thermometer, any convenient thermometer of suitable range with 0.1° or 0.2° subdivisions. Standardize carefully, preferably by comparison with a thermometer calibrated by the U. S. Bureau of Standards.

B. Procedure:

a) *Specific Gravity of Oils up to 500 Stokes Viscosity*

1. Cool the sample to 20° to 23°C. and fill the specified specific gravity bottle to overflowing, holding the bottle on its side in such a manner as to prevent the entrapment of air bubbles. (See Note 2.)
2. Insert the stopper, immerse, and hold in the water bath at 25° ± 0.2°C. for 30 minutes.
3. Carefully wipe off any oil which has come through the capillary opening, cap tightly, and remove from bath. Clean and dry thoroughly.
4. Weigh the bottle and contents, and calculate the specific gravity as directed in C, 1.

b) *Specific Gravity of Oil With Viscosities From 500 to 2,500 Stokes*

1. Place the sample in the Hubbard specific gravity bottle using extra care to prevent entrapment of air bubbles. (See Note 3.)
2. Cool to 20° to 23°C., stopper and continue as directed under B (a).

c) *Specific Gravity of Oils With Viscosity Over 2,500 Stokes*

1. Carefully pour a 10 to 15 g. sample in the Hubbard specific gravity bottle and weigh accurately. Remove the entrapped air as directed in D, 3.
2. Cool to 20° to 22°C. and add recently boiled and cooled distilled water by pouring gently down the side of the bottle.
3. Insert the stopper, immerse, and hold in the water bath at 25° ± 0.2°C. for 30 minutes.
4. Carefully remove any water which has come through the capillary opening. Remove from bath, clean and dry thoroughly.
5. Weigh the bottle and contents, and calculate the specific gravity as directed in C, 2.

C. Calculation:

1. Specific gravity at 25/25°C. for oils up to 2,500 Stokes.

$$\text{Specific gravity at } 25/25^\circ\text{C.} = \frac{C-A}{\text{Weight of water at } 25^\circ\text{C.}}$$

2. Specific gravity at 25/25°C. for samples having viscosities over 2,500 Stokes.

$$\text{Specific gravity at } 25/25^\circ\text{C.} = \frac{C-A}{(B-A)-(D-C)}$$

A = Weight of empty specific gravity bottle.
B = Weight of specific gravity bottle plus water.
C = Weight of specific gravity bottle plus sample.
D = Weight of specific gravity bottle plus sample plus water.

D. Notes:

1. If the specific gravity bottles are not protected by caps, care must be taken so that no oil or water is lost in the interval between removal from the bath and weighing. If the temperature of the room is above 25°C. this is likely to happen. Even the warmth of the hand surrounding the bottle is sufficient to cause expansion of the contents of the flask.
2. In the determination of the specific gravity of oils approaching 500 Stokes viscosity, special care must be taken to avoid entrapment of air bubbles. The oil should be poured slowly down the side of the specific gravity bottle, and the uncapped sample should stand until free of air bubbles. This will take from 0.5 hour to 1 hour. Alternately, the sample may be centrifuged for a few minutes at 1,000 r.p.m.
3. In order to remove entrapped bubbles, the sample should be centrifuged at approximately 1,000 r.p.m. for 5 to 15 minutes. Do not attempt to remove by standing because the time required is so long that there is danger of change in gravity due to absorption of oxygen.

SUBCOMMITTEE ON ANALYSIS OF LECITHIN:

(J. K. Gunther, chairman)

The members of this committee have carried out collaborative work on the determination of total acidity, acetone soluble acidity, and phosphorus content of one sample of lecithin.

The total acidity (acid value) was determined by titration with potassium hydroxide solution of the lecithin in a benzene-alcohol solvent.

The acidity of the acetone soluble fraction which contains the glycerides and fatty acids but not the phosphatides was ascertained by potassium hydroxide titration of the acetone solution. The results are expressed as per cent of oleic acid.

Phosphorus content of the sample of lecithin was found by the well known volumetric method, which consists of digestion in a mixture of sulfuric and nitric acids followed by precipitation of the phosphorus as the molybdate and subsequent titration with standard acid.

The results obtained on the sample sent to the committee members are shown in the table below:

Laboratory	Acid Value	Acidity of Acetone Soluble (as oleic acid) %	Phosphorus %
1	33.4	6.1	2.04
2	34.5	6.5	2.13
3	35.3	7.2	2.04
4	34.3	6.6	2.08
5	36.1	6.6	.....

The data are encouraging and with some further committee consideration and possibly additional collaborative tests it appears that suitable methods can be evolved.

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