Adapting Some Official Methods to the Semi-Micro Range[1,2]

R. P. A. SIMS and BERNARD STONE, Canada Department of Agriculture, Ottawa, Ontario

OCCASIONALLY only a limited amount of material is available for analysis and although chromatography could be used to determine composition, conventional indices are required. This situation can be met by extending the range of established micro techniques or by adapting official methods to smaller sample size. The latter alternative was taken in this laboratory to determine oil content, iodine value, saponification and neutralization equivalents, polyunsaturated fatty acids, and solid fatty acids. Although foreign language reviews of semi-micro analytical methods have been published (1, 4), no comprehensive survey of the analysis of fats and oils on a reduced scale appears to be available in English. These notes have therefore been assembled.

Materials and Methods

In general, samples of less than 20 mg. were weighed on a semi-micro analytical balance and used directly. In some cases the samples were dissolved in dodecyl alcohol, made up to volume, and aliquot samples were used as suggested by Herb and Riemenschneider (2). Titration volumes greater than 0.2 ml. were measured with Koch micro-burettes; smaller volumes were dispensed from a Gilmont Ultramicro burette (Emil Greiner Co., Chicago, Ill., U.S.A.). Specific experimental details are given with each method of analysis.

Experimental and Results

Oil Content. The following method for determining oil content is used in our Analytical Laboratory. Samples weighing 1 g. are extracted in a Goldfisch apparatus for 2 hrs. at high heat with petroleum ether, B.P. 30–60°C. (Skellysolve F). Then, after grinding with sand, they are extracted again for 2 hrs., the solvent is removed, and the oil is dried at 100°C. in an air oven. The modifications introduced consist of reduced sample weight. Soxhlet extractors in place of Goldfisch apparatus, a nitrogen sweep during extraction, and vacuum-stripping of the solvent at 40°C.

Unless extreme care was used in cleaning the glassware of the Goldfisch apparatus, oil content was found to vary inversely with sample size. However, when the glassware was cleaned with chromic acid and fresh thimbles were used for each analysis, reproducible results, independent of sample weight, were obtained. Similar precautions gave like results with the Soxhlet extractors. When the Soxhlet extractors were heated individually so that rate of distillation could be closely controlled, reproducible results for oil content were obtained without grinding which agreed well with the values obtained by the macro method. The time required however was 7 hrs. as compared to a total time of 4 hrs. with the Goldfisch apparatus.

The oil obtained by use of a nitrogen sweep during extraction and vacuum-stripping of the solvent was of such high quality that it could be used for subsequent analysis (Table I).

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[2] Presented at the 29th fall meeting of the American Oil Chemists' Society, Philadelphia, Pa., Oct. 10–12, 1955.

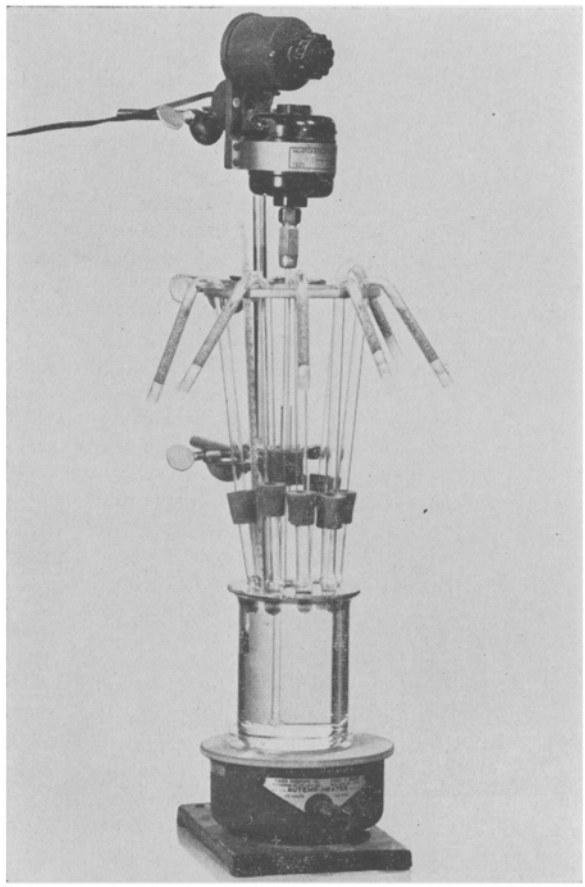

FIG. 1. Saponification equivalent apparatus.

Saponification Equivalent. The Analytical Laboratory uses the A.O.C.S. Official Method Cd. 3-25, with 4- to 5-g. samples, 0.5M alkali and acid, and a reflux time of 45 min. The procedure was modified in the following ways: use of boron-free flasks and tubing, protection of solutions by Ascarite, and stirring with nitrogen during titration. The double indicator method of Reiman (5) was also tried.

For samples smaller than 0.2 g., modified test tubes were used for weighing, refluxing, and titrating. They were made from 9.0-cm. lengths of 12-mm. boron-free glass tubing by sealing one end and blowing a 15-mm. bulb 2 cm. from the closed end. The tubes, air condensers, Ascarite tubes, and heating bath are shown in Figure 1. Greater accuracy was obtained by weighing the alcoholic KOH solution rather than measuring it volumetrically.

The results obtained (Table II), using the conven-

TABLE I

Effect of Extraction Technique on Oil Quality

	Carver Press	Soxhlet, 7 hrs.	Goldfisch, 4 hrs.	
			Sand grind	
	2,000 lb. p.s.i.	N_2 sweep vacuum strip	Air oven	Vac. strip
P.V.[a]	2.55	2.91	30.19	5.53
I.V.[b]	181.0	182.0	181.0	181.1

[a] Peroxide value, milli-equivalents per kg.
[b] Iodine value, Wijs.

TABLE II

Saponification Equivalent of Benzyl Benzoate
[(M.W. 212.25) 0.5N alcoholic KOH; 0.3N aqueous HCl]

Sample wt., g.	2.0	0.2[a]	0.02[a]
Single indicator	210.7	209.4	213.8
Double indicator	208.8	208.5	215.8
Unheated blank (ml. 0.3N HCl)	38.90	10.66	0.5966
Heated blank (ml. 0.3N HCl)	38.39	10.59	0.5932

[a] Protected by Ascarite during saponification and by nitrogen during titration.

tional single indicator method and Reiman's double indicator method, showed that the values were practically independent of sample weight, provided that care was taken to exclude atmospheric carbon dioxide. However, unless the color change of the bromphenol blue was matched against samples of the indicator brought to neutrality with the aid of a pH meter, there was little chance of success. Moreover the blanks caused so little trouble that the single indicator method was preferred. The results were also found to be independent of the presence of dodecyl alcohol. As anticipated, more satisfactory titrations were made with concentrated solutions measured accurately than with dilute solutions measured with conventional burettes.

Neutralization Equivalent. The A.O.C.S. Official Method Cd. 5a-40 was adapted for use with small samples by conducting the titrations in 8 x 40-mm. test tubes stirred by a nitrogen stream, using a Gilmont ultramicro burette for the alkali. The indicator was α-naphtholphthalein. The method developed here resembles that of Stetten and Grail (6) except that aqueous HCl and a nitrogen stream are used. Oleic acid was found superior to stearic acid as a standard.

Iodine Value. In the Analytical Laboratory iodine value is determined by the Wijs method as modified by Hiscox (3). With reduced sample size the following modifications were necessary: sealed reaction vessels (30 x 50-mm. weighing bottles with internal ground glass stoppers, stirred magnetically during titration), elimination of the catalyst, and weighing rather than pipetting the Wijs solution. Except that potentiometric or "dead-stop" titrations were found unnecessary, the method developed bears a close resemblance to that of Whalley and Ellison (8).

The data (Table III) show that the measured iodine value is somewhat less than the calculated value. However the coefficient of variation is satisfactorily low. Dodecyl alcohol is immiscible with Wijs solution and, unless present in very small amounts, causes low results.

Polyunsaturated Acids. The Analytical Laboratory uses the A.O.C.S. Official Method Cd. 7-48, as modified by Vandenheuvel (7). The method was further modified by the use of Teflon plugs in the reaction bottles, improved stirring and temperature control, and a reaction temperature and time of 165°C. and 60 min.

TABLE III

Iodine Values of Small Samples

Sample wt., mg.	Iodine value
5.62	180.4
5.85	180.4
5.96	180.6
3.49	180.4
6.12	180.5
3.90	180.1
4.10	182.4
5.87	179.2
4.92	179.9
5.61	182.1

Mean = 180.6. Macro I.V. = 181.1. σ = 0.956. Coefficient of variation = 0.531.

A summary of specific absorption coefficients (Table IV) shows that the value of the blank is very low, that of dodecyl alcohol low (after heating with alkali), and the coefficients for bromination-debromination linoleate and linolenate slightly dependent on concentration.

Solid Fatty Acids. The A.O.C.S. Official Method Cd. 6-38, in which 5-g. samples of fatty acids are used, has been modified in the following manner: sample reduced to 50 mg., use of centrifuge tubes for solution, precipitation and recrystallization, and use of centrifugation for washing of precipitates. It was also found that, for best precipitation, the mixture of lead acetate and fatty acids should be held at the boiling point for at least one minute. Good mixing at this stage is essential.

TABLE IV

Specific Absorption Coefficients, l./g./cm.
1 Hour at 165°C.

Wavelength	Glycol-KOH (6.5%) vs. Et OH	Dodecyl alcohol vs. Et OH	Linoleic acid[a] Sample wt. g.			Linolenic acid[a] Sample wt. g.		
			0.1	0.01	0.005	0.1	0.01	0.005
268	0.001	.....	.....	.....	.....	54.54	55.01	54.97
233	−0.003	0.0039	87.82	87.23	87.94	58.80	57.64	56.97

[a] Prepared by bromination-debromination.

Mixtures of known composition were analyzed by the modified lead salt method (Table V), and oleic acid was found to contribute to the apparent solid-acid content. In the two mixtures studied, where the oleic acid was only 6%, the solid-acid content showed a dependence on sample size. With higher oleic acid content however less dependence on sample size was noted, but the iodine value of the "solid" acids was considerably higher. Tests with pure oleic acid showed that about 18% of it formed an insoluble lead soap.

TABLE V

Variation in Apparent Solid Acids Content with Sample Size and Composition

Mixture No. 1: 12.05% saturated; 6.04% oleic; I.V. = 148.3		
Method	% Solid	I.V.
Macro, 5.0 g.	11.2	8.0
Micro, 0.5 g.	10.5	7.5
Micro, 0.05 g.	8.9	....
Mixture No. 2: 5.5% saturated; 13.5% oleic; I.V. = 156.9		
Method	% Solid	I.V.
Macro, 5.0 g.	9.8	42.4
Micro, 0.5 g.	7.5	20.8
Micro, 0.05 g.	7.5	....

Suggestions

1. It is generally better to measure accurately small quantities of strong reagents than larger volumes of more dilute solutions.
2. Where small amounts of primary reagent are required, *i.e.*, Wijs solution or alcoholic KOH, it is more accurate to weigh than to measure volumetrically.
3. Exclusion of atmospheric carbon dioxide and the use of boron-free glassware are necessary when alkali is used quantitatively.
4. Lead-salt precipitation is more conveniently done with smaller samples in centrifuge tubes.

Appendix

Saponification Equivalent

Weigh approximately 20 mg. of oil into an alkali-resistant tube. Weigh 0.5 ml. of 0.5N alcoholic KOH solution from a tuberculin syringe into the test tube,

and immediately fix in place an air condenser-Ascarite tube (see illustration). Heat the reaction mixture in an oil bath at 100 to 105°C., and allow it to reflux for 45 min. Run two blanks in the same way.

To titrate, add one drop of 0.2% phenolphthalein solution and seven drops of benzene. Stir the solution with a stream of nitrogen bubbles and titrate with 0.5N HCl, using a Gilmont Ultramicro burette.

Iodine Number

Weigh between 3 to 5 mg. of oil to ±0.02 mg. into a 30 x 50-mm. weighing bottle and dissolve in 1 ml. of chloroform. Using a 1-ml. tuberculin syringe, weigh rapidly, to ±0.02 mg., 1 ml. of 0.2N Wijs solution; add it to the oil and seal the bottle by applying 15% KI solution to the stopper. Let stand for 30 min. in the dark, then add 0.5 ml. of 15% KI solution and 2 ml. of water. While the contents of the bottle are stirred magnetically, titrate the excess iodine with 0.01N sodium thiosulphate solution, using Thyodene indicator. Run two blanks with the samples.

Solid Fatty Acids

Into a 50-ml. graduated contrifuge tube, weigh a 50-mg. sample of fatty acids and into another tube 0.15 g. of lead acetate. To each tube add 7.5 ml. of 95% ethanol and boil in a water bath. Add the lead acetate solution to the sample, letting it boil for a further 60 seconds, mixing well with a stirrer. (A convenient stirrer that can be left in the centrifuge tube can be made from a 15-cm. length of 16-gauge Nichrome wire.)

Cool the mixture to room temperature, then place it in an ice bath for 2 hrs. Centrifuge at 3,000 r.p.m. for 3 min., and then decant the supernatant layer. Wash the precipitate 3 times with cold 95% ethanol, mixing well with a stirrer and centrifuging each time. Test filtrate with sulfuric acid for excess lead acetate.

Add 1 drop of acetic acid and boil until the precipitate has dissolved. Cool to room temperature and place in an ice bath for 2 hrs. or in a refrigerator overnight. Centrifuge and decant, then wash with 10 ml. cold 95% ethanol, centrifuge and decant three more times.

Transfer the precipitate to a 30-ml. separatory funnel, washing with 10 ml. ether and finally with 2 ml. of 1:4 nitric acid. Separate the water and wash repeatedly with 7-ml. volumes of water until methyl orange shows no acid present. Transfer to a tared flask and carefully strip under vacuum at 40°C. until almost dry. Dry in an oven at 100°C. until weight is constant.

REFERENCES

1. Gorbach, G., Malissa, H., and Vioque, A., Grasas y Aceites, *3*, 187 (1952).
2. Herb, S. F., and Riemenschneider, R. W., Anal. Chem., *25*, 953 (1953).
3. Hiscox, D. J., Anal. Chem., *20*, 679 (1948).
4. Komori, S., J. Chem. Soc. Japan Ind., Chem. Sect., *51*, 120 (1948).
5. Reiman, W., Ind. Eng. Chem., Anal. Ed., *15*, 325 (1943).
6. Stetten, D., and Grail, F. G., Ind. Eng. Chem., Anal. Ed., *15*, 300 (1943).
7. Vandenheuvel, F. A., and Richardson, G. H., J. Am. Oil Chemists' Soc., *30*, 104 (1953).
8. Whalley, C., and Ellison, M., J. Oil and Colour Chemists' Assoc., *35*, 596 (1952).

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Report of the Spectroscopy Committee 1955-56

DURING THE PAST YEAR the Spectroscopy Committee has been concerned with three problems:

a) modifiations of the present A.O.C.S. Tentative Method Cd 7-48, both to simplify it and to extend its scope. At the beginning of the year work on this problem was entering its final phase;

b) investigations to establish a satisfactory method for the quantitative determination of polyunsaturated fatty acids in samples containing large quantities of conjugated constituents. This problem had advanced to the stage where we were about ready for active collaborative measurements;

c) proposed investigation of an infrared absorption method for the determination of *trans*-acids in the presence of nonconjugated *cis*-unsaturated and of saturated constituents. This problem was, at the beginning of the year, still in the planning stages.

Accomplishments During the Year

Recommended Changes in Method Cd 7-48. Collaborative results in the annual report of the Spectroscopy Committee for 1954–55 had shown that the present A.O.C.S. Tentative Method Cd 7-48 could be simplified and its scope extended. If a sample is known to have no constituent more highly unsaturated than diene, measurements and calculations can be restricted to the diene region; if no more unsaturated than triene, to the triene and diene regions, etc. The scope of the method can be increased to include the determination of pentaenoic acids by means of 21% alkali reagents. Preliminary drafts of the proposed modifications in the method to achieve these purposes were prepared and submitted to each committee member for comment, correction, and modification. Based on suggestions received, a final draft was submitted to each member for approval for recommendation to the Uniform Methods Committee for adoption. By a vote of 8–0, with one member not voting, the nine-man Spectroscopy Committee approved submission of the proposed modification to the Uniform Methods Committee for incorporation into the present A.O.C.S. Tentative Method Cd 7-48.

Collaborative Work on Attempts to Establish a Satisfactory Method for Samples Containing Large Quantities of Preformed Conjugation. To test a recently published procedure for the determination of polyunsaturated acids in the presence of large quantities of preformed conjugation (J. Am. Oil Chemists' Soc., *30*, 182–186 [1953]) a sample of *alpha*-tung oil and two samples of mixtures of known proportions of *alpha*- and *beta*-tung oils were submitted to each committee member with specific instructions for collaborative measurements and calculations of linoleic and *alpha*- and *beta*-eleostearic acids. Included with these three samples were urea adducts of both *alpha*- and *beta*-eleostearic acids. Collaborative reports were received from eight of the nine-man committee. However, before a composite tabulation of the results could be compiled, reports had been received from two laboratories of committee members which indicated that the absorptivities for the determina-