ably present not as a phosphatide but as some other compound or compounds as yet not identified. It is also interesting to note that the ratio of phosphorus to nitrogen of an alkali refined oil is different from that of the crude oil. The determination of nitrogen in oils in p.p.m. leaves much to be desired. The degree of alkali refining may be inferred from the phosphorus of the oil.

In conclusion, it may be remarked that the performance of the Clayton Sodium Carbonate Refining Process in practise during the past few years has been such as to justify the use of the Wesson Absolute Refining Loss as a yardstick with which to compare plant refining losses, proposed when the process was first introduced.

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Colorimetric and Potentiometric Determination of Acid Numbers of Vegetable and Marine Oils[1]

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A COLORIMETRIC method for the determination of acid numbers has been developed, utilizing isopropanol-benzene as the titration solvent and offering several advantages over either of the present A.S.T.M. or A.O.C.S. colorimetric methods. The A.S.T.M. potentiometric method has been satisfactorily applied to vegetable and marine oils, particularly to dark or colored oils. Close correlation was found between the acid numbers obtained by these two procedures, making it possible to compare directly colorimetric and potentiometric results. It is suggested that these two procedures be investigated for possible adoption as A.O.C.S. Official Methods.

Methods for the determination of acid numbers in petroleum products are given in the 1946 A.S.T.M. Standards both by colorimetry (A.S.T.M. D663-46T) (1) and by potentiometry (A.S.T.M. D664-46T) (2). In the colorimetric method the oil is added to a titration solvent consisting of denatured ethanol (10% methanol), the mixture is heated to boiling and is titrated while hot with aqueous KOH solution until a pink color with phenolphthalein is obtained. The potentiometric method follows the procedure devised by Lykken, Porter, Rullifson, and Tuemmler (3). The oil is added to an isopropanol-benzene titration solvent, and the effect of the addition of alcoholic KOH is followed by means of a glass electrode-calomel electrode system. The potentiometric method is recommended for dark or colored oils where the colorimetric endpoint is difficult to ascertain. It is stated in a note (1, 2) that acid numbers obtained by these two different methods may or may not be numerically the same. Lykken *et al.* (3) presented data referring to oxidized petroleum oils, indicating that no correspondence existed between acid numbers determined colorimetrically and potentiometrically.

Contrasted with these procedures, latest A.O.C.S. Official Methods give only a colorimetric method, A.O.C.S. Ca 5-40 (4), for the determination of acid numbers of vegetable and marine oils. This procedure resembles the colorimetric A.S.T.M. method except that hot titration solvent is added to the oil rather than the alcohol-oil mixture heated. In the case of dark-colored oils the use of another indicator, Dr. Grubler's Aniline Blue, is suggested to replace phenolphthalein.

Colorimetric Determination of Acid Numbers

Neither of the existing official colorimetric methods is entirely satisfactory since many oils are not completely soluble in the denatured ethanol titration solvent, and heating the mixture may introduce errors. Titrations are not too reliable when carried out in a two-phase system formed with an insoluble oil. The indicator endpoint depends on the rate of shaking and on the time of shaking. Within the limited time for carrying out the titration, equilibrium conditions may not be established resulting in fading endpoints and poorly reproducible acid numbers. Heating the oil may cause sufficient modification to alter the acid number. This is particularly true in the case of oils which can be saponified; acid numbers which are too high result, accompanied by fading endpoints. Variation in the temperature causes changes in the amount of saponification and leads to poorly reproducible values. The hydrogen-ion activity varies inversely as the absolute temperature so that titrations carried out at different temperatures would yield different acid numbers. These variable factors may partially compensate each other, but this does not alter the conclusion that the experimental methods are not entirely satisfactory. Curve II in Fig. 1 illustrates the result obtained when a partially insoluble oil separates on cooling and the effect of reheating on the potentiometric titration curve. In curve III of Fig. 1 an example is given of the result of slow titration of a saponifiable oil.

Most of these difficulties may be traced to the choice of denatured ethanol as a titration solvent. Substitution of a solvent in which most oils were completely soluble should overcome many of these undesirable effects and should result in a more satisfactory method. An isopropanol-benzene mixture [as originally developed for the potentiometric method

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[1] Communication No. 124 from the Laboratories of Distillation Products, Inc., Rochester, N. Y.

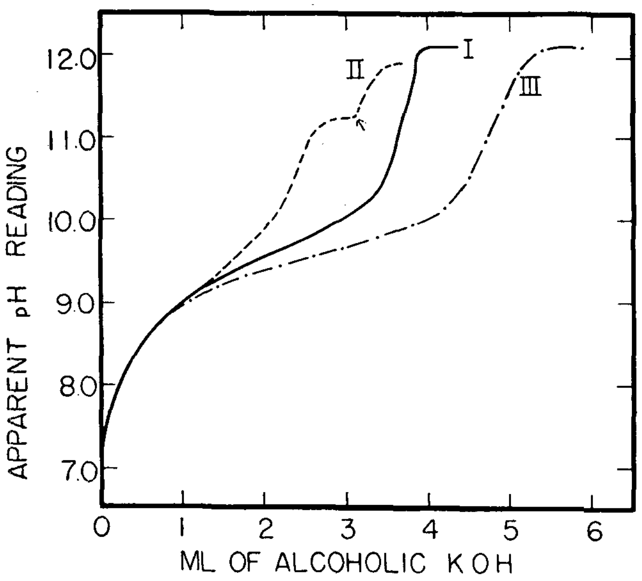

FIG. 1. Representative Titration Curves. I. Titration curve of a soybean oil distillate obtained with either potentiometric or modified colorimetric procedures. II. Titration curve of above oil obtained with present official colorimetric procedure. Arrow indicates point at which sample was reheated. III. Titration curve obtained with an easily saponifiable oil (such as a vitamin A acetate concentrate) using present official colorimetric procedure (with modified colorimetric or potentiometric methods, a curve equivalent to curve I was obtained).

(2)] is such a titration solvent, and its use has been found to be eminently satisfactory. The use of this solvent has necessitated a few other modifications, such as the use of alcoholic instead of aqueous KOH. All the vegetable and marine oils we have tested proved to be completely soluble at room temperature. This results in a single phase system to be titrated. Fading endpoints are practically non-existent, and easily-saponifiable oils can be assayed at room temperature with little or no error. A comparison of the results obtained with the two methods may be seen in Table I and, although the acid numbers are similar, the characteristics of the titration indicate that better results are obtained with the isopropanol-benzene solvent. That the use of isopropanol-benzene as titration solvent yields reproducible results may be seen in the data in Table II. An outline of the proposed method follows:

TABLE II

Reproducibility of Acid Numbers
Determined Colorimetrically and Potentiometrically

Colorimetric Determination (Modified method)
Soybean oil distillate, Sample A; Solubility, ++++; endpoint, ++++

Determination	Acid Number	
1	7.75	
2	7.70	Mean = 7.71
3	7.70	σ = 0.025
4	7.70	

Potentiometric Determination
Soybean oil distillate, Sample B; Solubility, ++++; endpoint, ++++

Determination	Acid Number	
1	7.40	Mean = 7.39
2	7.45	σ = 0.043
3	7.36	
4	7.36	

The details of the modified colorimetric procedure are given in the text. The solubility and endpoint codes are given in the legend of Table I. The potentiometric determination was performed according to the A.S.T.M. Official Method (2) under the following conditions: These determinations were carried out with a Beckman Laboratory Model G pH Meter using the five-inch shielded glass and calomel electrodes as supplied by the National Technical Laboratories for use with the above pH meter. After addition of a portion of KOH and stirring, equilibrium conditions were reached within 30 seconds. The pH meter with the shielded electrodes was standardized to pH 4.0 with aqueous phthalate standard buffer. The instrument performance was then checked by comparison with aqueous phosphate buffer, pH 7.0. Use of non-aqueous buffers and conversion to cG units were avoided and values were reported as "apparent pH readings." About ten minutes were required to determine the acid number of an average sample.

Proposed Colorimetric Method

Scope. This method has been found to be satisfactory for the determination of acidic constituents in marine and vegetable oils, including chemical modifications of these oils and products and residues of refining processes.

Reagents. a) Standard potassium hydroxide solution (approximately 0.1 N) in anhydrous isopropanol. This solution is stable for several weeks if stored in a glass container and protected from atmospheric CO_2.

b) Titration solvent consisting of 49.5% C. P. anhydrous isopropanol, 50% C. P. benzene, and 0.5%

TABLE I

Comparison of Colorimetric Methods for the Determination of Acid Numbers

Type of Oil	Approx. Wt. of Sample (grams)	A.O.C.S. Official Method			Modified Method			Deviations	
		Acid No.	Solubility	Endpoint	Acid No.	Solubility	Endpoint	Numerical	%
Refined fish liver oil	50	0.089	+[1]	+[2]	0.075	++++[1]	+[2]	—0.014	—15.8
Crude fish liver oil	10	1.38	++	++++	1.38	++++	++++	0.0	0.0
Crude fish liver oil	10	1.78	++	++	1.71	++++	++++	—0.07	—3.8
Fish liver oil distillate	10	0.70	++	++	0.65	++++	++++	—0.05	—7.0
Fish liver oil distillate	10	0.54	++	++	0.52	++++	++++	—0.02	—3.8
Soybean oil distillate	10	2.00	++	++++	1.98	++++[3]	++++	—0.02	—1.0
Vegetable oil by-product	2	98	++	++++	103	++++	++++	+5.0	5.1
Vegetable oil by-product distillate	10	9.9	++++	++	9.7	++++	++++	—0.2	—2.0
Fatty acid fraction from distillation of vegetable oil	2	167	++++	++++	179	++++	++++	+12.0	7.2
Soybean oil distillate	10	0.99	++++	++	1.03	++++	++++	+0.04	4.0

[1] 100 ml. solvent used.
[2] Reddish-brown oil: very hard to detect endpoint.
[3] Turbid: due to inorganic constituents.

Procedures followed are given in the text. Omitting the determination of the acid value of refined fish liver oil whose very low acid value and dark color would cause an abnormally large per cent error, the mean deviation of the remaining determinations is —0.2%. The code for solubility is as follows: ++++, completely soluble; +++, almost completely soluble; ++, fairly soluble; +, poorly soluble; and 0, not soluble. The code for end point characteristics is as follows: ++++, sharp endpoint and no fading; +++, good endpoint; ++, fair endpoint; +, poor endpoint; and 0, no endpoint visible.

water. This solution is neutralized with alkali to a permanent faint pink color just before using.

c) Phenolphthalein indicator, 1% in anhydrous isopropanol. This solution is neutralized with dilute alkali to a permanent faint pink color.

Procedure. Approximately 10 grams of a representative sample of oil is weighed into a 250-ml. Erlenmeyer flask. Fifty ml. of neutralized titration solvent and 1 ml. of neutralized indicator solution are added and the flask contents agitated by swirling to ensure thorough mixing. The solution is titrated at room temperatures with the standard alcoholic potassium hydroxide solution until a permanent faint pink color is obtained.

Calculations. The acid number is calculated from the following equation:

$$\text{Acid number in mg. KOH per gram} = \frac{\text{ml. KOH} \times \text{Normality KOH} \times 56.1}{\text{weight of oil in grams}}$$

Remarks. a) The size of the sample is determined by the acid number of the oil. If the oil has a very low acid number, increase the size of the sample up to 50 grams. If the oil has a very high acid number, decrease the size of the sample accordingly (for examples see Tables I and II).

b) For dark or colored oils in which the endpoint is difficult to determine, use the potentiometric procedure.

Potentiometric Determination of Acid Numbers

The colorimetric titration of dark or colored oils has presented a problem of considerable industrial importance since many oils fall into this category. The solution offered by the A.O.C.S. official method, that of a different indicator, only solves the problem in the case of partially colored oils. The use of thymolphthalein in place of phenolphthalein has been tried in these laboratories with similar results. Substitution of a different indicator always raises the question of variable shift in the pH of the endpoint when using titration solvents containing non-aqueous materials (see Fig. 2).

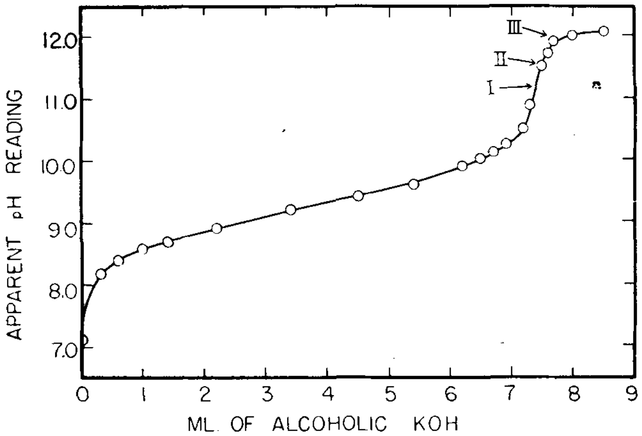

FIG. 2. Representative titration curve of a soybean oil distillate obtained with either modified colorimetric or potentiometric procedure. Arrows indicate (I) inflection point of titration curve, (II) phenolphthalein endpoint, and (III) thymolphthalein endpoint.

The potentiometric method offers a technique which is readily reproducible (see Table II) and which can be applied to an oil irrespective of its color. The advantages obtained by complete solubility without

TABLE III

Comparison of Colorimetric and Potentiometric Methods for the Determination of Acid Numbers

Type of Oil	Approx. Weight (grams)	Modified Colorimetric Method		Potentiometric Method		Deviations	
		Acid No.	Endpoint	Acid No.	Endpoint	Numerical	%
Soybean Oils							
Distillate	10	2.73	++++	2.73	++++	0	0
By-product	10	1.64	++++	1.64	++++	0	0
Distillate	10	3.82	++++	3.80	++++	—0.02	—0.53
Degummed oil	10	0.41	++++	0.41	+++	0	0
Refined oil	50	0.050	++++	0.050	+++	0	0
Vegetable Oil By-Products							
Sample A	2	105	++++	104	++++	—1.0	—0.95
Distillate	10	3.64	++++	3.59	+++	—0.05	—1.4
Sample B	2	99	++++	99	++++	0	0
Acid fraction	2	162	++++	162	++++	0	0
Acid fraction	1	165	++++	166	++++	+1.0	+0.60
Fish Liver Oils							
Refined fish liver oil	50	0.061	++++	0.070	+	—0.09	—13.0
Vitamin E Concentrates							
Distillate, crude	10	14.8	++	14.6	++++	—0.2	—1.4
Distillate	10	3.42	++++	3.29	+++	—0.13	—3.8
Distillate	10	2.11	++++	2.11	+++	0	0
Vitamin A Concentrates							
Distillate, fatty acid fraction	10	14.7	++++	14.7	++++	0	0
Stearine	10	0.80	++++	0.83	+++	+0.03	+3.6
Vitamin A acetate concentrate	10	1.09	++++	1.15	++	+0.06	+5.2
Vitamin A acetate concentrate	10	1.34	++++	1.34	+++	0	0
Distillate	10	0.25	++++	0.25	++	0	0
Vitamin A alcohol concentrate	10	1.80	++++	1.77	+++	—0.03	—1.7
Drying oils							
Distillate of bodied fish oil	1	4.5	++++	4.8	++	+0.3	+6.2
Distillate of bodied fish oil	5	0.49	++++	0.49	++	0	0
Bodied fish oil	10	4.88	++++	4.69	+++	—0.19	—4.0
Bodied fish oil	10	6.74	++++	6.74	+++	0	0
Tall Oils							
Total acids	1	164	++++	163	++++	—1.0	—0.61
Total acids	1	191	++++	189	++++	—2.0	—1.1
Rosin acids	0.5	191	++++	74	++++	—2.0	—2.7
Rosin acids	0.5	76	++++	64	++++	—3.0	—4.5

Procedures followed are given in the text and in the legend of Table II. The solubility and endpoint codes are given in the legend of Table I. Omitting the refined fish liver oil sample, the mean deviation of the remaining 27 samples is —0.3%. The sample of refined fish liver oil possesses a very low acid value, and a deep color which makes the colorimetric determination subject to greater errors than others of the group. Expressing all the values obtained by the colorimetric method as 100 and converting values obtained by the potentiometric method to the same base, the standard deviation calculated by analysis of variance is 2.4.

heating, which have been already referred to, apply fully to the potentiometric method. One factor which must be considered is that the potentiometric method is more time-consuming than the colorimetric technique. The shape of a typical titration curve is given in Fig. 2 along with a comparison of the inflection point with the colorimetric endpoints. A rather good correspondence is obtained with the phenolphthalein endpoint and the inflection point of the titration curve, but the thymolphthalein endpoint is considerably higher. A comparison of the colorimetric and the potentiometric methods both employing isopropanol-benzene as a titration solvent is given in Table III for a variety of fatty oils. These oils include a number of special interest to these laboratories, but their unusual nature and wide variety serve to emphasize the wide applicability of the methods. As may be seen, the correspondence between the colorimetric and potentiometric procedures is uniformly satisfactory.

With the exception of omitting the use of the cG nomenclature and expressing results in either "apparent pH readings" or "pH meter readings," the A.S.T.M. Official Method D 664-46T has been followed and may be referred to for more extensive detail. This method has been found to be satisfactory for the determination of acidic constituents in marine and vegetable oils, including chemical modifications of these oils and products and residues of refining processes. It is particularly suitable for dark or colored oils for which the colorimetric determination is difficult or impossible.

To avoid the confusion of titration data obtained in non-aqueous media with those in water, the term cG was introduced by Lykken *et al.* (3), to replace the term pH when using non-aqueous media. The use of cG units in millivolts involves calibration of two non-aqueous buffer standards. The value of these additional operations is questionable since there is a direct relationship between the pH meter reading and the cG scale. The A.S.T.M. potentiometric method (2) gives a factor of 0.000198 multiplied by the absolute temperature to convert pH meter readings to cG units. If this direct conversion is valid, then nothing is to be gained by the use of the term cG, and reference to "pH meter readings" or "apparent pH reading" should be satisfactory and avoid needless computation.

Summary

A discussion is presented of present official methods of determining the acid number of oils. A colorimetric method is presented for the determination of acid numbers of marine and vegetable oils and related products. It involves the use of alcoholic KOH, phenolphthalein, and a titration solvent consisting of 49.5% anhydrous isopropanol. 50% benzene, and 0.5% water. Most oils are completely soluble in the titration solvent, thus avoiding the disadvantages of two phase systems and of heating the oil. It compares satisfactorily with present official methods.

A potentiometric method which is at present an A.S.T.M. Standard Method for petroleum products is discussed. It has proved to be satisfactory for use with marine and vegetable oils.

A close correspondence has been shown with a number of different oils between acid numbers obtained colorimetrically and potentiometrically. This makes it possible to compare directly results obtained by the two methods.

Acknowledgment

Appreciation is expressed to H. W. Rawlings of the Products Control Laboratory, at whose suggestion this project was undertaken, for his cooperation and advice during the course of this investigation.

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Rice Bran Oil. I. Oil Obtained by Solvent Extraction[1]

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RICE BRAN is a by-product of the rice milling industry and a potential source of edible oil. At present rice bran oil is produced by several mills in this country and by a considerably larger number in the Orient. In recent years Japan has produced as much as 17,000 tons of rice bran oil per year, for the most part by inefficient methods; and in pre-war years between 5 and 10 million pounds were imported into the United States, principally from Japan. Rice bran oil enters the United States at an import duty of 20% *ad valorem* in accordance with schedule 1, paragraph 53, of the Tariff Act of 1930 for expressed or extracted oils, not especially provided for. There are no established price quotations for this oil.

While rice bran oil will always be of minor importance in the vegetable oil industry of the United States, so far as total production of this commodity is concerned, it possesses considerable potential importance in certain limited areas.

The bran, which consists essentially of the outer portions and germ of the dehulled rice grain, amounts on an average to 8.5% of the rough rice. The approximately 1,600,500 tons of rough rice produced in the United States in the crop year 1945-1946 is equivalent to approximately 136,042 tons of bran. Assuming an extractable oil content of 10 to 14%, this amount of bran is equivalent to 27 to 38 million pounds of oil.

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[1] Presented at the 38th Annual Meeting of the American Oil Chemists' Society, New Orleans, Louisiana, May 20-22, 1947.

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