Evaluation of the Modified Renard and Kerr Tests for the Determination of Peanut Oil

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The presence in peanut oil of arachidic acid, which was first reported by Gössman (1), has resulted in many attempts to develop a method based on the isolation of this acid as a means of quantitatively identifying peanut oil in a mixture of oils. Renard (2), in 1871, applied to the analysis of peanut oil Gusserow's (3) method of separating solid fatty acids from liquid fatty acids on the basis of the differential solubility of the lead soaps in ethyl ether. The separated solid fatty acids were then fractionally crystallized from 90 percent alcohol. By the application of this method Renard obtained a product which was designated as the "arachidic acid fraction" melting at 70-71° C. This fraction was reported to consist of a mixture of palmitic acid, m. p. 64°, and arachidic acid, m. p. 75°. Based on the observation of the analyses of a number of peanut oils from different sources, that the arachidic acid content varied only slightly from 5 percent, Renard suggested that the arachidic acid content, obtained by this method, multiplied by twenty could be used as a means of quantitatively determining peanut oil in an admixture with other oils. Following the application of the method to the detection of peanut oil as an adulterant in olive oil, Renard later (4) modified the factor to twenty-two.

Other methods for the detection of peanut oil have been proposed. These include the potassium-soap acetone method of Fachini and Dorta (5); the magnesium-soap alcohol method of Kerr (6) originally reported as a qualitative test only but later modified somewhat by Thomas and Yu (7) and designated as a quantitative method; and various modifications (8) of Renard's original method. All of these methods and modifications thereof consist essentially of two steps; first, the separation of the solid fatty acids from the unsaturated fatty acids, and second, the separation of the arachidic acid fraction from the total solid acid fraction. The latter step is dependent upon fractional crystallization of the solid acids from dilute alcohol in which the longer chain length saturated fatty acids (arachidic, etc.) are somewhat less soluble than are the shorter chain length saturated acids (palmitic, stearic, etc.).

During the past four years the Fat Analysis Committee of the American Oil Chemists' Society has conducted cooperative work on the applicability of the Renard test to the determination of peanut oil in admixture with other oils. This work indicated that anomalous results were obtained with certain oil mixtures and that the method was not of universal application. In connection with this cooperative work the Southern Regional Research Laboratory investigated the applicability of the Renard test, as modified by the Association of Official Agricultural Chemists, to the determination of peanut oil in admixtures with various other oils such as cottonseed, hydrogenated cottonseed, soybean and olive. The work included an investigation of the Thomas and Yu modification of the magnesium-soap alcohol method because these authors claimed their method gave more accurate results than did the Renard method when applied to the determination of peanut oil in the presence of cottonseed or other oils containing a high percentage of saturated acids.

Experimental

Samples containing known amounts of peanut oil in cottonseed, soybean, hydrogenated cottonseed, and olive oils were prepared and analyzed for arachidic acid content by the Renard procedure as described in the Methods of Analysis of the Association of Official Agricultural Chemists (9).

Ten grams of the oil under examination was saponified with alcoholic potassium hydroxide, and neutralized with acetic acid (1 + 3) to phenolphthalein, after which it was poured into a 500 ml. flask containing 100 ml. of a boiling solution of 10 percent lead acetate. After boiling the solution for a few minutes the precipitated soaps were cooled by immersing the flask in water. After cooling, the solution of the excess lead acetate was decanted and the solid residue was washed first with water and then with cold 90 percent alcohol. One hundred ml. of ether was added to the soaps. The flask was then closed with a cork and allowed to stand until the soaps disintegrated, after which it was heated on a water bath under reflux for about 5 minutes. The solution was then cooled to 15 to 17° C. and allowed to stand overnight at this temperature. The insoluble lead soaps were filtered and washed with ether, and then transferred into a separatory funnel. The lead soaps suspended in ether were then acidified with nitric acid (1 + 3). The ether solution of the solid acids was washed with water until the wash water no longer gave an acid reaction to methyl orange. The ether was distilled and the solid acids dried by adding absolute alcohol and evaporating on a steam bath. The fatty acids were then dissolved in 50 ml. of 90 percent alcohol (by volume) with warming, and allowed to stand overnight at room temperature (25 to 30° C.) after which they were cooled at 20° C. in a water bath for one to two hours. The acids were then filtered and washed with 10 to 20 ml. of 90 percent alcohol (previously cooled to 20° C.) and then with 70 percent alcohol. The arachidic acid was then dissolved in absolute alcohol in a weighed dish, the alcohol evaporated and the residue dried and weighed. To the observed weight a correction of 0.0025 grams was added for each 10 ml. of 90 percent alcohol used in crystallization and washing if conducted at 15°, or if conducted at 20°, 0.0045 grams was added for each 10 ml. The percentage of peanut oil was calculated as twenty times the total weight of the arachidic acid fraction.

Analysis of the olive oil used in preparing the samples reported in Table 3 indicated that it was grossly adulterated. The determinations were repeated using a different sample of olive oil, apparently genuine, and these results are recorded in Table 4. The comparative analysis of these two olive oils and the peanut oil used in this work are recorded in Table 1. The ap-

TABLE 1

Properties of Oils

	Olive oil 0-44	Olive oil 0-112	Peanut oil 0-50
Refractive index N^{25}_{D}	1.4702	1.4659	1.4673
Iodine number	122.3	87	97.7
Thiocyanogen number	79.3	78.2	69.7
Saponification number	191.9	190.4	191.2
Acid number	0.26	4.15	0.23
Color	10Y/1.08R	35Y/2.74R	.....
Unsaponifiable matter, percent	1.32	0.90	0.38
Acetyl number	3.4	4.34	2.50

parent amounts of peanut oil which were present in known mixtures of cottonseed, hydrogenated cottonseed, soybean, and olive oils as found by the application of the above described method are recorded in Tables 2 to 5, inclusive. All recorded melting points are uncorrected for stem emergence.

Thomas and Yu modification of Kerr's method: Mixtures of peanut oil with cottonseed and soybean oils were also prepared and analyzed by the Thomas and Yu modification of Kerr's method. According to this procedure 10 grams of the oil were saponified with alcoholic potassium hydroxide, and the soap solution neutralized to phenolphthalein with acetic acid (1 + 3) and then just enough alcoholic alkali was added to produce a pink color. The soaps were precipitated from the warm solution by addition of alcoholic magnesium acetate, after which the solution was heated to boiling and then allowed to cool. After standing in refrigerator overnight at about 10° the insoluble soaps were separated and washed with precooled 90 percent alcohol. The washed soaps were acidified with dilute (1 + 3) nitric acid (Thomas and Yu used 5 M hydrochloric acid) and washed free of mineral acid as indicated by the reaction of the wash waters to methyl orange. The solid acids were dried and weighed and then redissolved in 90 percent alcohol with mild application of heat. The mixture was allowed to stand overnight at room temperature (25° to 30° C.) and then cooled in a water bath for one to two hours at 20° C. The solids were filtered, washed with a measured volume of precooled 90 percent alcohol and then with precooled 70 percent alcohol. The arachidic acid was dissolved in absolute alcohol in a weighed dish after which it was dried and weighed. To the observed weight there was added a solubility correction from tables of Thomas and Yu for the amount of 90 percent alcohol used. The total weight multiplied by 20 is reported as the percentage of peanut oil in the sample.

The results of the examination by this method of a number of mixtures of peanut with other oils are recorded in Table 6. All melting points are uncorrected for stem emergence.

Discussion

The results of the analyses recorded in Tables 2 to 6 indicate that neither method investigated is sufficiently accurate to warrant its use for the quantitative determination of peanut oil in vegetable oil mixtures.

The results are in most cases very erratic. The greatest discrepancies occur in the analyses of hydrogenated peanut-hydrogenated cottonseed oil mixtures (Table 5). The best results were obtained in the analyses of mixtures of peanut-soybean oil by the modified A. O. A. C. method where differences, from the theoretical, of minus 3.1 to plus 6.2 percent were obtained. However, the analysis of these same mixtures by the modified Kerr method (Thomas and Yu, Table 6) gave values varying from minus 13.7 to plus 8.8 percent from theory. The discrepancies noted in the analyses of these mixtures and with the peanut-adulterated olive oil mixtures may be due to the influence of the high content of unsaturated fatty acids in these oils on the solubility of the lead and magnesium soaps of the saturated acids. This phenomenon has also been observed by Lewkowitsch (11), who states that the solubilities in ether of the lead soaps of the saturated fatty acids are considerably greater in the presence of appreciable quantities of the lead soaps of the un-

TABLE 2

Determination of Peanut Oil by A. O. A. C. Modified Renard Method

Percentage composition of oil mixture *	Weight of solid acid	Weight of arachidic acid from 90% EtOH	Temperature of EtOH (60 ml.)	Solubility correction **	Total weight	Melting range	Peanut oil found
	g.	*g.*	*C°*	*g.*	*g.*	*C°*	*Percent*
Peanut-cottonseed, 35:65	2.36	0.1650	20	0.0387	0.2037	.....	40.7
Peanut-cottonseed, 35:65	Recry'd	0.0689	25	0.0708	0.1397	68-72	27.9
Peanut-cottonseed, 35:65	2.39	0.1942	20	0.0390	0.2332	.....	46.6
Peanut-cottonseed, 35:65	Recry'd	0.0964	25	0.0726	0.1690	73-74	33.8
Peanut-cottonseed, 35:65	2.47	0.0940	20	0.0344	0.1284	.....	25.7
Peanut-cottonseed, 35:65	2.35	0.2284	20	0.0396	0.2680	60-62	53.6
Peanut-cottonseed, 65:35	2.33	0.4093	25	0.0918	0.5011	.....	100.2
Peanut-cottonseed, 65:35	Recry'd	0.0778	20	0.0378	0.1156	68-72	23.1
Peanut-cottonseed, 65:35	2.17	0.4409	25	0.0936	0.5345	.....	106.9
Peanut-cottonseed, 65:35	Recry'd	0.0796	20	0.0342	0.1138	71-75	22.8
Peanut-cottonseed, 65:35	2.20	0.2130	25	0.0792	0.2922	68.5-71	58.4
Peanut-cottonseed, 65:35	2.01	0.1734	25	0.0792	0.2526	68.5-71.5	50.5
Peanut-soybean, 35:65	1.68	0.0825	25	0.0720	0.1545	66-70	30.9
Peanut-soybean, 35:65	1.49	0.0894	25	0.0720	0.1614	66-70	32.3
Peanut-soybean, 35:65	1.57	0.1048	25	0.0732	0.1780	69-70	35.6
Peanut-soybean, 35:65	1.54	0.0998	25	0.0732	0.1730	69-70	34.6
Peanut-soybean, 65:35	1.90	0.2519	25	0.0822	0.3341	69-73	66.8
Peanut-soybean, 65:35	1.85	0.2426	25	0.0816	0.3242	69-73	64.8
Peanut-soybean, 65:35	2.02	0.2737	25	0.0822	0.3559	67-68.5	71.2
Peanut-soybean, 65:35	1.86	0.2701	25	0.0822	0.3523	67.5-69	70.4

* 10 g. samples used for each determination. ** Determined from table of Thomas and Yu.

TABLE 3

Determination of Peanut Oil in Olive Oil by A. O. A. C. Modified Method

Percentage composition of oil mixture*	Weight of solid acid	Weight of arachidic acid from 90%EtOH	Amount and temperature of EtOH		Solubility correction	Total weight arachidic acid	Melting range	Peanut oil found
	g.	g.	ml.	C°	g.	g.	C°	Percent
Peanut-olive, 20:80**	1.40	0.0892	50	21	0.0225	0.1117	66-69.5	22.3
Peanut-olive, 20:80**	1.41	0.1058	50	21	0.0225	0.1283	67-70	25.6
Peanut-olive, 20:80**	1.42	0.1105	50	21	0.0225	0.1330	68-70.5	26.6
Peanut-olive, 40:60**	1.57	0.1620	50	21	0.0225	0.1845	68.5-70	36.9
Peanut-olive, 40:60**	1.46	0.1396	50	21	0.0225	0.1621	68.5-70	32.4
Peanut-olive, 40:60**	1.51	0.1672	50	21	0.0225	0.1897	69-70.5	37.9
Peanut-olive, 60:40**	1.73	0.2055	60	22	0.0270	0.2325	68-70	46.5
Peanut-olive, 60:40**	1.77	0.2217	60	22	0.0270	0.2487	68-71	49.7
Peanut-olive, 60:40**	1.73	0.1917	60	22	0.0270	0.2187	68-71	43.7

* 10 g. samples used for each determination. ** Olive oil No. 0-44, obviously adulterated.

TABLE 4

Determination of Peanut Oil in Olive Oil by A. O. A. C. Modified Method

Percentage composition of oil mixture*	Weight of solid acid	Weight of arachidic acid from 90% EtOH	Amount of EtOH temperature 20° C.	Solubility correction	Total weight	Melting range	Peanut oil Found
	g.	g.	ml.	g.	g.	C°	Percent
Peanut-olive, 20:80**	1.21	0.0642	60	0.0270	0.0912	69-70.5	18.2
Peanut-olive, 20:80**	1.41	0.0811	60	0.0270	0.1081	69-71	21.6
Peanut-olive, 40:60**	1.37	0.1500	60	0.0270	0.1770	69.5-71	35.4
Peanut-olive, 40:60**	1.40	0.1480	60	0.0270	0.1750	69.5-71	35.0
Peanut-olive, 60:40**	1.52	0.2238	70	0.0315	0.2553	68-70	51.1
Peanut-olive, 60:40**	1.48	0.2235	70	0.0315	0.2550	68-70	51.0
Peanut-olive, 80:20**	1.83	0.3574	70	0.0315	0.3889	68-69.5	77.8
Peanut-olive, 80:20**	1.89	0.3662	70	0.0315	0.3977	68-69.5	79.5
Olive oil, No. 0-112	0.88	trace	....	.....	.....	.....	.....
Olive oil, No. 0-112	0.90	trace	....	.....	.....	.....	.....
Peanut oil, No. 0-50	1.98	0.4692	70	0.0315	0.5007	68-69.5	100.1
Peanut oil, No. 0-50	2.03	0.4563	70	0.0315	0.4878	69-70.5	97.6

* 10 g. samples used for each determination. ** Olive oil No. 0-112.

TABLE 5

Determination of Peanut Oil by A. O. A. C. Modified Method

Percentage composition of oil mixture*	Weight of solid acid	Weight of arachidic acid from 90% EtOH	Amount of EtOH Temperature 22° C.	Solubility correction	Total weight arachidic acid	Melting range	Peanut oil found
	g.	g.	ml.	g.	g.	C°	Percent
Hyd. peanut-hyd. cottonseed,** 20:80	8.18	2.8373	120	0.0540	2.8913	66.5-68	578.0
Hyd. peanut-hyd. cottonseed,** 20:80	7.95	2.6836	120	0.0540	2.7376	65-68.5	547.0
Hyd. peanut-hyd. cottonseed,** 20:80	8.58	2.8577	120	0.0540	2.9117	64-67.5	582.0
Hyd. peanut-hyd. cottonseed,** 40:60	8.69	2.9654	120	0.0540	3.0194	64-66	604.0
Hyd. peanut-hyd. cottonseed,** 40:60	8.32	2.6321	120	0.0540	2.6861	64.5-67	537.0
Hyd. peanut-hyd. cottonseed,** 40:60	8.17	3.4493	120	0.0540	3.5033	64-66	701.0
Hyd. peanut-hyd. cottonseed,** 60:40	8.91	3.9278	130	0.0585	3.9963	62.5-65	799.0
Hyd. peanut-hyd. cottonseed,** 60:40	8.75	3.3817	130	0.0585	3.4402	62-65.5	688.0
Hyd. peanut-hyd. cottonseed,** 60:40	8.97	3.1696	130	0.0585	3.2281	64-66.5	645.0

* 10 g. samples used for each determination. ** Hydrogenated peanut oil, iodine value 19.4; hydrogenated cottonseed oil, iodine value 24.1.

TABLE 6

Determination of Peanut Oil by Method of Thomas and Yu

Percentage composition of oil mixture*	Weight of solid acid	Weight of arachidic acid from 90% EtOH	Amount and temperature of EtOH		Solubility correction**	Total weight	Melting range	Peanut oil found
	g.	g.	ml.	C°	g.	g.	C°	Percent
Peanut-cottonseed, 35:65	2.26	0.0712	60	20	0.0336	0.1048	68-70	21.0
Peanut-cottonseed, 35:65	2.24	0.0515	60	20	0.0324	0.0839	69-71	16.8
Peanut-cottonseed, 35:65	2.12	0.0719	70	20	0.0390	0.1109	67.5-68	22.2
Peanut-cottonseed, 35:65	2.10	0.0734	70	20	0.0390	0.1124	67-68	22.5
Peanut-cottonseed, 18.5:81.5	2.46	0.0223	70	15	0.0175	0.0398	64-66	7.96
Peanut-cottonseed, 18.5:81.5	2.24	0.0228	70	15	0.0175	0.0403	64-66	8.06
Peanut-cottonseed, 18.5:81.5***	.....	0.0633	120	20	0.0648	0.1281	68-69.5	12.8
Peanut-cottonseed, 65:35	2.37	0.2140	60	20	0.0396	0.2536	68-71	50.7
Peanut-cottonseed, 65:35	2.25	0.2118	60	20	0.0396	0.2514	68-71	50.2
Peanut-cottonseed, 65:35	2.21	0.2155	70	20	0.0469	0.2624	65-66	52.5
Peanut-soybean, 35:65	1.88	0.1809	60	20	0.0384	0.2193	64-69	43.8
Peanut-soybean, 35:65	1.12	0.0781	60	20	0.0336	0.1117	70-71.5	22.3
Peanut-soybean, 35:65	1.39	0.1145	70	20	0.0413	0.1558	70-70.5	31.2
Peanut-soybean, 35:65	1.40	0.1725	70	20	0.0434	0.2159	65-66	43.2
Peanut-soybean, 65:35	1.97	0.2529	60	20	0.0420	0.2949	66-69	59.0
Peanut-soybean, 65:35	1.90	0.2373	60	20	0.0414	0.2787	63-69	55.7
Peanut-soybean, 65:35	1.59	0.1935	70	20	0.0462	0.2397	70-71	47.9

* 10 g. sample used for each determination. ** Computed from table of Thomas and Yu. *** 20 g. sample used for this determination.

saturated fatty acids than when the latter are present only in relatively small amounts.

It was observed that where the mixtures contained large amounts of saturated acids, as in the case of cottonseed and hydrogenated cottonseed oils, the fractional crystallization of arachidic acid from a mixture of other solid fatty acids is affected by the amount and type of acids present. Holde and coworkers (10) have shown that the solid acids of peanut oil are composed of palmitic, stearic, arachidic, behenic, lignoceric and possibly cerotic acids and it is obvious that a variety of solid solutions and eutectics will result during the crystallization of such a complex and variable mixture of acids. The usual difficulties of fractional crystallization are certainly not lessened when the ratio of one or more of the components is greatly changed in either direction. In the present work it has been observed that where the yield of solid acids is low, as in the cases of mixtures of peanut oil with soybean oil, and of peanut oil with olive oil, the results are closer to the theoretical than in the case where the yield of solid acids is high.

Substantially similar results were recently reported by Pritzker and Jungkunz (12) who critically examined the methods for the determination of peanut oil in mixtures with other oils, fats and soaps. They concluded that ". . . satisfactory results were obtained by none of the methods." Pointing out the impossibility of obtaining even a reasonably pure "arachidic acid fraction" by one crystallization from 90 percent alcohol when appreciable quantities of other solid fatty acids were present, they suggested making repeated crystallizations from 90 percent alcohol until the acids obtained melted above 72°.

BIBLIOGRAPHY

(1) Gössman, Ann. d. Chemie u. Pharm. *89*, 1 (1854).
(2) Renard, M. A. Compt. rend. *73*, 1330 (1871).
(3) Gusserow, C. A. Arch. Pharm. *27*, 153 (1828).
(4) Renard, M. A. Wittsteins Vrtljschr. *21*, 571; Chem. Zentr., 3rd series *1872*, 692.
(5) Fachini, S., and Dorta, G. Chem. Ztg. *38*, 18 (1914).
(6) Kerr, R. H. J. Ind. Eng. Chem. Ind. Ed. *8*, 904 (1916).
(7) Thomas, A. W., and Yu, Chai-Lan. J. Am. Chem. Soc. *45*, 113 (1923).
(8) a. Tortelli, M., and Ruggeri, R. Chem. Ztg. *22*, 600 (1898).
b. Archbutt, L. J. Soc. Chem. Ind. *17*, 1124 (1898).
c. Tolman, L. M. U. S. Dept. Agri. Bur. Chem. Bul. No. 65, p. 33 (1902).
d. Tolman, L. M. U. S. Dept. Agri. Bur. Chem. Bul. No. 81, p. 64 (1904).
(9) Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 5th edition, p. 439, Washington, D. C. (1940).
(10) Holde, D., and Godbole, N. N. Ber. *59B*, 36 (1926); Holde, D., Bleyberg, W., and Rabinowitsch, I., Ber. *62B*, 177 (1929); Holde, D., and Bleyberg, W., Z. angew. Chem. *43*, 897 (1930); Holde, D., and Bleyberg, W., Metallborse *20*, 1855 (1930).
(11) Lewkowitsch, J. "Chemical Technology and Analysis of Oils, Fats and Waxes," 6th edition, Volume I, p. 554. Macmillan and Co., Ltd., London (1921). (Reprinted 1938.)
(12) Pritzker, J., and Jungkunz, Rob. Mitt. Lebensm. Hyg. *33*, 75 (1942).

Determination of Glycerol by the Pyridine-Acetylation Method

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In a comparatively recent paper Shaefer has described the determination of glycol in dilute solution by the removal of water or other low boiling solvent by distillation after addition of pyridine, and subsequent acetylation of the residue. (1) As Shaefer points out in his paper he was interested mainly in the determination of glycol. Using glycerol two preliminary experiments indicated that glycerol acetylated to the extent of 97.8 and 96.6%. On the basis of 6 runs on glycol the reaction was found to proceed to the extent of 97.9% and use of the latter factor is recommended in the determination of glycol.

During the past several years the present writers have run well over one hundred glycerol determinations by this method and have found it to be unusually practical and reliable. Possibly the most important single feature of the method and one which was perhaps insufficiently emphasized in the original paper, is the fact that it permits checking the dichromate method on dilute glycerol solutions (soaps, sweetwaters, soap lyes, etc.) by acetylation, a procedure which was formerly practical only on concentrated glycerol samples.

Before accepting the method for use in this laboratory a number of check runs were made to determine the accuracy of the procedure. A sample of C.P. glycerol assaying 98.83% glycerol on the basis of specific gravity determination and 98.26% glycerol by dichromate oxidation was determined ten times by the pyridine-acetylation method. The average glycerol value was 98.10 ± 0.11%. It is apparent that the pyridine acetylation method checks dichromate oxidation very closely and the new method therefore becomes extremely valuable as a means of checking oxidation values on dilute solutions of glycerol by acetylation.

Assuming the specific gravity determination to be the most accurate method of determining glycerol strength the factor representing the extent to which acetylation takes place when determining glycerol by the pyridine-acetylation method should be 0.993 and it is recommended that the latter value be employed in calculating analytical results when using this method.

LITERATURE CITED

(1) Shaefer, Ind. Eng. Chem., Anal. Ed. *9*, 449 (1937).