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THE DETERMINATION OF THE MIXTURE OF ARACHIDIC AND LIGNOCERIC ACIDS IN PEANUT OIL BY MEANS OF MAGNESIUM SOAPS

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Following the resolution of Gössman's[2] "arachidic acid" from peanut oil into arachidic and lignoceric acids by Kreiling[3] many attempts have been made to isolate the crude arachidic acid as a reliable means of identifying peanut oil. In 1898, Jean[4] proposed a method based upon the comparatively low solubility of "potassium arachidate" at 15° in 90% alcohol, previously saturated with the same soap. Bellier,[5] in 1899, separated crude arachidic acid from peanut oil by crystallizing the insoluble acids from 70% alcohol at 15°. This method was afterwards modified by Evers.[6] In 1914, Fachini and Dorta[7] suggested the potassium-soap-acetone method and two years later Kerr[8] proposed the magnesium-soap-alcohol method as qualitative tests for peanut oil. But none of these is well known or has been shown to be of quantitative application.

The most popular and repeatedly investigated one is the lead-salt-ether method. It was originated by Gusserow[9] who was the first to observe that the lead soaps of unsaturated acids are much more soluble in ether than those of saturated acids of high molecular weights. Since then various modifications have been made by Oudemans,[10] Kremel,[11] Röse,[12] Muter and de Koningh[13] and other investigators, but the fundamental basis, originated by Gusserow, remains the same. As early as 1871, Gusserow's principle was introduced by Renard,[14] in peanut oil analysis.

[1] Adapted from the thesis submitted by Chai-Lan Yu in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

[2] Gössman, *Ann.*, **89**, 1 (1854).

[3] Kreiling, *Ber.*, **21**, 880 (1888).

[4] Jean, "Les corps gras. ind.," **1888**, through Glikin's "Chemie der Fette, Lipoide und Wachsarten," Gebrüder Borntraeger, **1**, 544 (1912).

[5] Bellier, *Ann. chim. anal. appl.*, **4**, 4 (1899).

[6] Evers, *Analyst*, **37**, 487 (1912).

[7] Fachini and Dorta, *Chem.-Ztg.*, **38**, 18 (1914).

[8] Kerr, *J. Ind. Eng. Chem.*, **8**, 904 (1916).

[9] (a) Gusserow, *Ann.*, **27**, 153 (1828). (b) Lewkowitsch, "Chemical Technology and Analyses of Oils, Fats and Waxes," Macmillan Co., **1913**, 1, 543.

[10] Oudemans, *J. prakt. Chem.*, **99**, 407 (1866).

[11] Kremel, *Pharm. Zentralhalle*, **5**, 337, through Glikin Ref. 4, p. 744.

[12] Röse, *Z. anal. Chem.*, **27**, 533 (1888).

[13] Muter and de Koningh, *Analyst*, **14**, 61 (1889).

[14] Renard, *Compt. rend.*, **73**, 1330 (1871).

By means of Renard's method, he and other investigators found that the yields of "arachidic acid," with melting point 71–72°, varied from 4.30 to 5.00% and suggested the factor 20, that is, the yield of "arachidic acid" multiplied by 20 should give the percentage of peanut oil. In 1891, de Negri and Fabris[15] studied known mixtures of olive oil and peanut oil by this method, finding it uncertain when 10% or less of peanut oil was present.

Tortelli and Ruggeri[16] in 1898 showed that Renard's corrections for the solubilities of "arachidic acid" in 90% alcohol were too low. They obtained from peanut oil 4.32–5.00% of crude arachidic acid with melting point from 74.3° to 75.2°. In the same year, Archbutt[17] also made a careful study of the application of the method to the analysis of peanut oil but could not reproduce the results of Tortelli and Ruggeri in all respects. Archbutt recommended a method of partial precipitation of lead soaps, which is a rather long procedure and of the same general principle as Renard's. Renard's method was simplified by Tolman[18] in 1903.

Discussion of Lead-Salt–Ether Method

The solubility determinations (given later) make evident the impossibility of the sharp and complete separation of unsaturated acids from the saturated, and if the loss due to the solubilities of the lead soaps in ether could be diminished, greater accuracy would result. Besides this, the temperature, the amounts of unsaturated soaps present and the quality and quantity of ether employed also have a marked influence upon the solubilities of lead soaps.

At temperatures above 20°, a greater quantity of saturated lead soaps passes into solution. At too low a temperature, the unsaturated soaps previously dissolved separate from the solution and deposit with the unsaturated soaps.

The very volatile nature of ether makes it extremely difficult to maintain, in different experiments, exactly the same amounts of this substance, as required by the Renard-Tolman method. Lewkowitsch[19] noticed that lead soaps are more soluble in anhydrous ether than in moist ether. In confirmation of this, the present authors found that, when anhydrous ether is employed in the separation of known mixtures of lead oleate and lead stearate, the amount of the stearic acid recovered is 1 or 2% too low. Furthermore, in the customary use of ether containing a small amount of moisture, the solid acid cake obtained has an iodine number of about 20

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[15] de Negri and Fabris, *Ann. Lab. Chim. Centr. Gabelle*, **2**, 120 (1892–3), through Lewkowitsch, Ref. 9b, vol. **2**, p. 311.

[16] Tortelli and Ruggeri, *Chem.-Ztg.*, **22**, 600 (1898).

[17] Archbutt, *J. Soc. Chem. Ind.*, **17T**, 1124 (1898).

[18] Tolman, U. S. Dept. Agr., *Bur. Chem. Bull.*, **81**, 64 (1903).

[19] Ref. 9b, p. 179.

and its yield is several per cent. higher than expected, the presence of water decreasing the solubilities of the unsaturated lead soaps.

Filtration and washing of lead soaps is a difficult process. The washings pass through the filter very slowly, particularly when the quantity of soaps is large. In warm weather, the rapidly evaporating solvent leaves behind, on the filter paper as well as inside the funnel wall and stem, a coating of soaps. A few cases have been encountered in which, also, the formation of an emulsion makes filtration extremely slow.

After the decomposition of lead soaps, the insoluble lead chloride formed and occluded by the solid acid cake is difficult to remove, necessitating repeated washing with hot water.

Possibly the reasons given suggest why the lead-salt–ether method is reported in the literature as not giving concordant or reproducible results in the analysis of peanut oil, as it has been stated by many investigators using this method that peanut oil contains 3 to 5%, or 4 to 5% of arachidic acid. As will appear later in this communication, the results obtained by means of the new method proposed indicate that such wide variations are due to the errors in the "lead-salt–ether" method itself.

Experimental Conditions Governing the Separation of Saturated and Unsaturated Acids

The present authors have systematically and quantitatively studied the behavior of a number of pure soaps in various solvents, and found, for instance, that no exact separation of sodium stearate and oleate or barium stearate and oleate could be effected in 90% alcohol at 20° or 25°. Zinc oleate is quite soluble in boiling ether but the greater portion of it separates, when this solution is cooled to 15°. Thorium soaps behave like lead soaps. Lead stearate dissolved in hot toluol and cooled crystallized in nacreous scales, but lead lignocerate treated similarly deposited in the form of a soft mass. Potassium soaps of saturated acids of high molecular weight are slightly soluble in 95% alcohol at 15°, but they are too soft and gelatinous to be of any value for the purpose of quantitative estimation.

Magnesium soaps in 90 or 95% alcohol were found to afford the most satisfactory separation. At 25°, 100 g. of 90% alcohol dissolves only 0.007 g. of magnesium stearate, 0.006 g. of lignocerate but 8.60 g. of oleate, showing a greater differential in solubility than is the case with the corresponding lead soaps in ether.

Preparation and Purification of Fatty Acids

Stearic Acid.—A reagent stearic acid (m. p., 68–69°; acid number, 199.73) upon purification yielded a product which showed no ash and no iodine number and melted at 69.5°; titration with alcoholic potassium hydroxide using phenolphthalein as indicator showed that it had a molecular weight of 284.44 (calc., 284.39).

Lignoceric Acid.—A modification of Jean's method was employed. The following shorter and more economical procedure was used; it is based upon the principle of the greater solubility of potassium oleate as compared with that of stearate and lignocerate in 95% alcohol at 25°. The stearate, lignocerate and oleate dissolved in 100 g. of the alcohol to the extent of 0.63, 0.15 and 41.0 g., respectively. The density of the alcohol was 0.80935 at 25°/4°.

Technique.—About 300 cc. of peanut oil was saponified with 1 liter of saturated alcoholic potassium hydroxide solution by boiling for half an hour on a water-bath under a reflux condenser. The solution was allowed to stand overnight at about 10°. The insoluble soaps were separated, washed with 95% alcohol and decomposed with hot 2 *M* hydrochloric acid. After a clear oily layer had formed, cold water was poured into the flask; the resulting solid acids were separated and washed free from chloride. The dried acid cake was dissolved in hot 95% alcohol and crystallized from the same solvent.

These crystals melted at 75–76° and showed molecular weights of 350–355, but no ash or iodine number. Upon repeated recrystallizations from absolute alcohol a substance was finally obtained which melted at 79–80° with a constant molecular weight of 368.1 (lignoceric acid is 368.36). Its composition and properties were the same as those of Meyer, Brod and Soyka's *iso*lignoceric acid.[20] The yield was about 3 g.

Oleic Acid.—A specimen of imported oleic acid was twice cooled to 10°, which thus removed 8.6% of solids consisting of palmitic acid and unsaponifiable matter. The filtrate had an acid number of 204.3 and iodine number of 86.1 (Hanus). Thereafter, the purification was carried out essentially according to Lewkowitsch's suggestion,[21] the final product having a molecular weight of 282.65 (calc. 282.37), an iodine number (Hanus) of 88.96 (calc. 90.07) and showing no ash.

Preparation and Analysis of Soaps

Lead and Magnesium Soaps.—These salts of lignoceric and stearic acid were prepared by treatment of their alcoholic solutions with aqueous solutions of the corresponding acetates. Lead stearate was recrystallized from hot toluol, while lead lignocerate, and magnesium stearate and lignocerate were recrystallized from 95% alcohol. The crystals in each case were dried in an oven at 80° to constant weight.

The oleates of lead and magnesium were prepared by double decomposition of their acetates with ammonium oleate. The soaps were washed and dried in a vacuum at 40°.

ANALYSES

	Lead soaps		Magnesium soaps	
	Pb in lead soaps		Mg in magnesium soaps	
Soap	Found %	Calc %	Found %	Calc. %
Stearate	26.73	26.78	4.12	4.11
Lignocerate	21.96	21.99	3.22	3.20
Oleate	26.79	26.92	4.10	4.14

Potassium Soaps.—Potassium soaps of the 3 acids were prepared by titrating 10 g. of the acid dissolved in 100 cc. of 95% alcohol with 0.5 *N* alcoholic potassium hydroxide solution using phenolphthalein as indicator, and recrystallizing from 95% alcohol.

Determination of Solubilities

The solvents used were redistilled (the alcohol over potassium hydroxide and silver oxide, the ether over sodium, etc.) and only the portions distilling at the proper boiling points were utilized.

[20] Meyer, Brod and Soyka, *Monatsh.*, 34., 1113 (1913).

[21] Ref. 9b, p. 544.

Technique.—An excess of the solid was placed in a 250cc. tightly stoppered bottle and covered with 150 cc. of the solvent. A set of bottles so prepared was placed on a rotating platform machine and the mixtures they contained were violently agitated overnight at 25°. The bottles were then immersed for 36 hours in a water thermostat,

TABLE I

SOLUBILITIES

Soap used	Strength of alcohol Percentage by weight	Strength of alcohol Density 25°/4°	Solid dissolved in 100 cc. of solution G.	Solid dissolved in 100 g. of solvent G.
(a) Stearic acid in mixtures of water and ethyl alcohol				
	91.53	0.80935	1.803	2.28
	86.16	0.82356	1.014	1.25
	63.07	0.87783	0.087	0.099
(b) Lignoceric acid in mixtures of water and ethyl alcohol				
	91.53	0.80935	0.182	0.226
	86.16	0.82356	0.092	0.112
	63.07	0.87783	0.011	0.013
(c) Potassium stearate, lignocerate and oleate in mixtures of water and ethyl alcohol				
Soap used				
Stearate	91.53	0.80935	0.483	0.633
Lignocerate	91.53	0.80935	0.123	0.153
Oleate	91.53	0.80935	25.2	41.1
(d) Magnesium stearate in mixtures of water and ethyl alcohol				
	91.53	0.80935	0.006	0.007
	86.16	0.82356	0.006	0.007
	72.17	0.85821	0.005	0.006
	63.07	0.87783	0.004	0.005
	44.13	0.92275	0.003	0.003
	27.29	0.95792	0.003	0.003
	19.09	0.97122	0.004	0.004
	Water	0.99709	0.004	0.004
(e) Magnesium lignocerate in mixtures of water and ethyl alcohol				
	91.53	.....	0.005	0.006
	86.16	.....	0.005	0.006
	72.17	.....	0.006	0.006
	63.07	.....	0.004	0.005
	44.13	.....	0.003	0.003
	27.29	.....	0.002	0.002
	19.09	.....	0.002	0.002
	Water	.....	0.003	0.002
(f) Magnesium oleate in mixtures of water and ethyl alcohol				
	86.16	.....	6.64	8.60
	72.17	.....	5.35	6.64
	63.07	.....	3.89	4.60
(g) Lead stearate, lignocerate and oleate in anhydrous ether				
Soap used				
Stearate	...	.....	0.015	0.021
Lignocerate	...	.....	0.018	0.025
Oleate	...	.....	6.46	9.59

maintained at 25° ± 0.005°. During the first 12 hours they were occasionally rotated gently, but they were left quiet after that, so that at the end of 36 hours a clear solution could be pipetted out. As a precautionary measure, the tip of the calibrated pipet used was covered with extracted filter paper. Twenty-five cc. of the solution at 25 ± 0.005° was pipetted into a weighed 30cc. beaker. The solution was covered and slowly evaporated to dryness at a temperature much below the boiling point of the solvent. The residue in each beaker was dried in an oven at 80° to constant weight. The oleates were dried in a vacuum oven at 40°.

In the meantime, another portion of the solution was pipetted into a 25cc. specific-gravity bottle. After it had stood for 20 minutes in the thermostat at 25° ± 0.005° it was wiped dry and weighed quickly.

After an additional 1 or 2 hours, another 25cc. portion of the saturated solution in the original bottle was pipetted out, evaporated and the amount of dissolved solid again determined. If this result did not check with the first one, the whole experiment from the beginning was discarded and a new one performed, until a close check was obtained between two successive determinations.

It was assumed, in the foregoing experiments, that the principle of the greater solubility of the magnesium soap of oleic acid in 90% alcohol would apply to that of linolic acid and that the arachidate would have the same low solubility as stearate and lignocerate. This was afterward found to be true.

Preparation of Reagents

Alcoholic Potassium Hydroxide Solution.—Fifty g. of potassium hydroxide ("purified by alcohol") was dissolved in 1 liter of purified alcohol and filtered to remove impurities. Fifty cc. of this solution is more than sufficient for saponification of 10 g. of vegetable oil.

Alcoholic Magnesium Acetate Solution.—Fifty g. of magnesium acetate was dissolved in 100 cc. of distilled water, heated to boiling and filtered. Three volumes of 95% alcohol was then added to each volume of the cold filtrate.

Alcoholic Acetic Acid.—Twenty cc. of glacial acetic acid was dissolved in 80 cc. of 95% alcohol. This reagent is quite stable, and only slight esterification was observed after a month's standing.

Alcohol.—Solutions prepared from time to time varied in strength from 90.0 to 90.5% by volume (densities, 0.82473 to 0.82293 at 25°/4°), and 70.0 to 70.6% by volume (densities, 0.88117 to 0.87977 at 25°/4°).

Separation of Known Mixtures of Acids

Influence of Temperature upon the Separation of Magnesium Soaps.—At 25°, 100 g. of 90.3% alcohol (by volume) dissolves only 0.007 g. of magnesium stearate and 0.006 g. of magnesium lignocerate. At 35°, more than 0.03 g. of stearate is dissolved, while at 10°, it approches a minimum. On the other hand, magnesium oleate, linolate and ricinoleate remain in complete solution at a temperature as low as 10°.

Known mixtures of stearic or lignoceric acid with oleic acid were neutralized with alcoholic potassium hydroxide solution. Magnesium soaps were precipitated by the addition of excess of the alcoholic magnesium acetate reagent and allowed to stand overnight at different temperatures. The insoluble soaps were separated and washed with 90% alcohol, until the washings no longer gave a turbidity with water. They were decomposed with hot 5 *M* hydrochloric acid. The solid acids were washed free from magnesium and chloride ions, dissolved in small amounts of absolute alcohol and quantitatively filtered into a 50cc. weighed beaker, the solvent was evaporated, and the residue was dried to constant weight at 80° and weighed.

Temp. °C.	Stearic acid taken G.	Oleic acid taken G.	"Stearic" acid recovered G.	Iodine number of recovered acid
25	1.551	3.937	1.543	0.1
20	1.549	3.948	1.567	3.5
10	1.556	3.940	1.603	6.9
	Lignoceric acid taken		Lignoceric acid recovered	
20	1.571	3.942	1.598	4.1

Examination of these figures reveals the fact that although a little of the stearic acid is lost at 25°, the part recovered is practically pure, as shown by the iodine number of approximately zero, while at 20° and 10° all of the stearic acid is recovered, contaminated, however, with a small amount of oleic acid, more so at 10° than at 20° as indicated by the iodine numbers. For the present purposes it is better to recover all of the stearic acid, even though slightly contaminated, and consequently the lower temperatures are preferable for this separation.

Influence of Acidity and Alkalinity.—Only the solubilities of 3 normal magnesium soaps, namely magnesium stearate, lignocerate and oleate, have been determined with accuracy. Therefore, in separating magnesium oleate from magnesium stearate and lignocerate, it is essential to keep the soaps as much in the form of normal salts as possible.

In ascertaining the influence of a slight excess of acetic acid or potassium hydroxide upon the separation of magnesium stearate and oleate, the first part of the experiment was carried out at 20° in the same manner as described above. The filtrate and washings from the solid acid cakes were collected, evaporated to small bulk and the magnesium content determined.

1.513 g. of stearic acid taken in every case

Oleic acid taken G.	Acid or alkali (alcoholic) added Cc.	*M*	Subs.	Stearic acid recovered G.	Mg from recovered "stearic acid" Found G.	Calc. G.
3.917	5	0.5	HAc	1.483	0.0629	0.0634
3.896	0.5	0.5	HAc	1.507	0.0641	0.0646
3.889		Neutral		1.528	0.0665	0.0653
3.803	0.5	0.5	KOH	1.601	0.0781	0.0684
3.909	3	0.5	KOH	2.105	0.1407	0.0900

From the foregoing data, it appears that on the acid side there is formation of either free fatty acids or acid soaps which, being more soluble in the menstruum, are responsible for the loss of stearic acid. On the alkaline side, the excess of magnesium comes from the precipitated magnesium hydroxide, the absorbed magnesium oleate and perhaps also the basic soaps. This experiment shows that it is safer to keep the soap solution slightly on the alkaline side, which can be attained best by first neutralizing the alkaline soap solution with alcoholic acetic acid, using phenolphthalein as indicator, and then titrating it back to the appearance of the first permanent pink color.

Separation of the Saturated and Unsaturated Acids

Saturated magnesium soaps in peanut oil are almost entirely soluble in boiling alcoholic soap solution. But they separate nearly completely upon standing at 10°, particularly magnesium stearate, arachidate and lignocerate. This complete separation, however, depends upon two important factors: (1) time of cooling, and (2) amount of available magnesium acetate present in the solution. These factors again bear direct relationship to the amount of saturated glycerides in a given sample of peanut oil.

About 10 g. of the oil (later referred to as "Refined U. S. Peanut Oil"), accurately weighed, was saponified by a mixture of 50 cc. of alcoholic potassium hydroxide solution and 50 cc. of 95% alcohol. The soap solution was neutralized with alcoholic acetic acid and titrated back to the appearance of the first permanent pink color. To this was added 50 cc. of alcoholic magnesium acetate reagent. The mixture was allowed to stand in a refrigerator at 10° for different intervals of time. The insoluble magnesium soaps were separated and washed almost free from unsaturated soaps, as indicated by the production of only traces of turbidity in mixing the last part of the washings with water. They were then decomposed and analyzed for total solid acids. It was found that although the deposition is complete within 4 to 6 hours, it is best to allow the mixture to stand overnight, to produce a coarser or more granular precipitate which permits rapid filtration and washing.

Alcoholic Magnesium Acetate Required for Complete Precipitation of Saturated Soaps in Peanut Oil.—This series of experiments was carried out as above described, except that instead of using 50 cc. of alcoholic magnesium acetate solution, various amounts of the reagent were added to the soap solutions. The mixtures were allowed to stand overnight at 10°. Samples of oil varied in weight from 10 to 10.5 g.

It was found that 10 cc. of 0.8 *M* alcoholic magnesium acetate solution was just sufficient for this particular oil, but due to variation in solid acid content of peanut oils in general, it is safer to use 25 cc. of the reagent to insure a complete precipitation of the saturated soaps.

Separation of the Mixture of Arachidic and Lignoceric Acids from Peanut Oils.—Renard[14] used 90% alcohol at 15° or 20° to separate arachidic and lignoceric acids from palmitic and stearic acids as a quantitative means of identifying peanut oil. To ascertain the validity of the method, experiments were made by the present authors upon known mixtures of fatty acids. The acids were dissolved in 60 cc. of hot 90.3% alcohol (by volume) in 150cc. covered beakers and allowed to stand overnight in a thermostat at 20°. The insoluble acids were separated, washed twice with 10 cc. of 90.3% alcohol and then with 70.6% alcohol (by volume) until the washing showed no turbidity with water. The material thus obtained was dissolved with absolute alcohol into a weighed dish, the solvent evaporated and the residue dried to constant weight. The results are tabulated below.

Mixture of stearic acid taken G.	Arachidic acid taken G.	Arachidic acid Recovered G.	Arachidic acid Molecular weight Found	Calc.
0.3163	0.2078	0.1971	309.6	312.4
Stearic acid taken	Lignoceric acid taken	Lignoceric acid	Lignoceric acid	
0.3163	0.2394	0.2301	365.1	368.4

The losses shown by the figures above are due to the amounts of the acids dissolved by 90.3% alcohol. They were found to increase or decrease, as the temperature is elevated or lowered. With application of solubility corrections, a good quantitative separation can be made at 20° or 25°, when the amounts of the interfering fatty acids present are not too great. Their solubilities are enhanced by the presence of small amounts of unsaturated acids, which will not however be present to any detrimental extent in actual analysis, provided the magnesium soaps are carefully washed. At 15° too much stearic acid is precipitated with the arachidic or lignoceric acid and the separation becomes impracticable.

Corrections for Solubilities of Arachidic and Lignoceric Acids in 90.3% Alcohol.—Renard[14] made solubility corrections by adding to the weight of "arachidic" acid 0.0045 g. for each 10 cc. of 90% alcohol in the filtrate and washings at 20°, or 0.0025 g. for each 10 cc. at 15°. He apparently assumed that his weighed acid was a pure chemical compound and had, therefore, a definite solubility equilibrium at a given temperature and pressure, irrespective of the quantities of solid phase present in the saturated solution. Tortelli and Ruggeri,[16] in 1898, showed that, first, Renard's corrections were too low and, second, at a given temperature the solubilities of the "weighed acids" in 90% alcohol increased with the amounts of the mixed acids in the saturated solution. His data were confirmed by Archbutt.[17]

We have shown that every 100cc. portion of saturated 90.3% alcoholic solution at 25° dissolves 0.092 g. of lignoceric acid and 1.01 g. of stearic acid. Arachidic acid as an intermediate member has a higher solubility than lignoceric acid and a lower solubility than stearic acid. Hence, with 0.100 g. of homogeneous mixture of arachidic and lignoceric acids no saturated solution can be formed at 25°. With a 0.200g. mixture, saturation takes place. If it were a binary system, any increase in the solid phase from now on, under the same condition, would not alter the solubility equilibrium, but the system is ternary, having 3 components, arachidic acid, lignoceric acid and alcohol. There are two solid phases, one vapor phase and one liquid. Hence the system is unvariant, according to the formula: $P + F = C + 2$. As the temperature and pressure are fixed in these experiments, change of the quantities of the 2 solid phases will alter the equilibrium. As the increase in the homogeneous mixture is constant, the increase in the solubility will also have a constant value. This conclusion is warranted by experimental results.

TABLE II

SOLUBILITIES OF A MIXTURE OF ARACHIDIC AND LIGNOCERIC ACIDS[a] M. P. 72–73°; 90.3% (BY VOLUME) ALCOHOL

Mixture taken G.	Dissolved solid in 100 cc. of solution at 20° G.	at 25° G.
0.600	0.099	0.172
0.500	0.091	0.162
0.400	0.083	0.152
0.300	0.075	0.144
0.200	0.068	0.132
0.100	0.058	[b]

[a] As isolated from peanut oil.

[b] No saturated solution is formed.

TABLE III

SOLUBILITY CORRECTIONS FOR THE WEIGHED ACID MIXTURE[a] PER 100 CC. OF ALCOHOLIC SOLUTION

Weight of acid mixture obtained G.	Solubility correction at 20° G.	at 25° G.
0.60	0.099	0.172
0.55	0.096	0.167
0.50	0.091	0.162
0.45	0.087	0.157
0.40	0.083	0.152
0.35	0.079	0.147
0.30	0.075	0.142
0.25	0.070	0.137
0.20	0.066	0.132
0.15	0.062	0.127
0.10	0.059	0.122
0.05	0.054	0.117

[a] As isolated from peanut oil.

The solubility corrections in Table III would appear to be too small for the actual analysis of peanut oil, because the "weight of acid mixtures obtained" really represents the "acid mixture taken" in Table II. But we must consider that: (1) solubility corrections in Table II were made for use with the mixture of arachidic and lignoceric acids alone, while in peanut oil analysis the alcoholic solution contains considerable quantities of palmitic and stearic acids, which undoubtedly lower the solubilities of arachidic and lignoceric acids, and (2) the solubility corrections in Table III were determined with exactly 90.3% (by volume) alcohol. In practical work, due to loss of alcohol vapor through slight evaporation upon standing, the final strength of alcohol would be somewhat less than 90% and the solubilities of arachidic and lignoceric acids would be diminished. Hence, for practical purposes the given corrections represent perhaps a state nearer to truth than one would otherwise obtain.

Technique of the Proposed Quantitative Method

About 10 g. of oil, accurately weighed, is saponified in a 300cc. Erlenmeyer flask by a mixture of 50 cc. of alcoholic potassium hydroxide solution and 50 cc. of 95% alcohol. The saponification is carried out by heating the mixture for half an hour under a reflux condenser. Upon the completion of saponification, the soap solution, while still warm, is neutralized with the alcoholic acetic acid reagent, using phenolphthalein as indicator. Thereupon, just enough alcoholic potassium hydroxide solution is added to give a permanent pink color. Twenty-five cc. of alcoholic magnesium acetate reagent is added. This mixture is heated just to boiling and then allowed to stand overnight in a refrigerator at about 10°. The insoluble soaps are then separated and washed with 30 cc. of 90% (by volume) alcohol which has been used to rinse out the soaps adhering to the flask.

The greater part of the filtered and washed magnesium soaps is then transferred to the 300cc. Erlenmeyer flask and the remainder is removed by 100 cc. of boiling 5 *M* hydrochloric acid. The magnesium soaps are decomposed by boiling them for about 5 minutes. After a few minutes, cold water is poured into the flask slowly, so as to produce shreds of solid acids, which are then filtered off and washed free from magnesium and chloride ions. The moisture in the flask is removed by drying at 80–100°. The solid acids are transferred to a 150cc. beaker. Sixty cc. of 90% alcohol is warmed in 3 successive portions in the flask and poured over the filter paper, dissolving out completely the remaining acid residue, the filtrate passing into the beaker containing the acids previously transferred. The covered beaker is allowed to stand overnight in a thermostat at 20° or 25°. The crystals which have formed are separated and washed twice with 10 cc. of 90% alcohol and then with 70% (by volume) alcohol (the temperature of 70% alcohol for washing should be about 20°), until 10 drops of the washing liquid produce no turbidity with water. The filtrate and washings from 90% alcohol are collected and accurately measured. The residue is transferred into the original beaker, and dissolved with small portions of absolute alcohol. The solution is carefully poured over the filter paper and the filtrate is caught in either a weighed dish or a 50-cc. beaker. The original beaker and filter paper are quantitatively washed with another small portion of absolute alcohol. The solvent is evaporated and the residue is dried to constant weight at 80°. This weight is corrected for the total amount of the mixture of arachidic and lignoceric acids dissolved in the filtrate and washings of 90% alcohol at the selected temperature. The mixed acids thus obtained melt between 71° and 73°. Upon analysis, they show no ash, no iodine number and a mean molecular weight of about 340. Upon recrystallization from another 60cc. portion of 90% alcohol, the mixed acids melt at 75–76°, or occasionally slightly higher. This recrystallization is valuable as a confirmatory test.

TABLE IV

APPLICATION OF THE PROPOSED METHOD TO THE ANALYSIS OF PEANUT OIL

Sample	Analyses Taken G.	 Acids found G.	Correction for acids dissolved at 25° G.	(90% alcohol taken) Cc.	Total acids G.	%
Spanish...........	10.40	0.4743	+0.0960	60	0.5703	5.48
Texas (DeLeon)[a]...	10.45	0.4695	+0.1033	65	0.5728	5.47
Java..............	10.21	0.4335	+0.1089	66	0.5424	5.41
Virginia...........	10.80	0.4843	+0.0912	57	0.5755	5.31
Alabama..........	10.14	0.4232	+0.1088	66	0.5318	5.24
Mexican..........	10.04	0.4281	+0.0977	64	0.5257	5.23
China (Canton)....	10.35	0.4404	+0.0952	61	0.5356	5.17

[a] Probably expressed from Spanish peanuts.

The crude oils were directly expressed from peanuts without refining. They represent nearer the true contents of arachidic and lignoceric acids than refined oils. With the exception of the China crude oil, the remaining 6 oils do not differ more than 0.25% in their arachidic and lignoceric acid contents. By taking into consideration the experimental errors and variations in the expressing and handling of these oils by different persons, the difference in the contents of these 2 acids among the oils is surprisingly small.

It is of interest to note that the Texas crude oil was obtained by expressing hot, while the Spanish and Virginia samples (kindly furnished by Dr. Jamieson, of the Oil, Fat and Wax Laboratory of the U. S. Bureau of Chemistry), were cold pressed; we are not sure of the temperature of expressing the others, although informed that they were cold pressed. The Texas crude peanut oil was dark brown and contained an abundance of suspended peanut solids which were removed by the centrifuge before analysis.

TABLE V

ANALYSES AT 20°

Sample	Taken G.	Acids found G.	Correction for acids dissolved G.	Cc.	Total acids G.	%
Spanish....................	10.26	0.5010	+0.0611	66	0.5621	5.47
Virginia....................	10.40	0.4949	+0.0573	63	0.5522	5.30
Chinese (Hongkong)........	10.90	0.4771	+0.0570	64	0.5341	4.89

Analyses of 3 unrefined oils were also carried out at 20° to show the influence of change of temperature upon the final result during the crystallization of arachidic and lignoceric acids from 90% alcohol.

Analysis of Refined Peanut Oils.—From the following table it is noted that refined oils contain a lower amount of arachidic and lignoceric acids.

TABLE VI

ANALYSES OF REFINED OILS

10 g. was taken in each case

Sample	Total arachidic and lignoceric acids found %
Deodorized Chinese	5.13
Refined	5.01
"Refined U. S."	4.98
Refined Virginia	4.78
Refined Canton	4.79

Judging from the analyses, an average sample of peanut oil contains about 5% of the mixture of arachidic and lignoceric acids. Hence for practical purposes, the factor 20, first suggested by Renard,[14] can be retained.

As the saponification number is practically always determined in oil testing, advantage would be gained in saving the work of an extra weighing and saponification, particularly where only a qualitative determination is desired, by use of the titrated soap solution from the saponification number in testing for lignoceric and arachidic acids. Lead chloride or sulfate is insoluble in organic solvents, extraction with ether is bothersome and, therefore, nothing along this line of investigation has been attempted with the "lead-salt-ether" method, but tests have shown that the use of this procedure with the proposed new method is possible.

After the saponification number titration, 25 cc. of alcoholic magnesium acetate reagent is added, the mixture allowed to stand overnight at 10°, and the insoluble magnesium soaps are separated, etc., in the same manner as described above for the quantitative procedure. Using 5.765 g. of Spanish crude oil, 4.671 g. of Virginia oil and 5.106 g. of Refined U. S. oil, 4.86, 4.77 and 4.61%, respectively, of mixed arachidic and lignoceric acids were obtained. It is evident that due to the small initial sample taken this alternative procedure gives about 10% lower yield than does the quantitative procedure. In instances where speed is more essential than accuracy this affords a very convenient means for the approximate determination of peanut oil. In this shortened procedure the factor 22 should be used.

Analytical Results Obtained with the Lead-salt-ether Method.—With Renard's method[14] and Tortelli and Ruggeri's modifications[16] Archbutt,[17] using both ordinary and recrystallized lead acetate, obtained mixed acids varying from 4.3 to 1.5% with melting points between 70° and 73° with the same sample of peanut oil. With different samples of peanut oils, Tolman and Munson[22] obtained 3.41 to 4.24% of acids, having melting points between 72° and 73°. Heiduschka and Felser[23] obtained 4.74 to 5.66%, with melting points varying from 67° to 69°. The following analyses are by the lead-salt-ether method, for three of the oils reported above, given for comparison.

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[22] Tolman and Munson, U. S. Dept. Agr., *Bur. Chem. Bull.*, **77**, 35 (1903).

[23] Heiduschka and Felser, *Z. Nahr. Genussm.*, **38**, 242 (1919).

	Acids found		Correction for acids dissolved, 20°		Total acids	
Sample	Taken	G.		Cc.	G.	%
Spanish	10.40	0.5015	+0.0573	65	0.5588	5.37
Virginia	10.90	0.4661	+0.0660	75	0.5321	4.89
Chinese	10.50	0.4542	+0.0450	55	0.4992	4.75

Application of Proposed Method to Mixtures

French olive oil and American cold drawn cottonseed oil were analyzed both singly and admixed with pure peanut oil, to determine the validity of the proposed method. These oils contained 11.6% and 19.6% of solid acids (corr.), respectively.

Since genuine peanut oil contains about 5.0% of the characteristic acids, this method as well as the "lead-salt–ether" method would not be expected, at 20°, to detect less than 10% of peanut oil in a mixture if one started with only 10 g. of oil for analysis. In order to increase the sensitiveness of the method, a larger sample of oil mixture must be taken. Hence, previous to studying the known mixtures of oils, experiments were first carried out with different amounts of the olive and cottonseed oils to see whether their solid acids would interfere. Ten- and 30g. samples of each were taken and treated with 60 cc. of 90% alcohol (100 cc. used for the 30g. sample of cottonseed oil), and in no case was crystallization of solid acid observed.

TABLE VII

ANALYSIS OF PREPARED MIXTURES OF FRENCH OLIVE OIL AND VIRGINIA CRUDE PEANUT OIL[24]

Sample G.	Composition of mixture Peanut %	Olive %	Mixed acids found G.	Correction for mixed acids dissolved at 20° G.	Cc.	Total acids G.	Peanut oil found %
10	100	..	0.4769	+0.0535	57	0.5304	100
10	90.0	10.0	0.4280	+0.0576	67	0.4856	91.7
10	50.2	49.8	0.2240	+0.0456	68	0.2606	49.2
10	20.1	79.9	0.0620	+0.0388	68	0.1008	19.0
30	10.0	90.0	0.1263	+0.0354	60	0.1617	10.1
50	5.9	94.1	0.0508	+0.0568	97	0.1076	4.1

TABLE VIII

ANALYSIS OF PREPARED MIXTURES OF AMERICAN COTTONSEED OIL AND SPANISH CRUDE PEANUT OIL[25]

Sample G.	Composition of mixture Peanut %	Cottonseed %	Mixed acids found %	Correction for mixed acids dissolved at 20° G.	Cc.	Total acids G.	Peanut oil found %
10.0	100	..	0.4891	+0.0615	64	0.5506	100
10.0	90.3	9.7	0.4363	+0.0559	65	0.4922	89.4
10.0	50.1	49.9	0.2260	+0.0483	70	0.2743	49.9
10.0	20.0	80.0	0.0642	+0.0414	74	0.1056	19.2
30.0	10.9	79.1	0.1101	+0.0597	97	0.1698	10.3
50.0	5.6	94.4	0.0350	+0.0742	141	0.1092	3.9

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[24] The factor for this particular peanut oil was known to be 18.9.

[25] The factor for this particular peanut oil was known to be 18.2.

TABLE IX
ANALYSIS OF PREPARED MIXTURES OF AMERICAN COTTONSEED OIL WITH "REFINED U. S." PEANUT OIL[26]

Sample G.	Composition of mixture		Mixed acids found G.	Correction for mixed acids dissolved at 25°		Total acids G.	Peanut oil found %
	Peanut %	Cottonseed %		G.	Cc.		
10.0	100	..	0.4086	+0.0896	60	0.4982	100
10.0	90.2	9.8	0.3452	+0.1029	70	0.4481	90.1
10.3	50.0	50.0	0.1709	+0.0740	60	0.2449	49.2
10.0	40.9	59.5	0.1140	+0.0860	70	0.2000	40.2
10.6	30.7	69.3	0.072	+0.0738	62	0.1458	29.2
20.0	20.1	79.9	0.0566	+0.110	93	0.1666	16.2

The above 3 peanut oils contained different amounts of arachidic and lignoceric acids—5.30% for Virginia crude oil, 5.49% for Spanish crude oil and 4.98% for Refined U. S. oil. Hence in order to test the accuracy and validity of the proposed separation, the 3 respective factors, 18.9, 18.2 and 20.1 of Tables VII, VIII and IX are used instead of the general factor 20. Using the general factor 20, we find that the maximum deviation of the percentages found from those in the prepared mixtures is not more than 5%, except for one case in Table VIII.

The foregoing tabulated data for arachidic and lignoceric acids from pure peanut oils and their prepared mixtures are easily reproducible. The proposed method gives a higher percentage and more consistent yields than the lead-salt–ether method. Solid acids from common vegetable oils such as refined castor, corn, cottonseed, linseed, olive, rape, sesame, soya bean and tung oils, do not give any precipitation in 90% alcohol upon standing overnight in a thermostat at 25°. At 20°, rape oil is an exception, giving about 0.5% of the insoluble acids, melting at 70°, a phenomenon discussed in a following paper.

Experimental errors involved in the slight alterations of working conditions are very small and are negligible. Experiments were carefully carried out with the same sample of peanut oil, with the following alterations: (1) use of 10 cc. more or less of 95% alcohol than the amount specified in saponifying the oil; (2) prolonged cooling of the magnesium soap solution; (3) slight excess of alcoholic acetic acid or alcoholic potassium hydroxide solution during neutralization; (4) crystallization of the arachidic

Temp. of cryst. of acids from Spanish crude peanut oil °C.	Total mixed acids found %	Temp. of cryst. of acids from Refined U. S. peanut oil °C.	Total mixed acids found %
25	5.49	25	5.00
25	5.45	25	4.97
20	5.48	20	5.01
20	5.50	25	4.87
25	5.41	25	4.99

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[26] The factor for this particular peanut oil was known to be 20.1.

and lignoceric acids at 20°. These slight alterations did not affect the final results to any appreciable extent. Some values obtained on a given sample at different times and temperatures are shown on the preceding page.

Constant errors in the proposed method are (1) loss of arachidic and lignoceric acids due to the slight solubilities of their magnesium soaps in 90% alcohol and (2) loss due to the solubilities of these 2 acids in 90% alcohol at 20° or 25° during their crystallizations. In the first case, the loss is theoretically less than 0.02 g. The loss in the second case is corrected by the solubilities as determined and shown in Table II.

Summary

1. The solubilities of the magnesium soaps of various fatty acids in 90% by volume ethyl alcohol have been accurately measured. These measurements indicate that an excellent separation of magnesium stearate, arachidate and lignocerate from magnesium oleate, linolate and linolenate is possible in this solvent.

2. The solubilities of potassium stearate, lignocerate and oleate in 91.5% by weight (94.3% by volume) alcohol and of the corresponding lead salts in ether have been carefully determined.

3. The solubilities of stearic and of lignoceric acids in 3 strengths of alcohol have been determined showing that a quantitative separation of arachidic and lignoceric acids from other saturated acids can be best accomplished with 90% by volume alcohol after standing for one night at 20° or 25°.

4. A new method for the quantitative determination of peanut oil is proposed, based on the separation of the magnesium soaps of saturated and unsaturated acids in 90% (by volume) alcohol.

5. The proposed method has the following advantages: (1) the *constant* errors such as are involved in the solubilities of lead soaps in ether are reduced to a minimum; (2) a direct and more rapid separation of saturated and unsaturated acids in peanut oil is accomplished without the use of a second solvent; (3) higher percentage yields of arachidic-lignoceric acid mixture than those from the "lead-salt-ether" method are obtained; (4) common vegetable oils, including cold drawn cottonseed oil, do not interfere with the quantitative estimation of peanut oil at either 20° or 25°.

This proposed method is not intended for the separation of saturated from all unsaturated acids in all fatty oils, as magnesium erucate and an unsaturated soap from tung oil (probably eleomargarate) were found to be insoluble in 90% by volume alcohol, as noted in a following paper.

6. The solution left after the determination of saponification number may be utilized for the separation of the mixture of arachidic and lignoceric acids by the proposed method, affording an excellent qualitative and a fair quantitative method for the identification of peanut oil.

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