[CONTRIBUTION FROM THE CHEMISTRY LABORATORY, UNIVERSITY OF VERMONT]

THE COMPOSITION OF SOYA BEAN OIL

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Introduction

Determination of the composition of animal and vegetable oils and fats is extremely difficult because of the close similarity in chemical and physical properties of many of the ingredients of these complex mixtures. Any addition to the more usual methods of study is therefore of interest.

Such an addition is that contributed by Twitchell,[1] in which measurements are made of the lowering of the melting points of relatively large amounts of the individual pure fatty acids that are represented in the oil, through addition to them of weighed amounts of the mixed acids obtained by hydrolysis of the oil. Since the acid in the mixture that is identical with the pure acid does not contribute to the lowering, a comparison of this lowering with the depression constant obtained by the addition to the pure acid of a different pure acid gives a measure of the amount of the first pure acid in the mixture. The calculation is approximate, as it assumes equality in the molecular weights of these higher fatty acids. It also depends upon the existence of a linear relation between melting point and concentration of added acid. In case of the saturated fatty acids, and within the concentration limits involved, this linear relation holds with sufficient accuracy.

Through the kindness of Dr. George S. Jamieson, opportunity was given us to examine by this method a portion of the same sample of soya bean oil that he and W. F. Baughman[2] subjected to more detailed analysis at the Fat and Wax Laboratory of the United States Bureau of Chemistry. The analysis here presented was made during the winter and spring of 1922; the oil, therefore, did not have opportunity to change through age, and corresponded perfectly with that used by Baughman and Jamieson. The physical and chemical constants of the oil as determined in this Laboratory are very close to those recorded by them.

Experimental Part

In essentials, the experiments were carried out as described by Twitchell. His melting-point determinations were made through direct observations of the samples in capillary tubes attached to the thermometer bulb. In the present experiments it was found difficult to choose by such direct observation the exact point of melting, and on this account the following method of observation was adopted. A thin-walled capillary tube of

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[1] Twitchell, *J. Ind. Eng. Chem.*, **6**, 564 (1914); **9**, 581 (1917).

[2] Baughman and Jamieson, THIS JOURNAL, **44**, 2947 (1922).

about 1mm. internal diameter, open at both ends, was warmed and dipped into the melted acids and a column about 0.5 cm. was withdrawn. While the sample was still molten, the tube was thrust into mercury. When the acid had solidified, the tube was removed. It now contained a layer of acid above a layer of mercury, with no air space between. The melting-point tube was next attached to the bulb of a Beckmann thermometer which was then suspended in a test-tube containing mercury. The bulb of the thermometer was completely covered with the mercury, and the melting-point tube was so placed that the column of fatty acid was just out of sight, below the mercury surface. The test-tube was placed in a larger one, forming an air jacket, and the whole was immersed in a 2-liter beaker of water into which dipped a second thermometer. The water was then so warmed that in the neighborhood of the melting point the thermometer bearing the capillary did not increase in temperature more rapidly than 0.05° per minute. The melting temperature was taken as that at which the layer of acid first came into sight above the surface of the mercury, through yielding to the slight pressure from beneath. Readings checking to 0.01° were obtained in this way, and the strain on the eye, as well as the feeling of uncertainty, was relieved.

Preparation of Materials

Stearic Acid.—This acid was prepared from beef tallow through hydrolysis and fractional precipitation with magnesium acetate. The acid so obtained was crystallized six times from aldehyde-free absolute alcohol; m. p., 69.26°.

Palmitic Acid.—Crude commercial palmitic acid was fractionally distilled under 10mm. pressure and suitable fractions were repeatedly crystallized from absolute alcohol; m. p., 62.00°.

Myristic Acid.—The acids obtained by the hydrolysis of nutmeg butter were fractionally distilled under reduced pressure, with subsequent repeated crystallization from alcohol; m. p., 53.8°.

For distillation, the acids were heated by means of a gas burner, with an electrically heated platinum coil immersed in the acid itself, to prevent bumping.

Arachidic Acid.—This acid was prepared from the erucic acid of rape seed oil through fusion with potassium hydroxide. The acids from 200 g. of the oil were fused with the alkali at 300° for six hours, when evolution of hydrogen ceased. The acids were set free with hydrochloric acid, dissolved in alcohol, decolorized with boneblack, and crystallized several times from alcohol. A final rehydrolysis to decompose any ethyl ester formed, with a single subsequent crystallization, gave an acid melting at 74.59°.

Oleic Acid.—An attempt was made to prepare pure oleic acid by distillation under reduced pressure of crude oleic acid. In this way it was easy to obtain an acid of iodine number 90.7, which is quite close to the value 90.07 calculated for pure oleic acid. But the melting point of the acid obtained was 10.06° instead of 14.00° recorded for oleic acid. When this acid was again distilled, the first portion collected became solid at room temperature. The iodine number was still 90.7. It appears, therefore, that distillation changed a portion of the oleic, to its isomer, elaidic acid. From the lowered melting point, one may judge the transformation to have been about 20%.

The oleic acid used in the preliminary experiment recorded in Table II was prepared from crude acid through the lead and barium salts, the latter being thrice crystallized from alcohol.

The Mixed Soya Bean Oil Acids.—The acids obtained from the oil by use of potassium hydroxide and hydrochloric acid were washed with hot water until free from chlorides and then dried at 120° by passing dry hydrogen through them.

Determination of Saturated Acids

The constants for the depression of the freezing point used in the calculations are those given by Twitchell; they were checked by use of our own samples of acids.

The melting-point measurements that relate to the determination of the saturated fatty acids are summarized in Table I.

Table I

Measurements

Sample in m. p. tube	Beckmann thermometer readings °C.		Depressions found °C.		Depression constant	Percentages of acids found		
	(a)	(b)	(a)	(b)		(a)	(b)	
Stearic acid	4.25	4.25	3.86	3.85	3.93	1.7	2.1	Av. 1.9% stearic acid
80% Stearic, plus 20% mixed acids	0.39	0.40						
Palmitic acid	4.73		3.74		4.16			10.1% palmitic acid
80% Palmitic, plus 20% mixed acids	0.99							
Myristic acid	5.04		4.52		4.53			myristic acid probably not present
80% Myristic, plus 20% mixed acids	0.52							
Arachidic acid	3.73		3.69		3.73			1.1% arachidic acid
80% Arachidic, plus 20% mixed acids	0.04							

Determination of Unsaturated Acids

As might be expected, the melting-point method cannot be applied directly to the determination of the unsaturated acids. As shown in Table II, the melting point of oleic acid is raised by the addition of small amounts of stearic acid.

Table II

Melting Points of Mixtures of Oleic and Stearic Acids

Oleic acid %	Added stearic acid %	Melting point °C.
100	...	13.54
99.5	0.5	13.90
99	1.0	14.25
98	2.0	14.58

After hydrogenation of the mixed acids, the unsaturated acids originally present may be determined as saturated acids of the same number of carbon atoms. Following Twitchell, we adopted this procedure. In the present instance, hydrogenation at 190–200° with nickel as catalyst was carried out at atmospheric pressure, and on this account was very protracted; its progress is shown in Table III.

TABLE III

RATE OF HYDROGENATION OF MIXED ACIDS

Time, hours	0	6	9	12	15	18	21
I. no. of mixed acids	134	84	57	36	16[a]	7	2

[a] Fresh catalyst added.

Unsaturated acids of 20 carbon atoms have not been found in soya-bean oil. However, a test was made for such acids through the use of pure arachidic acid, and on account of their anomalous nature it is necessary to record the results. Arachidic acid alone gave a thermometer reading of 4.56; but when 80% of arachidic was mixed with 20% of hydrogenated acids the reading was 0.45, showing a lowering of 4.11. The normal lowering shown by Twitchell for 20% of acid not arachidic added to 80% arachidic is 3.99. The present lowering leads to the anomalous calculated result of —3% arachidic acid, a discrepancy of at least 4%, since 1.1% of arachidic acid was found to be present before hydrogenation.

We are inclined to attribute this anomaly to the possible presence of decomposition products of lower molecular weight resulting from prolonged hydrogenation. Other evidence of such decomposition is lacking and Dr. Jamieson has advised us that much decomposition is doubtful. Relatively slight amounts of side products would, however, lead to the values found. In any case, the same source of error affects in like amount the determination now to be considered of the percentage of unsaturated acids of 18 carbon atoms. Thus stearic acid alone has a thermometer reading of 4.25, while a mixture of 80% of stearic and 20% of hydrogenated acids gave a reading of 3.62, showing a lowering of 0.63.

Again employing the depression constant of 3.93°, there is found to be 83.9% of stearic acid in the hydrogenated oil. Deducting the 1.9% of stearic acid found to be present originally, leaves 82% as resulting from hydrogenation of the unsaturated acids of 18 carbon atoms.

Taking account of the discrepancy of 4% shown above to exist in the effect of the hydrogenated acids on the melting point of arachidic acid, and attributing to acids of 18 carbon atoms the residual 2% of unsaturation in the hydrogenated acids, as shown in Table III, the final approximate value for unsaturated acids of 18 carbon atoms is 88%.

Summary

The approximate composition of the insoluble fatty acids of soya-bean oil as determined by the melting-point method of Twitchell, and an abbreviated summary, for comparison, of the analysis of the mixed acids from the same oil by Jamieson and Baughman, are given below. The results

of Jamieson and Baughman are here recalculated from percentages in the oil to percentages in the mixture of acids.

PERCENTAGE COMPOSITION OF FATTY ACIDS

Acid	J. and B. %		W. and B. %
Myristic	..		0
Palmitic	6.8		10
Stearic	4.4		2
Arachidic	0.7		1
Lignoceric	0.1		..
Linolenic	2.3	87.7	88
Linolic	51.8		
Oleic	33.6		

The chief discrepancy between the two sets of results lies in the distribution between palmitic acid and stearic acid of the saturated acids. No explanation of this discrepancy is offered.

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REDUCTION OF SITOSTEROL
PREPARATION OF DIHYDROSITOSTEROL OR SITOSTANOL

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Introduction

The properties of the saturated sterols that we have isolated from the endosperm of corn[1] and wheat[2] as previously described resembled to a considerable degree those of dihydrocholesterol or β-cholestanol, which substance was first prepared by Willstätter and Mayer[3] by reducing cholesterol with hydrogen in the presence of platinum black. In order that the properties of these substances might be compared we decided to make some attempts to reduce sitosterol with hydrogen using platinum black as the catalyst. The dihydrositosterol that should be formed if the reduction were successful might prove to be identical with the natural dextrorotatory saturated sterol occurring in the endosperm of different grains.

So far as we are aware, dihydrositosterol has never been prepared by reduction with hydrogen. The substance, prepared by Windaus and Hauth[4] by boiling a solution of sitosterol in amyl alcohol with metallic sodium and described by these authors as dihydrophytosterol, melted at 175°, and it was not saturated since it absorbed bromine. It was probably

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[1] Anderson, THIS JOURNAL, **46,** 1450 (1924).

[2] Anderson and Nabenhauer, *ibid.*, **46,** 1717 (1924).

[3] Willstätter and Mayer, *Ber.*, **41,** 2199 (1908).

[4] Windaus and Hauth, *Ber.*, **40,** 3681 (1907).