SOME NOTES ON THE TWITCHELL SEPARATION

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BY the adoption of standard methods for the proximate analysis of fatty acid mixtures the Society has simplified greatly the problem of the analytical chemist engaged in such operations. The Fat Analysis Committee[1] in recommending these methods suggests that "the constituent fatty acids . . . may be calculated from the thiocyanogen value and the iodine value of the mixed fatty acids" and further points out that its "recommendation of the modified Twitchell Method is based on the fact that, if it is desired to determine the isoöleic acid, it is necessary to make the solid acid separation." Thus is succinctly stated one of the two chief reasons for any of the various methods for separation of solid from liquid fatty acids. The other application of such separation methods is as a research tool for obtaining liquid and solid fractions for further examination, a use less often required in ordinary routine.

The Committee notes that "calculations of constituent acids (by the thiocyanogen method) are based on the assumption that no acids more unsaturated than linoleic are present." While no restrictions as to applicability of the Twitchell method are specified it is obvious that the assumption above noted must also apply and it is well established that higher or lower homologues of oleic, e.g., palmitoleic and erucic acids, not only render the calculation formulae invalid but may, in the case of erucic, cause serious confusion in the isoöleic acid reported, unless its presence is known and precaution is taken against such error. Though no mention is made in the Committee report it is equally well known that, in the presence of saturated acids of lower molecular weight than palmitic, the Twitchell separation is imperfect and even palmitic acid, if present in large amount, may contaminate the liquid acids seriously. Fortunately Mr. Sheely's method of calculation, which assumes the accuracy of the saturated acids determined by the thiocyanogen analysis, obviates this error entirely. Otherwise, in the absence of such correction, each one per cent of saturated acids which passes inadvertently into the liquid fraction, will cause a lowering of the apparent linoleic acid by one per cent and a corresponding raise in apparent oleic acid of two per cent.

These considerations force the conclusion that the thiocyanogen method[2] is preferable to any of the commonly employed separation methods except for the two reasons stated in the opening paragraph. Our chief interest in any separation method will be its value in the estimation of the so-called "new solid unsaturated acids formed during hydrogenation"—those commonly designated by the term "isoöleic."

METHODS OF ANALYSIS

The estimation of isoöleic acid by any known means involves a quantitative separation of solid acids and a determination of their iodine value from which the percentage of isoöleic acid may be calculated. At the present time there are in general use four methods for making the solid-from-liquid fatty acids separation.

Lead Salt-Ether Method

This method, known originally as the Gusserow-Varrentrapp Method, is discussed in detail by Lewkowitsch.[3] Its historical importance and the fact that it is the official method of the Association of Official Agricultural Chemists[4] for the determination of saturated acids are the chief reasons for its consideration here. In brief the method is as follows:

The sample to be analyzed is saponified in alcohol with KOH and the excess caustic is neutralized with acetic acid, the solution being left faintly alkaline to phenolphthalein. The neutralized soaps in alcohol are added to a boiling aqueous 20% lead acetate solution, the lead soaps formed are washed with water, dissolved in warm ether and allowed to stand for fifteen hours at about 15° C. The crystallized lead soaps are separated by filtration using suction, are washed with ether and transferred to a separatory funnel in which they are decomposed with dilate HCl and the solid fatty acids are extracted with ether. After drying and evaporating the ether the dry solid acids are weighed and their iodine value is determined.

Twitchell Method

This procedure, in modified form, has been adopted by both the American Oil Chemists' Society and the American Chemical Society on recommendation of their joint Committee on Analysis of Commercial Fats and Oils.[1] Twitchell's purpose[5] in devising his method apparently was not to achieve greater accuracy than that obtainable by the old lead salt-ether method, which was then in general use, but was rather to increase the ease and simplicity of operation, with attendant improvement in the agreement between results by different analysts. The analysis is made on fatty acids, prepared from the sample to be analyzed by saponification with KOH, acidification and extraction with ethyl ether. The solid acids are precipitated from a weighed amount of the mixed fatty acids (2 to 5 gms.) in 50 ml. hot alcohol by addition of 50 ml. hot alcohol in which are dissolved 1.5 gms. lead acetate. This mixture is allowed to stand overnight at approximately 15° C. and filtered. The lead salts of solid acids are dissolved in 100 ml. hot alcohol containing 0.5% acetic acid and are recrystallized by a second overnight cooling at 15° C. After the second filtration and washing the lead soaps, free from alcohol, are decomposed with dilute nitric acid, extracted with ether and transferred to a tared flask from which the ether is evaporated and the solid acids are weighed. Approximately 1.2 gms. of solid acids should be obtained if the proper weight of mixed fatty acids has been taken. An iodine value is obtained on the original fat and on the separated solid acids.

Cocks-Christian-Harding Method

Cocks, Christian and Harding[6], believing that the Twitchell method of analysis on hydrogenated oils yields low and inaccurate isoöleic acid content, developed a new method of separation based upon

the precipitation of lead salts of solid acids in alcohol and their subsequent washing with petroleum ether. Their method is as follows:

The analysis is made upon mixed fatty acids prepared in exactly the same manner as that employed in the Twitchell procedure. An exactly weighed 3.5 gm. sample is precipitated from 50 ml. hot alcohol by addition of 50 ml. hot alcohol in which are dissolved 3.45 gms. lead acetate (1.0 gm. if the sample contains less than 25% solid acids). The combined solutions are cooled overnight at 15°-20° C. and filtered. The insoluble lead salts are washed on the paper with 100 ml. petroleum ether, the ether washings being collected, freed from solvent by evaporation on steam bath and the precipitate dissolved by refluxing with 20 ml. alcohol containing one drop acetic acid. This solution is cooled for three hours at 15°-20° C and filtered. The precipitate, if any, is washed with cold alcohol and added to the main bulk of insoluble lead soaps. The entire precipitate is then decomposed with dilute nitric acid, the solid fatty acids are extracted with ether, dried, weighed and their iodine value is determined. An iodine value is also obtained on the original sample.

Baughman-Jamieson Method

Baughman and Jamieson[7] have not attempted to improve the accuracy of the solid fatty acid determination. Their objective appears to have been an improvement in ease of operation and a considerable saving in time by making the analysis on the original oil instead of on prepared fatty acids. Their modification of the Twitchell procedure is as follows:

A quantity of the oil containing approximately 1.0-1.5 gms. solid fatty acids (in no case over 6.0 gms. oil) is saponified in alcoholic KOH, the excess KOH is neutralized with acetic acid, one drop in excess being used, and 5.0 gms. lead acetate in 50 ml. hot alcohol are added. The total volume should be 200 ml. This solution is cooled slowly and allowed to stand overnight at 15° C. The precipitate is filtered, washed free from Pb, dissolved and reprecipitated from alcohol containing 0.5% acetic acid by volume. After standing overnight at 15° C. the lead salts of solid acids are filtered and washed as before. The solid acids are obtained by decomposing with dilute HCl and extraction with ether. After removal of the solvent the solid acids are weighed and if it is desired to determine the isoöleic acid an iodine value should be obtained.

Comparison of Methods

It will be noted that the most serious objection to the lead salt-ether procedure is its difficulty in the hands of an analyst unskilled in its operation. Within the rather narrow limits of its applicability as a method for effecting the solid-liquid fatty acid separation we are not aware that its accuracy has been seriously questioned—certainly not by Twitchell, who used it as a standard for testing his own procedure. Baughman and Jamieson in turn judged their modification by the Twitchell method as a yardstick. No really critical appraisal of the accuracy of the Twitchell method was made until Cocks, Christian and Harding in 1931 challenged the accuracy of isoöleic acid determinations made by that method of separation. They made a very careful and painstaking study of isoöleic acid estimation and claimed for their procedure higher and more accurate figures.

Though precipitation conditions in the Baughman-Jamieson modification are somewhat different from those of Twitchell's method, in that lead salts of solid acids are precipitated by double decomposition of potassium soaps and lead acetate in the presence of the potassium acetate formed during neutralization of excess KOH, and other factors, such as volume of alcohol and quantity of lead acetate might be expected to affect the results obtained, the Baughman-Jamieson Method has not been shown to be less reliable than the Twitchell.

While there has always been considerable uncertainty concerning the estimation of isoöleic acid in hydrogenated oils by the Twitchell Method, the simplicity of the operation and the unquestioned reproducibility of results have commended its use to chemists for that purpose. Granted that isoöleic figures may be low the values for different samples probably are comparable and afford a convenient index to the effect on an oil of variation of hydrogenation conditions. A recent paper by Bertram[8] has indicated a further complexity to the problem which cannot be ignored. In the course of an investigation of hazelnut oil as a readily available source of pure oleic acid, Bertram noted iodine values as high as 70 on solid acids obtained by the Twitchell separation from unhydrogenated oil. Suspecting the presence of an oleic acid isomer or homologue the unsaturated solid acids were subjected to an exhaustive investigation which revealed beyond any possibility of doubt that the unsaturated acid in question was really the usual 9:10 oleic acid.

Bertram's explanation for the anomalous behavior of hazelnut oil in the Twitchell separation seems quite convincing. "It appears that linoleic acid raises greatly the solubility of the oleic acid lead salt, and that a fixed (minimum ?) amount of linoelic acid is necessary for the lead oleate to remain in solution." Data are given showing Twitchell separations made upon mixtures of pure oleic and palmitic acids which amply substantiate his conclusions.

If Bertram's conclusions and proof are to be accepted they have a most important bearing upon the estimation of isoöleic acid in all oils, particularly those in which the linoleic acid content has been lowered by hydrogenation. Contrary to the claims of Cocks, Christian and Harding it is probable that such oils, by the Twitchell separation, actually show more than their true content of isoöleic acid.

EXPERIMENTAL WORK

Hazelnut oil was obtained by extraction of the ground nuts with petroleum ether. Olive oil of the finest edible grade was obtained from a source in which we have the utmost confidence. The other oils used were such as could be secured conveniently.

Table I shows the result of thio-

TABLE NO. 1

		Thiocyanogen Analyses					Twitchell Analyses							
	Oil	I. V.	T. V.	% Linoleic Acid	% Oleic Acid	% Saturated Acids	I. V. Mixed Acids	% Solid Acids	I. V. Solid Acids	% Liquid Acids	% Saturated Acids	% Isoöleic Acids	% Oleic Acid	% Linoleic Acid
1	Hazelnut oil	85.4	79.1	7.3	84.6	8.1	89.3	34.4	75.1	65.6	5.8	28.6	60.6	5.0
2	Olive oil	84.3	76.1	9.5	78.9	11.6	88.2	31.6	58.5	68.4	11.1	20.5	59.4	9.0
3	Almond oil	94.8	78.2	19.2	71.6	9.2	99.2	10.1	22.4	89.9	7.6	2.5	72.3	17.6
4	Red oil	92.5	78.0	16.0	70.6	13.4	93.6	7.9	34.6	92.1	4.9	3.0	83.3	8.8
5	Hydrogenated cottonseed oil	9.2	9.0	0.3	9.8	89.9	9.7	83.0	2.4	17.0	80.8	2.2	17.0	0.0
6	Corn oil	128.8	79.8	56.6	35.5	7.9	134.7	11.0	21.0	89.0	8.4	2.6	31.6	57.4
7	50% Red oil (4); 50% Hydrogenated cottonseed oil (5)	50.8	43.3	8.3	39.8	51.9	50.8	45.3	4.8	54.7	42.9	2.4	54.7	0.0

cyanogen and Twitchell analyses (uncorrected) on seven samples. The findings of Bertram on hazelnut oil are abundantly corroborated. There appears to be considerable discrepancy between Twitchell and thiocyanogen analyses on samples 4, 5 and 7. Otherwise fairly good agreement is shown. That this disagreement is a result of the solubility of lead salts of saturated fatty acids in alcohol in the Twitchell separation is shown if the saturated acids obtained by Twitchell are corrected by the difference in saturated acids shown by the two methods and the Twitchell percentages are recalculated. Corrected results are shown in Table 2. This demonstration becomes more convincing when it is pointed out that the palmitic acid content of all three oils is considerable. It is obvious that the usefulness of the Twitchell method alone for estimating saturated, oleic and linoleic acids is rather limited.

TABLE 2

Sample No.	Thiocyanogen Linoleic	Thiocyanogen Oleic	Thiocyanogen Saturated	Twitchell Corrected Linoleic	Twitchell Corrected Oleic*	Twitchell Corrected Saturated
4	16.0	70.6	13.4	17.2	69.4	13.4
5	0.3	9.8	89.9	0.7	9.4	89.9
7	8.3	39.8	51.9	8.3	39.8	51.9

*Including isoöleic.

The results on olive oil (sample 2) are consistent with Bertram's theory. High apparent isoöleic values on other samples of olive oil have been rather puzzling but now seem explicable. The analysis of sample 7 appears at variance with Bertram's reasoning, since a low linoleic content here fails to cause high apparent isoöleic. Perhaps the absolute linoleic acid concentration is not so much of a factor as the ratio linoleic/oleic.

As a further check lead salts of solid fatty acids from the first alcohol precipitation of hazelnut fatty acids were introduced into the liquid fatty acid—alcohol filtrate from an equal weight sample of corn oil.[A] Conversely, the solid fatty acid lead salts from the corn oil were introduced into the liquid acid filtrate from hazelnut oil analysis.[B] Finally olive oil (sample 2) and corn oil (sample 6) were mixed in equal proportions and analyzed by Twitchell separation method.[C] The results, in duplicate, are shown in Table 3. No explanation is offered for the failure of duplicates to check more closely, but in general it is felt that these figures are in harmony with Bertram's theory.

TABLE 3

	A Hazelnut Solid in Corn Oil Liquid	B Corn Oil Solid in Hazelnut Liquid	C Olive Oil 50% + Corn Oil 50%
Per cent Isoöleic acid ...	8.1	1.6	3.2
	3.7	1.2	2.3

A comparison of the Twitchell method with that of Cocks, Christian and Harding was made in this laboratory shortly after publication of the latter method. The oils used were hydrogenated cottonseed. Some of the results, hitherto unpublished, are presented in condensed form in Table 4. While the differences in apparent isoöleic content between the two methods are quite striking, thiocyanogen analyses made upon the solid acids showed at once the chief reason for the higher values by the Cocks method. Apparently the separation, using their procedure, between liquid and solid acids is quite imperfect, resulting in serious contamination of the latter. When the solid acids are corrected for this contamination, shown by thiocyanogen analysis and assuming the same linoleic/oleic ratio as in the liquid acids, the isoöleic figures are in much better agreement and, in our opinion, the differences between the two methods are of no real consequence.

TABLE 4

Hydrogenated Cottonseed Oils	Per cent Isoöleic Acid Twitchell*	Cocks	Corrected Cocks
1	5.4	11.3	6.1
2	4.2	12.2	6.1
3	4.9	10.3	6.3
4	5.1	10.3	6.0
5	5.4	12.6	8.1
6	5.4	12.2	7.6
7	7.7	13.9	8.6
8	8.8	13.5	8.4

*In every case thiocyanogen analysis of Twitchell solid acids showed no correction for liquid acid contamination.

In the course of the present investigation analyses of hazelnut and olive oil were made by both Twitchell and the Baughman-Jamieson methods. The results are shown in Table 5. Contrary to expectation the two methods are in wide disagreement. Though the less important linoleic and saturated acids are low by Twitchell they are far more in error by the Jamieson modification. On the other hand the enormous variation between apparent isoöleic content, judged from previous experience with the Twitchell on unhydrogenated oils, seems distinctly in favor of the procedure of Baughman and Jamieson. In an effort to throw more light on these findings the chief differences between these modifications were examined briefly. The effects of neutralizing fatty acids, presence of potassium acetate, increasing volume of alcohol, varying lead acetate concentration, lengthening time of crystallization and higher crystallization temperature upon isoöleic yield obtained by Twitchell separation, were determined. Table 6 shows the results of these experiments.

TABLE 5

Per cent	Hazelnut Oil Twitchell	Hazelnut Oil Jamieson	Olive Oil Twitchell	Olive Oil Jamieson
Solid	34.4-31.1	2.9- 3.9	31.6-30.7	7.7- 8.1
Liquid	65.6-68.9	97.1-96.1	68.4-69.3	92.3-91.9
Saturated	5.8- 5.8	2.6- 3.0	11.1-11.0	7.3- 7.5
Isoöleic	28.6-25.3	0.3- 0.9	20.5-19.7	0.4- 0.6
Oleic	60.6-63.9	95.3-93.9	59.4-60.4	87.1-86.5
Linoleic	5.0- 5.0	1.8- 2.2	9.0- 8.9	5.2- 5.4

TABLE 6

Exp.	Fatty Acids Neut.	Twitchell Analysis Modifications Gms. $Pb(Ac)_2$	Gms. KAc*	Ml. Alcohol	° C.	Hrs.	% Isoöleic Acid Olive Oil	Hazelnut Oil
1	Yes	5.0	0	100	15	24	1.6	23.5
2	Yes	1.5	0	100	15	24	17.8	8.7
3	Yes	5.0	0	100	15	72	4.1	17.0
4	Yes	5.0	0	100	15	24	16.3	15.0
5	No	1.5	1.0	100	15	24	0.0	0.0
6	No	1.5	5.0	100	15	24	0.0	0.0
7	No	1.5	1.0	100	15	24	0.0	0.0
8	No	1.5	5.0	100	15	24	0.0	0.0
9	No	1.5	0.2	100	15	24	0.0	0.0
10	No	1.5	0	100	15	24	...	20.8
11	No	1.5	0	150	15	24	...	26.1
12	No	1.5	0	200	15	24	15.0	28.6
13	No	1.5	0	100	27-30	24	0.7	0.9
14	No	1.5	0	100	15	72	27.8	27.5

*In every case added KAc resulted in no precipitate of lead salts of solid acids—neither saturated nor unsaturated.

Though the figures are very confusing and the data are too meager to justify conclusions the following

observations are made for what they may be worth:

(1) Neutralization of fatty acids and especially presence of potassium acetate are very important factors.
(2) The temperature is critical.
(3) Time of crystallization, volume of alcohol and concentration of lead acetate are less important factors.

CONCLUSIONS

1. The linoleic, oleic and saturated acid content of most common oils, except for limitations stipulated, may be estimated satisfactorily by the thiocyanogen method of analysis. None of the four methods generally employed for separation of liquid and solid fatty acids, which depend upon the insolubility of lead salts of saturated fatty acids, are entirely trustworthy.

2. The determination of isoöleic acid, by the Twitchell method of separation, is subject to the error of partial solubility of lead isoöleate. In some cases, a low linoleic/oleic ratio may cause a partial precipitation of lead oleate with the lead salts of isoöleic and saturated acids.

3. The Cocks-Christian-Harding method, for estimation of isoöleic acid, is no improvement on the Twitchell method.

4. While the Baughman-Jamieson modification is apparently free from the second error mentioned above, more work on hydrogenated oils is needed to establish the reliability of its isoöleic acid figures.

5. Further work should be done toward development of a dependable method for isoöleic acid estimation. It should be borne in mind that a method to be satisfactory need not necessarily concern itself with the determination of linoleic or saturated acids.

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CHEMICAL CONSTITUTION OF THE OILS FROM SUPERIOR AND INFERIOR FLAXSEEDS. (a) (b)

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THE oil of flaxseed consists of a mixture of glycerides of saturated and unsaturated fatty acids, and about 1% unsaponifiable matter. Substances composing the unsaturated fatty acid portion of linseed oil include the various isomers of oleic acid, α- and β-linoleic acid, and α- and β-linolenic acid. The change in the degree of unsaturation of an oil ordinarily is presumed to be caused by a change in the relative amounts of the three general types of unsaturated fatty acids present.

This paper describes a detailed study made on the unsaturation of two varieties of flaxseed oil grown in different localities; it shows the extent of variation of the iodine number with the change in the index of refraction, and the percentages of the unsaturated fatty acids composing the oils.

The two kinds of linseed oils studied were those obtained by extracting ground Abyssinian Yellow and Bison flaxseed in the customary Soxhlet fat extractor with petroleum ether. Abyssinian Yellow linseed oil is a superior oil having a relatively high iodine number, while Bison linseed oil is an inferior oil and usually has a somewhat lower iodine absorption value.

Experimental

Five samples each of the two varieties, grown in as many localities, were ground in a roller mill having 40 corrugations to the inch. The oil content of the seed was determined by extraction with petroleum ether (30°-60° C. B. P.). On occasion, samples of 50-60 grams in weight were extracted with the same solvent in order to obtain larger quantities of oil for examination.

Index of refraction was determined by an Abbé refractometer at 20° C., or corrected to this temperature.

The iodine numbers were determined according to the Rosenmund and Kuhnhenn[1] method, using a solution of bromine and pyridine sulfate in glacial acetic acid as the active halogen reagent. Such determinations were made on the oils as well as on the saturated and unsaturated fatty acid fractions obtained by the use of the Twitchell lead salt alcohol procedure. In each case, the lead salts were decomposed by treatment with dilute nitric acid and the free fatty acids were extracted with diethyl ether. An aliquot of the ether solution containing from 0.2 to 0.4 gram of fatty acids was evaporated to dryness under vacuum, and accurately weighed. The residue was dissolved in 10 cc. of chloroform and treated in a similar manner as the linseed oil, or unsaturated fatty acids.

Thiocyanogen numbers were determined according to the procedure of Kaufmann and Keller[2] with the added precaution that all the glass apparatus used in these determinations was dried in an electric oven at about 110° C. overnight, or longer before being used. By having all the glassware perfectly dry, very good check values were obtained among individual determinations of the thiocyanogen numbers for any one variety of linseed oil.

For the preparation of the free fatty acids, approximately 30 gram samples of linseed oil were saponified with a 0.75N solution of potassium hydroxide in alcohol. The ethyl alcohol used in the saponification process was made aldehyde-free by the method described by Dunlap.[3] Nitrogen saturated with alcoholic vapors by bubbling it through ethyl alcohol was passed through the saponification flask to prevent any

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(b) Condensed from a thesis presented to the Graduate School of the University of Minnesota by R. A. Gross, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, March, 1937.