# Crystallization at Low Temperatures as a Technique in the Analysis of Oils[1]

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In determining the unsaturated fatty acids in glycerides in which the presence of linolenic acid is suspected, it is necessary first to separate and to determine the saturated fatty acids. The two methods most commonly employed for this are the Twitchell (1) lead salt separation, or one of its modifications and the Bertram permanganate oxidation (2). These methods are laborious and require at least 5 grams of the glyceride. It appeared that low temperature crystallization from acetone solution suggested by Brown and Stoner (3) and adapted to quantitative use by Earle and Milner (4) might constitute an improvement over these older techniques. With the content of the saturated fatty acids established, the amounts of oleic, linoleic and linolenic acid could then be calculated from the iodine and thiocyanogen values.

## Experimental

### I. Apparatus and Reagents

The apparatus required included: 50 cc. narrow-neck centrifuge tubes, low temperature (—100° C.) thermometer, 125 cc. iodine absorption flasks, a capillary pipette, and an insulated low temperature bath of 600 cc. capacity containing a mixture of alcohol and dry ice. Other apparatus consisted of pipettes, burettes, etc. commonly found in every laboratory.

The reagents required were: a 20% solution of KOH in alcohol, concentrated HCl, petroleum ether (40 to 60° C.); dried, redistilled acetone; 95 percent alcohol; 1 to 2 pounds of dry ice and the reagents employed for the determination of iodine values (Wijs) and thiocyanogen values. A .2N thiocyanogen solution was used in preference to other concentrations.

### II. Separation of Saturated Acids

A one-gram sample of fat was saponified by boiling for 12 minutes under a reflux condenser with 2 cc. of a 20 percent solution of KOH in alcohol. Following saponification, the fatty acids were liberated by the addition of 4 cc. HCl and 10 cc. of water. After the water phase had been removed with a capillary pipette, the fatty acids were transferred to a 15 cc. centrifuge tube with the aid of small portions of petroleum ether. Five to 8 cc. of water were added, the mixture was agitated and then centrifuged for 5 minutes at approximately 2000 r.p.m. The washed fatty acids were transferred to a weighed 50 cc. centrifuge tube by means of the capillary pipette with the aid of small portions of petroleum ether. The solvent and traces of water were removed by shaking and heating under reduced pressure in a hot water bath until constant weight was obtained.

The total fatty acids were separated into three fractions by low temperature crystallization from acetone. They were dissolved in 45 cc. dried, redistilled acetone in a 50 cc. centrifuge tube which was then immersed in the low temperature alcohol bath. The temperature was lowered to —40° C. by the addition of solid $CO_2$. After standing 15 to 20 minutes, the tube was placed in a metal centrifuge tube which had been cooled to the same temperature and was centrifuged briefly (one minute or less), to throw out the crystalline saturated acids (Fraction A). The centrifuge tube was returned immediately to the low temperature bath and the supernatant solution (Fraction B) was transferred to a weighed tube by means of the capillary pipette. Ten cc. of acetone at —40° C. was blown from a pipette into the tube containing the saturated acids and the tube was rotated rapidly to wash the precipitate. After standing in the low temperature bath for 5 minutes, centrifugation was accomplished as before. The supernatant solution was pipetted off and was designated Fraction C.

The crystalline fatty acids (Fraction A) were dissolved in 10 cc. of acetone and were then recrystallized. The supernatant solution pipetted off following the recrystallization was added to Fraction C.

All fractions were freed from solvent under reduced pressure at a maximum temperature of 50° C. They were placed under vacuum in a desiccator over $P_2O_5$ before weighing or determining their iodine and thiocyanogen values.

Whatever traces of unsaturated acids were present in the saturated Fraction A was revealed by the iodine values which ranged from 1 to 7, with an average value of 3 for the 15 oils investigated. The weight of the saturated fatty acids was corrected for the presence of unsaturated acids according to the iodine number found, on the assumption that all of the contamination was due to oleic acid. This seemed justifiable since, at the temperature used, oleic acid, of all the unsaturated acids, would be the most likely to persist through the recrystallization.

Fraction C, which constituted only 2 to 7 percent of the total, was weighed and its iodine value determined. Its iodine value, which averaged about 75, indicated that in addition to the saturated acids, considerable amounts of oleic and linoleic acids were present. The presence of linolenic acid was discounted because of its great solubility in acetone at —40° C. For purposes of calculation, it was assumed that the iodine value of Fraction C was due to a mixture of equal amounts of oleic and linoleic acid. The corrected weight of Fraction C plus the corrected weight of Fraction A was taken as the amount of saturated acids.

The justifiability of the assumption that Fraction C contained substantial amounts of oleic and linoleic acids was verified by an analysis of such a fraction prepared from corn oil. It was found to contain 41 percent of saturated acids and to have an iodine value of 77. This iodine value was found to be due to a mix-

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ture of 58 percent oleic acid and 42 percent of linoleic acid. Since the average amount of Fraction C as obtained in our analyses represented less than 5 percent of the total fatty acids, and the average iodine number of the fractions was approximately 75, any assumptions made in the correction for unsaturation did not affect the values for the saturated acids by more than 1.5 units percent.

III. Examination of Unsaturated Acids

Iodine Values were determined in duplicate by the Wijs method and thiocyanogen values by the Kaufmann method. In the latter determination, a 0.2N thiocyanogen solution, 150 to 200 percent in excess of the amount required by theory was allowed to react for 24 hours at 20° to 25° C.

IV. Calculations

After determining the saturated acids and the iodine and thiocyanogen values of the unsaturated acids, the percentages of oleic (O), linoleic (L), and linolenic (Le) acid were calculated by the formulae given below which are modifications of Kaufmann's formulae (5). I and T are iodine and thiocyanogen values, respectively, of the total fatty acids calculated from the values determined on the unsaturated acids. S is the percentage of saturated acids.

$$(1)\ \ Le = 1.59\ T - .123\ I - 1.30\ (100\text{-}S)$$
$$(2)\ \ L = 1.35\ I - 3.19\ T + 1.63\ (100\text{-}S)$$
$$(3)\ \ O = 100 - (S + L + Le)$$

The formulae are based upon thiocyanogen values for pure oleic, linoleic, and linolenic acids of 89.4, 96.8, and 167.5 respectively as they have been established recently through an extensive study by Riemenschneider and Wheeler (6). These values were shown to be more nearly accurate than the values originally given by Kaufmann (8). The theoretical constants and the calculated formulae given above for the conditions employed differ only slightly from those determined at a lower reaction temperature by Matthews, Brode and Brown (7).

When the solution of equation 1, given above, resulted in a zero or negative value, no linolenic acid was present and therefore these formulae were not applicable. In that event, the content of oleic and linoleic acid was calculated by means of the iodine value of the unsaturated acids alone, as follows:

$$O = \frac{181.1 - I}{91.2}\ (100 - S)$$

$$L = 100 - (O + S)$$

## Discussion

Speed and applicability to small samples are the advantages claimed for the proposed method of determining the distribution of fatty acids. By the crystallization technique presented, the saturated acids can be determined in 3 to 4 hours and only one gram of fat is required. However, its application is limited. It cannot be used with oils containing appreciable amounts of short-chain saturated acids nor with unsaturated acids other than oleic, linoleic and linolenic acids. For example, erucic acid in rape seed oil was found to act like a saturated acid.

Results of an analysis of a known mixture of pure fatty acids (Table I) indicate that the proposed method was accurate within ± 1.5 units percent for saturated acids and within ± 2 units percent for oleic and linoleic acids. It therefore compares favorably with other methods for saturated acids. For the unsaturated acids, the accuracy is limited by that of the iodine-thiocyanometric technique which may be less precise but certainly is much more rapid than other techniques such as oxidation or distillation.

TABLE I

Analysis of Corn Oil and a Mixture of Pure Fatty Acids

| | Corn Oil | Synthetic Mixture * |
|---|---|---|
| A. Analytical Data | | |
| (1) Total Acids | 0.9645 gm. | 1.0030 gm. |
| (2) Fraction A (Unsaturated) | 0.8340 gm. | 0.8597 gm. |
| Iodine Value in percent | 142.9 | 144.6 |
| (3) Fraction B (Saturated) | 0.1070 gm. | 0.1079 gm. |
| Iodine Value in percent | 4.8 | 1.2 |
| (4) Fraction C (Mixed Saturated-Unsaturated) | 0.0368 gm. | 0.0354 gm. |
| Iodine Value in percent | 65.2 | 68.7 |
| B. Composition Calculated from Analytical Data | | |
| (1) Percent Saturated Acids | | |
| Present | ..... | 12.1 |
| Found | 11.8 | 12.4 |
| (2) Percent Oleic Acid | | |
| Present | ..... | 37.0 |
| Found | 36.9 | 35.1 |
| (3) Percent Linoleic Acid | | |
| Present | ..... | 51.0 |
| Found | 51.3 | 52.5 |

* The synthetic mixture was composed of recrystallized palmitic and stearic acids, plus distilled ethyl oleate and linoleic acid in amounts to approximate the composition of corn oil.

The results obtained by the proposed method are consistently reproducible as indicated by the analyses of 15 seed oils recorded in Table II. The variation between duplicate samples in the determination of the saturated acid content was 2 units percent or less, with the exception of cherry seed oil. Similarly, the variation in the linoleic acid content determined on duplicate samples was 4 units percent or less with the exception of perilla and sesame oils. The results for oleic and linolenic acid approached the same degree of precision.

TABLE II

The Fatty Acid Composition of Seed Oils

| | Iodine Value of Oil | Saturated Acids | Oleic Acid | Linoleic Acid | Linolenic Acid |
|---|---|---|---|---|---|
| | *Pct.* | *Pct.* | *Pct.* | *Pct.* | *Pct.* |
| Alfalfa | 173.0 | 7.5 | 30 | 22 | 42(40.4) |
| Cherry* | 109.1 | 6.0 | 61 | 34 | .... |
| Clover | 134.8 | 13.2 | 35 | 36 | 18 |
| Cucumber | 126.7 | 15.3 | 24 | 61 | .... |
| Hemp | 168.2 | 6.3 | 30 | 31 | 33(29.1) |
| Linseed* | 174.0 | 6.7 | 28 | 27 | 39(39.4) |
| Muskmelon | 139.5 | 10.2 | 21 | 70 | .... |
| Perilla* | 209.2 | 5.1 | 12 | 20 | 63(60.0) |
| Poppy | 141.2 | 8.2 | 24 | 68 | .... |
| Pumpkin | 119.6 | 11.7 | 40 | 49 | .... |
| Raisin* (Crude) | 132.3 | 9.7 | 28 | 63 | .... |
| Raisin* (Refined) | 132.8 | 9.5 | 29 | 61 | .... |
| Sesame | 110.1 | 13.5 | 47 | 39 | 3? |
| Squash | 115.4 | 15.4 | 36 | 49 | .... |
| Sunflower | 132.5 | 9.9 | 31 | 58 | 1? |
| Watermelon | 133.7 | 14.1 | 23 | 67 | .... |

* Obtained from commercial sources. All other oils were extracted in this laboratory. The values in parentheses were calculated from hexabromide values obtained by the usual technique (Vegetable Fats and Oils. G. S. Jamieson, The Chemical Catalogue Co., New York, 1932), using the factor 96.0 (Norman L. Mathews, Wallace R. Brodie, and J. B. Brown, J. Amer. Chem. Soc. 63, 1064, 1941.)

In general, the results of the analyses fell within the range of values reported in the literature (Table III). Outstanding exceptions were the data on alfalfa seed and perilla oil. However, the published data on alfalfa seed oil are rather limited and those on perilla oil spread over such a wide range that ours are probably as acceptable as any. Published data on clover

TABLE III

Comparison of Analytical Results With Analyses in the Literature *

| Seed Oil | Percent Linoleic Found | Percent Linoleic Reported in the Literature | | |
|---|---|---|---|---|
| | | Single Value or Range of Linoleic Acid Content | Number of Analyses | References |
| Alfalfa | 22 | 68 | 1 | (9) |
| Cherry | 34 | 42 | 1 | (10) |
| Clover | 36 | .... | .... | .... |
| Cucumber | 61 | .... | .... | .... |
| Hemp | 31 | 18-69 | 4 | (10) |
| Linseed | 27 | 23-62 | 15 | (10, 11) |
| Muskmelon | 70 | 68 | 1 | (12) |
| Perilla | 20 | 33-59 | 5 | (10, 11) |
| Poppy | 68 | 62, 65 | 2 | (10) |
| Pumpkin | 49 | 45, 46 | 2 | (10) |
| Raisin | 61 | 46-73 | 6 | (10) |
| Sesame | 39 | 37-47 | 3 | (10) |
| Squash | 49 | 44 | 1 | (10) |
| Sunflower | 58 | 54, 59 | 2 | (11) |
| Watermelon | 66 | 68 | 1 | (13) |

* Comparisons are limited to linoleic acid content since this acid was the major fatty acid present in most of the oils.

and cucumber seed oils were also found to be very meager. All comparisons were limited to the linoleic acid content since two-thirds of the oils tested contained linoleic acid as the major component.

## Summary

1. A rapid method for determining the saturated, oleic, linoleic and linolenic acids in a 1-gram sample of fat has been described.

2. Saturated fatty acids were separated by quantitative crystallization from acetone solution at —40° C.

3. The three unsaturated acids, oleic, linoleic and linolenic, were calculated from the iodine and thiocyanogen values of the remaining liquid acids.

4. Analysis of known mixtures of fatty acids demonstrated the accuracy of the method to be approximately ± 2 units percent.

5. Duplicate determinations of the fatty acid distribution in natural oils agreed within 0 to 4 units percent.

6. Data on the oleic, linoleic, and linolenic acid content of 15 seed oils were presented.

## LITERATURE CITED

(1) Twitchell, E. Precipitation of solid fatty acids with lead acetate in alcoholic solution. J. Ind. Eng. Chem. *13*, 806 (1921).

(2) Bertram, S. H. Determination of water-insoluble saturated fatty acids in fats or fatty acid mixtures. Chem. Weekblad *24*, 226-9 (1927).

(3) Brown, J. B., and Stoner, G. G. Studies on the chemistry of the fatty acids. I. The purification of linoleic acid by crystallization methods. J.A.C.S. *59*, 3-6 (1937).

(4) Earle, F. R., and Milner, R. T. A crystallization method for the determination of saturated fatty acids in soy bean oil. Oil and Soap *17*, 106 (1940).

(5) Kaufmann, H. P., and Keller, M. The field of fats. X. The thiocyanate method of analysis for fats containing linolenic acid. Analysis of linseed oil. Z. angew. Chem. *42*, 73-6 (1929).

(6) Riemenschneider, R. W., Swift, C. E., and Sando, C. Thiocyanogen values of the methyl esters of oleic, linoleic and linolenic acids. The application of these values in the analysis of mixtures. Oil and Soap, *18*, 203 (1941).

(7) Matthews, N. L., Brode, W. R., and Brown, J. B. Studies on the chemistry of the fatty acids. VIII. The reaction of thiocyanogen with linoleic and linolenic acids. The application of the thiocyanogen reaction to the determination of these acids in fatty acid mixtures. Oil and Soap, *18*, 182-87 (1941).

(8) Kaufmann, H. P. Thiocyanometry of fats and fat mixtures. Z. Untersuch. Lebensm. *51*, 15 (1926).

(9) Schuette, H. A., Vogl, H. A., and Wartinbee, C. H. Alfalfa seed oil. Oil and Soap *15*, 35 (1938).

(10) Hilditch, T. P. "Chemical constitution of natural fats, oils and waxes." John Wiley and Sons, Inc. (1940).

(11) Dean, H. K. "Utilization of the fats." Chemical Publishing Company of New York, Inc. (1938).

(12) Baughmann, W. F., and Jamieson, G. S. Cantaloup seed oil. J.A.C.S. *42*, 2398 (1920).

(13) Nolte, A. J., and von Loesecke, H. W. Characteristics and composition of watermelon seed oil (Cuban Queen variety). J.A.C.S. *61*, 889 (1939).

# The Preparation of Standard Soil Material for Testing Detersive Efficiency

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## Introduction

During the several years that we have been working upon a test for the evaluation of detergency in this laboratory we have tried every soiling formula that has come to our attention. It is, however, very rare to find in the published article a description of the method of applying the soiling solution or mixture to the fabric.

A brief survey of the literature discloses that Brauner (1) (2) used a Standard Soil consisting of various oils with cocoa, coffee, wine, milk, blood, rust and ink. Bergell (3) used linden charcoal in a fat ether solution and determined the amount of "dirt" deposited by weighing the goods before and after soiling. Gehm (4) prepared a mixture of oil, lanolin, egg yolk, egg albumin, milk, cocoa, soot, starch and sugar in water and applied this to desized cotton and rayon fabric with a viscose sponge. Hill (5) used a mixture of Oildag, olive oil, tallow and mineral oil and applied it to the goods in a small agitator type washing machine, claiming uniform deposition of the mixture over the surface of the fabric. Hoyt (6) prepared a mixture of lubricating oil, edible tallow and lamp black but made no mention of the method of application. He also reported (7) the use of an emulsion of lanolin, white mineral oil and deflocculated graphite in water, but again failed to state how he applied it. Morgan (8) studied the technic of applying a mixture of carbon tetrachloride, Russian Tallow, Nujol and lamp black to white cotton sheeting. He also wrote (9) about a test for detergency but gave no data on his soiling formula or its application. Schreive and Stiepel (10) applied mixtures of mineral oil, fatty oil, fatty acid and linden charcoal to linen cloth by means of a brush. Rhodes and Brainard (11) applied the A. O. C. S. soiling mixture by placing 100 ml. in a porcelain evaporating dish and drawing strips of desized cotton sheeting through the mixture until