# A Comparison of Various Methods of Determining Iodine Values, and Their Effect on the Calculated Results of Fat Analysis

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## Introduction

THE "iodine value" of fats and oils is one of the most valuable and widely used characteristic for the evaluation and differentiation of these compounds, representing as it does a measure of the degree of unsaturation. In addition to its use in identifying and classifying the fats, the iodine value may be combined with other determinations, such as the thiocyanogen value or the separation of fatty acids by the lead salt-alcohol or lead salt-ether methods, and it thus serves as a means of calculating the proportions of the various fatty acids which are present in the sample.

A number of methods have been devised for the estimation of the iodine value, and it is well known that all the proposed methods do not yield identical results. In official work, it is expressly required that the report of an iodine value should always be accompanied by a statement of the method used. For the identification of a fat it is usually sufficient to determine the iodine value accurate to one or two per cent. and in such a case the method used is of little importance. However, if the iodine value is used as a basis for further calculations, the accuracy of the determination becomes very important, since a difference of one per cent. in iodine value may make a difference of several per cent. in the calculated proportion of one of the constituents (for example linolic acid).

Although these differences are realized by all workers in fat chemistry, there is little data in the literature regarding the actual magnitude of the differences in iodine values by different methods. Several investigators have compared two or three of the existing methods, and hold various opinions as to their value. Thus Delaby and Charonnat,[1] Croxford[2] and van Loon[3] prefer the Wijs method, while Stewart and Banerjea[4] and Kubelka and his co-workers[5] consider the Hanus method to be better. The most extensive investigation is that of Yushkevich,[6] who compared the results by eight methods on a variety of fats and oils, and considered the method of Kaufmann to be the most satisfactory as well as the most convenient. None of these investigators, however, has considered the effect of using the results of the various methods for further calculations.

The work to be described was undertaken to compare the results by the commonly used methods on a typical group of common edible fats, and then to use the results by each method for calculation of the proportions of fatty acids present in each.

## Methods Used

The iodine value methods which were tested comprise those in common use at the present time. The methods are all similar in principle, involving the treatment of a solution of the fat or oil in a suitable solvent with a solution containing a free halogen, for a definite length of time; then the conversion of the excess halogen to iodine by treatment with potassium iodide, followed by titration with thiosulphate in the usual way. They are as follows:

*Wijs Method.*[7] This is the official method of the American Oil Chemists' Society, the American Chemical Society, one of the official methods of the Association of Official Agricultural Chemists, and recommended by the 9th International Union of Pure and Applied Chemistry. It is the method most widely used in English-speaking countries.

Reagent: Iodine chloride dissolved in glacial acetic acid.
Fat Solvent: Chloroform or carbon tetrachloride.
Time of Reaction: 30 minutes.

*Hanus Method.*[8] One of the official methods of the Association of Official Agricultural Chemists and of the German "Wizöff" (Fat Investigation Committee). The most widely used method in continental countries.

Reagent: Iodine Bromide dissolved in glacial acetic acid.
Solvent: Chloroform.
Time of Reaction: 30 minutes.

*Kaufmann Method.*[9] One of the official methods of the "Wizoff"; widely used in Europe, especially in Germany and the U. S. S. R.

Reagent: Bromine dissolved in a saturated solution of sodium bromide in anhydrous methanol.
Solvent: Chloroform.
Time of Reaction: 30 minutes.

*Rosenmund-Kuhnhenn Method.*[10] Not an official method, but of considerable interest particularly to research workers, since it is claimed to give theoretical iodine values with cholesterol and the other sterols ([11], [12], [13], [14], [15]). (It should be noted that Werner[16] has recently made the same claim for the Kaufmann method).

Reagent: Pyridine sulphate dibromide dissolved in glacial acetic acid.
Solvent: Chloroform.
Time of Reaction: 5 minutes.

*Margosches Method.*[17] Not an official method, but coming into wide use in plant control work, owing to its rapidity and the fact that it requires no special reagent.

Reagent: N/5 alcoholic iodine solution.
Solvent: Warm absolute alcohol.
Time of Reaction: 5 minutes.

## Experimental

The samples of fat used were as follows:

Refined Cottonseed Oil
Refined Peanut Oil
Lard
Hydrogenated Shortening

The Wijs and Hanus reagents were prepared according to the A.O.A.C. Official Methods[18]: the Kaufmann reagent according to the Deutsche Einheitsmethoden,[19] and the Rosenmund-Kuhnhenn reagent according to the instructions in the authors' original paper.[10]

The iodine values of the fats were determined on the glycerides, in triplicate, following exactly the specifications for each method. Chloroform was used as solvent in the first four methods.

The iodine value of the mixed fatty acids was calculated from the glyceride iodine value, assuming a fatty acid content of 95.5% in each case.

The "solid acids" were separated from fifteen samples of each fat by the lead salt-alcohol method of Twitchell[20] as modified by Baughman and Jamieson.[21] In each case the iodine value of the solid acids was determined in triplicate by the same method used for the glycerides. The results were used as a basis for calculating the content of saturated acids and the iso-oleic acid, the iodine value of the liquid acids, and the content of oleic and linolic acids according to Lewkowitsch.[22]

The thiocyanogen values were determined by the method of Kaufmann[23] modified as in the Deutsche Einheitsmethoden.[19] The thiocyanogen value for the mixed fatty acids was calculated from the glyceride value, as in the case of the iodine value. The proportions of saturated acids, oleic acid and linolic acid were calculated as described by Kaufmann.

TABLE I—COTTONSEED OIL

Results of Analysis by Various Methods
*Solid Acids* (Baughman-Jamieson): 21.6% of Total Fatty Acids. $\sigma = 0.2$
*Thiocyanogen Value:* Glycerides 67.3; Fatty Acids (Calc.) 70.5

| Iodine Value Method: | Wijs | Hanus | Kaufmann | Rosenmund-Kuhnhenn | Margosches | Average All Methods |
|---|---|---|---|---|---|---|
| Iodine value, glycerides | 103.8 | 101.7 | 103.2 | 99.6 | 102.9 | 102.2 |
| Iodine value, fatty acids (calc.) | 108.7 | 106.5 | 108.1 | 104.3 | 107.8 | 107.0 |
| Iodine value, solid acids | 0.8 | 0.8 | 0.7 | 0.7 | 0.5 | 0.7 |
| Iodine value, liquid acids (calc.) | 138.4 | 135.6 | 137.6 | 132.8 | 137.4 | 136.4 |
| Calculated Composition | | | | | | |
| Baughman-Jamieson Method: | % | % | % | % | % | % |
| Saturated acids | 21.4 | 21.4 | 21.4 | 21.4 | 21.5 | 21.4 |
| Apparent iso-oleic acid | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 |
| Oleic acid | 36.7 | 39.3 | 37.6 | 41.2 | 37.7 | 38.5 |
| Linolic acid | 41.7 | 39.1 | 40.8 | 37.2 | 40.7 | 39.9 |
| Thiocyanogen Method: | | | | | | |
| Saturated acids | 21.7 | 21.7 | 21.7 | 21.7 | 21.7 | 21.7 |
| Oleic and iso-oleic acid | 35.8 | 38.3 | 36.5 | 40.7 | 36.8 | 37.6 |
| Linolic acid | 42.5 | 40.0 | 41.8 | 37.6 | 41.5 | 40.7 |

TABLE II—PEANUT OIL

Results of Analysis by Various Methods
*Solid Acids* (Baughman-Jamieson): 18.8% of Total Fatty Acids. $\sigma = 0.1$
*Thiocyanogen Value:* Glycerides 69.2; Fatty Acids (Calc.) 72.5

| Iodine Value Method: | Wijs | Hanus | Kaufmann | Rosenmund-Kuhnhenn | Margosches | Average All Methods |
|---|---|---|---|---|---|---|
| Iodine value, glycerides | 96.5 | 95.4 | 95.8 | 93.4 | 97.1 | 95.6 |
| Iodine value, fatty acids (calc.) | 101.0 | 99.9 | 100.3 | 97.8 | 101.6 | 100.1 |
| Iodine value, solid acids | 1.7 | 1.8 | 1.4 | 1.3 | 1.3 | 1.5 |
| Iodine value, liquid acids (calc.) | 124.0 | 122.6 | 123.2 | 120.2 | 124.9 | 123.0 |
| Calculated Composition | | | | | | |
| Baughman-Jamieson Method: | % | % | % | % | % | % |
| Saturated acids | 18.4 | 18.4 | 18.5 | 18.5 | 18.5 | 18.5 |
| Apparent iso-oleic acid | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 |
| Oleic acid | 51.0 | 52.3 | 51.7 | 54.4 | 50.2 | 51.9 |
| Linolic acid | 30.2 | 28.9 | 29.5 | 26.8 | 31.0 | 29.3 |
| Thiocyanogen Method: | | | | | | |
| Saturated acids | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 |
| Oleic and iso-oleic acid | 48.8 | 50.1 | 49.6 | 52.4 | 48.2 | 49.8 |
| Linolic acid | 31.7 | 30.4 | 30.9 | 28.1 | 32.3 | 30.7 |

TABLE III—LARD

Results of Analysis by Various Methods
*Solid Acids* (Baughman-Jamieson): 37.2% of Total Fatty Acids. $\sigma = 0.3$
*Thiocyanogen Value:* Glycerides 50.9; Fatty Acids (Calc.) 53.4

| Iodine Value Method: | Wijs | Hanus | Kaufmann | Rosenmund-Kuhnhenn | Margosches | Average All Methods |
|---|---|---|---|---|---|---|
| Iodine value, glycerides | 61.7 | 61.5 | 61.4 | 60.2 | 61.7 | 61.3 |
| Iodine value, fatty acids (calc.) | 64.6 | 64.4 | 64.3 | 63.0 | 64.6 | 64.2 |
| Iodine value, solid acids | 1.1 | 1.1 | 1.0 | 0.9 | 0.5 | 0.9 |
| Iodine value, liquid acids (calc.) | 102.2 | 101.9 | 101.8 | 99.7 | 102.5 | 101.6 |
| Calculated Composition | | | | | | |
| Baughman-Jamieson Method: | % | % | % | % | % | % |
| Saturated acids | 36.7 | 36.7 | 36.8 | 36.8 | 37.0 | 36.8 |
| Apparent iso-oleic acid | 0.5 | 0.5 | 0.4 | 0.4 | 0.2 | 0.4 |
| Oleic acid | 54.4 | 54.6 | 54.7 | 56.1 | 54.2 | 54.8 |
| Linolic acid | 8.4 | 8.2 | 8.1 | 6.7 | 8.6 | 8.0 |
| Thiocyanogen Method: | | | | | | |
| Saturated acids | 40.7 | 40.7 | 40.7 | 40.7 | 40.7 | 40.7 |
| Oleic and iso-oleic acid | 46.9 | 47.2 | 47.3 | 48.7 | 46.9 | 47.4 |
| Linolic acid | 12.4 | 12.1 | 12.0 | 10.6 | 12.4 | 11.9 |

TABLE IV—HYDROGENATED SHORTENING

Results of Analysis by Various Methods

*Solid Acids* (Baughman-Jamieson): 41.5% of Total Fatty Acids. $\sigma = 0.4$

*Thiocyanogen Value:* Glycerides 58.7; Fatty Acids (Calc.) 61.5

| Iodine Value Method: | Wijs | Hanus | Kaufmann | Rosenmund-Kuhnhenn | Margosches | Average All Methods |
|---|---|---|---|---|---|---|
| Iodine value, glycerides | 63.3 | 63.1 | 62.5 | 62.5 | 63.7 | 63.0 |
| Iodine value, fatty acids (calc.) | 66.3 | 66.1 | 65.5 | 65.5 | 66.7 | 66.0 |
| Iodine value, solid acids | 24.0 | 24.0 | 23.6 | 24.0 | 23.4 | 23.8 |
| Iodine value, liquid acids (calc.) | 96.3 | 95.9 | 95.2 | 94.9 | 97.4 | 95.9 |
| Calculated Composition | | | | | | |
| Baughman-Jamieson Method: | % | % | % | % | % | % |
| Saturated acids | 30.4 | 30.4 | 30.6 | 30.4 | 30.7 | 30.5 |
| Apparent iso-oleic acid | 11.1 | 11.1 | 10.9 | 11.1 | 10.8 | 11.0 |
| Oleic acid | 54.5 | 54.7 | 55.2 | 55.4 | 54.0 | 54.8 |
| Linolic acid | 4.0 | 3.8 | 3.3 | 3.1 | 4.5 | 3.7 |
| Thiocyanogen Method: | | | | | | |
| Saturated acids | 31.7 | 31.7 | 31.7 | 31.7 | 31.7 | 31.7 |
| Oleic and iso-oleic acid | 63.0 | 63.2 | 63.9 | 63.9 | 62.5 | 63.3 |
| Linolic acid | 5.3 | 5.1 | 4.4 | 4.4 | 5.8 | 5.0 |

TABLE V—STANDARD DEVIATIONS (a)

| Method | Standard deviation of individual iodine value determinations from the averages for each method: | Standard deviation of percentages of all constituents from the average of results by all methods: |
|---|---|---|
| Wijs | 0.14 | 0.86 |
| Hanus | 0.20 | 0.36 |
| Kaufmann | 0.14 | 0.47 |
| Rosenmund-Kuhnhenn | 0.07 | 1.68 |
| Margosches | 0.41 | 0.85 |

(a) Calculated from the relation $\sigma = \sqrt{\frac{\Sigma (v^2)}{n}}$

Where $\sigma$ = standard deviation; v = deviation of an individual result from the average; n = total number of results.

The results are shown in Tables I to IV.

As a measure of the variability of each method, the standard deviation (root mean square deviation) was calculated for all the iodine values by each of the methods, and shown in Table V. As a matter of interest, the standard deviation of the calculated results of analysis by each method from the average for all the methods was also calculated, and shown in the same table.

## Summary

In general, the Wijs and Margosches methods give the highest iodine values, the Rosenmund-Kuhnhenn the lowest,* and the Margosches the most variable. (The results by the Margosches method seem to depend greatly upon the size of the sample: the smaller the sample the higher the result, and vice versa.) The least variation between individual results was shown by the Rosenmund-Kuhnhenn method, while the results of analysis by the Hanus method approach most nearly to the average for all the methods.

The results by the thiocyanogen method show in all cases higher contents of saturated acids and linolic acid than are found by the lead salt-alcohol method. This is in agreement with previous work by the author[24] and with the results of Martin and Stillman.[25] This discrepancy is probably due to incomplete precipitation of the saturated acids in the lead salt-alcohol method, since the difference is greatest in the fat with the highest saturated acid content (lard). That the precipitation is in fact incomplete has been recently shown by Grossfeld.[26]

For edible fats of the type studied, the Rosenmund-Kuhnhenn and Margosches methods are not recommended, the first because of the low results obtained and the second because of its variability. Of the other three methods, the Kaufmann method is outstanding, both from the standpoint of ease of preparation and of convenience and pleasantness in use. On the average, our results by the Kaufmann method are intermediate between the Wijs and Hanus results, differing from the former by an average of 0.9% and from the latter by 0.3%. The reagent is much superior to the Wijs in stability, and to the Hanus in ease and convenience of preparation. It also would seem to possess an important advantage in the fact that the reagent is neutral, not acid, and accordingly does not show the recurrent end-point which is sometimes troublesome in the Wijs and Hanus methods. If the findings of Werner[16] can be confirmed, it would also seem a much better method than the Rosenmund-Kuhnhenn for the analysis of sterols, owing to the much greater difficulty of preparation and the unpleasant characteristics of the latter reagent. Our results thus agree in general with the findings of Yushkevich[6] in regard to the general superiority of the Kaufmann method.

The author wishes to express his indebtedness to Mr. Arthur Hurndall for his valuable assistance with the analytical work.

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*Since this paper was presented, Hawley (Analyst **48**, 601 (1933)) has published results showing that the Rosenmund-Kuhnhenn method gives values from 3 to 5% lower than the Wijs, Margosches and Hübl methods with a variety of oils.

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### Discussion of Paper

Mr. M. L. Sheely asked if corrections to the data had been made for the iodine and thiocyanogen values of the unsaponifiable matter in the fats.

Dr. Barbour replied that he had not taken these into account in his analyses.

Mr. Sheely commented that he had found the thiocyanogen and iodine values of the unsaponifiable matter in some soap fats to be rather high.

Dr. Barbour remarked that he had used fats of very low unsaponifiable content and that any such correction would consequently be small.

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# Some New Detergents

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### Soap

THE standard detergent of modern man is soap. In the chemical sense, a soap is a salt of a fatty acid. In the popular sense, the word applies also to any cleansing mixture containing true soap as its most important ingredient. Some authorities would broaden the term further and designate as soaps all organic compounds having soap-like properties. This broad meaning is not yet generally accepted; in the present paper, soap should be understood to refer only to salts of fatty acids or mixtures containing same as their principal ingredient.

### The Search for Soap Substitutes

For a long time there have been efforts to modify the essential properties of soap and to find outright substitutes for soap.

There have been many futile efforts to obtain cheaper cleansing agents. For instance, one popular pastime has been to prepare detergents, alleged to be efficient, from cheap cereals or from cheaper earths. Chemically combining true soap with cheap hydrocarbons has been developed at least to the stage of effective work by the press agent. Considering the intrinsic inferiority of most soap substitutes in past experience and allowing for the fact that much of the fat for soap making is essentially a by-product and hence susceptible to wide adjustment in price, one is tempted to predict that the soap business will never be revolutionized merely as the result of the low price of a soap substitute.

More impressive than the search for cheaper detergents has been the search for materials which combine the desirable properties of soap with distinctive properties of their own. Long ago turkey red oil afforded industry a wetting and emulsifying agent which, unlike soap, is soluble in neutral and even acid solutions. Also turkey red oil forms in hard water a precipitate which is less flocculent and has less tendency to adhere to textile fibres than calcium and magnesium soaps. Thus turkey red oil went a long way toward overcoming the two outstanding defects of soap. Along with similar sulfated oils, misnamed sulfonated oils, it found many industrial applications and has even found some favor as a shampoo.

While the sulfated fatty oils are too low in cleansing value to be a serious threat against soap, they have until recently constituted the outstanding class of soap-like materials, and turkey red oil has been the inspiration of the chemist searching for new and better detergents.

Attempts to avoid precipitation of soap in hard water by merely incorporating sulfated oil in the soap were without marked success. Even sulfated oils are appreciably affected by the hardness of the water, due to their soap content. Free fatty acid, even if not present in the original fatty oil, is liberated by the action of sulfuric acid and subsequently converted at least in part to soap when the sulfated oil is neutralized.

Naturally there were attempts to develop sulfated oils free from soaps, both by keeping the carboxyl groups of fatty oils in an esterified state and by using oils containing no fatty acid from which soap could be formed. The search for new wetting, emulsifying, and cleansing agents increased, and within the past five years has become a general scramble. The patent literature already reflects the intensified effort, and is so voluminous that it is altogether impractical to survey any large portion of the field in a brief paper such as the present one. The existence of most of the proposed reagents is still confined to the laboratory or, very likely in some cases, to the printed page. Some of them, developed primarily for use in treatment of textiles, are being exploited commercially. Of these, two groups are of outstanding interest to soap chemists because they can really do the work of soap in addition to some things that soap cannot do.

### Alkyl Sulfates

One class of interesting detergents consists of alkyl sulfates, for example, sodium lauryl sulfate. The principal method for making these involves the following three steps.

1. *Reduction.* The fatty acid radical of various fatty materials is reduced, e.g., by high pressure catalytic hydrogenation, with formation of the corresponding normal primary alcohol. For example, lauric acid or its ester yields lauryl alcohol.

2. *Sulfation.* The alcohol is sulfated to yield an alkyl sulfuric acid. For example, lauryl alcohol yields lauryl sulfuric acid, as shown in the equation:

$$C_{12}H_{25}OH + H_2SO_4 = (C_{12}H_{25})HSO_4 + H_2O$$

3. *Neutralization.* The alkyl sulfuric acid is neutralized to form the sodium or other salt of the alkyl sulfuric acid.

These alkyl sulfates were first developed commercially in Germany by H. Th. Böhme A.-G. A number of patents in the field have been granted in several countries to this company and to Deutsche Hydrierwerke. A still earlier patent application on the hydrogenation step had been filed in Germany by I. G. Farbenindustrie, and these three companies are understood to control jointly in Germany the manufacture and sale of wetting and cleansing agents consisting of the sulfuric acid derivatives of the higher alcohols corresponding to the fatty acids of fats and oils.

In the United States the E. I. du Pont de Nemours