The anomalous results obtained in calculating the saturated acids present complications of the utmost significance. It has always been assumed for the purpose of analyses that the products of hydrogenation were, or reacted like normal fat acids. In natural, unhydrogenated fats one can obtain a reliable fat acid analysis from the iodine number and spectrometric data. Such an analysis is obviously impossible with hydrogenated fats. With our present knowledge and techniques it will be necessary that a direct analysis of saturated acids and isoöleic acid contents be made in conjunction with the above analysis if a reasonably reliable composition is to be obtained.

In all three samples the heat-nickel treatment (Table II) caused a decrease in spectrometric linoleic acid and an increase in conjugated acids, evidently the result of induced isomerization. These changes, however, are not large enough to account for all the iso-linoleic acid produced in the hydrogenations, which leads to the suggestion that hydrogen may serve as an additional activator of the isomerization. In addition to the changes in spectrometric data there is a gradual decrease in both the iodine numbers and thiocyanogen numbers of the oils during the heat-nickel treatment. These changes might well have been anticipated from the isomerization of the polyunsaturated acids inasmuch as it is common knowledge that iodine and thiocyanogen reagents do not react normally with conjugated double bonds and, further, Lemon (12) has pointed out that his "isolinoleic" acid does not absorb thiocyanogen in the expected manner.

The above observations lend a marked degree of confusion to the fat acid analysis of hydrogenated fats. It appears certain that the heat-nickel treatment caused changes within the fat molecules which result in lowered iodine numbers, thiocyanogen numbers, and spectrometric linoleic acid determinations but not in decreased total unsaturation (i.e. total number of double bonds). Thus, at the moment it is difficult to conceive any reasonably simple and uncomplicated method for the analysis of hydrogenated fats which can lead to a true fat acid composition. The need for further intensive research in this direction is evident.

**Summary**

Spectrometric and iodometric analyses of hydrogenated fats and of heat-nickel treated oils indicate that during hydrogenation there are formed isomers of polyunsaturated acids which do not react normally upon analysis, making it impossible to obtain a valid fat acid composition by the usual methods.

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# A Modified Kreis Test Suitable for Photocolorimetry

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**Summary**

A sensitive form of the Kreis test for rancidity in fats and oils is described, in which the reaction occurs in a one-phase system, which is suitable for direct photometric measurement. The procedure is convenient and rapid and yields reproducible results.

* * *

BECAUSE of its sensitivity and simplicity, the Kreis test (1, 2) is extensively used to detect oxidative deterioration in fats and oils. While the results are not a definitive index of rancidity, the information obtained is a highly useful supplement to other evidence.

A number of techniques have been proposed for the more precise evaluation of the concentration of active substances (3, 4, 5). Of these, the methods that involve distribution of the color-forming substance into two liquid phases and separation and also those involving removal of the active material in a stream of inert gas and absorption require considerable manipulation and do not always yield reproducible results. The procedure of Walters, Muers, and Anderson (5) offers the advantage that the reaction is carried out in a one-phase system, which requires no separation for photometric measurement.

Trials with this latter method were, however, disappointing. The acid reagent (1 gram of trichloroacetic acid per 0.382 ml. of amyl acetate) is unstable, produces large, variable blank values, and is unpleasant to use. It was not possible to reduce the blank values by purification of the reagents. It was learned, however, that the color could be developed equally as

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well in a glacial acetic acid solution of trichloroacetic acid containing enough chloroform to dissolve the fat.

## Procedure

A sample of the fat weighing 1.5 grams or less is introduced into an 18 x 250-mm. test tube and diluted to 5.0 ml. with chloroform. To this mixture 5.0 ml. of a solution of 150 grams of trichloroacetic acid in 500 ml. of glacial acetic acid and 1.0 ml. of a solution of 1.0 gram of phloroglucinol in 100 ml. of glacial acetic acid are added. The resultant mixture is stirred by a bubbling stream of air for 2 or 3 seconds. The test tube is then placed in a water bath at 45° C. After exactly 15 minutes the tube is removed, and 4 ml. of 95% alcohol is added. The photometric measurement is made on this solution within a few minutes. The comparison solution for adjustment of the colorimeter is prepared like the test solution with the exceptions that the phloroglucinol is omitted and 1 ml. of glacial acetic acid is used. The colorimeter should be equipped with a yellow-green filter, preferably having its maximum transmission near 545 mμ.

## Discussion

THE quantity of fat used in comparative tests must be uniform since changing the weight of sample does not produce a corresponding linear change in the optical density of the color formed. It has been found convenient to use small quantities of fat in studies of material in which a wide range of rancidity was expected and larger weights of sample as a sensitive test for incipient rancidity. The weight of fat that can be used is limited by its solubility in the mixture when diluted with the alcohol. Samples weighing as much as 2 grams have been used, but some fats cause turbidities at that concentration. Weights of 1.5 grams or less have always been completely soluble. A turbid test solution can be cleared by addition of a few tenths of a milliliter of chloroform; the slight error caused by the dilution can ordinarily be neglected.

The rate and intensity of color formation increase with increasing temperature, and the intensity at any temperature increases for an indefinite period. The colored material eventually begins to decompose in the reaction mixture. The addition of alcohol almost completely stops the reaction and stabilizes the color at a practically constant value for at least 15 minutes. The conditions of time and temperature must therefore be chosen empirically if they are to yield satisfactory intensities of color within a convenient time. A period of 15 minutes at a temperature of 45° C. has been found to meet these requirements. If maximum sensitivity is not necessary, it is possible to dispense with the water bath and to carry out the reaction at room temperature.

The reagents, prepared as directed, are stable at room temperature for several months. If the phloroglucinol and trichloroacetic acid are combined in a single reagent, the resulting solution becomes discolored within a few hours.

TABLE I.

Comparison of Tests for Rancidity in Chicken Fat.

| Time aerated, hours | Peroxide value, m. equiv. /kg. | Proposed method, % transmission in triplicate | Walters *et al.* method % transmission | Taffel and Reavis method, appearance, visually |
|---|---|---|---|---|
| 0 | 0 | 87.8, 87.8, 88.0 | 85.3 | Negative |
| 1 | 1.5 | 77.6, 77.0, 77.5 | 76.0 | Negative |
| 3 | 2.5 | 69.5, 69.3, 69.0 | 66.6 | Faintly positive |
| 6 | 6.0 | 59.0, 59.4, 59.6 | 59.0 | Positive |
| 12 | 67.0 | 5.8, 5.7, 5.7 | 28.0 | Strongly positive |

Table I compares results of several methods of testing for rancidity. The material tested was chicken fat that was undergoing accelerated oxidation by being held in a steam bath while a slow stream of dry air was blown through the fat. The tests used were the Kreis test as modified in the procedure described above, as modified by Walters, Muers, and Anderson (5), and as modified by Taffel and Reavis (3). The peroxide values were determined by Wheeler's method (6). The data show satisfactory agreement between replicate determinations by the proposed method, and they show further that the values obtained by this method correspond closely with those obtained by the method of Walters *et al.* except for the more rancid fat. The intense colors formed with more oxidized fats seem to be less stable in the solvent mixture used in the older method. Both methods are seen to be more sensitive than that of Taffel and Reavis, in which aqueous acid solution is used.

TABLE II.

Kreis Test Intensities and Peroxide Values for Several Types of Fats.

| Type of material | Modified Kreis test, % transmission | Peroxide value, m. equiv./kg. |
|---|---|---|
| Chicken fat (rendered) | 69.3 | 2.5 |
| Pork fat (rendered) | 69.0 | 2.5 |
| Cottonseed oil (hydrogenated) | 41.9 | 3.5 |
| Corn oil (refined) | 10.6 | 3.5 |
| Pork fat (rendered) | 36.3 | 12.0 |
| Avocado oil | 7.6 | 14.0 |
| Cottonseed oil (refined) | 6.9 | 9.5 |

The parallel development of peroxides and formation of the substance responsible for the color in the Kreis test (Table I) are typical although, at any level of peroxide value, different fats may give quite different intensities of color, as is indicated by the data of Table II. Hence no quantitative significance has been attached to the numerical results of tests. The test as described is suitable as a qualitative index of the condition of the material.

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