

A New Sensitive Method for the Determination of Peroxides of Fats and Fatty Acids *

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For the determination of peroxides in fatty products, several methods have been devised:

The oldest and best known is that of Lea¹, which is based on the liberation of iodine from potassium iodide by fatty peroxides in a glacial acetic acid-chloroform solution, followed by titration of the liberated iodine with thio-sulfate after the addition of water. Various modifications of this method have found a widespread technical application. It has, however, several disadvantages. Apart from its failure to indicate very small amounts of peroxides, a more serious objection against it is that the iodine has a tendency to combine with the double bonds, especially those of the highly unsaturated fatty acids.

A method based on another principle has been developed by Chapman and McFarlane². The principle is the oxidation, by peroxides in acetone or methyl alcohol, of ferrous sulfate to ferric sulfate, which is determined colorimetrically by means of ammonium thiocyanate. This method is more sensitive and gives higher values than Lea's method. It has, however, the disadvantage that atmospheric oxygen or oxygen dissolved in the solvents can also oxidize the reagent and cause considerable errors.

A new method has been proposed by Fondarai³, who uses the principle of adding thiofluorescein by the iodometric method. The thiofluorescein, which in alkaline solution has an intensive blue colour, is oxidized into a colourless

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compound, and the difference in colour intensity with, and without, peroxides can be tested colorimetrically. The method is highly sensitive, but rather troublesome and complicated.

In a previous paper, the authors reported that peroxides can react, in the presence of heme, with certain leuco-dyes to form coloured compounds. In the course of this work it was found that 3,5-dichloro-4,4'-dihydroxyphenylenediamine, the leuco-base of the well known redox-indicator 2,6-dichlorophenolindophenol, could react with considerable velocity with organic peroxides, even though heme was not present. The reaction did not show any tendency to have an autocatalytic character as did the heme-catalyzed reaction, and the dye formed has the advantage of being fat-soluble. Based on these observations, the authors have developed a new chemical method for the determination of peroxides, the details of which are given below.

METHOD

Preparation of dichlorodihydroxyphenylenediamine: 2 g of a commercial preparation of 2,6-dichlorophenolindophenol, as a rule of 65 per cent purity, and 1 g of ascorbic acid are dissolved in 100 ml of 50 per cent ethyl alcohol. After 10 minutes with occasional shakings, 200 ml of saturated aqueous sodium chloride solution, containing 20 ml glacial acetic acid, are added. The mixture is left standing for about 2 hours at 5° C., then decanted and filtered through a glass filter. The precipitate is rinsed with distilled water and further purified by repeated dissolutions in portions of about 25 ml absolute ethyl alcohol, from which it is precipitated by the addition of distilled water (300 to 500 ml portions). Eventually, the leuco-compound is dried *in vacuo* at room temperature. It is obtained as bluish-white metallic needles which should not contain traces of either sodium chloride or ascorbic acid.

The other reagents used in the reaction, such as xylene, butanol, and glacial acetic acid should be of a high degree of purity. By heating samples of them for 10 minutes in a boiling water-bath with dichlorodihydroxyphenylenediamine, or with a minute quantity of dichlorophenolindophenol, they should neither intensify nor bleach the pink colour. The test tubes used for the reaction should be rinsed with alcohol.

Preparation of the reagent: 0.1 g of dichlorodihydroxyphenylenediamine is dissolved in 10 ml of butanol or ethyl alcohol. The reagent should be kept in the refrigerator, and be discarded as soon as the red color becomes so intense as to give a considerable blank.

Procedure for the determination: From the oil, the peroxide value of which is to be determined, a suitable dilution is made by means of xylene, containing 5 per cent glacial acetic acid. 0.2 ml of the reagent is added to 5 ml of the oil dilution in a test tube, and the mixture heated in boiling water for 10 minutes. At the same time a blank determination is run. After cooling and adjusting the volumes, the intensities of the red colours are read in a Beckman spectrophotometer at 520 $m\mu$.

Calculation of the results: A curve is constructed for the extinction of 2,6-dichlorophenolindophenol in relation to its concentration. The concentration of the indophenol can be determined by adding potassium iodide in acid solution, followed by titration with

thiosulfate. The peroxide content is calculated as milliequivalents per kg of oil. A content of 0.001 milliequivalent of peroxide in the reaction mixture will give an extinction, after adjustment of the volume to 5 ml, in a 1 cm layer of 1.17.

RESULTS

The method has been used for a considerable length of time in our laboratory with good results. It has very often been compared with the other methods mentioned in the introduction, especially the iodometric method in the modification of King *et al.*⁴. The method has been found to be by far much easier and less time consuming than any of the other methods. The results have been very reproducible, since the method is not much influenced by variations in external experimental conditions. All observations tend to indicate that the reaction is quantitative and specific. Therefore, especially in highly unsaturated compounds, considerably higher figures have often been found than when the same oil was tested by the method of King *et al.* As mentioned in the introduction, this discrepancy is probably explained by an addition of the iodine liberated by the peroxide to the double bonds of the oil. Table 1 presents typical examples of comparative results obtained by the two methods.

Table 1. Comparison of the methods of King *et al.*, and the authors, for the determination of peroxides in a number of fats and fatty acids.

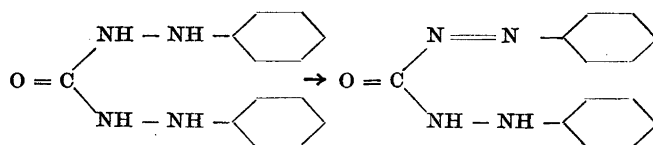
Type of fat	Milliequivalents of peroxide per kg fat by the method of	
	King <i>et al.</i>	The authors
Butterfat, oxidized	60	67
Margarine	4.8	5.0
Oleic acid, oxidized	1980	2500
Linseed oil	1800	4600
» »	500	1500
Arachid »	1.5	1.5

The method can be made still more sensitive by making the dichlorophenol-indophenol solution alkaline, since the blue colour, of the alkaline dye, is much more intense than the red colour in acid solution. The red colour will change into blue by the addition of potassium palmitate. In alkaline solution, however, the leuco-compound has a great tendency to become oxidized by atmospheric oxygen. Therefore, for most purposes it will be most convenient to use the

light absorption of the red colour. As a rule, this will be strong enough, so that a conversion into the blue component is not necessary.

DISCUSSION

There can be very little doubt that the chemical group in rancid fats which reacts with leucodichlorophenolindophenol is a peroxide or hydroperoxide. In 1926 Stamm⁵ proposed the use of diphenylcarbazide as an indicator for the decomposition of fats. In this reaction the colourless diphenylcarbazide is converted into diphenylcarbazone which is intensively red.



The reaction was explained by Stamm⁵ as caused by free fatty acids, aldehydes, and ketones, and by other investigators⁶⁻⁷ by oxyacids or catalytically acting unsaturated acids and aldehydes. It would appear to be a more simple explanation that this reaction is also caused by peroxide groups. In order to examine this question the authors synthesized diphenylcarbazide and studied its colour development with a variety of fats, the peroxide values of which had been determined by the indophenol method. A close parallelism was observed, which shows that the Stamm reaction is, in fact, also a peroxide reaction. The colour intensity of the two reactions is of the same order of magnitude. The diphenylcarbazide reaction can be carried out in exactly the same manner as the indophenol reaction, only a somewhat greater concentration of acetic acid must be present, *i. e.*, 10 per cent glacial acetic acid in xylene. However, the diphenylcarbazide reaction has the definite disadvantage that solutions of the diphenylcarbazide shows a much greater tendency to become oxidized by atmospheric oxygen than solutions of leucodichlorophenolindophenol.

SUMMARY

A new method for the determination of peroxides is described. The method consists of the oxidation of dichlorodihydroxyphenylenediamine by heating it with the peroxidized fat in a solution of an organic solvent. Dichlorophenolindophenol is thereby formed in a quantitative reaction, and can be determined colorimetrically.

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