The Determination of Peroxides by the Stamm Method[1]

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Abstract

A sensitive quantitative method for determination of the peroxide value of fats and oils is reported which is based on the color reaction with diphyenylcarbohydrazide discovered by Stamm.

Introduction

IN 1925 STAMM (9) REPORTED that rancidity could be detected in oils by the red color which developed when such oils were heated with *sym*-diphenylcarbohydrazide. He suggested the color was caused by reaction of the dye with acids, aldehydes and ketones. Later, Korpaczy (5) modified Stamm's test and attempted to make it quantitative. He believed the red color was caused by reaction with oxyacids. Roschen and Lehmann (8) applied Korpaczy's modification to various oils and fats with rather limited success. In 1938 Glimm et al. (2) reported an extensive study of the Stamm test. They studied the effect of solvents on the reaction and found that acetic acid greatly intensified the color. Pure aldehydes, ketones, acids, oxyacids, and hydrogen peroxide gave no color; but after autoxidation fats, aldehydes, and ketones gave a red color. They believed the reaction detected "active" or "molecularly bound" oxygen. Grant and Lips (3) used the Stamm test to follow the autoxidation of lard and found that it correlated well with the organoleptic evaluation. Hartmann and Glavind (4) suggested that the basis of the Stamm test was the oxidation of diphenylcarbohydrazide to diphenylcarbazone by peroxides since the Stamm test gave results which paralleled their indophenol test.

This paper reports a sensitive quantitative method for determining peroxide by the Stamm test.

Reagents

Commercially available 1,5-diphenylcarbohydrazide (Matheson) is usually pink or brown and gives a poor blank value. It was purified by recrystallization from ethanol which had been acidified to Congo Red with hydrochloric acid. The crystals were filtered off, washed with cold ethanol, and the ethanol clinging to the crystals was removed under reduced pressure to prevent reoxidation of the crystals. The material used in these experiments melted 173–174.5C. It gave one spot by thin-layer chromatography on silica gel (Absorbosil-2) plates developed with diethyl ether and sprayed with dichlorofluorescein.

Diphenylcarbazone was prepared from the commercially available 1:1 mixture of diphenylcarbohydrazide and diphenylcarbazone by the procedure of Krumholz and Krumholz (6). (This 1:1 mixture melts at 157C with decomposition and is often referred to as diphenylcarbazone.) The number of extractions with ethyl ether was increased from three to ten. It gave one spot on thin-layer plates developed as described above.

Procedure

A 0.5% solution of purified 1,5-diphenylcarbohydrazide is prepared in 1,1,2,2-tetrachloroethane with gentle heating. Technical grade tetrachloroethane is satisfactory if colorless. Fat or a solution of fat in tetrachloroethane is then added to a 15 ml centrifuge tube, and the volume is adjusted to 3 ml with tetrachloroethane. The 0.5% diphenylcarbohydrazide solution is diluted with an equal volume of acetic acid, and 2 ml of this mixture is pipetted into the 15 ml centrifuge tube and mixed immediately with a stream of air from a small tube. A blank is prepared in the same way by using 3 ml of tetrachloroethane in place of the fat. The order in which the reagents are added and rapid mixing are important, especially at high peroxide values. The blank and sample are heated in a boiling water bath for exactly 3 min and then cooled in a water bath at room temperature. The color intensity of the oxidized dye is quite sensitive to temperature. We have found it convenient to cool the tubes to 25C by shaking them 3 min in a large water bath. The optical density is then read at 565 mμ with a Beckman DU spectrophotometer fitted with constant temperature plates set for 25C. The peroxide value (meq/kg) is then calculated by the following formula:

$$PV = KA/W$$

where PV is the peroxide value, K is an empirical constant = 0.2827, A is the absorbance at 25C and W is the weight of fat added in grams.

The iodometric peroxide value was determined by the AOCS official method (1).

Results and Discussion

Lauroyl peroxide, 99.2% pure by the iodometric test, was assayed by our modification of the Stamm test, and the results are given in Figure 1. The ab-
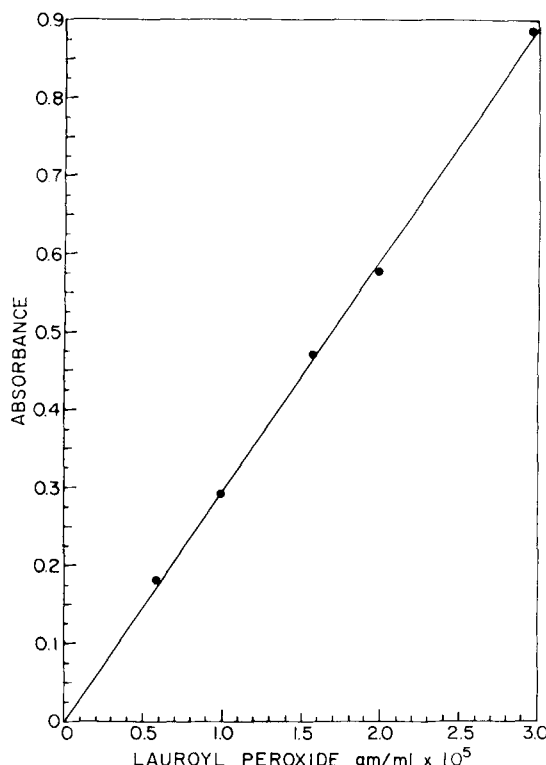

FIG. 1. The absorbance of the Stamm test versus lauroyl peroxide concentration in 20% acetic acid at 25C.

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sorbance was a linear function of the amount of peroxide over the entire usuable range of the spectrophotometer. The empirical constant relating the peroxide value with the absorbance was evaluated as 0.2827.

Diphenylcarbohydrazide can be oxidized to diphenylcarbazone by oxygen and hydrogen peroxide under certain conditions (6), and our results indicate that diphenylcarbazone is the reaction product produced in the Stamm test. In tetrachloroethane-acetic acid, the Stamm test gave a purple red color with a broad adsorption band which had a maximum at 565 mμ. Diphenylcarbazone gives an identical absorption spectrum. When the Stamm test was run under oxygen-free conditions, each mole of peroxide produced an absorbance equivalent to on mole of diphenylcarbazone.

When the test was performed without precautions to exclude oxygen, each mole of lauroyl peroxide oxidized 2.631 moles of diphenylcarbohydrazide. This indicates that oxygen, present in the solvents, enters into the reaction. However, this reaction with oxygen instead of being a drawback actually increases the sensitivity of the reaction. When the factor 2.631 is included in the calculation, the peroxide values of soybean oil by the Stamm test agreed with the iodometric value over a wide range of peroxide values as shown in Figure 2.

Evidently the reaction of each molecule of peroxide produces a chain reaction with the oxygen and diphenylcarbohydrazide, and, under the conditions of the test, the reaction chain length is quite reproducible. There is enough oxygen dissolved in the solvent so that oxygen does not become limiting as long as the amount of diphenylcarbohydrazide oxidized is in the range that can be read accurately in a spectrophotometer, i.e., absorbance < 0.8. The results with soybean oil show that the factor 2.631 holds for the type of peroxides found in soybean oil and that it is not altered by the natural antioxidants, trace metals, or variations in sample size. This factor may be altered by impurities in the diphenylcarbohydrazide. One batch of diphenylcarbohydrazide in spite of repeated crystallizations gave a melting point of 165.5–167.5C and contained two components by thin-layer chromatography. This gave a factor 16% smaller than pure diphenylcarbohydrazide. For the most accurate results the factor should be determined on each batch of reagent. Since the factor is empirical, one should exercise caution in extrapolating it to new conditions, particularly to samples with high concentrations of antioxidants and proxidants.

The absorbance is quite sensitive to the temperature. Figure 3 shows the change in absorbance of a solution of diphenylcarbazone at different temperatures. The data was fit with a straight line by the least squares method to give the equation:

$$A_t = 15.71 - 0.1806\ t$$

where A_t is the absorbance of a tetrachloroethane-acetic acid (4:1) solution containing 1 mg of diphenylcarbazone/5 ml and t is the temperature in °C. One can write a general equation for the peroxide value:

$$PV = 8.324\ A/FW(15.71 - 0.1806\ t)$$

where F is the empirical factor evaluated at 2.631 in the present experiments, and W is the weight of sample in grams.

The method is quite sensitive. Duplicate determinations agree within 0.005 absorbance. If we select 0.010 absorbance as the least value which can be detected
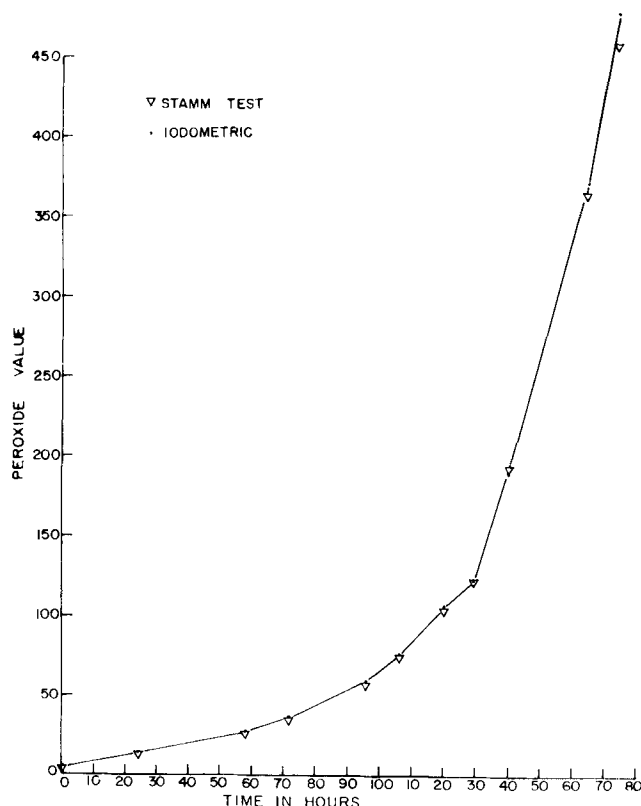

FIG. 2. Comparison of the Stamm and iodometric peroxide values on soybean oil oxidized at 60C.

with confidence and 1 g of fat as the largest sample that can be used, then one can detect peroxide values as low as 0.003 at 25C. Thus, the method should be particularly helpful in studying the early stages of autoxidation in fat. It should also be useful in following the autoxidation of milk fat and other fats where there is considerable flavor deterioration at very low peroxide values.

The sensitivity of the reaction is greatly enhanced by acetic acid as reported by Glimm et al. (2). The absorbance of diphenylcarbazone in tetrachloroethane increased with the concentration of acid up to about 20% acetic acid, so this concentration was used in the assay. The yield of diphenylcarbazone per mole of
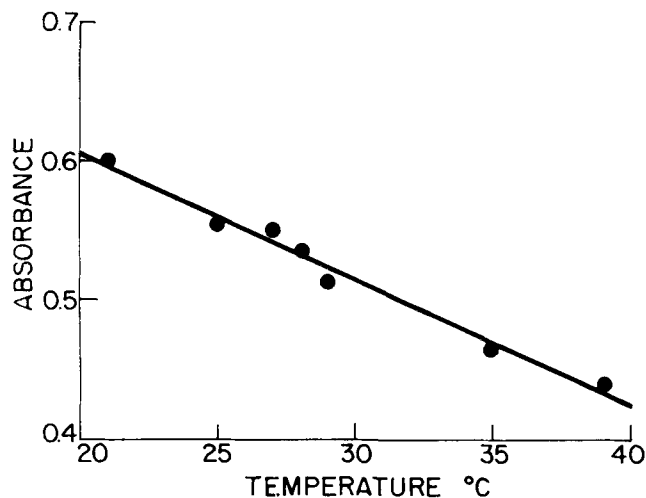

FIG. 3. The change in absorbance with temperature of a solution containing 1.10^{-5} g/ml of purified diphenylcarbazone (mp 125C).

peroxide is also affected by the acetic acid concentration, being 1.8 in a 5% solution compared with 2.631 in a 20% solution.

The polarity of the solvent also has an effect on the ease with which diphenylcarbohydrazide is oxidized by oxygen. In water, methanol, and ethanol, diphenylcarbohydrazide immediately forms a red solution unless oxygen is rigorously excluded. In these polar solvents, diphenylcarbazone acts as an indicator. It is red only above about pH 6; in more acid solutions, it is light yellow. This probably explains why Glimm et al. (2) were not able to get a Stamm test in ethanol and methanol or with hydrogen peroxide; their reagents may have been contaminated with acid.

The color produced in the Stamm test is not stable and should be read within 30 min. Solutions of diphenylcarbazone in tetrachloroethane are not stable for more than a few hours. According to Krumholtz and Watzek (7), diphenylcarbazone is oxidized by oxygen to the 5-hydroxy-2,3-diphenyl-2H-tetrazolium hydroxide inner salt.

Solutions of fat hydroperoxides in tetrachloroethane are not stable, and the peroxide value should be determined immediately after the solvent is added.

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Application of the Ritter Reaction to Petroselinic Acid

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Abstract

Substituted amidostearic acids were prepared from petroselinic acid by reaction, in the presence of sulfuric acid, with hydrogen cyanide, acetonitrile, propionitrile, acrylonitrile and benzonitrile. The products were crystalline solids. Attempts to obtain monoamides containing a nitrile group by the use of dinitriles were unsuccessful.

Introduction

AMONG THE OILSEED PLANTS selected for further study in the New Crops program of the U. S. Department of Agriculture are several members of the *Umbelliferae* family including parsley (*Petroselinum sp.*), wild carrot (*Daucus carota*), and fennel (*Foeniculum vulgare*). The characteristic fatty acid of the seed oil from these plants is petroselinic (6-octadecenoic) acid, and any industrial utilization of the oils would depend primarily on the petroselinic acid or its derivatives.

One means of modifying petroselinic acid to give potentially useful compounds is by modification at the site of the double bond. The formation of N-substituted amides by interaction of nitriles and olefins in the presence of strong acids, now designated the Ritter reaction, has been studied by numerous investigators (see references 1 and references cited therein) using a variety of nitriles and a variety of olefinic compounds. Many of the products of the Ritter reaction have been stated to be useful in the preparation of plasticizers, polymers, detergents and surface-active materials useful as wetting, emulsifying, or dispersing agents (2–5).

In the course of utilization studies with petroselinic acid, we have carried out its reaction with hydrogen cyanide and a variety of nitriles in the presence of sulfuric acid. This paper is a discussion of this work.

Materials and Methods

The petroselinic acid (6-octadecenoic) was obtained from parsley seed by the method of Fore, Holmes and Bickford (6). The nitriles were purchased from the Eastman Kodak Company and used without further purification. The acetamido-, propioamido-, acrylamido- and benzamidostearic acids were formed by the method of Roe and Swern (7,8), except that the waxy material, which formed in the diluted acid overnight, was separated, dissolved in ethyl ether, the ether solution washed with water to neutrality, dried with sodium sulfate and evaporated under aspirator vacuum at less than 40C to obtain the crude product. The crude product was wastefully recrystallized three times from acetone (1 g to 4–5 ml acetone) at −5C to give the purified products reported in Table I.

The formamidostearic acid was made by suspending two equivalents of powdered sodium cyanide in the petroselinic acid in a large test tube equipped with a stirrer. The sulfuric acid was added dropwise, the temperature of the reaction tube being kept above the melting point of petroselinic acid (31C) but below 40C as the sulfuric acid was added. More difficulty was encountered in crystallizing the formamidostearic acid and a small amount of it was crystallized only once from acetone.

The yields of the crude product and their nitrogen contents; and the analyses, molecular weights and melting points of the purified products are shown in Table I.

Results and Discussion

Roe and Swern (7) applied the Ritter reaction to oleic acid and obtained products which melted over wide ranges. They point out that considerable isomerization occurs in the sulfuric acid medium, resulting in a mixture of many isomers instead of only the two which would be expected if the double bond remained in the 9,10-position throughout. Their products were initially viscous, oily masses, which required as long

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