Estimation of Monocarbonyl Compounds in Rancid Foods

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A METHOD has been developed for the determination of monocarbonyl compounds in the benzene-soluble fraction of rancid foods. The determination involves the formation of the 2,4-dinitrophenylhydrazones of monocarbonyl compounds in benzene solution, removal of unreacted 2,4-dinitrophenylhydrazine reagent and any hydrazones of dicarbonyl compounds with alumina, and colorimetric measurement of the remaining hydrazones in alkaline solution.

Since monocarbonyl compounds (aldehydes) follow as secondary reaction products of the hydroperoxides initially formed in fat oxidative deterioration and contribute greatly to the off odors and flavors in rancid food (11, 17), there is, of course, a definite need for a convenient and reliable method for the estimation of these compounds. At the low levels of aldehydes found in rancid fats, previously available methods, such as differential titrations with sodium bisulfite (5) or hydroxylamine (6), or quantitative modifications of the Schiff test (2, 8, 13, 18), have failed to yield consistent results and occasionally have given high results with fresh foods.

2,4-Dinitrophenylhydrazine has been used in aqueous solution for the gravimetric determination of aldehydes and ketones (3) and in the colorimetric determination of dicarbonyl compounds (9, 10). Chromatographic separations of various aldehydes and ketones as their 2,4-dinitrophenylhydrazones have been reported (1, 7, 12, 14, 16, 19). No account of the use of this compound as a colorimetric reagent for simple aldehydes and ketones has been noted in the literature. The method as described below is applicable to many aldehydes, and particularly to the aliphatic, saturated or unsaturated, aldehydes which arise in fat deterioration.

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Reagents and Apparatus. a) Dissolve 500 mg. of 2,4-dinitrophenylhydrazine in 1 liter of benzene by heating gently and shaking occasionally. b) Dissolve 60 g. of potassium hydroxide in 1 liter of 99% aldehyde-free ethyl alcohol (15) and filter through a fritted-glass funnel or glass wool. The alcohol may be used without purification if the reagent is made up fresh daily. c) Activated alumina, F-20 grade, 80-200 mesh supplied by the Aluminum Ore Company, East St. Louis, Ill., was used throughout this work. It is too active as received and must be modified by mixing with 15% of fully hydrated material prepared by exposing the alumina in a thin layer to water vapor in a vacuum desiccator. Allow the mixed alumina to stand in a closed container several hours before using. If kept in an air-tight container, this mixed alumina is stable indefinitely. d) Chromatography tubes, 7 mm. inside diameter, 110 mm. long, with a short piece of 1-mm. capillary tubing sealed to the lower end and a 110-mm. length of 10-mm. tubing to the upper end. Insert a small loose plug of glass wool in the upper end of the capillary section to retain the alumina column. e) Twenty-five-ml. glass-stoppered graduated cylinders. f) Photoelectric colorimeter for measurements at 435 mμ. A Coleman photoelectric colorimeter was used in these studies.

Method of Analysis. Prepare the chromatograph column by pouring in alumina to a depth of 3 cm. Pipette in 10 ml. of the dinitrophenylhydrazine reagent and immediately sprinkle in sufficient additional alumina to make the total depth 10 to 11 cm. After all of the reagent solution has entered the top of the alumina column, add 5 ml. of fresh benzene. When all of this has entered the column, add the sample (containing the equivalent of 0.05-0.50 micromole of aldehyde) dissolved in 3-4 ml. of benzene and begin collection of the solution issuing from the column in respective concentrations of 10.5 ml. and 12.3 g. per liter (François & Sergent—Ann. nutrition et aliments, 3, 441). The preservation was further improved by adding 5 g. of potassium dichromate to the solution (François & Sergent—Bull. mens. ITERG 4, 151).

DECOMPOSITION BY ELECTRICAL DISCHARGE. Menzel et al. (Chem. Ber. 82, 418) recorded the reaction taking place on exposure of fatty materials to electric discharges. Stearic acid was converted to an unsaturated acid, and some moisture was split off and this corresponded to a decrease in the acid number. Methyl oleate subjected to electric-glow discharges in hydrogen atmosphere was hydrogenated, and some acidity developed. In nitrogen atmosphere the change in iodine value was less but more acidity developed. Splitting, condensation, and polymerization were also evident in the processes.
a 25-ml graduated cylinder. Add fresh benzene to the column until 19 ml. have been collected. Dilute to 25 ml. with alcoholic KOH, mix, and read the absorbancy at 435 m\(\mu\) immediately. The solution of dinitrophenylhydrazones in benzene is stable indefinitely, but the red color of the alkaline solution fades at a rate of 0.5% to 0.6% per minute. The importance of a uniform minimum delay in reading is apparent. A blank should be run with each series of determinations. Dilute the blank benzene solution, after passage through the alumina column, to 25 ml. with alcoholic potassium hydroxide and use for the comparison solution in setting the colorimeter to zero absorbancy (100% transmittance), thus automatically correcting for the small amount of carbonyl found in the usual reagent grade of benzene.

The simple procedure of carrying out the reaction between aldehyde and hydrazine on the alumina, in contrast to reaction in benzene solution followed by passage through the column, was adopted because it gave more nearly quantitative recovery of known aldehydes.

In the preparation of samples for analysis, fats and oils are easily dissolved in benzene and the appropriate aliquots used. Solid foods (e.g., potato chips) are ground as finely as is practical in a mortar or a mill. One- to two-gram samples are weighed into small beakers, benzene is added with stirring, and the solid plus benzene extract are transferred to the space above the alumina in the chromatograph tube with several small portions of benzene. The remainder of the procedure is the same as for fats and oils.

**Standardization and Evaluation of Method.** In order to express the absorbancies obtained by this method in terms of moles of carbonyl compounds or in terms of equivalent amounts of some known stable monocarbonyl compound, the spectral absorption of the 2,4-dinitrophenylhydrazones of a wide range of monocarbonyl compounds was measured. The 2,4-dinitrophenylhydrazones of butyraldehyde, isovaleraldehyde, heptaldehyde, nonylaldehyde, undecylaldehyde, crotonaldehyde, 2-decenaldehyde, and acetone were prepared and recrystallized several times. Various concentrations of these hydrazones in benzene solution were treated with alcoholic potassium hydroxide as described in the above method and the absorbancy at 435 m\(\mu\) determined. The resulting data, which are plotted in Figure 1, demonstrate the extreme dilution at which these substances will yield a measurable color, the satisfactory adherence to Beer's law over a considerable range of concentrations, and the relative constancy of the molar absorbancy index with change in the size and type of the carbonyl moiety. The molar absorbancy index calculated from the regression line for the saturated aldehydes is 19,200. The factor relating absorbancies to concentrations of hydrazones (and hence monocarbonyl compounds) will depend on cell thickness and spectral band width. With a Coleman photoelectric colorimeter (Model 11) and 1.3-cm. cells, the absorbancy is taken to be equal to the number of equivalent micromoles of saturated aliphatic aldehyde per 25 ml. of final volume of the test solution. This equality is based on a standard average value for the molar absorbancy index of the hydrazones of 19,200, with which the saturated aldehyde hydrazones agree quite closely, but from which hydrazones of certain unsaturated aldehydes and acetone show appreciable deviations. Since a complete quantitative determination of the carbonyl compounds in rancid fats has not yet been accomplished, it is impossible to determine the error introduced by these deviations. However, spectral absorption curves, in the visible region, of hydrazones of the total aldehydes in rancid fat closely resembled curves for the saturated aldehyde-hydrazones and differed markedly from the curves of unsaturated-aldehyde-hydrazones. This suggests a proportionately small contribution by the unsaturated aldehydes and a correspondingly small error.

The spectral absorption curves over the range 400 to 500 m\(\mu\) of the alkaline solution of the dinitrophenylhydrazones of heptaldehyde and of the carbonyl compounds isolated from rancid turkey fat are shown in Figure 2. The absorption maximum is in the region of 430 to 435. The measurements reported here were made at 435 m\(\mu\), which is near the maximum of the curve and at a point where the instrument sensitivity is slightly greater than at shorter wavelengths. The specificity of the method for monocarbonyl compounds was demonstrated by recovery experiments on known amounts of heptaldehyde, with and

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*Terminology of the National Bureau of Standards, as given in its circular 484, "Spectrophotometry," is used.*

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**Fig. 1.**

**Fig. 2.** Spectral absorption curve of the alkaline solution of the dinitrophenylhydrazones of (A) heptaldehyde (B) carbonyl compounds isolated from rancid turkey fat.