METHODS

A Colorimetric Microdetermination of Peroxide Values Utilizing Aluminum Chloride As the Catalyst

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ABSTRACT

A colorimetric microassay is described for the determination of lipid hydroperoxides. Hydroperoxides are reacted with potassium iodide in the presence of an acid catalyst and liberated iodine is measured. Aluminum chloride, an alcohol-soluble Lewis acid, is used as catalyst. Liberated iodine is measured colorimetrically at 560 nm after addition of starch in 0.01 N hydrochloric acid. The range of the measurement was 0.05-0.5 μmol of hydroperoxides.

INTRODUCTION

Peroxide values (PV) are usually determined by measuring the amount of iodine liberated from potassium iodide through oxidation by peroxides at room temperature in an acetic acid/chloroform medium (1-3). Colorimetric micromethods also have been reported. These include color formation with ammonium ferrous sulfate and ammonium thiocyanide (4), with ferrous chloride and ammonium thiocyanide (5-8), with ferrous chloride and 2,6-dichlorophenol indophenol (9), direct colorimetric determination of iodine (10) or triiodide (11), color formation with diphenylcarbazide (12,13), colored complex formation of titanium and hydroperoxides (14), and colored complex formation of iodine and cadmium ions (15).

In our previous papers (16-18), use of PV test paper was described for a simplified assay of peroxides in lipids. The test paper consisted of a potassium iodide-starch-silica gel sheet. Because silica gel is a Lewis acid, hydroperoxides can react with potassium iodide without an addition of acid as a catalyst. This reaction also was used in a colorimetric microdetermination of PV using a KI-silica gel reagent (19). However, the process was not simple and use of only liquid reagents appeared desirable.

Aluminum chloride, an alcohol-soluble Lewis acid, is used instead of silica gel in this procedure, which uses only reagents that are commonly available. The procedure is reasonably specific for hydroperoxides, is stoichiometric and is adequately sensitive.

MATERIALS AND METHODS

Reagents

Potassium iodide solution was prepared from 2 g of KI in 100 ml of ethanol; aluminum chloride solution was prepared from 2 g of $AlCl_3$ (anhydrous) and 0.02 g of *o*-phenanthroline in 100 ml of ethanol. Starch solution contained 1 g of soluble starch and 20 g of NaCl in 100 ml of water; the mixture was heated until it became clear. The potassium iodate (KIO_3) standard solution was 1 mM.

Procedure

The weighed sample (200 mg or less) or hexane-diluted sample (200 μl or less) was placed in a test tube. Potassium iodide solution (0.5 ml), aluminum chloride solution (0.5 ml) and hexane (1 ml) were added and the mixture was incubated for 5 min at 37 C in a dry block heater. Fifteen ml of 0.01 N HCl and 0.5 ml of starch solution were added and the mixture was shaken vigorously. The solution was transferred into a centrifuge tube, centrifuged for 3 min at 3000 rpm, and then the absorbance of the lower layer was measured at 560 nm. The total vol of the water layer was 16.5 ml. A blank test was run in parallel.

Calibration

Calibration was done by comparing the iodine liberated from potassium iodide through oxidation by potassium iodate standard solution.

Potassium iodate standard solution (0.2 ml),

aluminum chloride solution (0.5 ml) and potassium iodide solution (0.5 ml) were mixed; 0.01 N HCl (15 ml) and starch solution (0.5 ml) were added and the absorbance was read at 560 nm. The total vol was 16.7 ml.

The potassium iodate standard solution (0.2 ml) corresponded to 0.6 μmol I_2, because KIO_3 corresponds to 3 I_2. Therefore, the absorbance corresponding to 1 μmol active oxygen = A/1.2, because I_2 corresponds to 2-O (active oxygen), and PV (meq/kg) = active oxygen (μmol) × 1/sample (g). The A obtained may depend on the kind of starch, but in our experiments, it was 0.95.

This value was corrected for the difference in vol of the sample (16.5 ml) and the standard KIO_3 (16.7 ml). In our experiments, it was calculated to be 0.96.

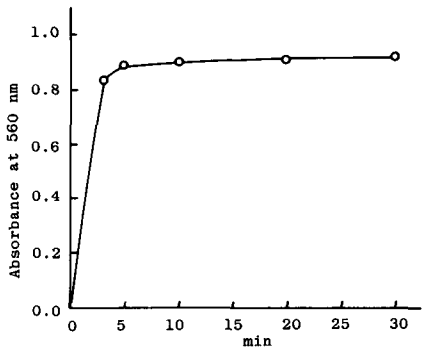

FIG. 1. Time course of iodine liberation with MLHPO at 37 C. A MLHPO hexane solution, 200 μl, was used. A concentration of 2.40 μmol/ml was obtained by titration with thiosulfate (3).

RESULTS AND DISCUSSION

The routine procedures (1-3) for the determination of PV depend on the reaction of potassium iodide with peroxides in acidic solution, followed by titration of the liberated iodine with sodium thiosulfate. Our method makes use of the same principle; however, titration is replaced by colorimetric assay. The use of an alcohol-soluble Lewis acid makes the colorimetric method particularly convenient compared to the KI-silica gel reagent used previously (19). Trifluoroborate also is a good catalyst, but is not commonly available. On the other hand, aluminum chloride is readily obtained and easy to handle. However, aluminum chloride tends to contain trace amounts of iron salts which may interfere in the reaction (11), and therefore, *o*-phenanthroline was added to the solution. Hexane added to the reaction mixture removes the oil after the reaction.

Figure 1 shows the time course of the reaction, i.e., the liberation of iodine, when pure methyl linoleate monohydroperoxides (MLHPO) (20) were used. A 5-min incubation period was sufficient to bring the reaction to completion.

As shown in Figure 2, a linear relationship exists between absorbance and sample size for 25-200 μl of MLHPO in hexane solution. The upper limit of the measurement was 2 μmol active oxygen, that is, PV 10. Larger samples must be diluted with nonoxidized oil or with hexane before PV determination or the sample size should be reduced. The scale of the experiments may be increased or reduced, as long as the ratio of reagents is kept constant.

In the oxidized soybean oil (Fig. 3), the amount of liberated iodine was exactly the

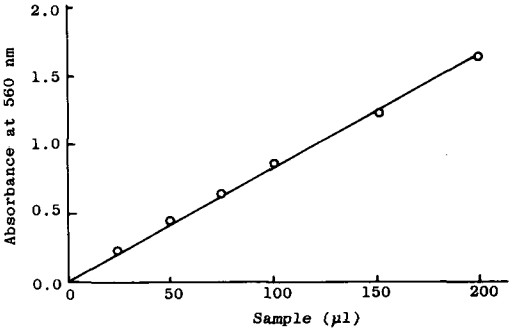

FIG. 2. Relationship between absorbance and sample size. A MLHPO hexane solution, 4.37 μmol/ml obtained from titration with thiosulfate, was used.

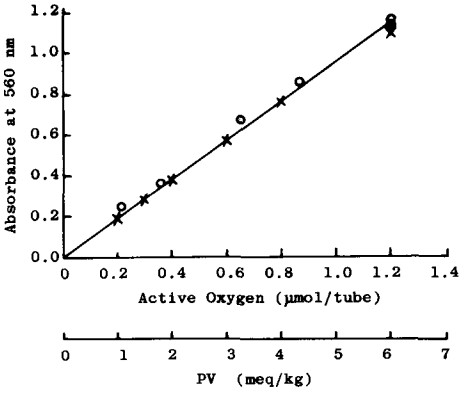

FIG. 3. Relationship between absorbance and different peroxide samples, hydrogen peroxide samples, and potassium iodate standard solution. For each sample, 200 μl was used. • = Potassium iodate standard solution; × = hydrogen peroxide; ○ = soybean oil; PV scale applies only when 200-mg sample is used.

same as that expected from the PV obtained by titration with thiosulfate (3). For calibration of the method, potassium iodate standard solution was used. In this case also (Fig. 3), the amount of liberated iodine in the mixture of potassium iodide, aluminum chloride and potassium iodate in ethanol was exactly the same as that obtained by the routine method, applied to a mixture of potassium iodide, sulfuric acid and potassium iodate in water. Hydrogen peroxide solution standardized with 0.01 N thiosulfate also liberated iodine quantitatively in ethanol. With potassium iodate, no incubation was necessary, but with hydrogen peroxide, the solution had to be incubated for 5 min.

The absorbance of the iodine-starch complex may vary with the source of starch (21), but it can be easily standardized with potassium iodate standard solution. Several kinds of starch were tested and an absorbance range of 0.74-0.95 (see Methods, calibration) was found.

When this method was applied to some arbitrary samples of hexane-diluted MLHPO and oxidized soybean oil, the obtained values (absorbance, $\bar{X} \pm SD$) were 0.916 ± 0.017 and 0.819 ± 0.010 from each of 16 experiments, respectively. Reproducibility was satisfactory.

REFERENCES

1. Lea, C.H., Proc. Soc. London Ser. B. 108:175 (1931).
2. Wheeler, D.H., Oil Soap (Chicago) 9:89 (1932).
3. The Standard Methods of Jpn. Oil Chem. Soc. 2.4.12-71.
4. Lips, A., R.A. Chapman and W.D. McFarlane, Oil Soap (Chicago) 20:240 (1943).
5. Chapman, R.A., and K.M. Mackay, J. Am. Oil Chem. Soc. 26:360 (1949).
6. Stine, C.M., H.A. Harland, S.T. Caulter and R. Jeness, J. Dairy Sci. 37:202 (1954).
7. Koch, R.B., B. Stern and C.G. Ferrari, Arch. Biochem. Biophys. 78:165 (1958).
8. Mitsuda, H., K. Yasumoto and K. Iwami, J. Jpn. Soc. Food Nutr. 19:210 (1966).
9. Hartman, L., J. Sci. Food Agric. 5:476 (1954).
10. Siddiqi, A.M., and A.L. Tappel, Chemist. Analyst 44:52 (1955).
11. Hicks, M., and J.M. Gebicki, Anal. Biochem. 99:249 (1979).
12. Stamm, J., J. Bull. Soc. Pharm. Estonia 5:181 (1925).
13. Hamm, D.L., E.G. Hammond, V. Parvanah and H.E. Snyder, J. Am. Oil Chem. Soc. 42:920 (1965).
14. Eskin, N.A.M., and C. Frankel, J. Am. Oil Chem. Soc. 53:746 (1976).
15. Takagi, T., Y. Mituno and M. Masumura, Lipids 13:147 (1978).
16. Asakawa, T., and S. Matsushita, J. Jpn. Soc. Food Nutr. 28:403 (1975).
17. Asakawa, T., and S. Matsushita, J. Jpn. Soc. Food Nutr. 29:408 (1976).
18. Asakawa, T., and S. Matsushita, Bull. Res. Inst. Food Sci., Kyoto Univ. 40:12 (1977).
19. Asakawa, T., and S. Matsushita, J. Am. Oil Chem. Soc. 55:619 (1978).
20. Terao, J., T. Ogawa and S. Matsushita, Agric. Biol. Chem. 39:397 (1975).
21. Baldwin, R.R., R.S. Bear and R.E. Rundle, J. Am. Chem. Soc. 66:111 (1944).

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