Comparison of Methods for Determining Peroxidation in Processed Whole Peanut Products

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ABSTRACT

Oxidation of fatty acids in peanut butter produces peroxides and changes in the conjugated diene contents. Two of the most widely used methods for measuring these oxidative effects, peroxide value and the spectrophotometric assay of conjugated diene hydroperoxides, were compared in determinations of shelf-life stability of peanut butters during short and long term storage. Results by the conjugated diene hydroperoxides method correlated with those by the peroxide value method over 4 and 12 week storage periods. The conjugated diene hydroperoxides method requires smaller samples and is quicker, more accurate, and simpler than the peroxide value method, nor does it require additional reagents nor depend upon a chemical reaction or color development.

INTRODUCTION

The three most widely used methods for determining degrees of rancidity, staling, or formation of lipid peroxides in oil containing food products are measurements of the peroxide value (PV), the increase in absorption at 234 nm due to increasing diene conjugation, and the thiobarbiturate (TBA) determination of malonaldehyde formation (1). All three are valid accepted methods depending upon the type of information sought, e.g. total amount of peroxides formed or change in diene conjugation in linoleate and higher polyunsaturated fatty acids. Conjugated double bonds are responsible for absorption at 234 nm, and the mechanism(s) causing peroxidation of polyunsaturated fatty acids produces conjugated diene hydroperoxides (CDHP).

During investigations on acceptable ways to retard or inhibit fatty acid peroxidation in peanut butters or roasted whole peanut products to increase their shelf-life, we required a rapid, simple technique for measuring lipid peroxidation because of the large number of samples to be assayed over long periods of time. In several series of experiments, all samples were analyzed simultaneously by modifications of both the PV and CDHP methods listed in the AOCS handbook (2).

The TBA method also has been used to determine oxidative rancidity in stored oils and oilseed products. However, it is based upon the reaction of oxidation products with TBA acid to produce a reddish color that is measured colorimetrically. The method is valid for analysis of oils, but, for quality evaluation of many lipid containing foods, some difficulties are encountered by reaction of the oxidation products with protein (or possibly carbohydrates) rather than with the reagent (3). In the CDHP and the PV methods, only the lipid peroxides are measured quantitatively. Therefore, the present comparison was confined to the latter two methods.

Although the PV method generally has been recognized as the method of choice for analysis of peroxide formation in edible oil products, the purpose of this report is to show that the CDHP method, used mostly to indicate *changes* in CDHP content of polyunsaturated fatty acids, also can be used as a rapid, simple alternate measure of PVs.

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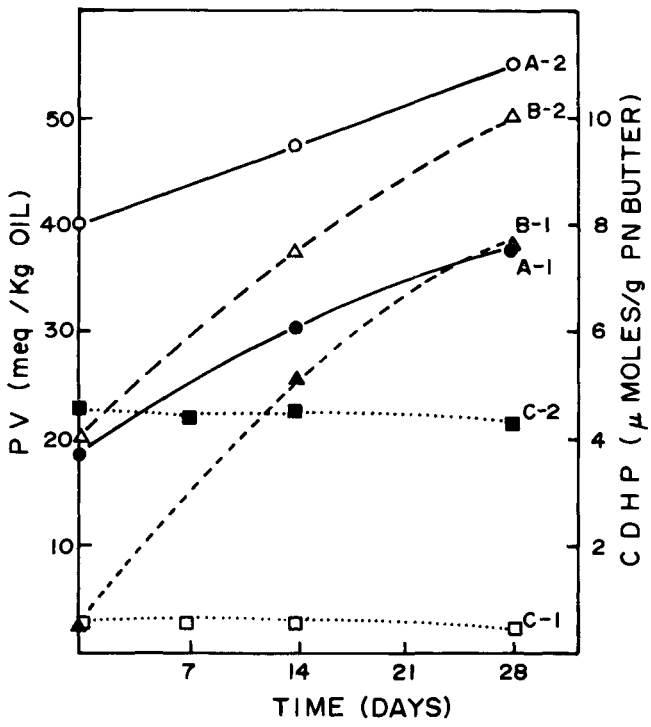

FIG. 1. Measurement of peroxidation of unsaturated fatty acids in a short term experiment. Curves A-1 and A-2 represent control samples; B-1 and B-2, peanut butter + 20 mg heat inactivated peroxidase; and C-1 and C-2, peanut butter + 0.02 mole sodium citrate. Peroxide values (PV) are plotted in curves A-1, B-1, and C-1; conjugated diene hydroperoxide (CDHP) values in A-2, B-2, and C-2. Each point is an average of three determinations.

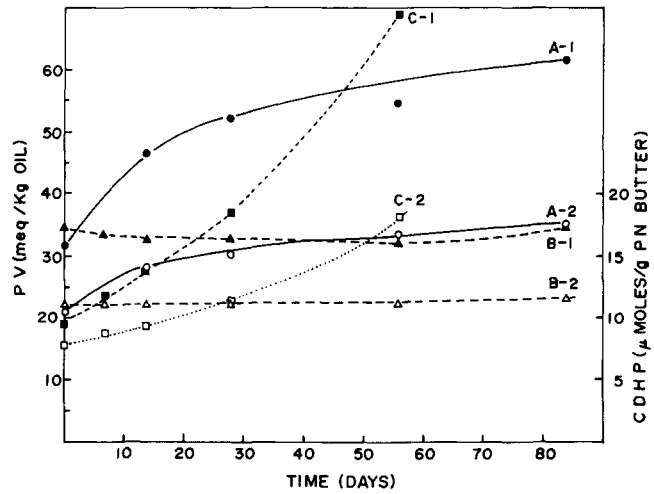

FIG. 2. Measurement of peroxidation of unsaturated fatty acids in a long term experiment. Curves A-1 and A-2 represent control samples; B-1 and B-2, peanut butter + 1.0 ml deionized water (for an antioxidant effect); C-1 and C-2, peanut butter + 0.02 mole cupric acetate (for a proxidant effect). Peroxide values (PV) are plotted in curves A-1, B-1, and C-1; conjugated diene hydroperoxide (CDHP) values in A-2, B-2, and C-2.

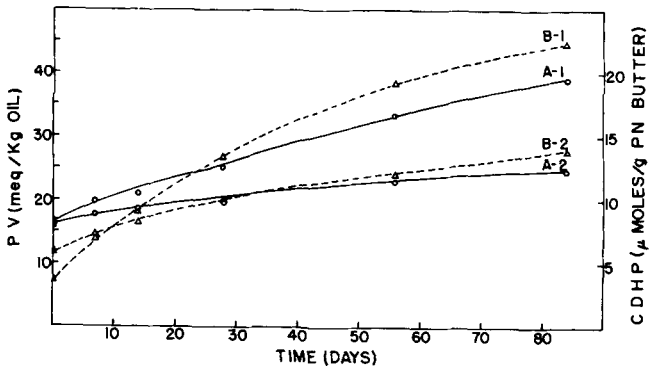

FIG. 3. Measurement of peroxidation of unsaturated fatty acids in a long term experiment. Curves A-1 and A-2 represent control samples; B-1 and B-2, peanut butter plus 20 mg heat inactivated peroxidase (a pro-oxidant). Peroxide values (PV) are plotted in curves A-1 and B-1; conjugated diene hydroperoxide (CDHP) values in A-2 and B-2.

EXPERIMENTAL PROCEDURES

In each series, 20 g commercial brand of peanut butter were weighed into autoclaved jars. Some served as controls (nothing added), and to others were added deionized water, sodium citrate, cupric ion, or peroxidase (Nutritional Biochemicals Corp., Cleveland, Ohio). Sampling procedures, PV, and CDHP values were determined as previously described (4). Briefly, samples (ca. 1.2 g) were removed at intervals, accurately weighed into centrifuge tubes, extracted with 30.0 ml spectrophotometric grade hexane for 1 hr, and centrifuged at 12,000 x g for 15 min at 4 C. The supernatants then were analyzed by both methods and the values plotted against storage time.

RESULTS AND DISCUSSION

Figure 1 illustrates the effects of additives upon peanut butters during a short term period (4 weeks), and Figures 2 and 3 show the effects over a longer period (12 weeks). All samples were stored at room temperature in the dark. During storage, control samples in all series (curves A-1 and A-2, Figs. 1-3) showed a gradual increase in oxidation, measured by either of the two methods. In a previous report (4), water and sodium citrate appeared to have antioxidant effects upon peanut butter. Peanut butters to which sodium citrate (Fig. 1, curves C-1 and C-2) or water (Fig. 2, curves B-1 and B-2) was added did not increase in peroxidation during storage, and values from both methods appeared to have a parallel relationship.

In the long term experiments (Figs. 2 and 3), correlation between the two methods was even more apparent. Curves for the controls and the peanut butter plus pro-oxidant materials, found to catalyze peroxidation of fatty acids in peanut butter (4), showed corresponding increases measured by both methods. The increase in peroxide formation catalyzed by added cupric ion in stored peanut butter (Fig. 2, curves C-1 and C-2) and by added heat denatured peroxidase (Fig. 3, curves B-1 and B-2) also yielded similar curves.

To test for possible correlation between the two methods, the PVs shown in Figures 1-3 were plotted against the corresponding CDHP values measured on the same samples (Fig. 4). The linearity of the curve indicates that results from the two methods are highly correlative. For samples in which there was a gradual increase in peroxide formation upon storage, the points fell along a straight line, the length of which is determined by the amount of increase in peroxides. Where there was little or no change in peroxidation (Fig. 1, curves C-1 and C-2; Fig. 2, curves B-1 and B-2), the points did not fall in a straight line in a PV vs CDHP plot, but rather in a cluster of points. The linear regression equation for CDHP and PV values from Figures

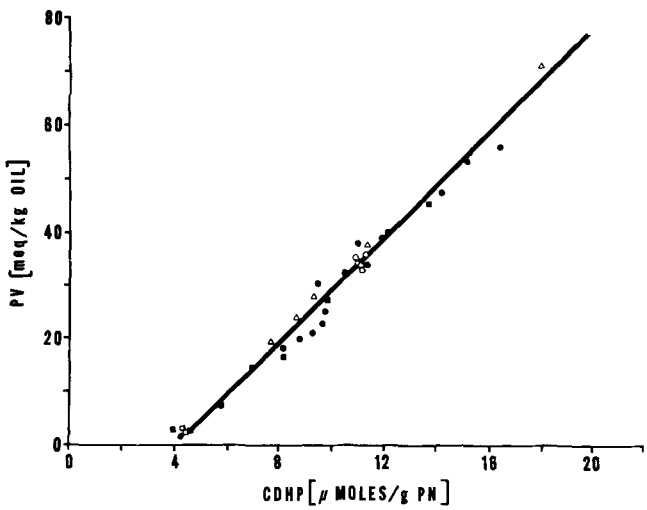

FIG. 4. Curves obtained by plotting peroxide value (PV) vs conjugated diene hydroperoxide (CDHP) values obtained from Figures 1-3: peanut butter controls (●); plus boiled peroxidase(⊠); plus water (○); plus cupric acetate (△); plus sodium citrate (□).

1-3, as plotted in Figure 4, is:

$$PV = -18.46 + 4.67\ (CDHP)$$

The standard error of estimate is ±2.29; the correlation coefficient is 0.98.

Brown, et al., (5) and Fore, et al., (6,7) have shown that roasted peanut and peanut butters produce certain volatile compounds that can be measured by a rapid gas chromatographic technique. Since the volatile components associated with stale flavors presumably are derived largely from peroxidized lipids, it is obvious that some of these peroxides break down further to volatile compounds detectable by gas chromatography but not by either the PV or the CDHP methods. However, the linear correlation between the latter two methods shown here suggests that the CDHP method also can be used to measure total peroxides present in whole oilseed products, such as peanut butter. The CDHP method could be used as an index of progressive staling in place of, or in additon to, PVs. The CDHP method, however, is faster than the PV method; it is much simpler, requires no chemical reagents, does not depend upon any chemical reaction or color development, and can be conducted on much smaller samples. This procedure should be applicable for the analysis of peroxides in vegetable oil products containing polyunsaturated fatty acids.

ACKNOWLEDGMENT

J.I. Wadsworth calculated the regression equation, standard error of estimate, and correlation coefficient.

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