Effect of Minor Constituents and Additives upon Peroxidation of Oil in Peanut Butter[1]

ALLEN J. ST. ANGELO and **ROBERT L. ORY**, Southern Regional Research Center[2], New Orleans, Louisiana 70179

ABSTRACT

Metalloproteins and ionic iron and copper salts are major catalysts of fatty acid peroxidation, but chelating agents, such as ethylenediaminetetraacetic acid and citric acid in water, can retard or reduce this catalytic effect. To extend shelf-life and retain the high quality of peanut butter, methods to prevent or decrease the catalytic effect of these metalloproteins and salts were conducted. Chelating agents suspended in inert solvents (mineral oil) were not as effective as when they were added in water. Depending upon the concentration, water can act as a prooxidant or an antioxidant in peanut butter.

INTRODUCTION

Peanuts are used in many food products, but one of their major uses in the U.S. is in the manufacture of peanut butter. Although some other countries produce larger quantities of peanuts, they use them mainly as a source of edible oil rather than in the manufacture of peanut butter. Peanut butter is a nutritious, high energy food containing 26-28% protein; it is high in B vitamins and minerals, and is a good source of polyunsaturated fatty acids (1,2). However, the main reason Americans eat peanut butter is because of its enjoyable flavor and aroma. Therefore, preserving these characteristics and increasing shelf-life of the product are of major importance.

In a preliminary study (3), we reported that certain metal containing proteins and salts of iron and copper could catalyze fatty acid peroxidation in peanut butter. This preliminary study showed that enzymatically active lipoxygenase, boiled cytochrome-C, and hemoglobin were not as active catalytically as were boiled peroxidase and boiled tyrosinase. Salts of iron and copper increased peroxidation more than did Raney nickel and salts of potassium, sodium, and calcium. The purpose of this paper is to present the results of extended studies on possible ways of increasing shelf-life of peanut butter by examining the more active catalysts (peroxidase and ferric and cupric salts) in combination with different amounts of water and chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and citric acid, which might be used to retard or decrease peroxide formation in samples stored up to 2 months.

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[2]ARS, USDA.

MATERIALS AND METHODS

Experimental Procedures

Commercial brands of fresh peanut butter were employed in all tests. These were various brands and lots of peanut butter prepared from different varieties of peanuts that were acquired from different sources. In each series of tests, a fresh, previously unopened jar of peanut butter was used. For each series, 20 g peanut butter was weighed into sterilized small glass jars. Additives were dissolved or suspended in either deionized water or mineral oil and the suspension added to the peanut butter and mixed by manual stirring. The jars then were closed tightly and stored in the dark at room temperature (24-26 C) until analyzed.

Additives were suspended in mineral oil by sonication for ca. 15 min. Peanut lipoxygenase (P/N enzyme prep) was prepared as described earlier (3). This was the 25-50% ammonium sulfate precipitate from the aqueous supernatant after centrifugation of a water extract of raw peanuts. Protein content is 0.4 mg/ml, equivalent to 0.12 units of lipoxygenase activity/ml (change in absorption measured at 234 nm). Horseradish peroxidase (Nutritional Biochemicals Corp., Cleveland, Ohio), metal salts, chelating agents, and mineral oil (Fisher Scientific Products Co., Fairlawn, N.J.) were commercial products. At weekly intervals (simulating normal home usage of opening and closing the jars in air), a small portion (ca. 1 g) of each peanut butter sample was weighed accurately into large centrifuge tubes, and 30 ml spectrophotometric grade hexane (Mallinckrodt Chemical Works, St. Louis, Mo.) was added to each tube. After being stirred thoroughly, the samples were allowed to stand for 1 hr, then centrifuged at 12000 x g for 15 min at 4 C. Precipitates were discarded, and the supernatants containing the oil, fatty acids, and peroxidized lipids were analyzed immediately for both peroxide value (PV) and conjugated diene hydroperoxide (CDHP) contents.

Results obtained on triplicate analyses of each sample showed the CDHP method to be reproducible within 0.2

TABLE I

Effect of Additives in Water upon Peroxidation of Fatty Acids in Peanut Butter: Results with Peanut Butter Sample A[a]

	ΔPV (meq/kg oil)			ΔCDHP (μmoles/g/PNBu)[b]		
	Days in storage					
Additive	7	14	28	7	14	28
None	---	11.9	19.2	2.0	1.5	3.0
2.5 ml water (11%)	-0.2	0.2	1.2	0	-0.5	-0.5
2.5 ml water P/N Enzyme[c]	2.9	4.5	3.7	1.5	1.0	1.0
2.5 ml water + boiled peroxidase[d]	15.5	23.2	35.6	3.5	3.5	6.0

[a]Values represent increases after 28 days storage.

[b]PV = peroxide value, CDHP = conjugated diene hydroperoxide , PNBu = peanut butter.

[c]1.0 mg (on protein basis).

[d]20 mg.

TABLE II

Effect of Additives in Water upon Peroxidation of Fatty Acids in Peanut Butter: Results with Peanut Butter Sample B[a]

Additive	ΔPV (meq/kg oil) Days in storage			ΔCDHP (μmoles/g PNBu) Days in storage		
	7	14	28	7	14	28
None	-4.1	14.6	20.4	-0.5	3.5	4.6
1.0 ml water (4.8%)	-1.2	-1.1	-1.7	0.1	0	0.2
1.0 ml water + boiled peroxidase[c]	2.2	3.6	7.3	1.0	1.1	1.8
1.0 ml water + boiled peroxidase[c] + EDTA[d]	-0.3	1.2	1.5	0.3	0.3	0.8

[a]Values represent increases after 28 days storage.

[b]PV = peroxide value, CDHP = conjugated diene hydroperoxide, and PNBu = peanut butter.

[c]20 mg.

[d]EDTA = ethylenediaminetetraacetic acid, 0.1 mmole.

μmole CDHP/g peanut butter for each sample within a given series. However, the preliminary study (3) showed some variations between O day values for control and test peanut butters removed from the same freshly opened jar. Since the time and manner of mixing additives in the 20 g sample and the periodic removal of aliquots was consistent throughout the tests, the procedure used in removal of 20 g samples from the original fresh jars was suspected as the source of these variations. Samples taken from the top (at the air space) already had begun staling, whereas later samples for the series removed from the bottom of the jar had less peroxidized oil. To avoid these possible sampling errors and differences in O day values and to better compare changes in peroxidation from the first day up through 2 months storage, net changes in PV or CDHP units (Δ PV or Δ CDHP) were employed in these and subsequent tests.

RESULTS AND DISCUSSION

Effects of Additives in Water

Table I shows the effects of materials added in 2.5 ml water (11%, v/w) upon peroxidation of fatty acids in peanut butter after a 1 month storage period. All values represent the net increase in PV and in CDHP content from the starting day of the experimental period. These results show that control sample A with no additives increased 19.2 meq/kg oil in PV and 3.0 μmoles/g peanut butter in CDHP content within the 28 days stored; but, when 2.5 ml water was added, there was virtually no increase in peroxide formation. This suggested that water, at this concentration, has an apparent protective function by preventing oxidation. Freshly prepared peanut lipoxygenase (P/N enzyme prep) showed a 3-fold increase in peroxidation over the sample with added water (compare PVs of 1.2-3.7 and CDHPs of -0.5-1.0). Boiled peroxidase, a metal containing enzyme that previously was shown to catalyze lipid peroxidation in peanut butter (3), significantly increased the rate of peroxidation. It, thus, appears that heat denatured peroxidase is able to overcome the apparent antioxidant effect of water much more than active lipoxygenase, the enzyme considered by many to be the primary catalyst in lipid peroxidation of unsaturated seed oils.

Peroxidase activity has been studied extensively by disc gel electrophoresis in several varieties of peanuts grown in different geographic areas (4,5). Five major isozyme patterns from mature peanuts comprised 78% of the 15 different patterns observed for 15 different varieties of Virginia, Spanish, and Runner peanuts examined (4). Five major isozymes were common to all varieties, indicating that peanuts contain many forms and amounts of peroxidase, which, after peanuts are roasted and homogenized during peanut butter manufacture, could contribute significantly to peroxidation, as nonenzyme hemeprotein catalysts.

TABLE III

Effect of Added Water upon Peroxidation of Fatty Acids in Peanut Butter[a]

Concentration (%)	Days in storage 16	28	56
0	0	1.4	2.5
0.6	1.3	2.5	5.8
1.2	0.3	0.8	2.4
2.4	-0.4	-0.2	0.1
4.8	-0.6	-0.3	-0.1

[a]Values are given as the change in conjugated diene hydroperoxide (μmoles/g).

Table II shows the effects of this hemeprotein in a lower concentration of water (4.8%) on peroxidation of peanut butter fatty acids. The control sample with no added water showed increases of 20.4 (PV) and 4.6 (CDHP). When 1 ml water/20 g peanut butter was added, these values dropped to -1.7 and 0.2, respectively, showing that water, at this concentration, also had a protective effect against oxidation. Boiled peroxidase (enzymatically inactive) added in 1 ml water was able to promote some oxidation but not as much as that found in the control. When a chelating agent, EDTA, was added, the prooxidant effect of the peroxidase was retarded greatly decreasing the 2 values to 1.5 and 0.8, respectively. This indicates that the EDTA probably binds the iron in the peroxidase.

Effects of Water Concentration

Since water had an apparent protective function and seemed to prevent oxidation (Tables I and II), the effects of different concentrations of water upon peroxidation were studied during the 56 day storage period (Table III). Peanut butter with no added water showed the expected normal increase in peroxidation of 1.4 and 2.5 μmoles/g. At only 0.6% water, CDHP values showed increases over the control at the same stages (values of 2.5 and 5.8 compared with 1.4 and 2.5). Thus, at this low concentration, water appeared to be a prooxidant. Increasing the water content to 1.2% gave results comparable to those for the control, but, at concentrations of 2.4% and 4.8%, there was a definite decrease in CDHP content. Thus, depending upon the concentration, peroxidation was either accelerated, remained the same, or decreased.

Labuza, et al., (6,7) have discussed this effect of water upon lipid oxidation in foods in terms of water activity or relative humidity. If water activity is plotted against relative activity (lipid oxidation), an inverted bell-shaped curve is obtained that indicates, at very low and at very high water

TABLE IV

Effect of Additives in Mineral Oil upon Peroxidation of Fatty Acids in Peanut Butter[a]

Additive	ΔCDHP (μmoles/g) Days in storage 16	28	56
None	0	1.4	2.5
Mineral Oil (1.0 ml)	0.9	2.5	3.5
Peroxidase[b]	1.2	2.6	3.2
Peroxidase[b] + EDTA (0.01 mmole)	1.5	2.5	4.0
Peroxidase[b] + EDTA (0.1 mmole)	1.5	2.6	4.5
Fe^{+3} (0.01 mM)	2.5	3.6	7.2
Fe^{+3} (0.05 mM)	3.6	4.0	7.1
Fe^{+3} (0.05 mM) + EDTA (0.1 mmole)	1.3	2.7	6.0
Cu^{+2} (0.01 mM)	1.2	2.6	5.8
Cu^{+2} (0.05 mM)	3.1	4.5	8.3
Cu^{+2} (0.05 mM) + EDTA (0.1 mmole)	2.4	3.7	7.4

[a]CDHP = conjugated diene hydroperoxide and EDTA = ethylenediaminetetraacetic acid.

[b]20 mg.

activities, water acts as a prooxidant. In between these two extremes (in the trough of his curve), water acts as an antioxidant. As Labuza (7) suggested, the protective function that water exhibited when the moisture content increased from the absolute dry state could be accounted for by two factors: water interacts with metal catalysts making them less effective through changes in their coordination sphere, or water hydrogen bonds with hydroperoxides tying them up so that they no longer are available for decomposition through initiation reactions. Salwin (8) previously attempted to explain how water interacted to decrease lipid oxidation by suggesting that it attached to sites on the food surface, thereby excluding oxygen from the lipid. Our results in Table III support Labuza's observations that water can act either as a prooxidant or as an antioxidant. The relative humidity in the air space above the peanut butter in the jars was not measured during our analyses. However, based upon estimations using the data of Labuzza, et al., (6) we assume that the water contents of samples in which oxidation was decreased are in the range in which water acts as an antioxidant.

Effects of Additives in Inert Solvent

In an attempt to examine the effects of additives without the apparent antioxidant influence of water, the additives were suspended in an inert solvent, mineral oil, then added to peanut butters that were to be sampled intermittently during the storage period. The results are shown in Table IV. After 56 days of storage, a peanut butter with added mineral oil had a CDHP content 3.5 μmoles/g as compared with 2.5 for the control. The reason for this increase in CDHP with mineral oil is unknown, and the increase was unexpected. It may have been due to something present in the oil which was a commercial product. However, peroxidase suspended in mineral oil showed almost no catalytic effect at all (value, 3.2). When EDTA was added at two concentrations, there was no apparent inhibition or chelating activity, as when EDTA was added in water (Tables I and II). Thus, it appears that peroxidase was not able to catalyze peroxidation in mineral oil; whereas, it readily does so in water. It is possible that the inability of the metal in the hemeprotein to ionize in an inert solvent may be the reason for this apparent loss of catalytic activity of the peroxidase; or the loss may be attributed to reasons previously suggested by Labuza (7).

When iron and copper salts were suspended in mineral oil and added to peanut butter, there was a small increase in CDHP values: 3.6 and 4.0 for iron, 2.6 and 4.5 for copper. However, in these cases, EDTA was able to reduce the catalytic effect from 4.0 to 2.7 for iron and from 4.5 to 3.7 for copper. Similar effects were obtained with citric acid as the chelating agent. Perhaps normal peanut butter contains sufficient water, even through the amount is small, for ionization of both the free metals and the EDTA. This suggests that metalloproteins may require a certain minimum concentration of water before they can catalyze lipid peroxidation, but free metals can increase peroxidation significantly in these lower water concentrations, even when suspended in the inert solvent, mineral oil.

It is interesting to note that water, depending upon the concentration, appeared to act either as a prooxidant or an antioxidant in peanut butter. Water, however, may have an effect upon other properties of peanut butter, i.e. texture, color, protein quality, microbial growth, etc. Therefore, before concluding that the addition of water could enhance the keeping quality of peanut butter significantly, a more detailed investigation on the effects of water is needed.

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