Disappearance of BHA and BHT in Relation to Peroxide Content in Breakfast Cereals[1]

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Abstract

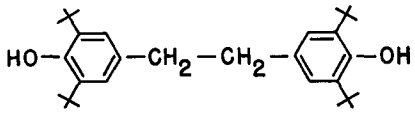
In ready-to-eat breakfast cereals the amounts of BHA and BHT remaining at any time during storage were inversely proportional to the amount of peroxides present in the cereal. At the point of organoleptic unacceptability of stored cereals the peroxide numbers were approximately 120, and contents of BHA and BHT were each approximately 10% to 20% of the initial levels.

No discernible amount of a dimeric oxidation product of BHA or BHT could be isolated from any stored cereal which contained BHA and BHT. It is likely that dimeric oxidation products do not occur in appreciable amounts as intermediates or final products in the reactions of BHA and BHT with peroxides in cereals. It is probable that each mole of BHA and BHT in cereals reacts with several moles of peroxide radicals to form hydroperoxides and complexes of antioxidants with peroxide radicals.

Introduction

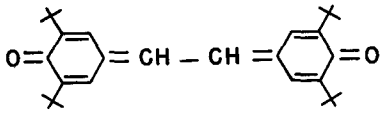
THERE HAVE BEEN a few reported studies of reactions of BHA (3-t-butyl-4-hydroxyanisole) and BHT (3,5-di-t-butyl-4-hydroxytoluene) with peroxides and other oxidizing agents. These reactions have been studied under conditions wherein isolation of products could be relatively easily effected. There have been no reports of studies of oxidation of BHA and BHT in food products.

Four products of the oxidation of BHT have been reported.



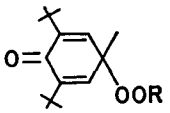
[I]

1,2-bis(3,5-di-t-butyl-4-hydroxyphenyl) ethane



[II]

3,5,3′,5′-tetra-t-butylstilbene-4,4′-quinone



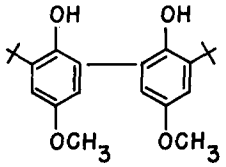
[where R is $-C(CH_3)_3$] [III]

1-me-1-t-butylperoxy-3,5-di-t-butyl-2,5-cyclohexadienone-4

[where R is $-C(CN)(CH_3)_2$] [IV]

1-me-1(2-peroxy-2-cyanopropyl)-3,5-di-t-butyl-2,5-cyclohexadienone-4.

There has been one report of the isolation of a product of the oxidation of BHA. Rosenwald and Chenicek (9) obtained 2,2′-dihydroxy-3,3′-di-t-butyl-5,5′-dimethoxybiphenyl (V) by the oxidation of BHA with potassium ferricyanide.



[V]

Compounds I and II were obtained by Cosgrove and Waters (8), and Yohe and co-workers (11) by the oxidation of BHT with benzoyl peroxide in boiling chloroform or with oxygen in alkaline, aqueous solution.

In this laboratory BHA was oxidized with benzoyl peroxide in the same manner as that employed by Cosgrove and Waters which resulted in dimeric products of the oxidation of BHT. Instead of a dimer, a benzoate of BHA was obtained as the principal product (1). This product is a monobenzoate, molecular weight 300, but it has not been established whether the benzoate group is in position 2,5 or 6 on the ring.

Campbell and Coppinger (6) obtained the crystalline peroxide (III) by the reaction of BHT with t-butyl hydroperoxide in a heated solution of t-butanol.

Boozer and co-workers (5) studied the inhibitory effects of a number of phenolic antioxidants on the autoxidation of cumene initiated by azo-bis-isobutyronitrile. From the reaction mixture containing BHT as the inhibitor a peroxide (IV) was isolated which was analogous to that obtained by Campbell and Coppinger. Since both peroxides were obtained in high yield, Campbell and Coppinger and Boozer and co-workers concluded that the formation of the peroxide represented the main course of the reaction and that no other product was formed in a significant amount. By measurements of the inhibition of the autoxidation of cumene by the various phenolic antioxidants, and by determinations of stoichiometric factors, Boozer and co-workers concluded that each mole of antioxidant reacted with two or more moles of peroxide radicals to form hydroperoxides and complexes of the antioxidants with peroxide radicals.

Since action of an antioxidant in a dehydrated food, such as a ready-to-eat cereal, may differ from its action in an oil or other solvent, we considered it worth while to attempt a preliminary investigation of the mechanism of the actions of BHA and BHT in breakfast cereals. The isolation of an adduct of BHA or BHT with a fatty acid peroxide moiety of a glyceride would probably be difficult. For that reason, we chose to investigate the mechanism by a "back door" approach to that of Boozer and co-workers. It was assumed that if no dimeric products of the oxidation of BHA and BHT were found in stored cereals containing those antioxidants, we might

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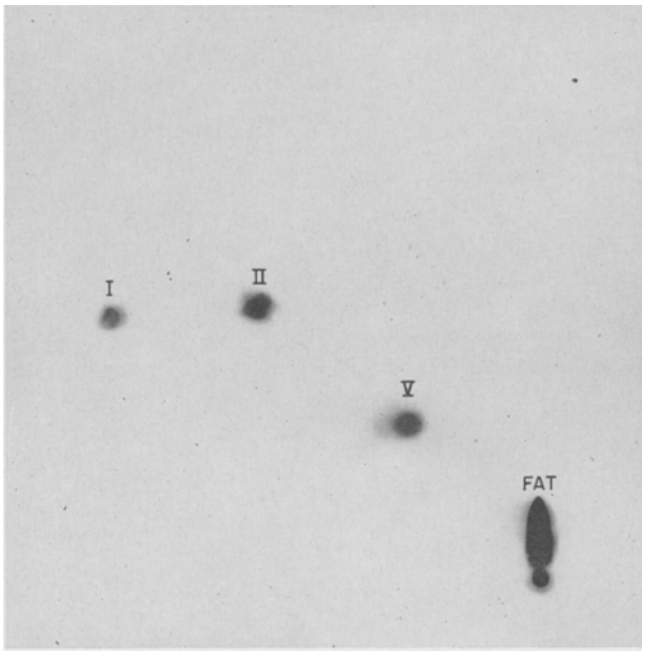

FIG. 1. Silica coated glass plate, coating 1 mm. Spots of I, II, V, and fat of wheat flakes in ether placed at origin. Developed in 90% hexane—10% ethyl ether. Sprayed with sulfuric acid and heated.

speculate that the antioxidants were forming adducts with peroxide radicals in a manner similar to that proposed by Campbell and Coppinger, and Boozer and co-workers. Measurements of the rate of disappearance of BHA and of BHT vs. appearance of peroxides were planned to test the supposition that BHA and BHT react with fatty peroxide radicals in breakfast cereals to form complexes and hydroperoxides.

The present investigation was undertaken with two objectives in mind. The first was to correlate the destruction of BHA and BHT in ready-to-eat breakfast cereals with organoleptic condition, peroxide content, and length of storage of the cereals. The second objective was to demonstrate whether or not dimeric oxidation products of BHA and BHT are intermediates or final products in the reactions of BHA and BHT with peroxides in cereals.

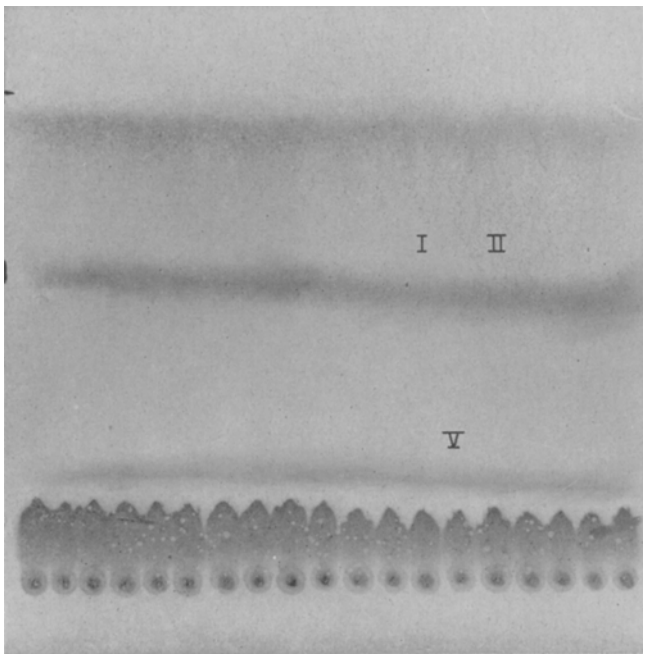

FIG. 2. Silica coated glass plate, coating 1 mm. Cereal fat (0.03 g in 1 ml ether) from corn flakes containing 5 ppm of each of I, II, and V placed in multiple spots at origin. Developed in 90% hexane—10% ethyl ether. Sprayed with sulfuric acid and heated.

Experimental

Compounds I and II were synthesized by the method of Cosgrove and Waters (8); compound I was also prepared by reduction of II with lithium aluminum hydride using the method of Bohn and Campbell (4). Compounds I and II had melting points corresponding to those previously reported and had infrared absorbance patterns similar to those reported by Bohn and Campbell.

Samples of ready-to-eat cereals of several types were sprayed with solutions of BHA and BHT in alcohol to several levels of antioxidants in each cereal. Cereals utilized were wheat flakes, corn flakes, and a puffed oat cereal. Other samples of the same cereals were sprayed with an alcoholic solution of I, II, and V to a level of 10 ppm of each oxidation product in each cereal. Portions of all the sprayed cereals were stored in regular cereal packages, double lined with glassine paper and in sealed jars. Duplicate samples were stored at 100F and 76F. Periodically, samples of stored cereals were examined organoleptically and levels of peroxides, BHA, BHT, and I, II, and V were determined.

Peroxide numbers were determined by the method of Smith (7). Samples for analyses were obtained by swirling 60 ml of benzene for 2 min in an Erlenmeyer flask with coarsely ground cereal (10 g puffed oat cereal, 20 g wheat flakes, or 30 g corn flakes). The mixture was filtered rapidly through a folded Whatman No. 1 paper, or decanted. A 10 ml aliquot was used to determine the fat content of the benzene solution and another 10 ml aliquot was analyzed for peroxide content. The intensity of red color which developed was read in a Coleman spectrophotometer at 490 mμ. The amounts of peroxides present were determined by comparison of the intensities of the unknowns with a standard curve prepared according to Smith's directions. Peroxide numbers determined in this manner have been shown by a number of workers (7) to be twice the magnitude of those obtained by the iodometric method. For closer comparison of peroxide numbers of cereal fats with those of fats determined by the iodometric method each peroxide number given in this report is half the value of the peroxide number actually obtained by the colorimetric thiocyanate method.

BHA and BHT were extracted from cereals by a method described by Anderson and Nelson (3). A mixture of 100 g ground cereal and 300 ml ethyl ether was shaken for 30 min. The mixture was rapidly filtered and the filtrate measured. The filtrate was concentrated to 20 ml by evaporation under water pump vacuum with just enough heat to keep the ether boiling. The concentrated filtrate was then reduced to 5 ml by blowing a gentle stream of nitrogen over it. To each filtrate was added 1 mg of 3,5-di-t-butyl-4-hydroxyanisole (di-BHA) as an internal standard. A suitable amount of the concentrated extract was injected into a model 609 F. and M. GLC instrument with a hydrogen flame ionization detector. The method used to determine BHA and BHT is described in detail elsewhere (10). It employs a 10 ft aluminum column packed with Chromosorb W, 70/30 mesh, coated with Tween 80 (1%) mixed with SE-30 silicone (2%).

Instrumental conditions: Detector and injection-port temperatures 250 C; helium and hydrogen each 50 ml/min, air 550 ml/min.

The area of each peak was determined by multiplying the peak height by the half band width. The ratio of the area of the di-BHA peak to the area of the BHA or BHT peak was calculated. The weight ratio of BHA or BHT present in each extract could be determined by comparing the respective area ratio to a calibration curve. The calibration curve was obtained by plotting known weight ratios of each antioxidant with di-BHA against area ratio responses calculated from chromatograms of the known solutions. By this method BHA or BHT may be measured in a cereal to as low a level as 0.5 ppm.

The dimeric oxidation products of BHA and BHT were also extracted from cereals by ether extraction. Aliquots of the concentrated ether extracts could not be directly injected into the GLC apparatus, because at the high column temperature necessary to vaporize the oxidation products there was considerable interference from products of the thermal decomposition of cereal fats. It was found that I, II, and V were separable from cereal fat by thin layer chromatography. Figure 1 shows a plate that has been developed in a solution of 90% hexane and 10% ethyl ether, sprayed with sulfuric acid and heated. At the origin were placed four spots: solutions of each of I, II, V, and cereal fat in ether. If such a plate were sprayed with a solution of sodium fluorescein, spots of I and V were purple and II was brown under UV light.

In order to effect separation of oxidation products from cereal fats, 0.03 g of fat from each extract was diluted to 1 ml with ether and streaked at the bottom of a square 200 mm glass plate coated with silica 1 mm in thickness. Plates were coated with a variable thickness coating apparatus obtained from Brinkmann Instruments Inc., Great Neck, N. Y.

The plate was immersed to a depth of approximately 0.5 in. in a mixture of 90% hexane and 10% ethyl ether in a covered glass jar, and allowed to remain until the solvent front was 1 in. from the top of the plate.

Figure 2 shows a plate prepared from cereal fat extracted from a corn cereal containing 5 ppm of each of I, II, and V, developed in the manner described, sprayed with sulfuric acid, and heated.

Compounds I and II were relatively nonpolar and advanced close to the solvent front. Compound V was less polar than triglycerides, and advanced slightly ahead of the oil front. Compounds I, II, and V were recovered from the plate by scraping off the silica layer in the proper areas and eluting the silica with ether. The ether extracts were concentrated and suitable aliquots injected into the GLC apparatus. The column used was a 5 ft aluminum column with Chromosorb W packing coated with 2% SE-30 (General Electric). Injection-port and detector temperatures and flow rates of helium, hydrogen, and air were the same as those used for BHA and BHT. One run was made at a column temperature of 210C to obtain a chromatogram of V, and a second run was made at a column temperature of 235C to obtain a chromatogram of I and II. Areas of peaks of I, II, and V were calculated by multiplying peak heights by half-band widths. Calculated areas in chromatograms of cereal extracts were compared to a standard curve obtained by plotting known weights of each compound injected into the GLC instrument against areas of peaks

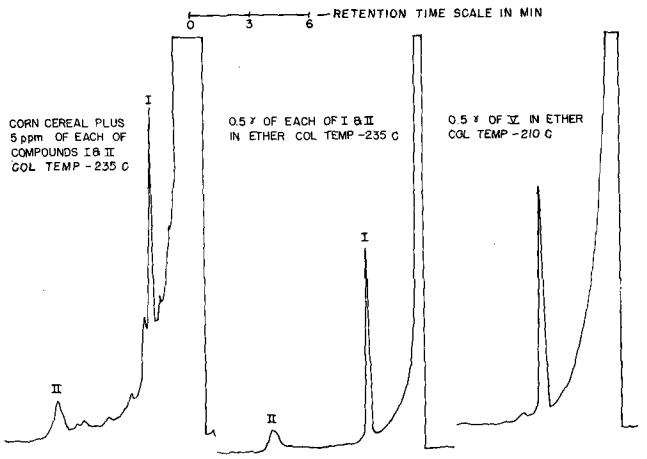

FIG. 3. Chromatograms of I and II and of V in ethyl ether, and chromatogram of fat of a corn cereal which contained 5 ppm of each of I and II, diluted in ether so that 0.5 γ of each compound was injected into the column.

obtained. Recovery experiments wherein oxidation products were determined in cereals to which known amounts had been added, showed that recovery of approximately 90% of each oxidation product was obtained, and that each of I, II, or V could be detected in a cereal at as low a level as 0.5 ppm.

Figure 3 shows chromatograms of ether solutions of I and II and V, and of I and II recovered from a cereal to which known amounts of the dimeric oxidation products were added. Compounds I and II were determined separately from V because at the higher column temperature used for I and II compound V vaporized too readily.

Discussion

In ready-to-eat cereals amounts of BHA and BHT present decreased linearly with time of storage and with increases in peroxide numbers of the cereal oils. These interrelationships are depicted for stored wheat flake cereals in Figure 4.

Data obtained from measurements of puffed oat cereals stored at 100F practically duplicated that of wheat flake cereals with comparable initial levels of BHA and BHT. Corn flakes responded more strongly to antioxidants, so the disappearance of antioxidants as well as the increase in peroxide content took place more slowly at 100F than in wheat flakes. At levels of added BHA and BHT of 15 ppm each, fat in corn flakes reached a peroxide number (PN) of 120 in

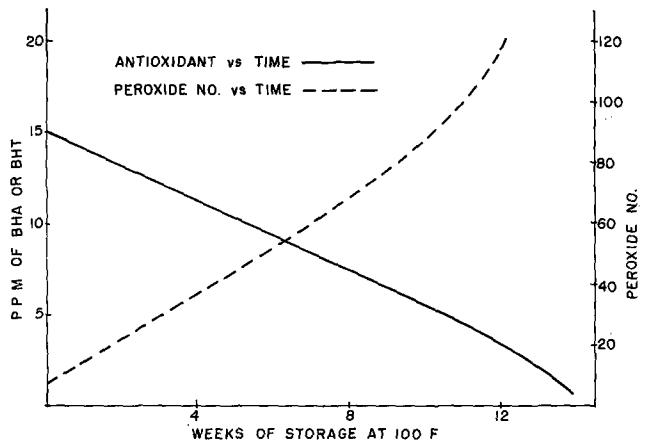

FIG. 4. Wheat flake cereals to which 15 ppm of each of BHA and BHT were added. Stored at 100F in packages with double glassine paper liners.

approximately 16 weeks. The relationships of remaining concentrations of BHA and BHT compared to PN were approximately the same as those in wheat flakes, in that each antioxidant was at approximately 20% of its initial concentration at the time that the PN of the fat in the corn flakes reached 120.

In cereals which contained initial levels of BHA and BHT ranging from 5 to 20 ppm of each antioxidant, the content of BHA or BHT of each cereal decreased linearly with an increase in the PN of the fat of the cereal during storage. When the fat reached a PN of 120, the content of BHA and BHT in each cereal was approximately 20% of the initial value. In the case of cereals with low initial levels of antioxidants, the contents of BHA or BHT at the point of rancidity were low and values determined probably had a large margin of error.

At 76F cereals were stored approximately three times as long as comparable samples stored at 100F before fats in the cereals reached PN of 120 and the cereals smelled rancid. At this point approximately 10% of the initial content of BHA and BHT remained in each cereal. The slightly lower concentration of BHA and BHT remaining at the point of rancidity in cereals stored at 76F may be due to the extended length of time of storage. Some BHA and BHT may be altered chemically by reactions with compounds other than free radicals of peroxides, and longer storage time at a lower temperature might increase the proportions of BHA and BHT destroyed in this manner.

In a few samples of stored cereals BHA was retained to a slightly greater degree than BHT, but in the main both antioxidants were destroyed at approximately the same rate.

Presumably, neither BHA nor BHT disappeared appreciably by volatilization from cereals stored in packages with double glassine liners. The rate of disappearance of either antioxidant from stored packages approximated that determined for cereals held in sealed jars at the same temperature.

In general, in all cereals included in these studies, the contents of each of BHA and BHT decreased linearly with increasing PN of the cereal fats, so that approximately 10% to 20% of the initial contents of the antioxidants remained at PN of 120. Storage lives of corn based cereals in general were extended to greater degree by addition of BHA and BHT than storage lives of wheat or oat based ready-to-eat cereals. Storage lives of individual samples varied widely and no generalization could be made that corn cereals were more stable than oat or wheat cereals without added antioxidants. Studies by Anderson et al. (2) have shown that antioxidants are formed in cereals during processing, presumably by browning reactions. Parameters in processing affect storage lives of cereals so that relative stabilities of corn, oat, or wheat cereals can be inverted by changing conditions of processing.

The molar disappearance of each of BHA and BHT was calculated and compared with the molar appearance of peroxide oxygen as measured by peroxide numbers at various points during the aging of cereals. An average value was obtained of approximately 5 moles of peroxide oxygen present for every mole of BHA or BHT oxidized in corn cereals. In wheat cereals and in oat cereals there were approximately 10 moles and 35 moles, respectively, of peroxide oxygen present for every mole of BHA or BHT oxidized. Corn, wheat, and oat cereals contain approximately 1%, 2%, and 7% fat, respectively. The amount of peroxide oxygen present in each cereal in relation to BHA or BHT destroyed was approximately proportional to the amount of fat present. This relationship indicates that in stored cereals the rate of disappearance of BHA and BHT was related to the moles of peroxides per unit of fat in the cereals, and was independent of the total amounts of peroxides or the amounts of fat present in the cereals. The concentrations of BHA and BHT in cereals decreased on storage at approximately the same percentage rate with increasing PN independently of the magnitude of the initial concentrations. Increases in the initial concentrations of BHA and BHT in cereals resulted in longer storage lives of those cereals before PN reached 120, and the concentrations of BHA and BHT were at 10% to 20% of the initial levels.

The ratio of the moles of peroxides formed to the moles of BHA or BHT lost during any period in stored cereals indicates that the reaction of BHA or BHT with peroxide radicals is more complex than a simple equimolar reaction which might be expected to give one mole of a dimeric oxidation product of BHA or BHT corresponding to the destruction of two oxidation chains.

No discernible amounts of I, II, or V were obtained from stored cereals containing BHA asd BHT at any periodic inspection. Storage tests of cereals containing added I, II, and V showed that II and V were retained in cereals without appreciable destruction until the cereals were organoleptically unacceptable. There was a measurable drop in content of I in several experiments; but losses of this compound were not consistent in different cereals at different temperatures and varied from no appreciable loss to 30% loss. On the supposition that I might be oxidized to II and replace any II lost, stored cereals fortified only with II were analyzed periodically. There was no indication of measurable loss of II in these cereals during the period of organoleptic acceptability.

Results obtained in this study did not disprove the supposition that BHA and BHT in cereals are destroyed by reactions with peroxide radicals in a manner analogous to that proposed by Campbell and Coppinger, and Boozer and co-workers. The dimeric oxidation products I, II, and V were not formed in appreciable amounts as intermediates or final products of the oxidation of BHA and BHT in stored cereals. If BHA or BHT had undergone equimolar hydrogen doning reactions with peroxide radicals, followed by coupling of the resulting semiquinones, I, II, or V should have appeared in an appreciable amount in stored cereals.

ACKNOWLEDGMENTS

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