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.

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

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

1-me-1-t-butylperoxy-3,5-di-t-butyl-2,5-cyclohexadienone-4
[where R is -C(CH₃)₃]

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

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-butyldihydroperoxide 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 worthwhile 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...
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.

**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 μm. 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 3/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%).