Alteration of Phenolic Antioxidants in Heated Vegetable Oil

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ABSTRACT

Studies were conducted to follow the fate of antioxidants in heated vegetable oil at temperatures approximating those of frying conditions. Samples of vegetable oils spiked with 0.02% [^{14}C] BHA or 0.02% [^{14}C] TBHQ were heated at ca. 180-190 C for 4.5 hr. It was found that 24 and 48% of the radioactivity added as BHA and TBHQ, respectively, remained in the oils. Normal-phase HPLC analyses revealed that 55% of the radiolabel in the heated BHA-oil and 95% in the heated TBHQ-oil were not eluted as parent substances. The as-yet unidentified alteration products in the heated oil appear to be less polar than the added antioxidants.

ABBREVIATIONS

BHA, 2- or 3-*t*-butylhydroxyanisole; BHT, 2,6-di-*t*-butylhydroxytoluene; TBHQ, *t*-butylhydroquinone; HPLC, high pressure liquid chromatography.

INTRODUCTION

Phenolic antioxidants such as BHA, BHT, TBHQ and propyl gallate play an important role in the manufacturing, packaging and storage of fats and fatty foods. These compounds have undergone extensive toxicological evaluations (1-6) and, in general, they show low orders of toxicity in laboratory animals. As mandated in the Food, Drug and Cosmetic Act, the addition of these antioxidants, alone or in combination, is limited to 0.02% by weight of the fat or oil content of the food (7).

In the safety evaluation of these compounds, or of direct food additives in general, a potential problem that has largely been ignored is the safety of the chemical alteration products of the additives produced during food processing and storage, and, in particular, during food preparation at high temperatures. To the extent that changes of the additives and subsequent reactions with food components occur in these processes, human exposure to anthropogenic compounds of unknown biological activity is inevitable.

There have been only a few reports in the literature concerning the detection, isolation, and identification of the alteration products of antioxidants in some food matrices. Mihara et al. (8) reported the formation of diphenylethane and diphenylether derivatives of BHA in soybean oil exposed to sunlight for 70 days. Leventhal et al. (9) isolated and identified stilbenequinone from edible oil with added BHT following heat treatment at 190 C for 11 days. However, the experimental conditions of these studies were exaggerated and investigations are needed which more closely approximate actual food storage and preparation conditions.

We have initiated a study of the stability of antioxidants in heated vegetable oil. In this report, we present evidence of the alteration of BHA and TBHQ.

EXPERIMENTAL PROCEDURES

Materials

A partially hydrogenated soybean oil with no added antioxidants (Crisco oil, Procter and Gamble, Cincinnati, OH) was employed for the study. BHA and TBHQ (food grade) were obtained from Eastman Chemical Co., Kingsport, TN. [^{14}C]-BHA (ring-labeled, sp act 9.7 mCi/mmol, 92.7% 3-isomer and 7.3% 2-isomer) and [^{14}C]-TBHQ (ring-labeled, sp act 9.7 mCi/mmol, 98.0% TBHQ and 2.0% *t*-butylbenzoquinone) were custom-synthesized and their purities established by Dynapol, Palo Alto, CA. All solvents used for extraction and chromatography were glass-distilled (Burdick and Jackson, Muskegon, MI). Ready-solve NA for nonaqueous samples (Beckman Instruments, Fullerton, CA) was used as the liquid scintillation counting cocktail.

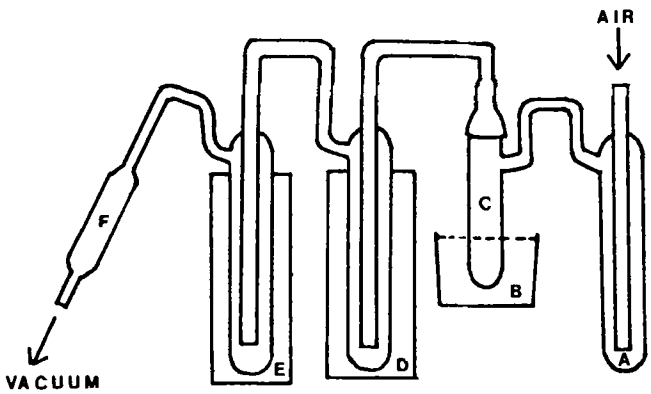

FIG. 1. The heating apparatus: A. moist air generator; B. silicon oil bath and heating mantle; C. reactor vessel; D. methanol trap immersed in ice water bath; E. Dry Ice/acetone trap; F. charcoal trap.

Spiking and Heating Procedures

Unlabeled and ^{14}C-labeled BHA or TBHQ dissolved in 200 μL of benzene were added to 4 g of oil to yield an antioxidant concentration of 0.02% and specific radioactivities of 7,600 dpm/mg oil and 2,700 dpm/mg oil for BHA and TBHQ, respectively. After removing the benzene with a stream of nitrogen, the antioxidant-spiked oil was divided into two portions. While one portion was used as the unheated control, the other was heated for 4.5 hr in a glass reactor vessel immersed in a silicon oil bath preadjusted to 200-210 C. The vegetable oil itself was found to be 17 ± 5

TABLE I

Radioactivity Distribution after Heating Oils Spiked with [^{14}C] Antioxidant[a] at 180-190 C for 4.5 hr

Description	Radioactivity, dpm × 10^6 (% Recovery)	
	BHA	TBHQ
Starting ^{14}C	13.55	5.74
Total volatiles[b]	8.79 (64.8)	2.75 (47.9)
Nonvolatiles[c]	3.27 (24.1)	2.73 (47.6)
Recovered ^{14}C	12.06 (88.9)	5.48 (95.5)

[a]Antioxidants were added to a concentration of 0.02%.

[b]Total radioactivity found in the methanol trap, Dry Ice/acetone trap, and connecting tubing rinses.

[c]Radioactivity remaining associated with the heated oil.

TABLE II

Methanol Extraction[a] of the Control and Heated Oil Samples

Antioxidants	Oil samples	(x 10^5 dpm) Total ^{14}C	^{14}C in methanol extracts	% Extracted
BHA	Heated oil	14.54	6.53	44.8
	Unheated control	42.65	36.89	86.4
	Postheating control[b]	1.97	2.09	105.0
TBHQ	Heated oil	7.69	0.97	12.6
	Unheated control	16.72	17.04	101.9
	Postheating control[b]	16.69	17.08	102.3

[a]Each sample was extracted with four 1-mL portions of methanol.

[b]Radiolabeled antioxidant was added as a methanolic solution to oil heated with the corresponding unlabeled antioxidant.

C cooler than the silicon bath in a separate experiment not involving ^{14}C. The heating apparatus (Fig. 1) not only allowed the collection of volatile materials, but also provided a continuous supply of moist air through the reactor. Volatile materials collected in the methanol and Dry Ice/acetone traps as well as methanol rinses of the traps and connecting tubing were pooled, and the radioactivity in the combined methanol solution was determined using a Packard Tricarb Scintillation Spectrometer. The radioactivity remaining in the heated oil was determined by scintillation counting. Counting efficiencies were determined by the sample channels ratio method.

In attempts to recover the antioxidant and/or its alteration products from the heated oil and the unheated control for HPLC analyses, samples were each extracted with four 1-mL portions of methanol at room temperature. Methanol and oil layers were separated by centrifugation at 1,000 rpm at 25 C for 10 min, using a Sorvall RC-3 centrifuge. The four methanol extracts from each sample were combined and the total radioactivity in the methanol was determined by scintillation counting.

HPLC Analyses

Due to the fact that the radioactive material in the heated oil could not be quantitatively recovered with methanol, as will be seen in Results, the oil samples were directly analyzed by HPLC.

A Spectra-Physics Model 3500B liquid chromatograph equipped with a rotary valve injector was used. One hundred μL of an oil sample containing at least 50,000 cpm was introduced into a stainless steel column (28 cm x 4.6 mm) packed with DuPont Zorbax silica (6-8 μm). Separation of the radioactive components was achieved by programmed gradient elutions at 2 mL/min with hexane (solvent A) and methylene chloride (solvent B), each containing 0.5% isopropyl alcohol. For continuous monitoring of radioactivity in the column eluate, an LC radiodetector (Berthold Radiation Measuring Instruments, Wildbad, Germany) was used. Tentative identifications of radioactive peaks corresponding to BHA isomers and TBHQ

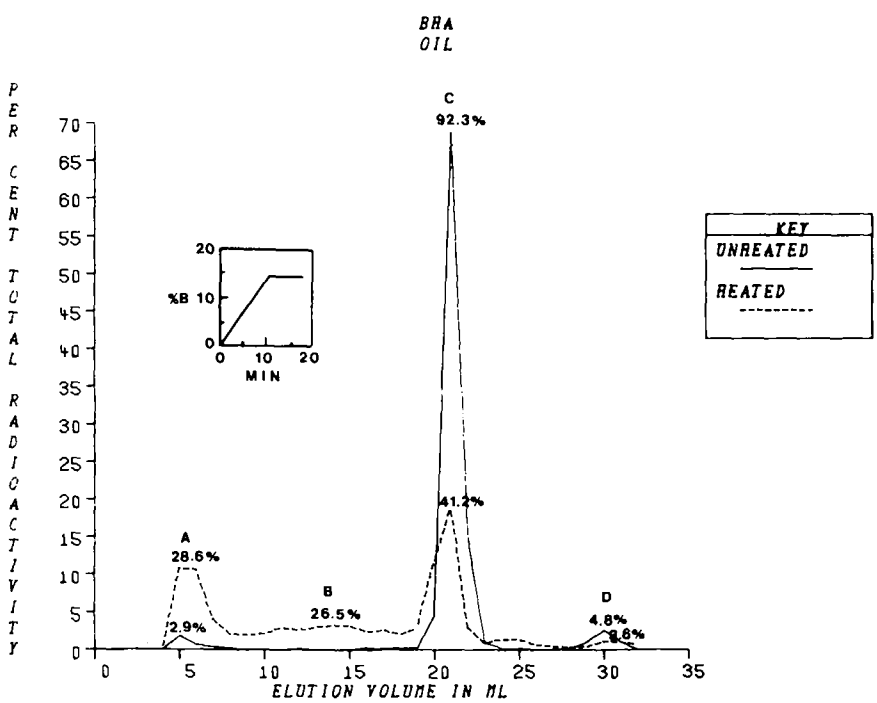

FIG. 2. Solvent-programmed HPLC separation of radioactive components in oil samples from BHA-spiking experiment. Column: DuPont Zorbax Silica (6-8 μm), 28 cm x 4.6 mm. Mobile phase: solvent A, hexane/0.5% isopropyl alcohol; solvent B, methylene chloride/0.5% isopropyl alcohol; solvent program shown as inset. Flow rate: 2 mL min^{-1}.

were based on the comparison of their retention times with those of ^{14}C-labeled reference standards. In order to detect low-level radioactivity, it was necessary to collect the eluate in fractions (1 mL) and measure the radioactivity in each fraction by scintillation counting.

RESULTS

Table I shows the radioactivity distribution between volatiles and nonvolatiles after the [^{14}C] antioxidant-spiked oils were heated at 180-190 C for 4.5 hr. In the case of BHA-spiked oil, 65 and 24% of the applied ^{14}C were found in the volatiles and nonvolatiles, respectively. As for TBHQ-spiked oil, 48% of the applied ^{14}C was found in the volatiles, with 48% remaining in the nonvolatiles. Total recoveries of ^{14}C were 89 and 96%, respectively, of the amounts of BHA and TBHQ added.

As shown in Table II, extraction of the heated oils with methanol recovered only 45 and 13%, respectively, of the nonvolatile ^{14}C from the BHA and TBHQ-spiked oils. However, the same extraction procedure resulted in 86% recovery of the added [^{14}C] BHA and quantitative recovery of the added [^{14}C] TBHQ from the unheated oils (Table II). The altered extractability of ^{14}C after heating suggested possible chemical changes of the added antioxidants.

In order to examine the possibility that the antioxidant undergoes reaction after addition of the methanol, a postheating control experiment was done concurrently with each study. A sample of oil with 0.02% of the unlabeled antioxidant was heated under the conditions specified for the experimental samples. A methanolic solution of labeled antioxidant was then added and the extraction carried out immediately in a fashion identical to that used for all the other samples. In all cases, the added ^{14}C was quantitatively recovered from the postheating control oils (Table II), proving that the alteration which was observed in the sample heated with the labeled antioxidant did not occur during the extraction of the oil sample with methanol.

That the altered extractability of ^{14}C coincided with qualitative changes of the antioxidant was further confirmed by normal-phase HPLC. Figure 2 shows that when the unheated BHA control was chromatographed, 92% of the radioactivity was eluted as the 3-isomer of BHA (peak C) and 5% as 2-isomer (peak D). Also detected was a small amount (3%) of a less polar material (peak A) which was undetectable in the [^{14}C] BHA standard. The ^{14}C distribution in the heated oil differed markedly from that in the unheated control (Fig. 2). Peak C was reduced from 92% in the control to 41% in the heated oil. A more striking change occurred in the increase of peak A from 3% to 29%. In addition, there was unresolved radioactive material (collectively designated B) eluted between peaks A and C, which accounted for 27% of total ^{14}C from the column.

Figure 3 shows a similar chromatographic comparison of the unheated control and heated oil from the TBHQ experiment. In the unheated control, 97% of the ^{14}C was eluted as TBHQ (peak D). Peak C accounted for 3% of the label with only traces in peak B. It is evident that there were drastic compositional changes in the radioactive material present in the heated oil, with only about 6% of the total activity remaining as unaltered TBHQ (peak D). A large percentage of the activity was associated with material less polar than TBHQ, which was dominated by peak C (56%). It is interesting that peak B was increased from the trace (less than 1%) in the control to 24% in the heated oil. Additionally, the least polar material (peak A), which was absent in the control, constituted 14% of the total ^{14}C associated with the heated oil.

DISCUSSION

In this study, we have demonstrated the chemical alteration of the added BHA or TBHQ in oil heated at temperatures approximating those of frying conditions. This would have been difficult without ^{14}C-labeled substances because of the occurrence of numerous decomposition products from the

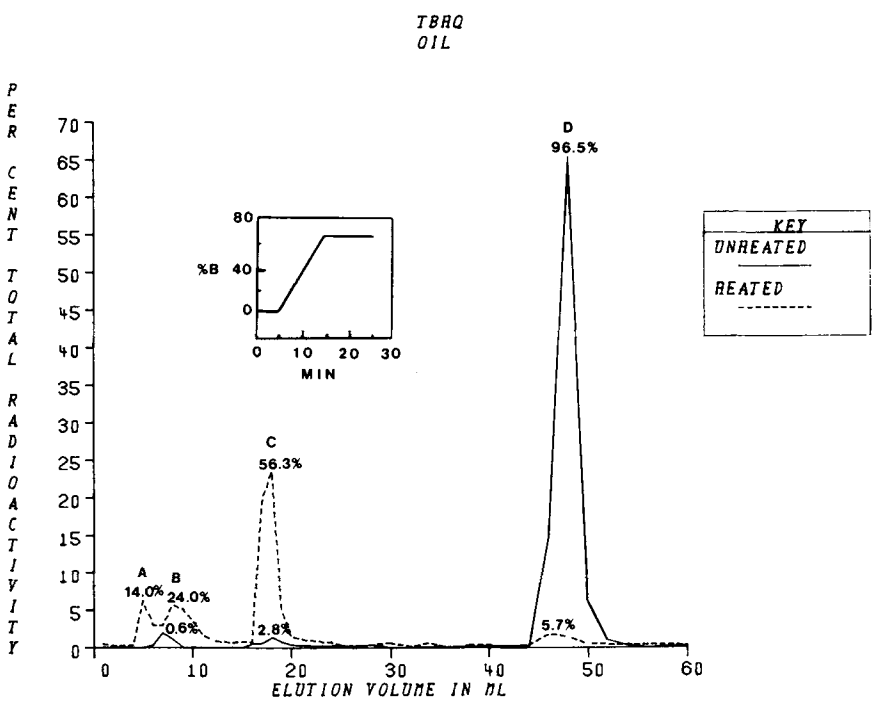

FIG. 3. Solvent-programmed HPLC separation of radioactive components in oil samples from TBHQ-spiking experiment. Chromatographic conditions same as those described in Fig. 2.

thermally oxidized oil (10,11). Chemical changes of the antioxidants were first indicated by the differential methanol extractabilities of the radioactive material present in the heated oil and in the unheated control. The presence of antioxidant alteration products in the heated oil was subsequently confirmed by the normal-phase HPLC analyses.

Although structures of the alteration products remain unidentified, it appears that the products are less polar than their corresponding parent antioxidants, as indicated by their elution sequence from the silica column. As shown by various model studies (12-14), autoxidation of phenolic compounds or their oxidation by other reagents produced compounds of quinone or epoxide nature. Monte and Maga (15) have demonstrated the formation of BHA dimers in water under stresses of sterilization. Also likely to occur in the frying oil is the radical addition of fatty hydroperoxides to the antioxidants, possibly in a manner similar to the adduct formation between linoleic hydroperoxides and α-tocopherol (16).

Whatever the nature of the antioxidant alteration products, the real concern is the inevitable human exposure to compounds of undefined toxicity from the use of the additives. As our future goal, we plan to conduct short-term in vitro bioassays of oil fractions that have been enriched with the antioxidant alteration products and, subsequently, to identify and synthesize the ones which gave positive responses for further toxicological testing in vivo.

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⁂Flavor and Oxidative Stability of Hydrogenated and Unhydrogenated Soybean Oil: Effect of Tertiary Butyl Hydroquinone[1]

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ABSTRACT

The efficacy of tertiary butyl hydroquinone (TBHQ) treatment for enhancement of the storage stability of soybean oil has been studied by flavor evaluation and chemical analysis. Soybean oils (I) unhydrogenated (IV = 137.7; % linolenate = 8.3), (II) hydrogenated with nickel catalyst (IV = 109.1; % linolenate = 3.3), and (III) hydrogenated with copper-chromium catalyst (IV = 112.8, % linolenate = 0.4) were each deodorized. In the cooling stage of the deodorizer, each oil was treated with citric acid plus TBHQ. These freshly deodorized oils were compared to separate batches of each oil treated with citric acid alone or with citric acid plus butylated hydroxyanisole and butylated hydroxytoluene. An analytical taste panel performed sensory evaluations by a paired sample test using an intensity rating scale system. The oils were also evaluated after being subjected to accelerated storage tests (4 days and 8 days at 60 C) and a fluorescent light exposure test (4 hr, ambient temperature). Peroxide development during storage was beneficially reduced in oils treated with TBHQ. The flavor stability of the three oils was not enhanced by treatment with TBHQ under any test conditions.

INTRODUCTION

Monotertiary butyl hydroquinone (TBHQ) was approved as a food grade oil soluble antioxidant in 1972 (1). It has been reported that treatment of polyunsaturated oils with TBHQ could effect oxidative stabilization to the same extent as could be achieved by partial hydrogenation of the oils (2). Our previous investigation of stored soybean oils (3) showed that treatment with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) antioxidants did improve the oxidative stability of citrated oils but did not impart improved flavor stability. The flavor and oxidative stability of citrated soybean oils treated with TBHQ now has been evaluated and compared to that of oils treated with citric acid (CA) only and with CA plus BHA/BHT.

EXPERIMENTAL PROCEDURES

The oils used in this study were prepared the same as those for our previous study (3), i.e., refined and bleached soybean oil (I), iodine value (IV) = 138, linolenate (Ln) = 8.3%; nickel-catalyzed hydrogenated soybean oil (II), IV = 109, Ln = 3.3%; copper-catalyzed hydrogenated soybean oil (III), IV = 113, Ln = 0.4%. Crystalline BHA, BHT and TBHQ (Eastman Chemical Products Inc., Kingsport, TN) were used for antioxidant treatments. One-liter portions of each oil were deodorized (210 C, 1 mm Hg, 5% sparge steam for 3 hr) (3) and treated with additives on the cooling side of deodorization at about 100 C (3). Additive

[1]Presented at ISF-AOCS Meeting, New York, NY, April 27-May 1, 1980.