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Fate of Antioxidants and Antioxidant-Derived Products in Deep-Fat Frying and Cookie Baking

Charles R. Warner, Daniel H. Daniels, Francis S. D. Lin,* Frank L. Joe, Jr., and Thomas Fazio

Studies with ring-labeled [^{14}C]BHA (2-(1,1-dimethylethyl)-4-methoxyphenol), ring-labeled [^{14}C]TBHQ (2-(1,1-dimethylethyl)-1,4-benzenediol), and [7-^{14}C]BHT (2,6-bis(1,1-dimethylethyl)-4-methylphenol) were undertaken to determine the fate of these compounds and the associated decomposition products in deep-fat frying and cookie baking. After the equivalent of 12 batches of french-fried potatoes was fried, over 80% of the ^{14}C initially added as ring-labeled BHA was retained by the lard. BHT was more volatile. All three antioxidants underwent extensive decomposition in deep-fat frying. Three conceivable phenolic oxidation products—2-(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione, and 3,3′-bis(1,1-dimethylethyl)-5,5′-dimethoxy-1,1′-biphenyl-2,2′-diol—were not found in the heated lard. High-performance liquid chromatographic analysis demonstrated that, in cookie baking, BHA and BHT are retained by the cookie as intact antioxidants, in contrast to TBHQ, which undergoes 28% decomposition.

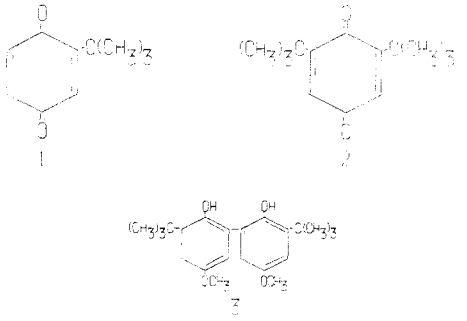
The fate of phenolic antioxidants and the associated reaction products in food processing has been the subject of speculation since these substances were authorized for food use. It has been postulated that antioxidants escape from the frying medium by volatilization and steam distillation due to the high temperatures characteristic of frying and the large amounts of water boiled out of the fried food. Many authorities have adopted this theory to explain the loss of antioxidants from frying oil (Fritsch, 1981; Stevenson et al., 1984). In spite of the disappearance of the parent antioxidant, the use of antioxidants in frying oil does yield a food product with a longer shelf life than the identical product fried in antioxidant-free oil (Min and Schweizer, 1983; Augustin and Berry, 1984). In a study with radiocarbon-labeled 2-(1,1-dimethylethyl)-1,4-benzenediol (TBHQ), Furia and Bellanca (1977) showed that the radiocarbon taken up by the potato was 80–85% of the expected level based upon the quantity of absorbed fat. Leventhal et al. (1976) isolated and identified a stilbenequinone derivative of BHT from vegetable oils heated at 190 °C for 11 days. In a study of photolytic effects on food additives, Mihara et al. (1974) demonstrated the formation of diphenyl ether derivatives from BHA in soybean oil or in lard after prolonged exposure to sunlight. More recently, we reported the decomposition of BHA and BHT in a partially hydrogenated vegetable oil heated at 190 °C for 4.5 h (Lin et al., 1981). The photochemically induced oxidations of BHA (Kurechi and Kunugi, 1983a), TBHQ (Kurechi et al., 1983; Kurechi and Kunugi, 1983b), and BHT (Kurechi and Kato, 1980) have been reported.

Many of these studies utilized conditions that are not typical of food processing or use relatively high concentrations of antioxidants to simplify the analyses of the reaction mixtures. Therefore, the relevance for humans is nonexistent or very obscure. For this reason, we undertook the studies described in this report utilizing actual food with antioxidant concentrations near the permissible levels. The samples were heated at temperatures typical of deep-fat frying. Our objectives were as follows: (1) to determine the extent of volatilization and decomposition of the antioxidants; (2) to determine the extent to which unknown antioxidant decomposition products are retained by the food.

Ring-labeled [^{14}C]BHA, ring-labeled [^{14}C]TBHQ, and [7-^{14}C]BHT in deep-fat frying and cookie baking were studied under actual cooking conditions. Simulated deep-fat frying was used to facilitate the determination of the radiocarbon-labeled products by high-performance liquid chromatography (HPLC) and thin-layer chroma-

Division of Chemistry and Physics, Food and Drug Administration, Washington, D.C. 20204.

tography (TLC). The quantity of radiocarbon absorbed by the french-fried potatoes was determined by combustion analysis. HPLC analysis for 2-(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione (TBBQ) (1) and 2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione (DBBQ) (2), and TLC analysis for 3,3′-bis(1,1-dimethylethyl)-5,5′-dimethoxy-1,1′-biphenyl-2,2′-diol (DHDP) (3) demonstrated



that these substances, which are oxidation products of phenolic antioxidants, are not found among the decomposition products. Normal-phase HPLC analysis indicated that BHA and BHT survived the cookie baking intact, while TBHQ underwent 28% decomposition. In deep fat, however, all three antioxidants formed dozens, perhaps hundreds, of reaction products.

EXPERIMENTAL SECTION

Materials. Esskay brand lard shortening (Schluerberg-Kurdle Co., Baltimore, MD) was used for the frying and baking studies. The antioxidant levels were estimated by TLC to be 70 and 50 ppm for BHT and BHA, respectively. Propyl gallate could not be detected even though the TLC method has a lower limit of quantitation of 10 ppm. The peroxide value was 6 mequiv/kg by AOCS Method Cd 8-53 (Walker, 1983). Bleached flour, eggs, and molasses for the cookie mix were purchased from a local supermarket.

Food-grade BHA, BHT, and TBHQ were supplied by Eastman Chemical Co., Kingsport, TN. TBBQ was synthesized by a modification of the procedure for benzoquinone (Blatt, 1943). The purity was checked with several TLC systems. DHDP was prepared as previously described (Hewgill and Lee, 1968). The nuclear magnetic resonance spectrum of the crystalline product was consistent with the structure. DBBQ was purchased from Aldrich Chemical Co. Inc., Milwaukee, WI, and used without further purification. Ring-labeled [^{14}C]BHA (sp act. 9.7 mCi/mmol, 93.7% 3-isomer and 7.3% 2-isomer), ring-labeled [^{14}C]TBHQ (sp act. 12.8 mCi/mmol, 98.0% TBHQ and 2.0% *tert*-butylbenzoquinone), and [7-^{14}C]BHT (sp act. 12.8 mCi/mmol) were custom synthesized, and their purity was established by Dynapol, Palo Alto, CA. Preparations with added carrier were checked for purity with three different TLC systems.

Glass-distilled solvents were purchased from Burdick & Jackson Laboratories, Muskegon, MI. Insta-Gel (Packard Instrument Co., Downers Grove, IL), a universal liquid scintillation fluid, was used for both aqueous and nonaqueous samples. Flexible silica gel 1B sheets for TLC were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ.

Radioactivity Determination. The activity in each sample was determined with a Packard 460 CD scintillation spectrometer, and the combustion was carried out with a Tri-Carb sample oxidizer (Packard Instrument Co., Downers Grove, IL).

Deep-Fat Frying of Potatoes. The lard was placed in a 100-mL triple-neck round-bottom flask equipped with a magnetic stirrer. Kontes Bantamware distillation apparatus was used with a series of traps. The first trap was chilled in an ice bath and the next two consisted of gas washing traps with Carbo-Sorb and dimethylformamide in traps 2 and 3, respectively. Finally, the last trap contained activated charcoal. A potato slice, 1.5 g, was impaled on a metallic hook attached to a tapered glass joint. To accomplish the frying, the tapered glass joint stopper (plug) in the center neck of the flask was replaced by the plug bearing the potato slice. After the cooking was complete, the plug with the potato slice was removed and replaced by the conventional plug.

Preparation of Lard with Radiolabeled Antioxidant. A 60-g sample of lard was melted under a stream of nitrogen and stirred in the molten state for at least 1 h to deoxygenate it. The radiolabeled antioxidant was introduced with unlabeled carrier as a 0.5-mL benzene solution. Under a nitrogen atmosphere, the lard was heated to 80 °C and mixed to ensure homogeneous dissolution of the added antioxidant. Before the sample for the frying studies was heated, the sweep gas was changed to air, which was passed through a column of Ascarite to remove the carbon dioxide. As soon as the lard temperature reached ca. 190 °C, frying of the potato was begun.

Simulated Deep-Fat Frying. A lard sample (1.5 g) dissolved in 15 mL of hexane–methylene chloride (1:1, v/v) was spiked with 200 μL of a BHA, BHT, or TBHQ stock solution in benzene to yield an antioxidant concentration of 0.013% based on lard weight (not including endogenous antioxidants in the lard). An aliquot of the corresponding ^{14}C-labeled antioxidant stock solution in benzene (2.2 μCi of BHA or BHT; 2.8 μCi of TBHQ) was then added to the lard solution. A portion of the solution was saved as the unheated control. After removal of the solvent with a stream of nitrogen, the lard was heated for 1 h in a 50-mL round-bottom flask immersed in a silicone oil bath preadjusted to 195–200 °C with a series of traps similar to those previously described (Lin et al., 1981).

Cookie Spiking and Baking Procedures. Cookie mix stocks were prepared with the following ingredients: bleached flour 91.3 g, eggs 9.2 g, NaCl 0.5 g, and molasses 9.5 g. For the baking experiment, 1 g of the cookie mix was placed in a glass reactor (Lin et al., 1981) containing 0.04 g of $NaHCO_3$ and 1 g of lard with added ^{14}C antioxidant (0.013%). Separately, a duplicate mixture was prepared, except in a capped glass centrifuge tube (125 cm × 16 cm), to be used as the unheated control. After thorough mixing of the contents with glass rods, the glass reactor was placed in a silicone oil bath at 195 °C for 10 min. After completion of the baking, the volatiles collected in the traps were recovered and analyzed as previously described (Lin et al., 1981). The heated cookie solids and the unheated control were extracted with methylene chloride–methanol. To minimize nonspecific absorption of the radiolabel by the cookie solids, the unlabeled antioxidant was included in the extracting solvent at a concentration of 0.1%. Entrapment was minimized by thoroughly triturating the sample with the extracting solvent. The resulting suspension was centrifuged at 4 °C at 2000 rpm for 10 min, and the supernatant was decanted. Successive extractions were performed until less than 0.1% of the initial ^{14}C was recovered in the last extract. All the extracts were combined and diluted to 25 mL, and the radioactivity was determined.

The amount of ^{14}C remaining in the extracted cookie solids was determined by scintillation counting after combustion of 50–200 mg of each sample. Cookie solids that were shown to contain radioactive material after exhaustive

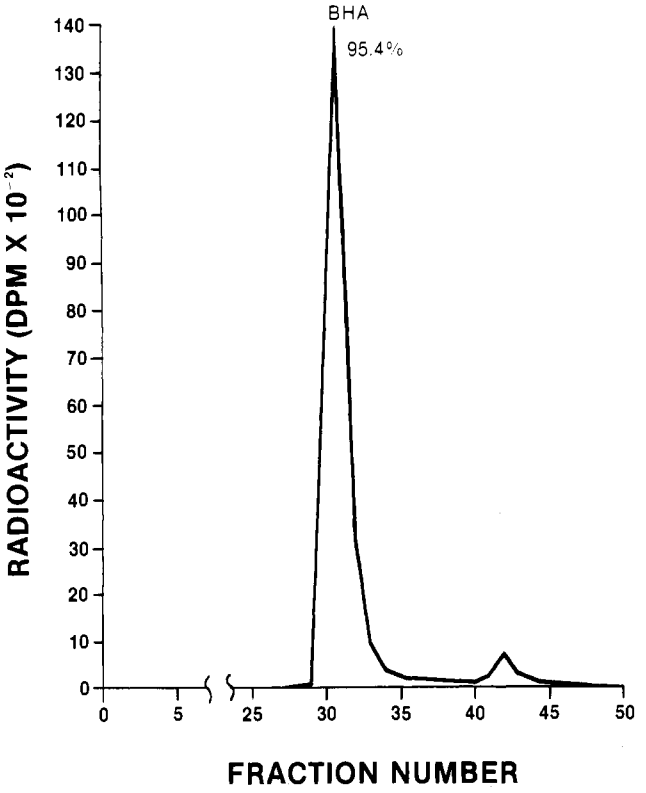


Figure 1. Solvent-programmed HPLC separation of radioactive components in the unheated lard of BHA-spiking experiment: column, Du Pont Zorbax silica; mobile phase, solvent A = hexane–0.25% 2-propanol, solvent B = methylene chloride–1.5% 2-propanol, programmed from 100% A to 80% A/20% B, 10 min; flow rate, 2 mL/min.

extractions with organic solvents were further extracted with water until less than 0.1% of the ^{14}C was found in the last aqueous extract.

Post-Heating Controls. Concurrent with each baking experiment, a post-heating control was included to determine whether alteration products were formed during solvent extraction. A cookie sample spiked with unlabeled antioxidant (0.013% of the lard content) was baked under the conditions specified for the experimental samples. A methylene chloride–methanol solution of the corresponding radiolabeled antioxidant was then added and the extraction carried out immediately in the same manner as that for the heated and unheated control cookies. In each case the label was quantitatively recovered as the parent antioxidant, proving that the extraction process did not introduce decomposition.

HPLC Analysis. Normal-phase HPLC was carried out as previously described (Lin et al., 1981). Operating conditions are given in Figures 1 and 2. Reversed-phase HPLC utilized a Spectra Physics Model 8100 liquid chromatograph with a Model 8400 spectrometric detector to determine TBBQ and DBBQ in heated lard. The mobile phase consisted of methanol–water (78:22, v/v). Ultraviolet detection at 254 nm was used. A 1.0-g sample of lard was partitioned between 5 mL each of mutually saturated acetonitrile and hexane. A 1.0-mL aliquot of the acetonitrile layer was diluted to 10 mL with acetonitrile. This solution was subjected to HPLC. The minimum measurable quantities of TBBQ and DBBQ were 0.1 and 0.5 μg/mL, respectively.

TLC Analysis. Water from the aqueous cookie extracts was removed azeotropically with methanol under reduced pressure, and the residues were dissolved in methanol. An aliquot of the methanol solution was spotted on a pre-coated plastic TLC plate, together with an appropriate reference standard of ^{14}C antioxidant. The plate was developed in a solvent system of methylene chloride–methanol–acetic acid (80:20:1, v/v/v). The plate was cut into three 1-cm portions, and the ^{14}C associated with each portion was determined by liquid scintillation. DHDP was determined by TLC on silica gel developed with hexane–benzene–diethyl ether (90:30:3). The lard sample was dissolved in benzene, and 1–10 μL was spotted on a silica gel TLC plate. Autoradiography was used to locate the spots.

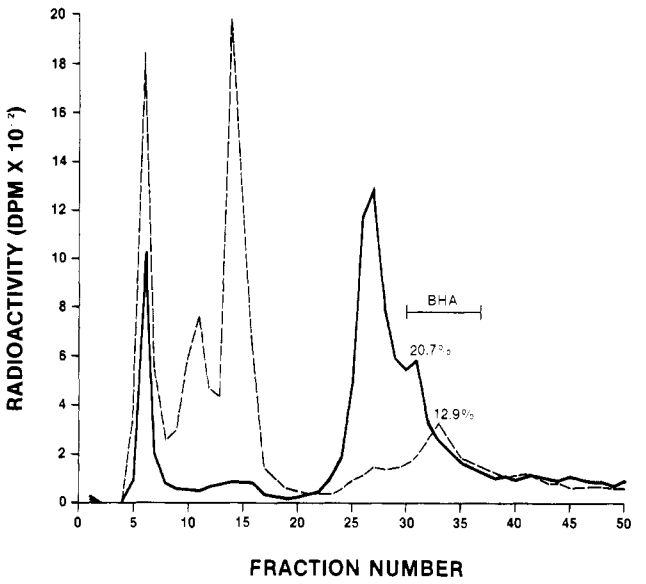


Figure 2. Solvent-programmed HPLC separation of radioactive components in the volatiles and heated lard of BHA-spiking experiment. Conditions as in Figure 1. Key: —, cooked; ---, volatiles.

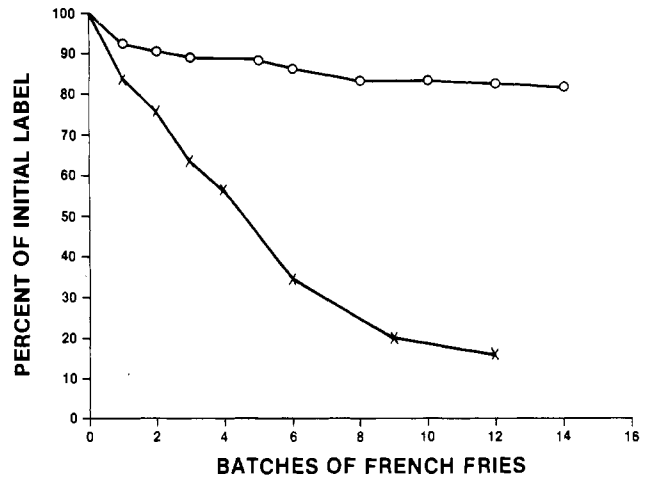


Figure 3. Level of radiocarbon activity remaining in lard as a function of number of potato fryings: O, BHA; ×, BHT.

RESULTS

Initial Studies. This work duplicated as closely as possible, consistent with the need to contain the radioisotope, the actual preparation of french-fried potatoes and cookies. The radiolabeled antioxidant was added to the lard that was used to prepare the food items. The headspace above the cooking food was continuously flushed with air to ensure that the volatiles were swept into the traps rather than returned to the cookie or lard by condensation.

In deep-fat frying the level of radiocarbon activity in the lard throughout the frying operation is shown in Figure

Table I. Extraction from Baked and Control Cookies of Radiocarbon Originally Introduced as Radiolabeled Antioxidant

	radioactivity, dpm × 10^{-5} (% rec)								
	BHA			BHT			TBHQ		
description	heated	unheated	postheated control	heated	unheated	postheated control	heated	unheated	postheated control
total ^{14}C in cookie	8.3	10.1	20.1	10.9	14.6	19.6	18.0	19.4	12.1
methylene chloride–methanol extr[a]	7.6 (92)	9.8 (97)	21.1 (105)	9.5 (87)	15.4 (105)	18.9 (97)	12.9 (72)	17.4 (90)	12.6 (105)
antioxidant peak (% of extr act.[b])	95[c]	95[c]	95[c]	97	99	99	96	96	97
extr cookie solids	0.3 (3.5)	0.3 (3.0)	*d*	0.1 (0.5)	*d*	*d*	3.7 (21)	1.7 (8.6)	*d*
total rec	7.9 (96)	10.1 (100)	21.1 (105)	9.6 (88)	15.4 (105)	18.9 (96)	16.9 (92)	19.1 (98)	12.6 (105)

[a] Cookies were repeatedly extracted with methylene chloride–methanol (2:1, v/v) until <0.1% of the total ^{14}C was found in the last extract. [b] Samples were subjected to normal-phase HPLC (see Experimental Section). [c] Approximately 4.4% of the total was incorporated in the 3-isomer. [d] Not determined.

3. BHA or its decomposition products were largely (over 80%) retained by the lard. Assuming the same molecular weight for BHA and its decomposition products, the french-fried potatoes absorbed 20–40 ppm of BHA and its decomposition products.

For [7-^{14}C]BHT, the radioactivity decreased markedly with frying. After the equivalent of four batches of french-fried potatoes, 50% of the label was retained, while after 12 batches, less than 20% remained in the lard.

The results with [7-^{14}C]BHT must be interpreted with caution because, in contrast to [^{14}C]BHA, which is ring-labeled, the BHT is side-chain labeled. Therefore, cleavage of the methyl group could lead to substantial reduction of activity in the lard even if the ring portion of the molecule is retained by the lard. Simulated deep-fat frying experiments were carried out with antioxidants present in the same concentration range but with much higher specific activities.

The radiocarbon introduced as a radiolabeled antioxidant was largely retained by the cookie. BHT, which is thought to be the most volatile of the phenolic antioxidants, volatilized to the extent of 28%, while the corresponding losses for BHA and TBHQ were 11 and 6.1%; respectively. The first step in the analyses of the radiolabeled substances in the baked cookies as well as the unheated and postheated controls was normal-phase HPLC analysis of the extracts (see the Experimental Section). HPLC analysis demonstrated that the radioactivity associated with the BHA, BHT, and TBHQ peaks accounted for 95, 97, and 96% of the activity of the corresponding methylene chloride–methanol extracts of the baked cookies. In contrast to BHA and BHT, a substantial percentage (21%) of the radiocarbon introduced as [^{14}C]TBHQ in the baked cookies could not be extracted by the organic solvent (Table I). Another extraction of the cookie solids with water recovered an additional 8% of the radiocarbon, and combustion of the solid residue remaining after the aqueous extraction accounted for another 11% of the initially added radiocarbon. Postheating controls (see the Experimental Section) were included to establish that the observed decomposition did occur in the cookie preparation and not after the solvent was added for the extraction.

Simulated Deep-Fat Frying. The frying was accomplished by the general procedure of Lin et al. (1981). Air, which had been bubbled through water to increase its water vapor content, was bubbled into the lard to provide both the air and the moisture typical of frying. Table II shows the ^{14}C distribution between volatiles and nonvolatiles after heating the lard samples spiked with ^{14}C antioxidants for 1 h at 195–200 °C. Under these heating conditions, 38–55% of the ^{14}C remained with the lard. Taking into account the volatiles collected in the traps, the recoveries of the added ^{14}C were 90, 86, and 97% for BHA-, BHT-, and TBHQ-spiked lards, respectively.

Table II. Distribution of ^{14}C After 1-h heating of ^{14}C Antioxidant-Spiked Lard[a] at 195–200 °C

	radioactivity, dpm × 10^{-6} (% rec)		
description	BHA	BHT	TBHQ
initial ^{14}C	4.3	4.7	4.0
total volatiles[b]	1.5 (35)	2.3 (49)	2.0 (50)
nonvolatiles[c]	2.3 (55)	1.8 (38)	1.8 (47)
recovered	3.8 (90)	4.1 (86)	3.8 (97)

[a] Antioxidants added to a concentration of 0.013%. [b] Total ^{14}C found in traps. [c] ^{14}C remaining associated with the heated lard.

Chemical alterations of the antioxidants in lard during the heating process were investigated using normal-phase HPLC. As shown in Figure 1, analysis of the unheated control used in the BHA-spiking experiment resulted in two well-separated peaks, corresponding to the 3- and 2-BHA isomers. When the volatiles and nonvolatiles obtained from heating the BHA-spiked lard were analyzed (Figure 2), we found that extensive changes of the antioxidant had occurred during heating. On the basis of the ^{14}C distribution between volatiles and nonvolatiles (Table II), as well as ^{14}C associated with the peaks corresponding to the unaltered BHA (Figure 2), we estimated that 84% of the added BHA was altered as the result of heat treatment. In fact, the extent of alteration may have been underestimated due to the observed peak overlap (Figure 2).

Similar chromatographic results with the volatiles and nonvolatiles obtained from heating the BHT-spiked and TBHQ-spiked lards confirmed the complexity of the products derived from the parent antioxidants. Chemical alteration of BHT in lard was clearly evident. At most, 5 and 19% of the radioactivity in the heated lard and the volatiles, respectively, could be accounted for by the unaltered parent antioxidant. Calculations based on the data in Table II and the chromatograms show that, overall, only 14% of the added BHT remained unchanged by the heating process. The alteration products of BHT, in contrast to those of BHA, seem to be more polar than the parent compound.

Unlike BHA and BHT, which were stable in unheated lard throughout the experiment, TBHQ appeared to be unstable in lard even at room temperature. Only 81% of the ^{14}C in the unheated control was attributable to TBHQ, as opposed to 97% in the ^{14}C reference standard. More interestingly, TBHQ was undetectable in the heated lard, and was present only in a minute amount (2%) in the volatiles. Overall, the heat treatment resulted in virtually total decomposition (ca. 99%) of the added TBHQ in lard, and the resultant products appeared to be less polar than TBHQ.

The three compounds, TBBQ, DBBQ, and DHDP, are likely decomposition products of TBHQ, BHT, and BHA. An HPLC procedure was developed that could quantitate 10 ppm of TBBQ or DBBQ in heated lard. Lard samples

that initially had 170 ppm of BHT and 208 ppm of TBHQ proved to have no detectable levels of TBBQ or DBBQ after heating. DHDP was not detected by TLC autoradiography of extracts of heated BHA-containing lard samples.

DISCUSSION

HPLC has been used to demonstrate the extent of decomposition of all three antioxidants under simulated deep-fat frying conditions. Because of the complexity of the products formed in heated fats and oils (Freeman 1969; Aitzetmuller, 1973; Paulose and Chang, 1978), it was essential to use radiolabeled antioxidants to detect the products derived from the antioxidants.

Under the simulated cookie-baking conditions employed, BHA and BHT were unaffected. However, TBHQ was still subject to chemical changes, albeit to a lesser degree than under the deep-fat frying conditions. In addition to the less polar alteration products present in the volatiles, derivatives of TBHQ with increased polarity were detected by TLC in the water extracts of the heated cookie. Furthermore, about 10% of the total radioactivity in the heated cookie was not extractable by organic solvents or water.

HPLC and TLC demonstrated that TBBQ, DBBQ, and DHDP do not appear in the heated lard at levels above 5% of the initial antioxidant concentration. The quinones, TBBQ and DBBQ, are reactive substances that, if formed, would probably undergo Thiel–Winter type reactions (acylation of quinones catalyzed by acids). TBHQ is very unstable under normal conditions of food storage and processing. Nevertheless, it is efficacious in extending the shelf life of products. TBHQ breakdown products may also be effective antioxidants. Identification of decomposition products of antioxidants is desirable not only because of food safety considerations but also because a great deal can be learned about the mechanism of the antioxidant protection provided in high-temperature processes such as deep-fat frying and baking. The complexity of the reaction product mixtures suggests that determination of the principal structural aspects of the compounds will require studies with model food systems.

Registry No. BHA, 25013-16-5; TBHQ, 1948-33-0; BHT, 128-37-0.

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