Stabilization of Brown Rice Products Using Ethanol Vapors as an Antioxidant Delivery System

ELAINE T. CHAMPAGNE[1] and CASEY C. GRIMM[1]

Cereal Chem. 72(3):255-258

ABSTRACT

Brown rice kernels and their flours were stabilized to lipolytic hydrolysis by exposing the kernels to vapors from boiling aqueous ethanol (EtOH). EtOH vapors were also effective in delivering butylated hydroxytoluene (BHT) to the kernels. Oxidative stability achieved from BHT depended on the oxygen permeability of the rice package. BHT provided oxidative stability to kernels and flours stored in glass jars but not to kernels stored in polyethylene bags. BHT was rapidly oxidized in kernels stored in bags, and thus did not protect them from oxidative deterioration. Flours stored in polyethylene bags were stable to oxidation.

Brown rice has a short shelf life (three to six months) because of hydrolytic and oxidative deterioration of bran oil. Dehulling rough rice to produce brown rice disrupts the outer bran layers, allowing oil to diffuse. The oil makes contact with both endogenous lipases and those of microbial origin, and the hydrolysis of triglycerides to free fatty acids (FFA) readily proceeds. Oil that diffuses to the kernel surface readily oxidizes.

Processes have been developed for stabilizing brown rice kernels and their flours to lipolytic hydrolysis by liquid ethanol (EtOH) extraction (Champagne et al 1990, 1991; Champagne and Hron 1992a) and by EtOH vapor treatment (Champagne et al 1992b, Champagne et al 1993) of the kernels. Inclusion of an antioxidant or iron chelator in the liquid extraction process yields products that are also stable to oxidative deterioration (Champagne and Hron 1993).

The objective of this investigation was to determine whether oxidation in EtOH-stabilized brown rice and its flour may be slowed by using EtOH vapors as a carrier for delivering antioxidants to the rice. Butylated hydroxy-toluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroxyquinone (TBHQ) were evaluated. These antioxidants were selected because of their steam volatility (Dziezak 1986).

MATERIALS AND METHODS

Rice Samples

Rough rice samples of Lemont (1992 crop) were obtained from Nolan J. Guillot, Inc. (Crowley, LA) and dehulled in a sheller (H.T. McGill, Houston, TX).

EtOH Vapor Treatment

A 40-g sample of freshly dehulled brown rice was placed in a jacketed glass butt tube (3 cm in diameter, 12 cm high) fitted with a wire mesh sample-retaining screen (Fig. 1). Water from a bath set at 83°C was circulated through the jacket. When the temperature of the sample reached 78°C (25 min), the glass butt tube was inserted into the neck of a 1,000-ml round-bottom flask containing 500 ml of boiling aqueous EtOH (95%, v/v, bp 78°C) and 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 g of BHT, 10.0 g of BHA, 10 g of TBHQ, or no antioxidant. Samples were exposed to EtOH vapors for 5 min. After treatment, the samples were transferred to shallow stainless steel pans and allowed to cool in room-temperature (24°C) air. Eight or 16 batches of rice treated at each level of antioxidant were combined.

Flours were prepared by grinding kernels to a powder in a Udy cyclone mill (Udy Corp., Fort Collins, CO) with a 20-mesh sieve screen. The kernels and their flours were divided and stored in duplicate in capped half-pint glass jars with air headspace at 36°C. Each jar initially contained 80 g of rice kernels; ~100 ml of air headspace or 80 g of flour; ~80 ml of air headspace. Portions (80 g) of control kernels and kernels treated with EtOH containing

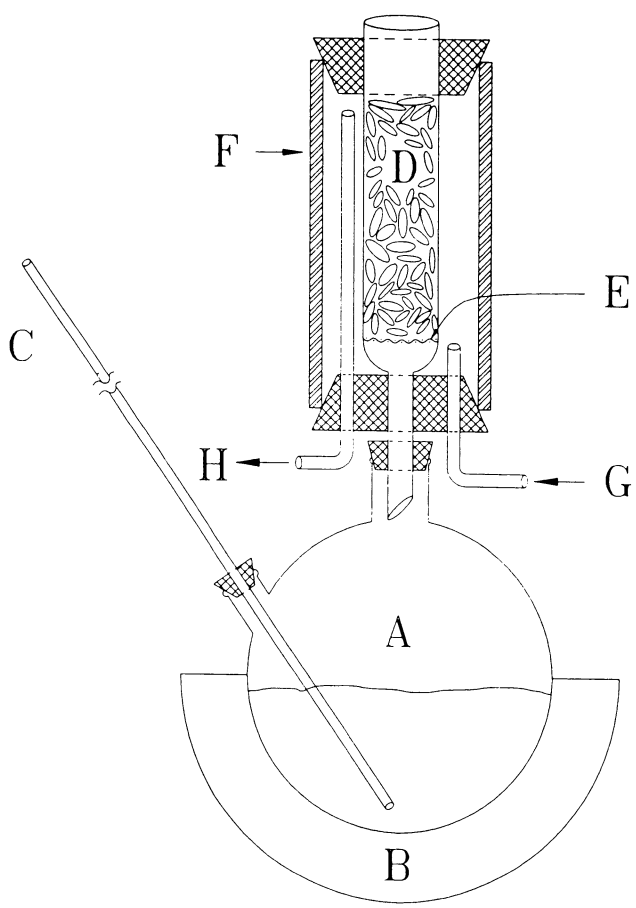

Fig. 1. Apparatus used to treat brown rice kernels with boiling aqueous ethanol (EtOH) vapors: 500-ml round-bottom flask with a side arm (A); heating mantle (B); glass tubing for venting (C); glass butt tube (3 cm in diameter, 12 cm high) holding 40 g of brown rice (D); wire mesh screen to retain the sample (E); plexiglass jacket (F); inlet from (G) and outlet to (H) circulating water bath.

[1]Southern Regional Research Center, ARS, USDA, New Orleans, LA.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

0, 10.0, 15.0, or 20.0 g of BHT and their flours were also stored in duplicate in two-layer, 2.25-mil polyethylene bags used by industry for packaging 2 lbs. of rice. Half a bag was used for each sample; the top was rolled down, removing air, and sealed with tape.

Antioxidant Content

Cargill Analytical Services (Cedar Rapids, IA) used inhouse gas-chromatography methods for determining BHT and BHA contents. TBHQ was determined by Nutritional International (Dayton, NJ) using gas chromatography.

FFA Contents

To measure the extent of lipolytic hydrolysis of brown rice kernels and flours during storage, FFA contents of treated and control samples were determined the day after vapor treatment and periodically thereafter by a micromethod (Hoffpauir et al 1947). Metacresol purple was substituted for the phenolphthalein as indicator. FFA were measured in oil extracted by petroleum ether from 5 g of ground rice using a Soxhlet extraction apparatus. FFA content was calculated as oleic acid and expressed as percent of oil.

Dynamic Headspace Analysis of *n*-Hexanal

n-Hexanal contents of vapor-treated and control brown rice kernels and their flours were determined as a measure of lipid oxidation. The amount of *n*-hexanal in stored brown rice is linearly proportional to the amount of oxidized linoleic acid with a correlation coefficient of 0.99 (Shin et al 1986). A concentrator (LSC 2000 Tekmar, Cincinnati, OH) equipped with a 25-ml straight-neck glass sample vessel was used for purge and trap analysis of *n*-hexanal in kernel and flour samples after one, four, and six months of storage. *n*-Hexanal was separated from other volatiles (HP Ultra 2 column, cross-linked 5% phenyl, 94% methyl, 1% vinylsilicon; 50m × 0.32 mm i.d. with 0.52 μm film thickness) using a HP5890 series II gas chromatograph (Hewlett Packard Co, Palo Alto, CA) equipped with an FID detector. Details of the method used and operating parameters for the concentrator and gas chromatograph are given elsewhere (Champagne and Hron 1993).

BHT Contents of Polyethylene Bags

BHT contents of the polyethylene bags used for the control and treated samples were determined after six months of storage. Each bag was cut into small pieces, placed in a 200-ml round-bottom flask, covered with ~100 ml of *n*-heptane and allowed to stand for 18 hr. The solvent was decanted and evaporated to ~100–150 μl with a steady stream of nitrogen. Final volume was estimated using a microliter syringe. Solution (2 μl) was then injected on a HP5988 gas chromatograph/mass spectrometer. Selected ion monitoring (SIM) of ions m/z 205 and 215 for BHT was performed. Standards were verified in linear mode and a calibration curve was generated in SIM mode. The total amounts of BHT in the bags were calculated from interpolation of the calibration curve and the final volume.

RESULTS AND DISCUSSION

Delivery of Antioxidants to Brown Rice by EtOH Vapors

EtOH vapors were effective in delivering BHT to brown rice kernels. The relationship between the amount of BHT added to the liquid EtOH and the amount of BHT carried in the vapors was described by the linear regression equation: $Y = 0.00606 \times X - 3.35011$, where Y = amount of BHT (ppm) in condensed EtOH vapors, X = amount of BHT (ppm) in liquid EtOH, correlation coefficient $(r) = 0.9905$. Approximately 0.6% of the BHT added to the liquid EtOH was carried in the vapors.

The retention of BHT by kernels treated with vapors carrying this antioxidant was described by the linear regression equation: $Y = 0.00211 \times X - 1.5298$, where Y = amount of BHT (ppm) in vapor-treated rice on a dry basis, X = amount of BHT (ppm) in liquid EtOH, correlation coefficient $(r) = 0.9976$.

No TBHQ and ~9 ppm of BHA were found in condensate from boiling EtOH containing 20,000 ppm of these antioxidants. BHA was not detected (limit 1 ppm) in kernels treated with vapors from boiling EtOH containing 20,000 ppm of this antioxidant. Thus, EtOH vapors were not effective in carrying BHA and TBHQ to the rice.

Effect of Storage on BHT Content

BHT contents of kernel and flour samples decreased during storage, as indicated in Table I. BHT loss was approximately the same in kernel samples and their corresponding flours. This loss during storage primarily resulted from oxidation, but may have been due to a small extent to volatilization or complexation with bran proteins (Cosgrove and Waters 1951, Cook et al 1955, Anderson et al 1963, Terada and Naito 1989).

BHT loss was markedly larger in samples stored in polyethylene bags compared to that of samples stored in glass jars. This large loss was not due to the antioxidant migrating into the polyethylene bags. The amounts of BHT that migrated into the bags (7–9 μg) were negligible compared to the amounts lost by the samples (3–6 mg). Oxygen supply may explain the differences in BHT loss in the glass jars and polyethylene bags (Saucy et al 1990). The consumption of BHT would decrease with time in the oxygen-limiting conditions in the glass jar, whereas the polyethylene bags are permeable to oxygen leading to a greater loss of BHT in samples as a result of its more rapid consumption.

Effects of EtOH Vapor Treatment on FFA Levels

The effectiveness of EtOH vapors in stablizing brown rice kernels and their flours to lipolytic hydrolysis has been previously reported (Champagne et al 1992b, Champagne et al 1993). Lipolytic hydrolysis, as indicated by FFA accumulation, was monitored in this study to determine the influences of BHT in the vapor and storage in jars versus polyethylene bags.

The effects of treating brown rice kernels with EtOH vapors for 5 min on the accumulation of FFA (as percent of oil) in the kernels and their flours during storage at 36°C are shown in Table II. During six months of storage in glass jars, FFA levels in EtOH vapor-treated kernels and their flours increased from 2.9 to 3.6 and 12.5%, respectively. In contrast, FFA levels in control kernels and flours stored in glass jars increased from 2.9 to 12.5 and 84.2%, respectively. The increases in FFA levels in EtOH vapor-treated and control kernel and flour samples stored in polyethylene bags were significantly ($P < 0.005$) smaller after four and six months than those in samples stored in jars. The FFA level in EtOH vapor-treated kernels stored in bags did not change; the level in flours prepared from EtOH vapor-treated kernels increased from 2.9 to 8.7% during six months. FFA levels in control kernels and flours stored in bags increased from 2.9 to 5.3 and 41.3%, respectively. The moisture contents of control

TABLE I
Effect of Storage Time on Butylated Hydroxytoluene (BHT) Content of Kernel and Flour Samples Stored in Glass Jars and Polyethylene Bags

Initial BHT Content	Storage Time					
	1 Month		4 Months		6 Months	
	Jars	Bags	Jars	Bags	Jars	Bags
Kernels						
1.7	<0.4	...	<0.4	...	<0.4	...
4.7	<0.4	...	<0.4	...	<0.4	...
21.5	14.4	...	0.4	...	<0.4	...
39.3	22.4	2.2	11.2	1.6	3.1	<0.4
65.3	38.8	6.1	29.5	2.3	17.4	0.5
81.0	64.6	13.2	53.6	2.6	36.5	0.9
Flour						
1.7	0.6	...	<0.4	...	<0.4	...
4.7	0.7	...	<0.4	...	<0.4	...
21.5	14.0	...	0.6	...	<0.4	...
39.3	26.0	3.7	9.8	<0.4	4.4	<0.4
65.3	39.8	5.4	30.9	<0.4	18.9	<0.4
81.0	62.4	8.9	55.4	<0.4	42.6	<0.4

TABLE II
Effects of Treatment[a] with Aqueous Ethanol (EtOH) Vapors on the Accumulation of Free Fatty Acids (FFA) in Brown Rice Kernels and Flours Prepared From Them[b]

	FFA %					
	1 Month		4 Months		6 Months	
Treatment	Jars	Bags	Jars	Bags	Jars	Bags
Control kernel	7.9 ± 0.2	5.5 ± 0.1	10.3 ± 0.3	4.7 ± 0.1	12.5 ± 0.3	5.3 ± 0.5
Control flour	28.5 ± 0.4	26.0 ± 0.0	55.6 ± 1.4	31.6 ± 0.1	84.2 ± 0.6	41.3 ± 0.1
EtOH-treated kernel	2.8 ± 0.1	3.0 ± 0.1	3.3 ± 0.1	2.9 ± 0.1	3.6 ± 0.1	3.0 ± 0.1
EtOH-treated flour	5.0 ± 0.1	5.0 ± 0.1	8.9 ± 0.1	6.6 ± 0.1	12.5 ± 0.3	8.7 ± 0.2

[a]5-min treatment.
[b]FFA expressed as percent of oil. Initial FFA content was 2.9 ± 0.1%. EtOH kernel and flour results are means of analyses on 14 samples; control kernel and flour results are means of analyses on 6 samples.

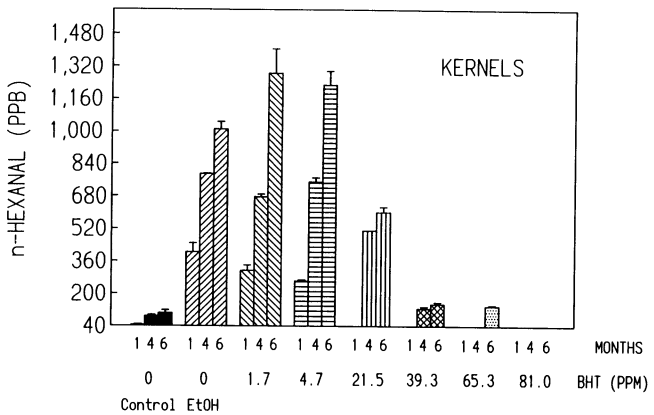

Fig. 2. Effects of butylated hydroxytoluene (BHT) on *n*-hexanal levels in vapor-treated kernels during storage in jars at 36°C. Means of duplicate analyses on two samples at each treatment level. Detection limit: 40 ppb of *n*-hexanal.

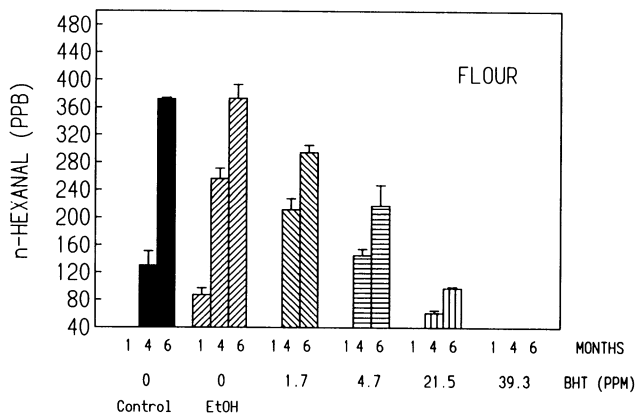

Fig. 3. Effects of butylated hydroxytoluene (BHT) on *n*-hexanal levels in flours prepared from vapor-treated kernels and stored in jars at 36°C. Means of duplicate analyses on two samples at each treatment level. Detection limit: 40 ppb of *n*-hexanal.

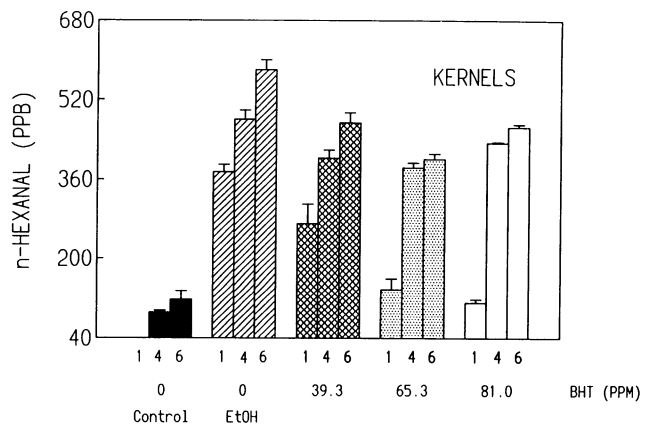

Fig. 4. Effects of butylated hydroxytoluene (BHT) on *n*-hexanal levels in vapor-treated kernels during storage in polyethylene bags at 36°C. Means of duplicate analyses on two samples at each treatment level. Detection limit: 40 ppb of *n*-hexanal.

(12.4%) and EtOH vapor-treated (11.0%) kernels and their flours in jars did not change during storage. However, the moisture contents of control and EtOH vapor-treated kernels and their flours in bags decreased to 7.5% following six months storage. The lower moisture contents of samples stored in bags led to smaller increases in FFA. Lipolytic hydrolysis proceeds at a lower rate at lower moisture levels (Galliard 1989).

No significant differences ($P > 0.05$) were observed in FFA levels during storage in kernels treated with EtOH vapors and those treated with EtOH vapors/BHT aerosol. Likewise, no significant differences ($P > 0.05$) existed in FFA levels during storage in flours prepared from these kernels. The low increases in FFA levels in EtOH vapor-treated kernels and their flours during storage indicate the presence of residual lipase activity.

Effects of BHT on *n*-Hexanal Levels

The effects of BHT on *n*-hexanal levels in vapor-treated kernels and their flours stored in glass jars for six months at 36°C are depicted in Figures 2 and 3, respectively. Figure 4 shows the effects of BHT on *n*-hexanal levels in vapor-treated kernels stored in polyethylene bags. The plotted values are means of duplicate analyses on two batches of rice.

n-Hexanal levels in kernels treated with EtOH vapors alone were markedly higher than that in control kernels. This increased susceptibility of EtOH vapor-treated kernels to oxidation can be attributed to disruption of the caryopsis coat by the vapors. Microscopy and differential scanning calorimetry (DSC) results (Champagne and Hron 1992) indicated that EtOH vapor treatments resulted in kernel fissuring, which increased kernel porosity, thus rendering the oil more susceptible to oxidation. The extent of oxidation in flours prepared from EtOH vapor-treated and control kernels was about the same after six months of storage.

n-Hexanal levels were markedly higher in EtOH vapor-treated kernels (with or without BHT) than in their flours during storage. Grinding the kernels to a flour dilutes the oil by a factor of 12 with starchy endosperm. When stored in a jar or bag, the flour particles pack tightly and exclude more air than would kernels similarly stored. Thus, more oil would be exposed to air and susceptible to oxidation in intact kernels than in flours.

Control kernels stored in jars were less susceptible to oxidation than their flours in this study. This is contrary to our observations in a recent study (Champagne and Hron 1993), in which control kernels had markedly higher susceptibility to oxidation than their flours. The lower susceptibility of the control kernels in this study to oxidation probably resulted from the bran layers of the kernels being disturbed to a lesser exent during shelling. The greater the

disruption of the bran layers during shelling, the more freely the oil migrates to the kernel surface, and the more readily it is oxidized.

Increasing BHT content of vapor-treated kernels and their flours resulted in lower *n*-hexanal levels during storage. Initial BHT contents of 81.0 and 39.3 ppm (corresponding to 36.5 and 4.4 ppm at six months) provided high oxidative stability to vapor-treated kernels and their flours, respectively, that were stored in jars. No *n*-hexanal (<40 ppb) was detected in these samples after six months storage at 36°C.

BHT provided oxidative stability to kernels stored in bags during the first month of storage. However, this stability was not observed (even at high initial BHT contents) after four and six months storage due to the rapid loss of BHT from the samples.

No *n*-hexanal (<40 ppb) was detected in control or vapor-treated flours stored in bags. As discussed earlier, the larger loss of BHT from these flours compared to flours stored in jars indicated a more ample oxygen supply. Thus, the expected result was lower oxidative stability in flours stored in bags, not higher. The lower moisture content of the flours stored in bags may have provided higher oxidative stability (Galliard 1989). Loss of hexanal by volatilization through the polyethylene bags may also explain the observed results.

In conclusion, EtOH vapors inhibit lipolytic hydrolysis in brown rice and its flour and serve as a carrier for delivering BHT to the kernels. The effectiveness of the BHT in inhibiting oxidation in vapor-treated kernels and their flours depended upon the oxygen permeability of the sample package. Unfortunately, the loss of BHT from samples stored at 36°C in typical polyethylene rice bags was too rapid to benefit from adding the antioxidant. To benefit from adding BHT, use of packaging that is less oxygen permeable (e.g., heavier gauge polyethylene or box with polypropylene liner) appears necessary.

ACKNOWLEDGMENTS

We acknowledge Carol Kelly and Olga Richard for technical assistance.

LITERATURE CITED

ANDERSON, R. H., HUNTLEY, T. E., SCHWECKE, W. M., and NELSON, J. H. 1963. Disappearance of BHA and BHT in relation to peroxide content in breakfast cereals. JAOCS 40:349-352.

CHAMPAGNE, E. T., HRON, R. J., SR., and ABRAHAM, G. 1990. Stabilizing unmilled brown rice be ethanol extraction. U.S. patent 07/557,882.

CHAMPAGNE, E. T., HRON, R. J., SR., and ABRAHAM, G. 1991. Stabilizing brown rice products by aqueous ethanol extraction. Cereal Chem. 68:267-271.

CHAMPAGNE, E. T., and HRON, R. J., SR. 1992a. Stability of ethanol-extracted brown rice to hydrolytic and oxidative deterioration. J. Food Sci. 57:433-436.

CHAMPAGNE, E. T., and HRON, R. J., SR. 1992b. Stabilizing brown rice to lipolytic hydrolysis by ethanol vapors. Cereal Chem. 69:152-156.

CHAMPAGNE, E. T., HRON, R. J., SR., and ABRAHAM, G. 1993. Stabilizing unmilled brown rice by ethanol vapors. U.S. patent 5,209,940.

CHAMPAGNE, E. T., and HRON, R. J., SR. 1993. Utilizing ethanol containing an antioxidant or chelator to produce stable brown rice products. Cereal Chem. 70:562-567.

COOK, C. D., NOSH, N. G., and FLANAGAN, H. R. 1955. Oxidation of hindered phenols (III). The rearrangement of the 2,6-di-tert-butyl-4-methylphenoxy radical. J. Am. Chem. Soc. 77:1783-1785.

COSGROVE, S. L., and WATERS, W. A. 1951. The oxidation of phenols with benzoyl peroxide. Part II. J. Chem. Soc. 388-391.

DZIEZAK, J. D. 1986. Antioxidants. The ultimate answer to oxidation. Food Tech. 40:94-102.

GALLIARD, T. 1989. Rancidity in Foods. J. C. Allen and R. J. Hamilton, eds. Elsevier: London.

HOFFPAUIR, C. L., PETTY, D. H., and GUTHRIE, J. D. 1947. Germination and free fatty acids in individual cottonseeds. Science 106:344-345.

SAUCY, F., DUCRET, F., LAMBELET, P., and LOLIGER, J. 1990. The fate of antioxidant radicals during lipid autoxidation. II. The influence of oxygen supply on lipid autoxidation. Chem. Phys. Lipids 55:215-221.

SHIN, M. G., YOON, S. H., RHEE, J. S., and KWON, T. 1986. Correlation between oxidative deterioration of unsaturated lipid and *n*-hexanal during storage of brown rice. J. Food Sci. 51:460-463.

TERADA, K., and NAITO, Y. 1989. Migration of BHT through airspace in food package systems. Packaging Tech. Sci. 2:165-171.

[Received October 3, 1994. Accepted January 23, 1995.]