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ANTIOXIDANT MEASUREMENT

Determination of 2,6-Di-*tert*-Butyl-4-Hydroxytoluene (BHT): Application to Edible Fats and Oils

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BHT is a very effective antioxidant for many organic substances such as edible fats and oils. Its wide use either alone or in combination with other antioxidants has indicated a need for a specific and effective method for its quantitative determination to maintain effective control over processing operations and to ensure adherence to regulatory requirements. The method presented is based on separation of BHT from fat or oil by steam distillation and colorimetric determination with a dianisidine–nitrous acid reagent. The method is capable of determining 10 to 200 p.p.m. BHT in the presence of other allowable antioxidants.

THE COMPOUND 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) is widely used as an effective antioxidant for a wide variety of organic substances, such as Vitamin A oils, edible fats and oils, rubber, petroleum, and plastics. The use of this compound has been permitted in the U. S. (*9*) and Canada (*2*).

There is a need for a specific and quantitative method for analysis of commercial products to maintain effective production control over processing operations employing this antioxidant and to ensure compliance to regulatory requirements. Butylated hydroxytoluene is used either alone or in combination with other permitted antioxidants; therefore, the method should be capable of determining 10 to 200 p.p.m. in the presence of other allowable antioxidants.

A survey of the literature showed that previous methods for the determination of BHT were based on colorimetry with ferric chloride-1,1′-bipyridine (*6*), ferric chloride and *o*-phenanthroline (*1*), and phosphomolybdate reagent (*8*).

Anglin, Mahon, and Chapman (*1*) separate butylated hydroxytoluene and butylated hydroxyanisole (BHA) from fats and oils by steam distillation. The distillate is analyzed for the sum of butylated hydroxyanisole and butylated hydroxytoluene with ferric chloride-2,2′-bipyridine and for butylated hydroxyanisole with 2,6-dichloroquinonechloroimide, thereby permitting butylated hydroxytoluene to be determined by difference. This method has been studied collaboratively (*3*). Phillips and Henkel (*7*) reported a method for the estimation of butylated hydroxytoluene by ultraviolet spectrophotometry after chromatographic separation on silicic acid, but tocopherol interferes in this analysis. A specific and rapid method by gas-liquid partition chromatography has also been reported (*5*). The colorimetric methods were not applicable in the presence of ascorbyl palmitate and natural tocopherols.

This study was initiated to develop a specific and rapid color reaction for the determination of BHT in the presence of butylated hydroxyanisole, ascorbyl palmitate, tocopherols, and other antioxidants. Preliminary experiments indicated that BHT treated with an alcoholic solution of dianisidine and nitrous acid formed a chromogen which could be extracted with a chlorinated solvent, such as chloroform or tetrachloroethane. This novel color reaction was studied intensively so that it could be applied to the quantitative determination of BHT.

Since water and alcohol are required for the reaction between the butylated hydroxytoluene and the dianisidine–nitrous acid reagent, the analysis cannot be carried out directly on an anhydrous fat or oil. Therefore, some means is necessary to separate the antioxidant from the fat or oil prior to final analysis. Various solvent extractions were studied and found to be unsatisfactory. The distillation technique of Anglin, Mahon, and Chapman (*1*) was modified and found to be applicable to corn, cottonseed, peanut, and soya oils, and to hydrogenated oil and lard.

Reagents

Unless otherwise indicated all reagents are reagent grade.

Magnesium Chloride Solution. Dissolve 100 grams of magnesium chloride, $MgCl_2 \cdot 6H_2O$, in 50 ml. of water.

Dianisidine Solution. Dissolve 250 mg. of dianisidine (3-3′-dimethoxybenzidine) in 50 ml. of methanol. Add 100 mg. of activated charcoal, shake for 5 minutes, and filter. Mix 40 ml. of the clear filtrate with 60 ml. of 1*N* hydrochloric acid. Prepare daily and protect from light.

Sodium Nitrite Solution. A 0.3% aqueous solution.

Reference Standard Solution of 2,6-di-*tert*-butyl-4-hydroxytoluene. Dissolve 50.0 mg. of pure (99.8%) 2,6-di-*tert*-butyl-4-hydroxytoluene in sufficient methanol to make 100.0 ml. Prepare working standards containing 1 to 5 μg. per ml. by diluting the reference standard stock solution with 50% (v./v.) methanol.

Apparatus

Distilling Apparatus. A microsteam distillation apparatus of the Jenden-Taylor (*4*) type, with a spiral coil con-

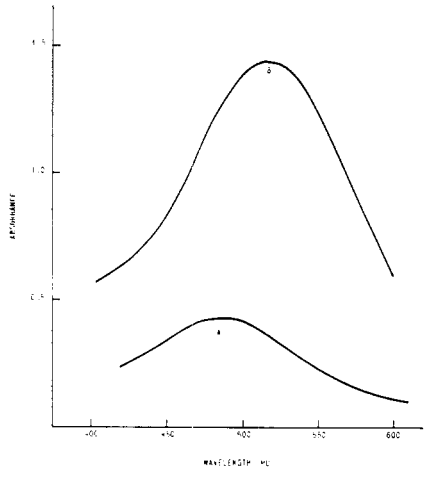

Figure 1. Absorption curves for direct and chloroform extraction procedures

A. Direct B. Chloroform

Table I. Comparison of Solvents as Extractants for the Chromogen

Solvent	
Chloroform	Chromogen extracted rapidly to yield solution. Absorption maximum at 520 mμ. Color stable for at least 1 hour when protected from light.
Methylene chloride	Chromogen extracted rapidly to yield purple solution. Absorption maximum at 525 mμ. Because of high volatility of solvent, difficult to obtain reproducible absorbance readings.
Ethylene dichloride	Chromogen extracted rapidly to yield purple solution. Absorption maximum at 540 mμ. Color stable for 1 hour.
Sym-tetrachloroethane	Chromogen extracted rapidly to yield purple solution. Absorption maximum at 530 mμ. Color stable for 1 hour.
Isoamyl alcohol	About 30% of chromogen extracted to yield an orange solution. Absorption maximum at 485 mμ.
Methyl chloroform	Extracts about 5% of the chromogen.
Trichloroethylene	Extracts about 5% of the chromogen.
Ether	Chromogen insoluble.
Isooctane	Chromogen insoluble.
Benzene, toluene	Chromogen insoluble.
Hexane, cyclohexane	Chromogen insoluble.
Carbon tetrachloride	Chromogen insoluble.

denser instead of a straight condenser, is used with a 100-ml., round-bottomed distilling flask. A 1-liter, round-bottomed flask containing distilled water and heated with a heating mantle is used as the steam generator. All connections are made with standard taper joints except for a short piece of seasoned Tygon tubing connecting the steam outlet with the distilling flask head.

Bath. A 1-liter beaker half-full of bath wax (Fisher bath wax, m.p. 60° C., smoke point 270° C., flash point 360° C.) is used.

Method

Colorimetric Procedure. After study of many variations in details, the following procedure was found to give the most accurate and reproducible results for solutions containing 1 to 3 μg. of BHT per ml.

Thoroughly clean and dry six 60-ml., Squibb-type, separatory funnels made of low actinic glass or rendered impervious to light by wrapping with black tape. Mark the funnels *B*, S_1, S_2, S_3, X_1, and X_2. Into funnel *B* pipet 25.0 ml. of 50% methanol (v./v.); into funnels S_1, S_2, and S_3 pipet 25.0 ml. of 50% methanol containing 25, 50, and 75 μg. of BHT, respectively; into funnels X_1 and X_2, pipet duplicate 25.0-ml. aliquots of a 50% methanol (v./v.) solution of the sample. To each funnel add 5.0 ml. of dianisidine solution and carefully mix the contents. Add 2.0 ml. of 0.3% sodium nitrite solution, stopper the funnels, and mix the contents. Let stand for 10 minutes. Then add 10.0 ml. of chloroform to each funnel. Extract the colored complex by vigorously shaking each funnel for 30 seconds. Let stand for 3 minutes to allow the two layers to separate completely.

Mark six clean, dry, 10-ml. volumetric flasks of low actinic glass B_1, S_1, S_2, S_3, X_1, and X_2, respectively. Pipet 2.0 ml. of anhydrous methanol into each flask. From the corresponding funnel draw off a sufficient volume of the chloroform extract (bottom layer) to reach the mark on the flask. Stopper and mix well. Avoiding any unnecessary exposure to light and air, transfer the colored chloroform solution to a 1-cm. cell and measure the absorbance in a suitable spectrophotometer or photoelectric colorimeter at 520 mμ with a mixture of 2.0 ml. of methanol and 8 ml. of chloroform as reference solution.

The absorbances S_1-*B*, S_2-*B*, and S_3-*B* are measures of the standards. The absorbances X_1-*B* and X_2-*B* are the measures of the BHT in the aliquot of the sample solution.

Procedure for Fats and Oils. Transfer 15.0 ml. of magnesium chloride solution into the 100-ml. distilling flask. Add sufficient weight of oil or fat equivalent to 400 μg. of BHT. (The sample weight should not exceed 5 grams.) Lightly grease the ground-glass joint and place the top on the distilling flask. Preheat the bath for the distillation flask to 160° ± 10° C. Adjust the steam generator to distill about 4 ml. of water per minute. Maintain these conditions throughout the distillation. Connect the condenser and steam generator to the distilling flask, and immediately immerse the latter in the bath. Collect the first 100 to 125 ml. of the distillate in a 200-ml. volumetric flask containing 50 ml. of methanol. Disconnect the distillation flask from the steam generator and remove the distillation flask from the bath. When the mouth of the condenser has cooled, disconnect it from the distillation flask and drain the water from the jacket. Wash the condenser with 5-ml. portions of methanol adding the washings to the volumetric flask. Cool to room temperature, adjust the volume to 200 ml. with methanol, and mix well. Proceed as described under Colorimetric Procedure.

Formation of Color and Absorption Spectra. Figure 1 shows the absorption spectra of the chromogen formed by the reaction of BHT with the dianisidine-nitrite reagents.

An orange-red color was formed when 200 μg. of BHT in 10.0 ml. of 50% methanol (v./v.) were allowed to react with 5.0 ml. of dianisidine and 2.0 ml. of 0.3% sodium nitrite solution for 10 minutes. The orange-red color exhibits a maximum absorption at 490 mμ as shown in curve *A*.

Curve *B* was obtained when 200 μg. of BHT in 10.0 ml. of 50% methanol were treated with 5.0 ml. of dianisidine and 2.0 ml. of a 0.3% sodium nitrite solution. After standing 10 minutes, the orange-red solution was extracted with 10 ml. of chloroform. The purple chromogen extracted with chloroform showed a maximum absorption at 520 mμ. The absorbance of the chromogen in chloroform is three times greater than that in the aqueous solution.

Choice of Solvent. Preliminary experiments indicated that the chromogen formed by the reaction of BHT with dianisidine-nitrite reagents was soluble in chlorinated solvents and could be extracted easily. Various other solvents were tried in an attempt to accomplish quantitative extraction of the colored complex into the organic phase with a single equilibration. Tests for the suitability of the solvent were carried out by extracting the chromogen produced by 250 μg. of BHT and the reagents under the conditions of the procedure with a single 10-ml. portion of the solvent. The extent of the extraction was estimated by the color in the solvent. Of all the solvents tested, chloroform,

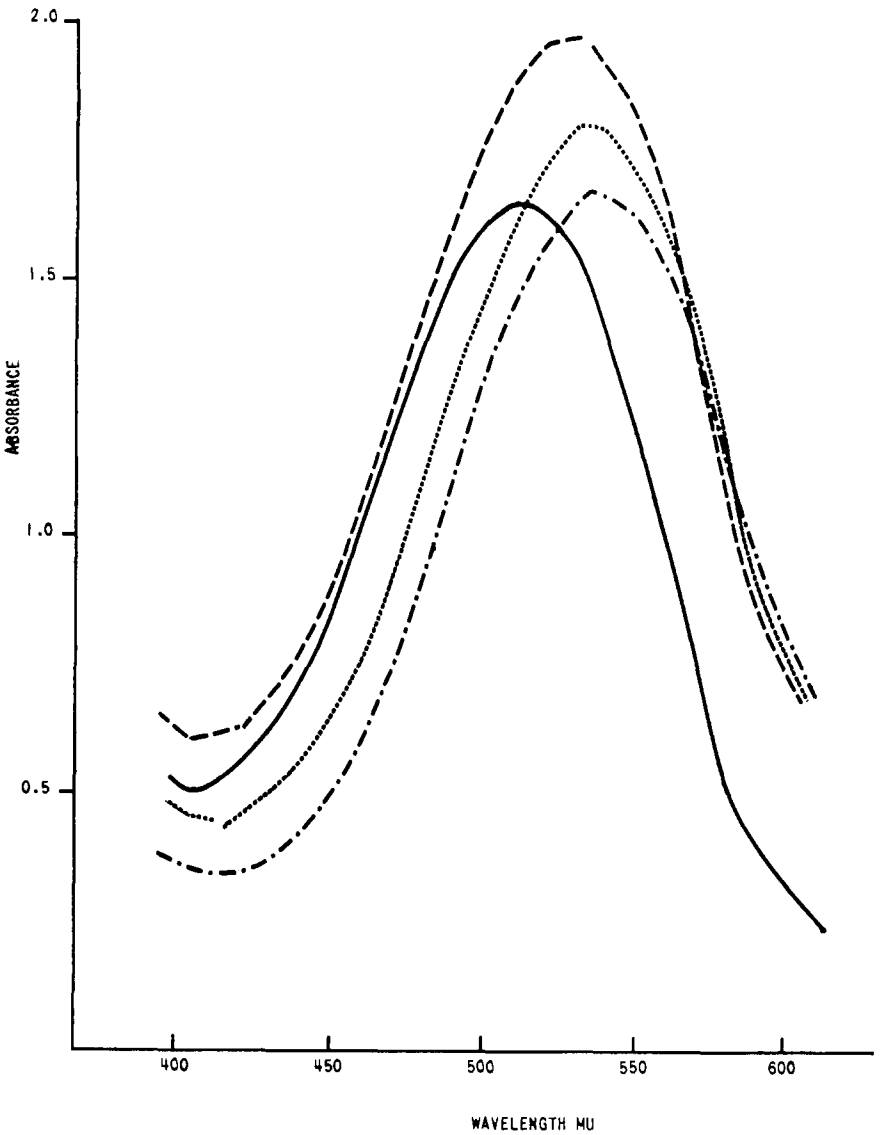

Figure 2. Absorption curves of BHT chromogen in various solvents

-----CH_2Cl_2; ——— $CHCl_3$; .....$CHCl_2.CHCl_2$; -·-·-·$CH_2Cl.CH_2Cl$

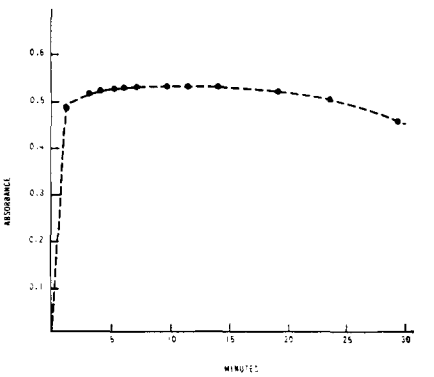

Figure 3. Time of reaction

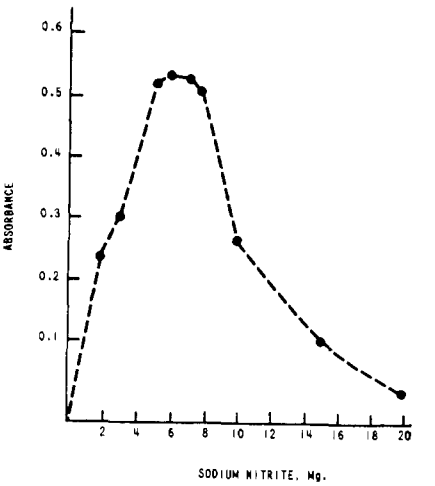

Figure 4. Effect of sodium nitrite concentration

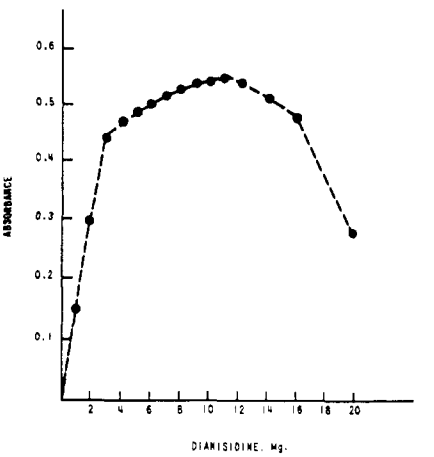

Figure 5. Effect of dianisidine concentration

methylene chloride, ethylene dichloride, and *sym*-tetrachloroethane were satisfactory (Table I).

Chloroform was selected as the solvent because it is readily available and has a rapid rate of extraction, sharp phase separation, and low solubility in water. The spectral characteristics of the chromogen in the various solvents are shown in Figure 2.

Relationship of Ratio of Volume of Reaction Solution to Volume of Solvent. The chromogen can be extracted from the reaction mixture with chloroform over a wide range of ratios of volume of reaction mixture to chloroform. When a constant volume of chloroform (10 ml.) is used and the volume of the reaction solution (15 to 40 ml.) is varied, equal absorbance readings are obtained. Conformity to Beer's law was indicated by the linear relationship between the micrograms of BHT and the absorbance.

Rate of Reaction. Figure 3 shows the rate of color development of BHT with the dianisidine-nitrite reagents at room temperature (25° C.). The reaction time was determined by treating 10.0 ml. (60 μg.) of a stock solution of the butylated hydroxytoluene in 50% methanol (v./v.) with 5.0 ml. of dianisidine and 2.0 ml. of a 0.3% sodium nitrite solution in separatory funnels of low actinic glass for 1 to 30 minutes. At the end of each period, the color was extracted with 10.0 ml. of chloroform, and the absorbance measured at 520 mμ. The intensity of the color reached its maximum in 5 to 7 minutes, remained constant for an additional 10 minutes, then gradually decreased.

Effect of Nitrite. In this series of experiments, 10-ml. (60 μg.) aliquots of the stock solution were treated with 5.0 ml. of the dianisidine solution and 2.0 ml. of nitrite solution containing varying quantities of sodium nitrite. At the end of 10 minutes, each colored solution was extracted with 10.0 ml. of chloroform, and the absorbance of the chloroform extract measured at 520 mμ. A similar series of blanks were prepared at the same time. Figure 4 shows that the amount of nitrite has a marked effect on color development and that this effect is a complex one, with maximal color when 6 mg. of sodium nitrite are present.

Effect of Dianisidine. The effect of the amount of dianisidine added on the color development is shown in Figure 5. To 10.0-ml. (60 μg.) aliquots of the stock solution, 5.0 ml. of dianisidine solution

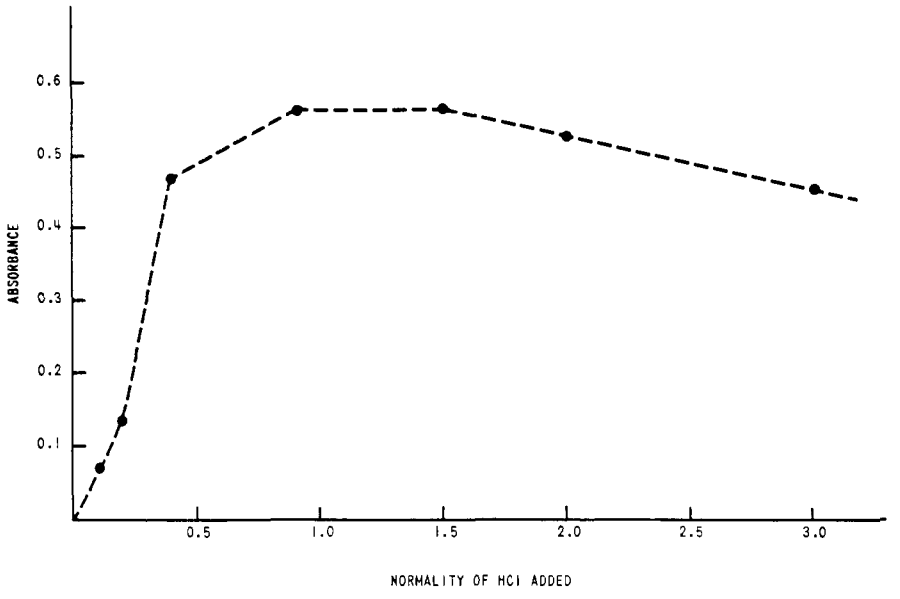

Figure 6. Effect of HCl concentration

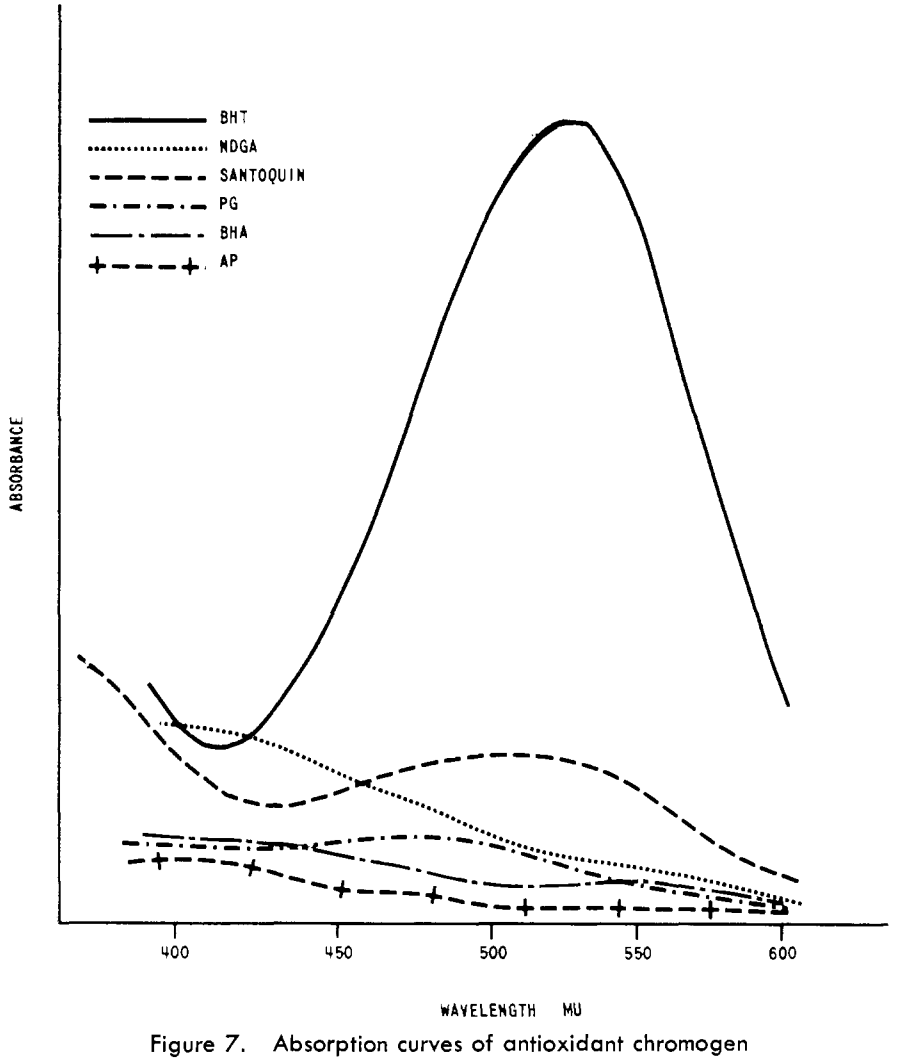

Figure 7. Absorption curves of antioxidant chromogen

containing varying amounts of dianisidine and 2.0 ml. of a 0.3% sodium nitrite solution were added, and after 10 minutes the colored solutions were extracted with 10 ml. of chloroform. The absorbances of the chloroform extracts were measured at 520 mμ. A similar series of blanks were prepared at the same time. Maximal color development requires the presence of 10 mg. of dianisidine.

Table II. Reproducibility of Absorbance at Three Concentrations at 520 mμ

25 μg.	50 μg.	75 μg.
0.173, 0.180	0.355, 0.365	0.525, 0.545
0.187, 0.183	0.358, 0.361	0.535, 0.530
0.177, 0.180	0.367, 0.355	0.540, 0.533
Av. 0.180	0.360	0.535
Std. dev. 0.005	0.005	0.007

Effect of Hydrochloric Acid Concentration. In this series of determinations, 10.0-ml. (60 μg.) aliquots of the stock solution were treated with 3.0 ml. of 0.1 to 3.0*N* hydrochloric acid, 2.0 ml. of a 0.5% dianisidine in methanol solution, and 2.0 ml. of a 0.3% sodium nitrite solution. At the end of 10 minutes, each colored solution was extracted with 10.0 ml. of chloroform, and the absorbance of the chloroform extract measured at 520 mμ. Figure 6 shows that the intensity of the color formed increased as the normality of the acid added increased, until maximum color intensity was obtained with 1*N* acid. A gradual decrease in intensity was obtained as the normality of the acid added became greater than 1.5

Light Stability. Solution of BHT along with blank solutions were treated with the reagents and extracted under conditions described in the procedure. The stability of the color was studied over a period of 3 hours. Colored solutions which were kept in amber flasks (low actinic flasks) and protected constantly from light showed no decrease in absorbance after 1 hour. Those colored solutions exposed to sunlight or normal laboratory lighting faded rapidly.

Precision of Color Measurement. A plot of absorbance against concentration of BHT was found to be linear and passed through the origin. Beer's law is obeyed over the range of 0 to 80 μg. Table II gives the absorbance obtained at three levels of BHT. Examination of the data in Table II shows that 18 determinations of *K* values (absorbance per microgram) resulted in a mean of 0.00718 with a standard deviation of 0.00013. To prevent errors due to the relatively high coefficient of thermal expansion of chloroform, all solutions were kept and read at 25° C.

Specificity. A number of antioxidants and related substances were treated with dianisidine-nitrite reagents under the conditions described in the procedure. Spectral characteristics were determined with a Cary recording spectrophotometer, and the absorbance of the color produced by each compound was measured at 520 mμ.

Data in Table III and Figure 7 show that propyl gallate and santoquin exhibit absorption maxima at 490 and 505 mμ, respectively. 4,6 - Di - *tert* - butyl - *m*-hydroxytoluene and 6-*tert*-butyl-*m*-hydroxytoluene do not give the color reaction. Butylated hydroxyanisole and nordihydroguaiaretic acid form a slight yellow color. Table IV lists a number of compounds which form a yellow color and those which give no color.

Results and Discussion

To determine the reliability of the general method for 2,6-di-*tert*-butyl-4-hydroxytoluene, synthetic mixtures of the butylated hydroxytoluene and various antioxidants were prepared and analyzed as directed in the general method. Results obtained are shown in Table V. These results indicated that only santoquin produced an error greater than 3%. Oxidizing substances, if present in the oil, react with the butylated hydroxytoluene and thereby cause low recovery of the antioxidant. To obtain an accurate determination of the recovery of the butylated hydroxytoluene from edible oils using the method described, the oil was prepared free of peroxides or other oxiding substances. U.S.P. grade corn, cottonseed, peanut, and soya oils free of oxidizing substances were prepared by adding 0.10% tocopherol to each oil and heating on a steam bath for 15 minutes.

Each sample was first tested as received to establish the presence or absence of 2,6 - di - *tert* - butyl - 4 - hydroxytoluene. Samples of oils which gave a negative test for BHT were first treated with tocopherol, then accurately determined amounts of BHT were added in various combinations with other antioxidants and tested according to the procedure described. The results are shown in Table VI.

Addition of various salts, such as potassium chloride, potassium carbonate, calcium chloride, sodium chloride, and magnesium chloride, to the water in the distillation flask to increase the temperature of the steam and hasten distillation of the antioxidant was studied, and magnesium chloride was found to give the most reproducible results. With the high temperature involved in the steam distillation, the presence of magnesium chloride in the distillation flask retained sufficient water to prevent the fat or oil from decomposing to acrolein. Using superheated steam at approximately 160° C. and magnesium chloride as the salt in the distillation flask, butylated hydroxytoluene distills quantitatively from the sample.

Steam distillation completely separates butylated hydroxytoluene from propyl gallate, nordihydroguaiaretic acid, ascorbyl palmitate, and tocopherol, as these antioxidants either are not steam distilled or are decomposed at the tem-

Table III. Absorption Maximum and Absorbance at 520 Mμ

	Abs. Max., Mμ	Abs./μg. at 520 Mμ
2,6-Di-*tert*-butyl-4-hydroxytoluene (BHT)	520	0.0075
4,6-Di-*tert*-butyl-*m*-hydroxytoluene	...	0.00002
6-*tert*-Butyl-*m*-hydroxytoluene	...	0.00002
2-*tert*-Butyl-4-hydroxyanisole	...	0.00002
3-*tert*-Butyl-4-hydroxyanisole	...	0.00002
Propyl gallate (PG)	490	0.0006
1,2-Dihydro-6-ethoxy-2,2,4 trimethylquinoline (santoquin)	505	0.0011
Nordihydroguaiaretic acid (NDGA)	...	0.0003
4-*tert*-Butylpyrocatechol	...	0.0001
2-*tert*-Butylhydroquinone	...	0.0006
α-Tocopherol (and mixed tocopherols)	...	0.00007
Ascorbyl palmitate (AP)	...	0.00007

Table IV. Specificity of Reaction

Substances Developing No Color

Butyl-*p*-hydroxybenzoate	Phenol
p-Ditertiary butyl benzene	Cresol
p-*tert*-Butylbenzoic acid	Resorcinol
p-Hydroxybenzoic acid	Hydroquinone
Ethyl-*p*-hydroxybenzoate	2,4-Dimethylphenol
Methyl-*p*-hydroxybenzoate	3,5-Dimethylphenol
Propyl-*p*-hydroxybenzoate	3,4-Dimethylphenol
2,6-Di-*tert*-butyl-*m*-hydroxytoluene	6-*tert*-Butyl-*m*-hydroxytoluene

Substances Forming Yellow Color

2-*tert*-Butyl-4-hydroxyanisole	Norhydroguaiaretic acid
3-*tert*-Butyl-4-hydroxyanisole	α-Tocopherol (and mixed tocopherols)
4-*tert*-Butylpyrocatechol	Ascorbyl palmitate
2-*tert*-Butylhydroquinone	*p*-Aminophenol
2,5-Di-*tert*-butylhydroquinone	2,5-Di-*tert*-amylhydroquinone

Table V. Recoveries of 2,6-Di-*tert*-Butyl-4-Hydroxytoluene from Various Mixtures of Antioxidants

Mixture	% BHT Recovered
50% BHT + 50% BHA	101.4, 100.4, 100.5, 100.7
+ 50% NDGA	101.7, 100.7
+ 50% PG	103.2, 102.1
+ 50% AP	100.8, 100.5
+ 50% Tocopherols (mixed tocopherols)	100.7, 100.4
+ 50% Santoquin	113, 114.2
33 1/3% BHT + 33 1/3% BHA + 33 1/3% NDGA	101.6, 101.7
33 1/3% BHT + 33 1/3% BHA + 33 1/3% PG	102.4, 102.6
25% BHT + 25% BHA + 25% PG + 25% NDGA	102.2, 103.2
25% BHT + 25% BHA + 25% AP + 25% Tocopherol	102.1, 101.4
50% BHT + 25% BHA + 25% Santoquin	105.0, 106.5

Table VI. Recovery of 2,6-Di-*tert*-butyl-4-hydroxytoluene (BHT) from Oils

	% Antioxidant Added	% BHT Found	% Recovered
Soya oil	0.00252 BHT	0.00247	97.8
Cottonseed oil	0.00252 BHT	0.00248, 0.00255	98.4, 101.2
Soya oil	0.00505 BHT	0.00496	98.2
Cottonseed oil	0.00505 BHT	0.00481, 0.0049	95.2, 97.0
Corn oil	0.0050 BHT	0.00497	99.7
Peanut oil	0.0050 BHT	0.00478	95.6
Hydrogenated vegetable oil	0.0050 BHT	0.0048, 0.00495	96.0, 99.0
Lard	0.00525 BHT	0.00505	96.2
Soya oil	0.00758 BHT	0.00723	95.5
Cottonseed oil	0.00758 BHT	0.0072, 0.00744	95.0, 98.1
Soya oil	0.0101 BHT	0.00965	95.5
Cottonseed oil	0.0101 BHT	0.0099, 0.00968	98.0, 95.8
Corn oil	0.0100 BHT	0.00977, 0.00956	97.7, 95.6
Peanut oil	0.0100 BHT	0.00957, 0.0096	95.7, 96.0
Hydrogenated oil	0.0100 BHT	0.00958, 0.00938	95.8, 93.8
Lard	0.0105 BHT	0.0099, 0.00994	94.3, 94.7
	0.0105 BHT	0.0100, 0.00982	95.2, 93.5
	0.0210 BHT	0.0201	95.5

(*Continued on page 495*)

Table VI. (continued)

	% Antioxidant Added	% BHT Found	% Recovered
Hydrogenated oil	0.0200 BHT	0.0196, 0.0191	98.0, 95.5
Soya oil	0.0202 BHT	0.0195	96.6
Cottonseed oil	0.0202 BHT	0.0201, 0.0197	95.5, 97.3
Corn oil	0.0200 BHT	0.0196	98.0
Peanut oil	0.0200 BHT	0.0194	97.0
Corn oil	0.0050 BHT + 0.0050 BHA	0.00475	95.0
	0.0100 BHT + 0.010 BHA	0.0099	99.0
	0.0100 BHT + 0.005 BHA	0.0098	98.0
	0.0200 BHT + 0.010 BHA	0.0191	95.5
Peanut oil	0.010 BHT + 0.010 BHA	0.0098	98.0
Soya oil	0.0101 BHT + 0.010 BHA	0.0097	96.1
Cottonseed oil	0.0101 BHT + 0.005 BHA	0.0097	96.0
	0.0101 BHT + 0.010 BHA	0.0101	100.0
Hydrogenated oil	0.010 BHT + 0.010 BHA	0.0097	97.0
	0.020 BHT + 0.020 BHA	0.0192	96.0
Lard	0.0105 BHT + 0.010 BHA	0.0102	97.0
	0.0105 BHT + 0.005 PG	0.0103	97.6
Hydrogenated oil	0.010 BHT + 0.005 PG	0.0098	98.0
	0.020 BHT + 0.005 PG	0.0193	96.0
Cottonseed oil	0.0202 BHT + 0.010 PG	0.0202	100.0
	0.0202 BHT + 0.020 PG	0.0197	97.2
Soya oil	0.0101 BHT + 0.010 AP	0.0095	94.2
	0.0101 BHT + 0.010 BHA + 0.010 AP	0.0097	96.0
Cottonseed oil	0.0101 BHT + 0.005 BHA + 0.005 PG	0.0094	93.4
	0.0101 BHT + 0.0025 BHA + 0.0025 PG	0.0097	96.5
Cottonseed oil	0.0101 BHT + 0.005 BHA + 0.0025 PG	0.0098	97.0
	0.0101 BHT + 0.075 BHA + 0.0025 PG	0.0098	97.0
Peanut oil	0.010 BHT + 0.005 BHA + 0.005 PG	0.0100	100.0
Hydrogenated oil	0.010 BHT + 0.005 BHA + 0.005 PG	0.0102	100.2
	0.010 BHT + 0.005 BHA + 0.0025 PG	0.0097	97.0
Lard	0.0105 BHT + 0.005 NDGA	0.0102	97.3
Peanut oil	0.010 BHT + 0.005 BHA + 0.005 NDGA	0.0099	99.0
Hydrogenated oil	0.010 BHT + 0.0025 NDGA	0.0099	99.0
	0.010 BHT + 0.005 BHA + 0.0025 NDGA + 0.0025 PG	0.0100	100.0
	0.010 BHT + 0.005 NDGA	0.0095	95.0
	0.020 BHT + 0.005 NDGA	0.0193	96.5
Corn oil	0.010 BHT + 0.005 BHA + 0.005 NDGA + 0.005 PG	0.010	100.0
	0.020 BHT + 0.010 PG	0.0198	99.0
Peanut oil	0.010 BHT + 0.005 BHA + 0.005 NDGA + 0.005 PG	0.0101	101.0
Lard	0.0105 BHT + 0.005 BHA + 0.005 NDGA + 0.005 PG	0.0104	98.8
	0.0105 BHT + 0.0025 BHA + 0.0025 NDGA + 0.0025 PG	0.0103	98.0
	0.0105 BHT + 0.005 BHA + 0.005 NDGA + 0.005 PG	0.0104	98.8
	0.0105 BHT + 0.0025 BHA + 0.0025 NDGA + 0.0025 PG	0.0103	98.0
	0.010 BHT + 0.010 santoquin	0.0118	118.0
	0.010 BHT + 0.005 santoquin	0.0113	113.0
		Av.	97.1
		Std. dev.	1.9

peratures involved. Santoquin is partially steam distilled and causes a positive interference. Table VI shows recoveries of butylated hydroxytoluene obtained from the oils.

Repeated determinations were made on oil and lard samples containing the antioxidants at various levels to determine the precision of the method. Results in Table VI indicate satisfactory agreement between the butylated hydroxytoluene added and amount recovered.

Butylated hydroxytoluene was added on the basis of currently permissible levels and in amounts and combinations of antioxidants beyond permissible limits to simulate a more severe test of the method.

The reaction with the dianisidine-nitrite reagents is specific for 2,6-di-*tert*-butyl-4-hydroxytoluene among the antioxidants currently added to edible oils, lard, or shortening.

The procedure given is based on a sample weight equivalent to about 400 μg. of the butylated hydroxytoluene and results in the use of 50 μg. of the butylated hydroxytoluene for development of sufficient intensity of color to minimize instrumental error. By use of cell paths greater than 1 cm. in depth, this sensitivity can be increased.

Best results are obtained when the color reaction is carried out strictly according to the recommended procedure. The method provides a rapid, accurate, and specific procedure for the determination of 2,6-di-*tert*-4-hydroxytoluene in edible oils, lard, and hydrogenated oils.

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