Determination of Antioxidants in Edible Fats

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Methods are presented for determining the antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), nordihydroguaiaretic acid (NDGA), and all combinations except those containing both propyl gallate and nordihydroguaiaretic acid. Butylated hydroxyanisole and butylated hydroxytoluene are separated from the fat and the other antioxidants by distillation with superheated steam. 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. Nordihydroguaiaretic acid and propyl gallate are extracted from a carbon tetrachloride solution of the fat using 50% ethyl alcohol and are determined with ferrous sulfate buffered to an appropriate pH. Butylated hydroxyanisole and butylated hydroxytoluene, although partially extracted with 50% ethyl alcohol, do not react with ferrous sulfate.

USE OF BUTYLATED HYDROXYTOLUENE (BHT; 3,5-di-*tert*-butyl-4-hydroxytoluene) as an antioxidant in foods has been permitted recently in Canada (*1*) and the United States (*9*). Previously butylated hydroxyanisole [BHA; 2-(and 3-)*tert*-butyl-4-hydroxyanisole], nordihydroguaiaretic acid [NDGA; 4,4′-(2,3-dimethyltetramethylene)dipyrocatechol], propyl gallate, [PG] and mixtures of butylated hydroxyanisole and propyl gallate; butylated hydroxyanisole and nordihydroguaiaretic acid were the common antioxidants found in lard and shortening. Dugan and coworkers (*2*) reported that butylated hydroxytoluene provides excellent lard stability and that combinations of butylated hydroxytoluene and butylated hydroxyanisole exhibit a higher "carry through" effect in baked goods than any other combination of permitted antioxidants. It is likely that in the future butylated hydroxytoluene will be used either alone or in combination with other permitted antioxidants. Therefore, a method is required for determining these four antioxidants alone or in whatever combination they may be used.

Mahon and Chapman (*5*) reported a method for determining propyl gallate, nordihydroguaiaretic acid, butylated hydroxyanisole, and mixtures of these antioxidants not including both propyl gallate and nordihydroguaiaretic acid when found in lard and shortening. In this procedure, nordihydroguaiaretic acid and butylated hydroxyanisole are extracted from a petroleum ether solution of the fat using 72% ethyl alcohol and made to react with ferric chloride–2,2′-bipyridine. Under these conditions butylated hydroxytoluene, if present in the fat sample, is partially extracted and therefore interferes in the analyses for butylated hydroxyanisole and nordihydroguaiaretic acid.

Koppers Co., Inc. (*3*), reported a method for analyzing butylated hydroxytoluene in lard. The lard sample, dissolved in cyclohexane, is chromatographed on a silicic acid column and the eluate is analyzed for butylated hydroxytoluene using the ultraviolet absorption curve between 240 and 320 mμ. Butylated hydroxyanisole, propyl gallate, and nordihydroguaiaretic acid do not interfere in this analysis. However, tocopherol does interfere and therefore this method could not be used for the determination of butylated hydroxytoluene in vegetable fat. It is claimed (*3*) that the use of a longer chromatographic column avoids the interference due to tocopherol. This revised method should therefore be satisfactory for the determination of butylated hydroxytoluene in lard and shortening but provides no means of analyzing for butylated hydroxyanisole, propyl gallate, and nordihydroguaiaretic acid that may also be present.

The present paper describes a method for determining butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, and nordihydroguaiaretic acid and all combinations except those containing both propyl gallate and nordihydroguaiaretic acid.

Development of Procedure

Nordihydroguaiaretic Acid and Propyl Gallate. In the method of Mahon and Chapman (*5*), propyl gallate is extracted from a petroleum ether solution of the fat with aqueous ammonium acetate (pH 7.0) and reacts with ferrous tartrate to form an intense purple color. These authors noted that, if nordihydroguaiaretic acid is present in high concentrations, it will react with ferrous tartrate to form a blue precipitate. Lundberg and Halvorson (*4*) stated that ferrous tartrate gives a colored reaction product with most, if not all, polyphenols but that these products are insoluble in many cases, and, therefore, cannot be estimated colorimetrically.

It was found possible, however, to obtain a soluble purple nordihydroguaiaretic acid–ferrous complex by conducting the reaction in a 25% ethyl alcohol medium buffered at pH 10 with sodium carbonate-bicarbonate. The intensity of the purple color is used for the quantitative estimation of nordihydroguaiaretic acid.

The reaction between ferrous tartrate and nordihydroguaiaretic acid or propyl gallate, is hindered by the presence of ethyl alcohol, as indicated by the failure to obey Beer's law. This difficulty is overcome by using ferrous sulfate in place of ferrous tartrate. However, without the tartrate in the reagent, there is a greater tendency toward precipitation of iron hydroxide and this tendency increases with increased ethyl alcohol concentration. It was found that 25% ethyl alcohol used in the reaction keeps the colored complex in solution and prevents the precipitation of iron hydroxide.

Mahon and Chapman (*5*) used petroleum ether as the fat solvent and extracted the propyl gallate with aqueous

ammonium acetate and the nordihydroguaiaretic acid with 72% ethyl alcohol. It was found that 50% ethyl alcohol will extract both nordihydroguaiaretic acid and propyl gallate from a petroleum ether solution of the fat. However, dilution of this 50% ethyl alcohol extract to 25% prior to analysis causes the separation of a small amount of dissolved petroleum ether, which remains suspended in the extract, cannot be removed, and interferes in the colorimetric analysis.

If carbon tetrachloride is used as the fat solvent, 50% ethyl alcohol completely removes propyl gallate and nordihydroguaiaretic acid in one extraction (see Figure 1). Although dilution of this extract to 25% ethyl alcohol causes the separation of some dissolved carbon tetrachloride, this suspension can easily be removed by centrifuging and a clear solution is obtained which can be used for colorimetric analysis.

If nordihydroguaiaretic acid and propyl gallate are present together in the fat sample, both will appear in the alcoholic extract. An attempt to adjust the pH of the reaction medium to permit the analysis of one of these antioxidants in the presence of the other was unsuccessful. However, propyl gallate and nordihydroguaiaretic acid have similar antioxidant properties; therefore, they are not likely to be used together.

Butylated Hydroxyanisole and Butylated Hydroxytoluene. There are two reagents available for the determination of butylated hydroxyanisole, the ferric chloride–2,2′-bipyridine reagent (*5*) and the 2,6-dichloroquinonechloroimide reagent (*6*). The former reacts with any reducing material but, among the common synthetic antioxidants, the latter is specific for butylated hydroxyanisole.

Commercial butylated hydroxyanisole is a mixture of two isomers, 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole. With the ferric chloride–2,2′-bipyridine reagent, these isomers exhibit similar absorbancies per microgram (k values)—namely, 0.0125 and 0.0116, respectively. But with the 2,6-dichloroquinonechloroimide reagent, the k values are 0.0025 and 0.0111, respectively. Since the 3-isomer is a better antioxidant than the 2-isomer, the trend in commercial production has been towards an increase in the percentage of 3-*tert*-butyl-4-hydroxyanisole. Recent analyses of commercial butylated hydroxyanisole preparations showed them to be from 91 to 96% of the 3-isomer. Therefore, the 2,6-dichloroquinonechloroimide reagent can now be used for the quantitative colorimetric analyses of butylated hydroxyanisole with only negligible errors resulting from slight fluctuations in the isomer ratios.

Butylated hydroxytoluene, like butylated hydroxyanisole, reacts with the ferric chloride–2,2′-bipyridine reagent to form the red ferrous-bipyridine complex. Under the conditions described for the determination of butylated hydroxyanisole in the method by Mahon and Chapman (*5*), the reaction between butylated hydroxytoluene and ferric chloride–2,2′-bipyridine does not reach completion even after 9 hours. However, by decreasing the ethyl alcohol concentration in the reaction medium, the reaction rate is increased (see Figure 2). An ethyl alcohol concentration of 33% was chosen for the reaction medium, since lower alcohol concentrations do not significantly increase the rate of reaction, and some alcohol is required to keep the butylated hydroxytoluene in solution. A reaction time of 30 minutes is used, but as this does not allow time for the reaction to reach completion (see Figure 2), variations in the temperature will cause fluctuations in the k value or absorbancy per microgram of butylated hydroxytoluene. Therefore, a calibration line must be prepared with each analysis.

As water is necessary to increase the rate of reaction between butylated hydroxytoluene and the ferric chloride–2,2′-bipyridine reagent, the analysis of butylated hydroxytoluene cannot be carried out directly in the anhydrous fat solution. Therefore, some means is necessary for removing the butylated hydroxytoluene from the fat prior to analysis. Solvent extraction was found unsatisfactory since butylated hydroxytoluene is more soluble in fat and fat solvents than in extracting solvents such as ethyl alcohol, acetone–ethyl alcohol (50 to 50 by volume), and methyl cellosolve.

It had been noted (*7*) that butylated hydroxyanisole distills from fat during the baking process; therefore, an attempt was made to separate butylated hydroxytoluene from the fat by distillation. Heating the fat directly was found unsatisfactory due to the formation of acrolein which interferes in the ferric chloride–2,2′-bipyridine reaction. Heating the fat in the presence of water prevents the formation of acrolein and the steam aids in the distillation of butylated hydroxytoluene, but even after 1 hour the distillation is not complete. The addition of salts such as sodium chloride, potassium carbonate, and calcium chloride to the water in the distilling flask increases the temperature of the steam and, therefore, hastens the distillation. However, high temperatures cannot be maintained for a sufficient period of time to complete the distillation, because of precipitation of the salt as the water distills. If superheated steam is continuously passed

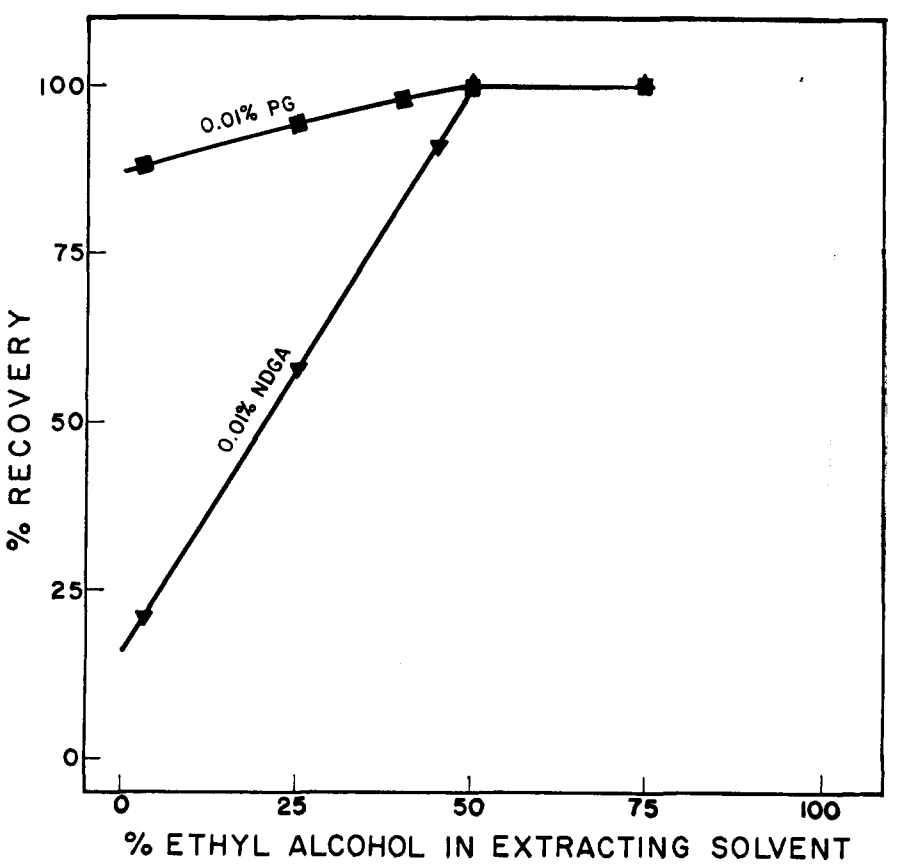

Figure 1. Effect of ethyl alcohol concentration in extracting solvent upon recovery of propyl gallate and nordihydroguaiaretic acid from fat dissolved in carbon tetrachloride

into the distilling flask, high temperatures can be maintained indefinitely without the precipitation of the salt. With the high temperature involved in the superheated steam distillation, a small amount of salt is necessary in the distilling flask to retain some water and thus prevent the fat from decomposing to acrolein. Using superheated steam at a temperature of approximately 160° C. and calcium chloride as the salt in the distilling flask, it is possible to distill butylated hydroxytoluene and butylated hydroxyanisole quantitatively from a fat sample.

During the distillation a small amount of fatty material distills. This material is filtered off and dissolved by washing the condenser and filter paper with hot ethyl alcohol. However, on mixing the alcoholic washings with the aqueous distillate, the fatty material again separates forming a dispersion in the distillate. Therefore, *n*-butyl alcohol is added to dissolve the dispersion before colorimetric measurements are made on the distillate.

The distillation procedure completely separates butylated hydroxytoluene and butylated hydroxyanisole from propyl gallate, nordihydroguaiaretic acid, and tocopherol, as these latter antioxidants either are not steam distilled or are decomposed at the high temperatures involved. Therefore, the distillate is analyzed for the sum of butylated hydroxyanisole and butylated hydroxytoluene using the ferric chloride–2,2′-bipyridine reagent and for butylated hydroxyanisole using the 2,6-dichloroquinonechloroimide reagent; and the concentration of butylated hydroxytoluene is determined by difference.

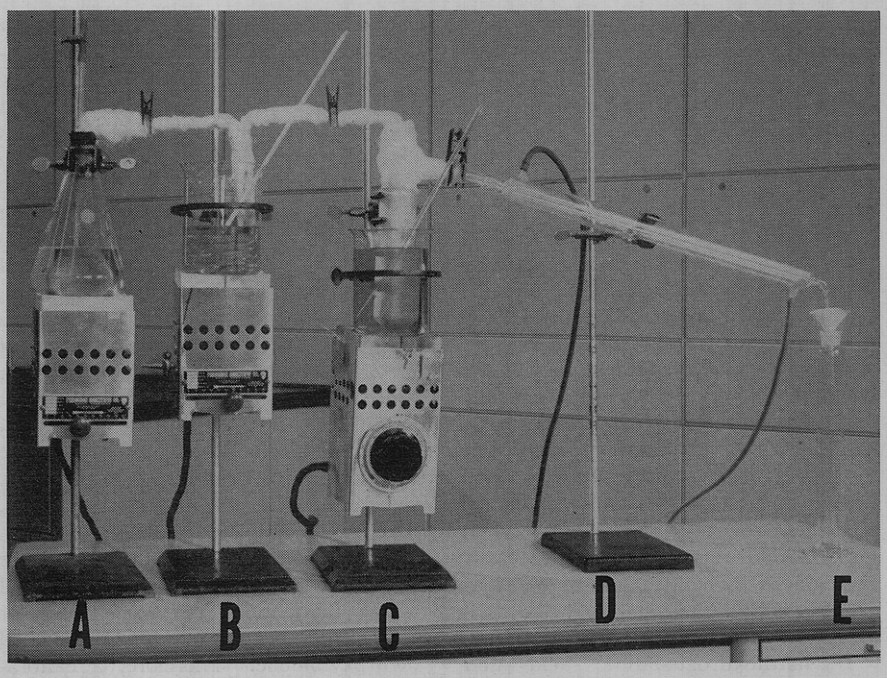

Figure 3. Distillation apparatus

A. Steam generator *B.* Superheater *C.* Distillation flask *D.* Condenser *E.* Filter and receiving cylinder

Reagents

Carbon tetrachloride, reagent grade.

Ethyl alcohol. Add approximately 0.1% potassium hydroxide and 0.1% potassium permanganate to commercial absolute alcohol and distill in an all-glass apparatus. The distillate is 100% alcohol and is diluted volume for volume to obtain the 50% and 25% alcohol.

Barium hydroxide. Prepare 1% barium hydroxide [$Ba(OH)_2.H_2O$] in boiled distilled water. This reagent must be kept in a tightly stoppered bottle.

Ammonium hydroxide, concentrated C.P. reagent.

Ferrous sulfate. 0.04% ferrous sulfate ($FeSO_4.7H_2O$) in distilled water, freshly prepared.

Sodium carbonate–bicarbonate buffer. Prepare 5.3% anhydrous sodium carbonate and 4.2% sodium bicarbonate in distilled water.

Ammonium acetate. Prepare a solution containing 2% of ammonium acetate in distilled water.

Calcium chloride, reagent grade 20 mesh.

Ferric chloride. Prepare fresh 0.2% of ferric chloride ($FeCl_3.6H_2O$) in distilled water.

2,2′-Bipyridine. Dissolve 200 mg. of 2,2′-bipyridine in 0.5 ml. of 100% ethyl alcohol and dilute to 100 ml. with distilled water. If the 2,2′-bipyridine is brownish, sticky, or possesses a strong odor, it should be purified (*8*).

Borax buffer. Prepare 2% borax ($Na_2B_4O_7.10H_2O$) in distilled water.

n-Butyl alcohol, reagent grade.

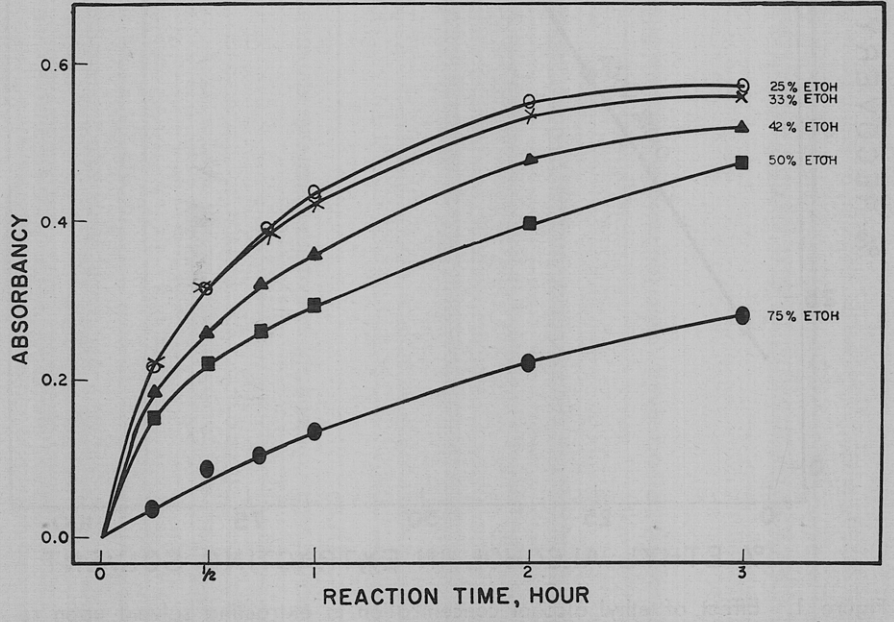

Figure 2. Effect of alcohol concentration in reaction medium upon rate of reaction between butylated hydroxytoluene and ferric chloride–2,2′-bipyridine reagent

Procedure

Analyses of Propyl Gallate and Nordihydroguaiaretic Acid. Weigh 20 grams of the fat into a 250-ml. separatory funnel and dissolve in 40 ml. of carbon tetrachloride. Gentle warming may be necessary to complete the solution. Extract this solution with 70 ml. of 50% ethyl alcohol by repeatedly inverting the funnel for 5 minutes, at the rate of approximately 120 inversions per minute. Run the entire contents of the separatory funnel into a 250-ml. centrifuge bottle and centrifuge for 10 minutes at 1500 r.p.m. Pour the upper alcoholic layer into a beaker. A portion of this 50% alcoholic extract is used for the qualitative analyses and the remainder is then diluted to 25% alcohol for the quantitative analyses.

Qualitative Analyses

Nordihydroguaiaretic Acid. Pipet 10 ml. of the 50% alcoholic extract into a

test tube (15 × 1.5 cm.). Add 1 ml. of barium hydroxide reagent, shake immediately, and look down the length of the tube against a white background. If there is more than 0.001% of nordihydroguaiaretic acid in the fat, a blue color will form and fade rapidly. If there is more than 0.002% of propyl gallate in the fat, a transitory green color forms. If both propyl gallate and nordihydroguaiaretic acid are present in the same sample, a green color forms first and quickly fades; then the blue nordihydroguaiaretic acid color forms and fades. To obtain a positive test in the presence of propyl gallate, at least 0.003% of nordihydroguaiaretic acid must be present in the fat.

Propyl Gallate. Pipet another 10 ml. of the 50% alcohol extract into a test tube and add 1 ml. of concentrated ammonium hydroxide. If 0.0001% of propyl gallate is present in the fat a pink to red color forms. This color is stable for 3 to 5 minutes depending upon the propyl gallate concentration.

Quantitative Analyses

Pipet 25 ml. of the 50% alcoholic extract into a 50-ml. centrifuge tube. Add 25 ml. of water, mix, and centrifuge at 2000 r.p.m. for 15 minutes, or until a clear solution is obtained. Pour off the clear 25% alcoholic solution into a beaker and use this solution for the quantitative analyses.

Nordihydroguaiaretic Acid. Pipet three different aliquots of the diluted alcoholic extract (25% ethyl alcohol) into 18-mm. colorimeter tubes and make up to 12 ml. with 25% ethyl alcohol. Add 1 ml. of ferrous sulfate reagent and 1 ml. of sodium carbonate-bicarbonate buffer. Measure the absorbancy after 10 minutes with an Evelyn photoelectric colorimeter using a 515 mμ filter. All absorbancies should be measured relative to a reagent blank.

Prepare a reference curve over a range of 50 to 500 γ of nordihydroguaiaretic acid by replacing the alcoholic extract in the above procedure with aliquots of a standard nordihydroguaiaretic acid solution in 25% ethyl alcohol. Under these conditions, the observed absorbancy divided by a k value of 0.00141 gave the concentration of nordihydroguaiaretic acid in micrograms per aliquot used.

Propyl Gallate. Pipet three different aliquots of the diluted alcoholic extract (25% ethyl alcohol) into 18-mm. colorimeter tubes and make up to 12 ml. with 25% ethyl alcohol. Add 1 ml. of ferrous sulfate reagent and 1 ml. of ammonium acetate buffer, and mix. Measure the absorbancy after 10 minutes with an Evelyn photoelectric colorimeter using a 515 mμ filter. All absorbancies should be measured relative to a water blank. Prepare a reference curve over the range of 30 to 300 γ of propyl gallate per aliquot. Using this procedure, the observed absorbancy divided by a k value of 0.00205 gave the concentration of propyl gallate in micrograms per aliquot used.

Analyses of Butylated Hydroxyanisole and Butylated Hydroxytoluene

Distillation Apparatus. A photograph of the distillation apparatus is shown in Figure 3. The steam generator consists of a 1000-ml. Erlenmeyer flask containing water and several boiling chips. The rubber stopper in the flask has an outlet tube ending in a 12/5 socket joint. The water is boiled on an electric heater.

The superheater consists of a glass coil (see Figure 4) placed in a 1000-ml. beaker half full of Fisher bath wax (melting point 60° C., smoke point 270° C., flask point 360° C.) and provided with a thermometer.

A detailed diagram of the distilling flask is shown in Figure 4. The flask is made from a 50/50 standard-taper joint, a 12/5 and a 28/15 ball joint. The distilling flask is heated in a 1000-ml. beaker containing approximately 800 ml. of bath wax. During a distillation the inlet and outlet tubes of the superheater and distilling flask are wrapped in glass wool. The condenser is 24 inches long with a 28/15 socket joint. The distillate is filtered and collected in a 250-ml. glass-stoppered graduate.

Distillation of Butylated Hydroxyanisole and Butylated Hydroxytoluene from Fat

Place 16 grams of anhydrous calcium chloride (reagent grade) and 10 ml. of distilled water in the distilling flask; cool to approximately room temperature and weigh in 5 grams of the fat sample. Lightly grease the ground-glass joint and place the top on the distilling flask.

Before starting the distillation, heat the bath for the distilling flask to 160° ± 10° C., and the superheater bath to 200° ± 20° C. and adjust the steam generator to distill approximately 4 ml. of water per minute. Maintain these conditions throughout the entire distillation.

As soon as these conditions are fulfilled, connect the superheater and the condenser to the distilling flask. Start the distillation by connecting the steam generator to the superheater and immediately place the bath around the distilling flask. Collect the distillate in a 250-ml. glass-stoppered graduate, filtering the distillate through a 9-cm. No. 54 Whatman filter paper as it collects. The rate of distillation should be such that 125 ml. of distillate collects in 30 ± 5 minutes.

When 125 ml. of distillate has collected, stop the distillation by disconnecting the distilling flask from the superheater and removing the bath around the distilling flask. When the mouth of the condenser has cooled, disconnect it from the distilling flask and drain the water from the water jacket. Wash the condenser and filter paper thoroughly, using six 10-ml. portions of hot (60° ± 5° C.) 100% ethyl alcohol, allowing the alcoholic washings to filter into the distillate. Continue washing the filter with hot alcohol until the combined volume of distillate and washings is 250 ml. when cooled to room temperature.

Analyses of Distillate

Butylated Hydroxyanisole (2,6-Dichloroquinonechloroimide Method). Pipet three different aliquots of the distillate (50% alcohol) into 18-mm. colorimeter tubes and make up to 12 ml. with 50% ethyl alcohol. Add 2 ml. of the 2,6-dichloroquinonechloroimide reagent and 2 ml. of borax buffer and mix. After 15 minutes, add 5 ml. of n-butyl alcohol to each tube, mix, and measure the absorbancy with an Evelyn photoelectric colorimeter using a 620 mμ filter. Measure all absorbancies relative to a reagent blank.

Prepare a reference curve over a range of 10 to 50 γ of butylated hydroxyanisole. The concentration of butylated hydroxyanisole, in micrograms per aliquot used, is obtained by dividing the observed absorbancy by a k value of 0.0102.

Butylated Hydroxyanisole plus Butylated Hydroxytoluene (Ferric Chloride–2,2′-Bipyridine Method). All solutions must be cooled to room temperature before starting this analysis.

Pipet duplicate aliquots of the alcoholic distillate (50% ethyl alcohol) into 50-ml. glass-stoppered Erlenmeyer flasks rendered impervious to light with black tape, and make up to 8 ml. with 50% ethyl alcohol. Add 2 ml. of ferric chloride reagent and 2 ml. of 2,2′-bipyridine reagent to each flask and mix. Thirty minutes after the addition of the ferric chloride reagent add 5 ml. of n-butyl alcohol and mix. Thirty-five minutes after the addition of the ferric chloride reagent, pour the contents of the flask into an 18-mm. colorimeter tube and, after a further 2 minutes, measure the absorbancy in an Evelyn photoelectric colorimeter using a 515-mμ filter. All measurements are made relative to a reagent blank. The absorbancy is a measure of the sum of butylated hydroxyanisole and butylated hydroxytoluene.

Prepare a reference curve for butylated hydroxytoluene, over a range of 10 to

Table I. Effectiveness of Procedure to Determine Individual Antioxidants in Presence of Others

Antioxidant in Fat Present, %	Found, %	Error in Analysis, %
0.0100 PG with 0.05 tocopherol + 0.02 BHA + 0.02 BHT	PG 0.0101	+0.0001
0.0050 NDGA with 0.05 tocopherol + 0.02 BHA + 0.02 BHT	NDGA 0.0051	+0.0001
0.02 PG + 0.01 NDGA + 0.10 tocopherol	Analyzed as BHA 0.0003	+0.0003
0.02 PG + 0.01 NDGA + 0.10 tocopherol	Analyzed as BHT 0.0002	+0.0002

50 γ with each set of analyses. This is necessary since the k value for butylated hydroxytoluene varies with temperature; k values were found to range from 0.0108 at 22° C. to 0.0142 at 30° C.

Prepare a reference curve for butylated hydroxyanisole over a range of 10 to 50 γ. Under the above conditions a k value of 0.0114 was obtained.

Divide the absorbancy obtained with the 2,6-dichloroquinonechloroimide reagent by the aliquot volume and by the 2,6-dichloroquinonechloroimide k value for butylated hydroxyanisole to obtain the concentration of butylated hydroxyanisole in micrograms per milliliter of distillate. Multiply this value by the ferric chloride–2,2′-bipyridine k value for butylated hydroxyanisole and by the number of milliliters of distillate used in the ferric chloride–2,2′-bipyridine reaction. This figure represents the absorbancy due to butylated hydroxyanisole in the ferric chloride–2,2′-bipyridine reaction. Subtract this latter figure from the measured absorbancy in the ferric chloride–2,2′-bipyridine reaction to find the absorbancy due to butylated hydroxytoluene. Calculate the amount of butylated hydroxytoluene in the distillate.

Example.

Weight of fat sample = 5.0 grams

Total volume, distillate + washings = 250 ml.

2,6-Dichloroquinonechloroimide Reaction

K_{BHA} = 0.0102

Aliquot volume = 12 ml.

Absorbancy = 0.260

Ferric Chloride–2,2′-Bipyridine Reaction

K_{BHA} = 0.0114 Aliquot volume = 8 ml.

K_{BHT} = 0.0122 Absorbancy = 0.398

Calculation. Concentration of BHA per ml. of distillate = $\frac{0.260}{0.0102 \times 12} = 2.27\gamma$

Absorbancy per aliquot due to BHA in $FeCl_3$–2,2′-bipyridine reaction = $2.27 \times 0.0114 \times 8 = 0.207$

Absorbancy per aliquot due to BHT in $FeCl_3$–2,2′-bipyridine reaction = $0.398 - 0.207 = 0.191$

Micrograms of BHT in distillate = $\frac{0.191}{0.0122} \times \frac{250}{8} = 489$

Concentration of BHT in fat sample = $\frac{489}{5 \times 10^6} \times 100 = 0.0098\%$

Concentration of BHA in fat sample = $\frac{2.27 \times 250}{5 \times 10^6} \times 100 = 0.0113\%$

Table II. Reproducibility of Analytical Results

Antioxidant Added to Fat, %	No. of Analyses	Antioxidant Found, %
BHA 0.0050	2	0.0049–0.0051
0.0100	3	0.0095–0.0098
0.0200	2	0.0197–0.0206
BHT 0.0050	2	0.0050–0.0050
0.0100	3	0.0098–0.0101
0.0200	2	0.0198–0.0199
PG 0.0050	2	0.0050–0.0050
0.0100	4	0.0098–0.0102
0.0200	2	0.0203–0.0203
NDGA 0.0025	2	0.0023–0.0028
0.0050	3	0.0049–0.0051
0.0100	2	0.0098–0.0100
Mixtures BHA + BHT		
1. 0.0100 BHA + 0.0200 BHT	2	0.0103–0.0106 0.0209–0.0212
2. 0.0100 BHA + 0.0100 BHT	3	0.0103–0.0104 0.0100–0.0107
3. 0.0200 BHA + 0.0100 BHT	2	0.0204–0.0206 0.0093–0.0101

Results and Discussion

The results in Table I show that the presence of butylated hydroxyanisole, butylated hydroxytoluene, and tocopherol in fat causes no error in the analyses for propyl gallate and nordihydroguaiaretic acid.

Fat containing propyl gallate, nordihydroguaiaretic acid, and tocopherol was steam distilled and the distillate analyzed for butylated hydroxyanisole and butylated hydroxytoluene. Data in Table I indicate that propyl gallate, nordihydroguaiaretic acid, and tocopherol cause no interference in the analyses for butylated hydroxyanisole and butylated hydroxytoluene.

Repeated determinations were carried out on lard samples containing the antioxidants, propyl gallate, nordihydroguaiaretic acid, butylated hydroxyanisole, and butylated hydroxytoluene, individually, at various levels to determine the precision of the procedure. Analyses were also conducted on lard samples containing mixtures of butylated hydroxyanisole and butylated hydroxytoluene. The results shown in Table II indicate close agreement between the amount of antioxidant added and the amount recovered. For practical purposes results should be reported to three places of decimals.

This method for analyzing the antioxidants added to fats is considered superior to the method previously published by Mahon and Chapman (*5*). The new procedure overcomes many of the difficulties in the previous procedure and, in addition, permits the quantitative analyses of antioxidant mixtures containing butylated hydroxytoluene.

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