♣Spectrophotometric Determination of BHA in Edible Fats and Oils

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ABSTRACT

A method is described for the analysis of 2- and 3-tert-butyl-4-hydroxyanisole (BHA) in edible fats and oils. The method is based on measurement of a specific color developed from the reaction of BHA with NN-dimethyl-p-phenylenediamine in the presence of a mild oxidizing agent in alkaline solution. The purple-violet color developed is extracted in carbon tetrachloride, and the absorbance is measured at 550 nm. The presence of other antioxidants also was examined, and the method was applied to various foodstuffs (e.g. olive oil, lard and butter). It gave satisfactory results. The detection limit was 0.4 ppm of BHA.

INTRODUCTION

The widespread use of antioxidants in foodstuffs resulted in the development of numerous techniques for their qualitative and quantitative determination. The most widely used analytical procedures involve spectrophotometric methods. Usually, a food extract, steam distillate or fat solution is treated with a specific reagent to produce a color with the antioxidant. The principal spectrophotometric methods used for the determination of BHA (mixture 2- and 3-t-butyl-4-hydroxyanisoles) are the following:

- The 2,6-dichloroquinone-4-chloroimide (Gibb's reagent) method. According to this method the steam distillate (1,2) is treated with Gibb's reagent (3) to produce a blue color which is measured at 620 nm. This reaction is specific for BHA in the presence of BHT (2,6-di-t-butyl-hydroxytoluene).
- The sulfanilic method (4). This method uses the color produced by the reaction of BHA with diazotized sulfanilic acid in basic solutions. BHT reacts with the reagent but at such a slow rate that little interference is encountered if the measurements are made within 5-10 min.

Propylgallate (PG) must be removed prior to the analysis, because it inhibits the color development.

The present work is concerned with the development of a quantitative spectrophotometric method for the determination of BHA. This is based on the fact that some p-phenylenediamines react with phenols in the presence of an oxidizing agent to produce color (5,6).

EXPERIMENTAL PROCEDURE

Reagents

All reagents used were of Analytical Reagent grade.

BHA, BHT (Sigma) standard solution 100 ppm in 1,4-dioxan; PG (BDH) standard solution 100 ppm in 1,4-dioxan, and OG (octylgallate), DG (dodecylgallate) (Fluka) standard solutions 100 ppm in 1,4-dioxan.

Sodium hydrogen carbonate solution 5% w/v in distilled water. NN-dimethyl-p-phenylenediamine oxalate. 0.17 g of this reagent was dissolved in 100 ml H_2O under a nitrogen atmosphere. The solution was prepared daily.

Potassium ferricyanide, 8% w/v in distilled water; carbon tetrachloride; tocopherol acetate, and α-tocopherol.

Apparatus

A Unicam SP1800 spectrophotometer with 1 cm cuvettes was used.

An apparatus similar to that described by Takahashi (7) was used for the steam distillation of BHA and BHT. A 250 ml round bottomed flask containing 2% magnesium oxide suspension in water was used for the removal of phenolic and acidic compounds from the sample.

Procedure

A known quantity of fat or oil (10 g) fortified with BHA was placed in the distillation flask together with 10 g of anhydrous calcium chloride and 40-50 ml of distilled water. About 200 ml of distillate were collected in about 30-35 min. Quantities of distillate ranging from 1 to 10 ml were pipetted into a 25 ml volumetric flask, followed by 2 ml of sodium hydrogen carbonate, 2 ml of N,N dimethyl-p-phenylenediamine solution and 2 ml of potassium ferricyanide, 5% w/v. After mixing the solution was made up to the mark with water and allowed to stand for 15 min. The deep blue-brown colored solution was extracted twice with 20 ml portions of carbon tetrachloride. The combined carbon tetrachloride extracts were dried with anhydrous sodium sulphate and filtered through a glass fiber filter paper. The filter paper was washed with a small volume of the solvent washings being added to the filtrate. The latter was made up to 50 ml with carbon tetrachloride. The absorbance of the solution was measured at a wavelength of 550 nm against a blank solution which contained all the reagents except the antioxidant and was extracted with carbon tetrachloride. Correction should be made for the dye absorbed by the filter paper. This is achieved by the use of a standard solution of the antioxidant. The correction, however, is very small.

RESULTS AND DISCUSSION

The derivative formed by reaction of BHA with N,N dimethyl-p-phenylenediamine has an absorption maximum at 550 nm. The other antioxidants, e.g. BHT, PG, OG and DG, gave brownish colors not extracted in the carbon tetrachloride layer, while the color of the product of the reaction with BHA was purple-violet. Accordingly, it was obvious that, of the antioxidants, only BHA gave a colored compound extractable with carbon tetrachloride.

The validity of the Beer-Lambert law was examined (Fig. 1) for quantities ranging from 5 to 100 μg. As can be seen from Figure 1, the Beer-Lambert law can be applied to quantities of BHA varying between 10 and 100 μg. For quantities of BHA less than 10 μg, the results deviate from the law.

Interference by Other Antioxidants

The presence of other antioxidants also was investigated, and the results are shown in Table I. It is obvious that the other antioxidants do not affect the determination of BHA even when they are present in a 10-fold excess. Natural antioxidants such as tocopherols did not affect the determination either.

Application of the Method

The isolation of antioxidants from high fat foods in various organic solvents has many disadvantages, owing to the fact that the extract contains substances which interfere in the

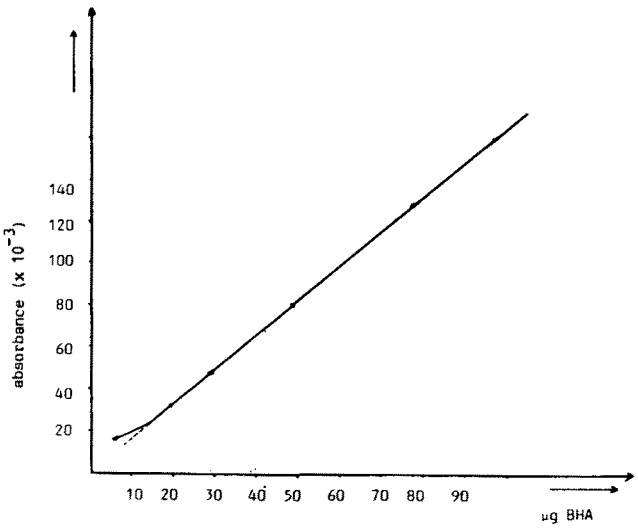

FIG. 1. Relationship between absorbance and quantity of BHA.

TABLE I

Effect of Antioxidants on BHA Determination

Antioxidant	µg added	ppm BHA calculated* with other antioxidant	ppm BHA calculated* without other antioxidant
PG	1000	95.7	97
OG	1010	97	97
DG	1008	94.7	97
BHT	1000	93	97
Tocopherol acetate	100	97	97
α-Tocopherol	100	97	97

*Each determination was carried out in duplicate.

TABLE II

Recoveries of BHA from Various Samples

Sample	ppm BHA Added	ppm BHA Found[a]	ppm BHT present	Recovery %	Standard deviation[b]
Olive oil	97	93	100	95.9	0.37
Olive oil	25	24.2	100	96.8	0.34
Corn oil	25	24	25	96	0.35
Corn oil	97	94	100	96.9	0.33
Lard	20	19.2	25	96	0.31
Lard	97	94.2	100	97.1	0.32
Butter	97	92	100	94.85	0.40
Butter	25	23.8	25	95.2	0.38

[a]Mean of 6 determinations.

[b]The standard deviation is based on n-1 degrees of freedom.

spectrophotometric determination. However, the distillation apparatus proposed by Sloman et al. (8) and modified by Takahashi (7) offers a solution to the problem. BHA and BHT can be steam distilled at 160 C, but gallates are destroyed by high temperatures. It also has been shown by Filipic and Ogg (2) that a 2% aqueous magnesium oxide suspension removes phenolic and acidic compounds which interfere with color reactions.

Thus, a 10 g sample fortified with antioxidants was stream distilled according to the procedure described previously. Superheated steam was used because BHA and BHT distill from the fat very slowly. To prevent the absorption of very small quantities of antioxidants, especially BHT, the magnesium oxide suspension also was heated. Table II shows the results of determination of BHA in various edible fats and oils.

Table II indicates that the method for determination of BHA in fats and oils is accurate and can be applied to food with a high fat content. The method does not give accurate results when the quantity of BHA present in the analyzed sample is less than 10 µg.

The procedure described above could be used for a rapid assessment of BHA in edible oils in the presence of other antioxidants. It possesses the disadvantage that it gives a color with some phenols such as o- and m-cresols; by using the special distillation apparatus referred to above, though, these compounds can be removed. This method offers a new spectrophotometric technique which can be used for BHA determination because of its specificity. The method gave reproducible results and can be carried out with facilities existing in most analytical laboratories.

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