# Analysis of Antioxidants in Oil

**D.B. MIN, D. TICKNOR** and **D. SCHWEIZER,** Department of Food Science and Nutrition, Ohio Agricultural Research and Development Center, 2121 Fyffe Road, Columbus, OH 43210

## ABSTRACT

Oils containing from 0 to 200 ppm of the most common phenolic antioxidants, BHA, BHT and TBHQ, were analyzed by a new gas chromatographic method. This method requires neither extraction of antioxidants from oil nor derivatization of antioxidants. The correlation coefficients (r) between gas chromatographic peak heights or peak area and their concentrations in oils were 0.99 for BHA, BHT and TBHQ. This simple gas chromatographic method can accurately determine as little as 10 ppm of BHA, BHT and TBHQ in oils in 1 hr.

## INTRODUCTION

Antioxidants are frequently added to foods to minimize the oxidation of fats and oils. Angeline and Leanardos (1) reported that more than 15 million lb of antioxidants was used by U.S. food manufacturers in 1967. The safety of BHA and BHT has been studied by numerous scientists. Brannen (2) thoroughly reviewed the toxicology and biochemistry of BHA and BHT. Brannen's review (2) clearly showed concerns over the observed pathological effects, carcinogenic potential, interactions of BHA and BHT with enzymes and lipids, effects of BHA and BHT on reproduction, and metabolism of BHA and BHT. Long-term safety effects of these antioxidants are subject to controversy among scientists. The maximal legal antioxidant level allowed in most food products is 200 ppm of the oil content in foods (3). Dugan (4) reported that this level was determined from clinical tests of both acute and chronic toxicity in a variety of animals.

Quantitative and qualitative determinations of antioxidants are very important for regulation, quality control and research in food products. Therefore, numerous analytical methods for determination of antioxidants in oils and food products have been developed.

Anglin et al. (5) described an analytical method which isolated BHA and BHT from oil by steam distillation and then analyzed these antioxidants quantitatively by a colorimetric method. Sahasrabudhe (6) reported a method for the analyses of BHA and BHT in oil by extracting them from oil with a solvent of 20% acetonitrile and 80% ethanol and then separating them by two-dimensional thin layer chromatography (TLC) and measuring the concentration by a colorimetric method after extracting the spots on the thin layer chromatogram.

Stoddard (7) reported a method which extracted antioxidants from oil with a solvent of 20% acetonitrile and 80% ethanol, then derivatized extracted antioxidants with dimethylsilane, and finally separated the derivatives by gas chromatography (GC). Jedrych and Karlowski (8) isolated BHA and BHT from oil by distillation, extracted them with N-butylalcohol, then analyzed them by GC. Austin and Wyatt (9) reported a gas chromatographic antioxidant analysis in which they extracted TBHQ from oil with acetonitrile and derivatized the isolated TBHQ using N,0-bis-trimethylsilyl-trifluoroacetamide, then analyzed the derivative by GC.

Pokorny et al. (10) reported a gel permeation liquid chromatographic method for antioxidant analysis in oil, which included extraction of antioxidants, removal of fatty acids and solvent evaporation.

Hammond (11) extracted antioxidants with methanol and then analyzed them by high pressure liquid column chromatography. Doeden et al. (12) reported a gel permeation chromatographic method where oil was weighed and diluted to the desired volume with chloroform, and an aliquot of diluted sample was filtered through a Swinney-type filter containing a 0.5-$\mu$ millipore filter element. A 0.25-mL aliquot of the filtered sample solution was then injected into the column.

Isolation of antioxidants from products by solvent extraction or steam distillation and then derivatization before they are analyzed by GC are commonly involved in the quantitative and qualitative analyses of antioxidants in foods.

Most of the reported procedures are time-consuming, tedious, complex, and generally give poor reproducibility due to the multisteps required for sample preparation. This paper reports a simple GC method which requires neither time-consuming extraction procedures nor derivatization of antioxidants for the determination of the most common phenolic antioxidants in oil.

## EXPERIMENTAL

### Preparation of Antioxidant Isolation Apparatus

The isolation apparatus used is essentially the same as the apparatus used to isolate flavor compounds in oil reported by Min (12). Two-and-one-half ft of GC quality aluminum tubing, 0.25 in. outside diameter, was bent into a U-tube as shown in Figure 1. The isolation U-tube was packed

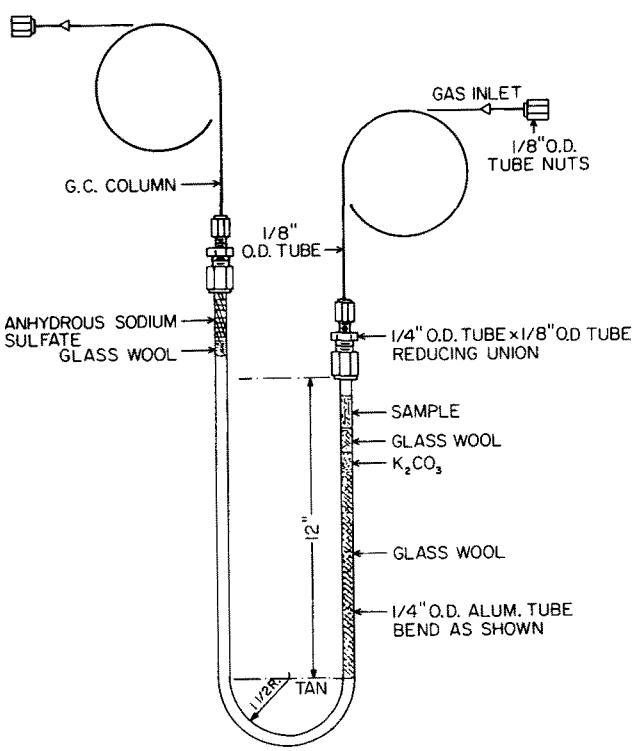


**FIG. 1. Apparatus for the isolation of BHA, BHT and TBHQ in oil. (Reprinted from *JAOCS* 59:278 [1982]).**

with 0.2 g anhydrous sodium sulfate, 0.2 g silanized glass wool, 0.6 g silanized glass wool, 0.2 g potassium carbonate, and 0.15 g silanized glass wool in sequence as shown in Figure 1. These different amounts of silanized glass wool were packed loosely and evenly into some portions of the U-tube apparatus as shown in Figure 1. After the U-tube apparatus had been packed, one side of the apparatus was connected to a nitrogen gas line (Fig. 1) and then lowered into a forced-air laboratory heating oven at 200 C. The heating oven was modified to accomodate the U-tube apparatus by cutting a 4 in. x 1 in. rectangular space on the oven top. The U-tube apparatus was purged with nitrogen at 30 mL/min for 10 min at 200 C in the oven to remove any contaminants in the apparatus. After the U-tube apparatus was purged, the apparatus was then removed from the oven and cooled to room temperature. The oven temperature was lowered to 160 C for the antioxidant isolation from oil as described later.

### Antioxidant Isolation

The stainless steel GC column packed with Tenax-GC coated with 10% polymetaphenoxylene was connected to the antioxidant isolation U-tube apparatus as seen in Figure 1. One mL of oil sample was introduced by a 1-mL syringe on the top of the glass wool in the U-tube apparatus and then connected to a 1/8 in. nitrogen gas inlet tube. The apparatus containing oil was lowered into the 160 C forced-air oven. Only the aluminum tube of the apparatus was in the oven, and the reducing unions were outside the oven. The sample in the antioxidant isolation apparatus was purged for 15 min by nitrogen gas at 30 mL/min. The compounds thus isolated from oil by nitrogen gas were collected on a Tenax coated with 10% polymetaphenoxylene. After antioxidant isolation, the GC column was disconnected from the U-tube apparatus and then connected to the gas chromatograph to separate the isolated compounds in the GC column. The aluminum U-tube apparatus was discarded after the antioxidant isolation; however, the reducing unions on the U-tube were saved.

### Gas Chromatography

A Hewlett-Packard model 5880A chromatograph with an electronic integrator for GC peak area calculation and a flame ionization detector was used.

A 10 ft x 1/8 in. stainless steel column packed with 80/100 mesh Tenax-GC that had been coated with 10% polymetaphenoxylene (Applied Science Laboratories, State College, PA) was used.

The initial temperature of 140 C was held for 2 min and then the temperature was programmed at 6 C/min to 250 C and held for 15 min. The nitrogen flow rate was 40 mL/min.

### Materials and Sample Preparation

Fresh, deodorized soybean oil was obtained from Capital City Products, Columbus, OH. BHA and TBHQ were obtained from Eastman Chemical Products, Inc., Kingsport, TN, and BHT was obtained from UOP, Inc., Des Plaines, IL. Soybean oils containing 0, 50, 100, 150 and 200 ppm (w/v) of BHA, BHT and TBHQ were prepared to obtain a wide range of antioxidant concentrations.

## RESULTS AND DISCUSSION

The preliminary study showed that the coefficient of variation of reproducibility of the isolation and separation of 100 ppm BHA in oil by the apparatus shown in Figure 1 was less than 2.5% when six replicates were analyzed. This indicated that the reproducibility of analytical method was good. Since no extraction and/or derivatization steps were required in the analysis of antioxidants in oil and the preliminary study showed a good reproducibility of isolation, separation and quantitation of antioxidants from oil, an internal standard was not used in this study. One percent dodecane in hexane (v/v) was used as an external standard to monitor the variation of GC responses during the analysis of samples.

The gas chromatogram of oil containing 100 ppm each of BHT, BHA and TBHQ is shown in Figure 2. These three most common phenolic antioxidants were baseline separated. BHT and BHA gave symmetric GC peaks, but the GC peak of TBHQ was a little skewed and had a wide baseline.

Figures 3 and 4 show that fresh soybean oils containing no added antioxidants gave an almost flat baseline under the GC conditions used. Figures 3 and 4 also show that no chemical compounds which could have the same retention time as any of the antioxidants used were present in the fresh oil. Preliminary experiments also showed that no chemical compounds which could have the same retention time as any of the antioxidants used were present in oxidized corn oil, soybean oil or hydrogenated soybean oil. The amounts of antioxidants isolated from hydrogenated soybean oil and nonhydrogenated soybean oil were the same when these oils had the same amounts of antioxidants. Figures 3 and 4 show that, as the concentrations of BHA and TBHQ increased from 0 to 200 ppm, the GC peak height for GC peak area increased. It also was observed that the GC peak height of BHT increased proportionately as the BHT concentration in oil increased from 0 to 200 ppm as was observed in Figures 3 and 4 for BHA and TBHQ.

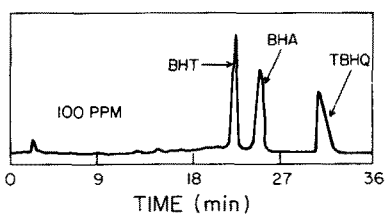


**FIG. 2. Gas chromatogram of oil containing BHT, BHA and TBHQ.**

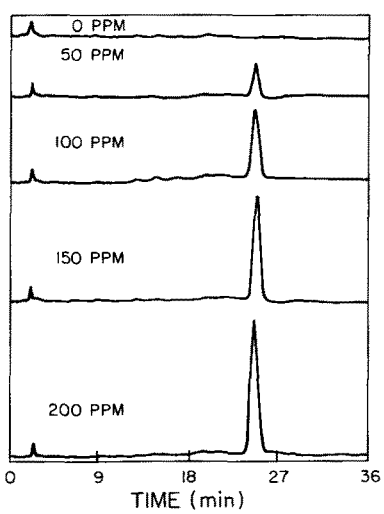


**FIG. 3. Gas chromatograms of different levels of BHA in oil.**

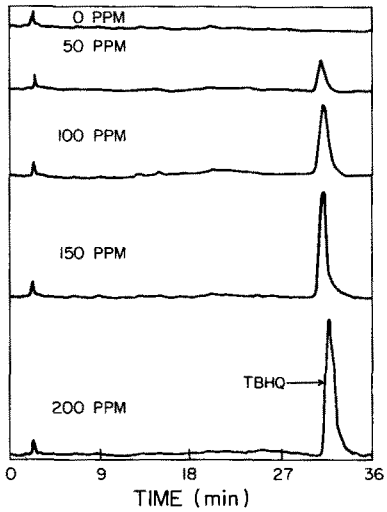


**FIG. 4. Gas chromatograms of different levels of TBHQ in oil.**

The standard calibration lines of oils containing 0, 50, 100, 150 and 200 ppm concentrations of BHA and TBHQ are shown in Figures 5 and 6, respectively. The correlation coefficient (r) between BHA concentrations and GC peak height of the standard calibration line in Figure 5 is 0.99. For the TBHQ standard calibration line, GC peak area of TBHQ was used instead of peak height because peak area gave a better r than peak height. This may be due to the skewed GC peak shape of TBHQ as shown in Figure 4. The r between TBHQ concentration and GC peak area is 0.99. Even though the figure for calibrated standard line between BHT content in oil is not presented here, the r between BHT concentration and GC peak height is 0.99.

To determine the reproducibility of isolation of antioxidants in oil, five replicate samples of soybean oil containing 200 ppm BHA and 200 ppm BHT were analyzed and the concentrations of the antioxidants in oil were determined

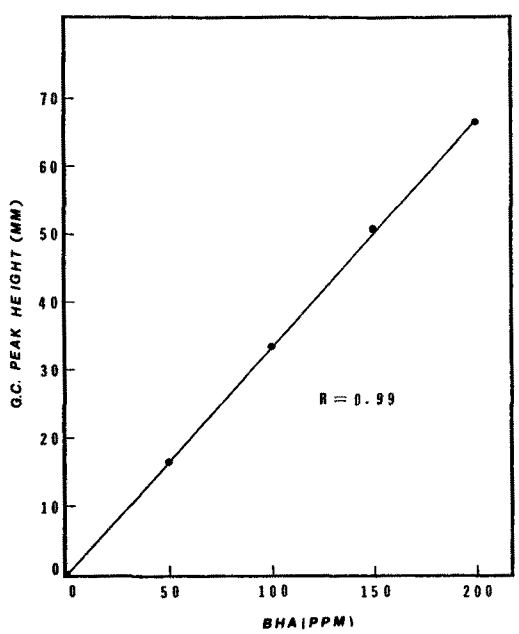


**FIG. 5. Correlation between BHA gas chromatographic peak height and BHA content in oil.**

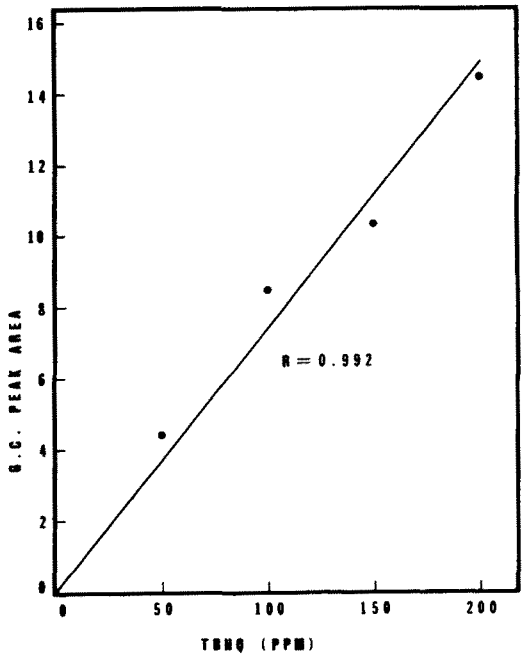


**FIG. 6. Correlation between TBHQ gas chromatographic peak area and TBHQ content in oil.**

using standard calibration lines. BHA content ranged from 193 to 203 ppm; BHT ranged from 195 to 207 ppm. The coefficient of variations for 200 ppm BHA and 200 ppm BHT were 2.2 and 2.9%, respectively.

To determine the lowest limit of detection of BHA, BHT and TBHQ in oil, studies were conducted with oils containing 0, 5, 10, 20, 30 and 50 ppm BHA, BHT and TBHQ. The results show that the method used can detect 10 ppm of these antioxidants in oil.

The relatively low coefficient of variation for the reproducibility of determining antioxidant content indicates that these three most common antioxidants in soybean oil can be determined by the GC analysis method described without requiring derivatization and extraction of antioxidants from oil.

ACKNOWLEDGMENTS

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