Food Chemistry **25** (1987) 159–164

Technical Note

Spectrophotometric Determination of Some Antioxidants in Oils and Fats

ABSTRACT

New spectrophotometric methods for the determination of antioxidants (tertiary butyl hydroquinone, butylated hydroxy anisole and gallic acid) have been developed using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and ceric ammonium sulphate. MBTH is oxidized and its resonance products are coupled with antioxidants to give coloured species which obey Beer's Law. The method is sensitive, reproducible and accurate and applicable to the assay of antioxidants in oils and fats.

INTRODUCTION

Antioxidants such as tertiary butyl hydroquinone (TBHQ), butylated hydroxy anisole (BHA), propyl gallate (PG) and gallic acid (GA) are permitted to be added to edible oils and fats to prevent oxidative rancidity in a concentration not exceeding 0·02%, either individually or in combination, under the provisions of the Prevention of Food Adulteration Act, India 1954 and Rules 1955 thereof as amended up to 1985.

Several spectrophotometric methods (visible region) have been reported for the determination of antioxidants such as propyl gallate (Vos *et al.*, 1957; Cassidy & Fischer, 1960; Pozo & Salazar, 1962; Schwien & Conroy, 1965; Sastry *et al.*, 1982), gallic acid (Mahan & Chapman, 1951; Anglin *et al.*, 1956; Conroy, 1959; Heidrich & Conroy, 1962; Bhatia *et al.*, 1971) and BHA (Austin, 1954; Kahan, 1954; Sloman *et al.*, 1962). We recently reported the determination of antioxidants with potassium permanganate and metol (Viplava Prasad *et al.*, 1985). We have now developed a simple, rapid,

Food Chemistry 0308-8146/87/$03·50

sensitive and accurate method of spectrophotometric determination of TBHQ, BHA and GA using MBTH and ceric ammonium sulphate.

MATERIALS AND METHODS

Spectral and absorbance measurements were made with a Systronics model 105 (MK 1) spectrophotometer.

All the solutions were prepared in double distilled water using GR or CP grade chemicals. MBTH (0·2%, Fluka), ceric ammonium sulphate (1% in 4% sulphuric acid, BDH, AnalaR), TBHQ (0·5 mg/ml Eastman Kodak, USA) and GA (0·5 mg/ml, BDH, AnalaR) were prepared in distilled water. BHA (0·5 mg/ml BDH, AnalaR) was dissolved initially in the minimum volume of alcohol and made up to 100 ml with double distilled water.

Preparation of standard curve

To a series of 10-ml volumetric flasks, different volumes of antioxidant, optimum volumes of MBTH and ceric ammonium sulphate (Table 1) solutions were added and diluted to 7 ml. The flasks were kept in a boiling water bath for optimum times (heating not necessary in the case of gallic acid), cooled and diluted to 10 ml with methanol (distilled water in the case of gallic acid). The absorbances were measured at appropriate wavelengths against a reagent blank after attaining colour stability (Table 1). A calibration curve was prepared relating concentration of antioxidant with absorbance.

TABLE 1
Experimental Conditions for the Determination of Antioxidants with MBTH and Ce (IV)

Anti-oxidant	*Optimum volume of Ce (IV) (1%) required (ml)*	*Optimum volume of MBTH (0·2%) required (ml)*	*Optimum temperature*	*Optimum time for maximum colour development (min)*	*Stability (min) after cooling*	λ_{max} *(nm)*
TBHQ	1·0[a] (0·5–1·5)	2·0[a] (1·5–3·0)	95–100°C	10	60	500
BHA	0·5[a] (0·3–0·7)	3·0[a] (2·5–4·0)	95–100°C	15	90	480
GA	0·5[a] (0·3–0·7)	2·0[a] (1·5–3·0)	28–33°C	1	60	440

[a] Used in the experiment.

Method for the determination of antioxidants in oils and fats

Ten grams of oil or fat were dissolved in 50 ml of carbon tetrachloride and extracted with four 20-ml portions of 50% aqueous alcohol. The combined alcoholic extracts were evaporated to 5 ml and then diluted with water to 100 ml in a standard flask and filtered through dry paper after neutralizing with 1 g of calcium carbonate. The filtrate was used for TBHQ, BHA and gallic acid determinations and their contents were computed from the standard curves.

RESULTS AND DISCUSSION

The optimum conditions for each method were established after a thorough systematic study of parameters such as concentration of MBTH and of Ce (IV), period of heating for maximum colour development and order of addition of reagents. The absorption maximum and the experimental conditions are given in Table 1. The optical characteristics such as molar absorptivity and Sandell's sensitivity for each antioxidant are given in Table 2. The slopes, intercepts and correlation coefficient obtained by linear least squares treatment of the results for the systems involving antioxidant with the reagent are also presented in Table 2. The reproducibility of the method was found by measuring the absorbances of six replicate samples containing a known amount of antioxidant (TBHQ, BHA or GA) and the results obtained are represented in Table 2.

To check the suitability of the method for the determination of antioxidants in oils and fats, recovery experiments were carried out by adding known quantities of antioxidants to the pre-analyzed ground nut,

TABLE 2
Optical Characteristics, Precision and Accuracy in the Determination of Antioxidants

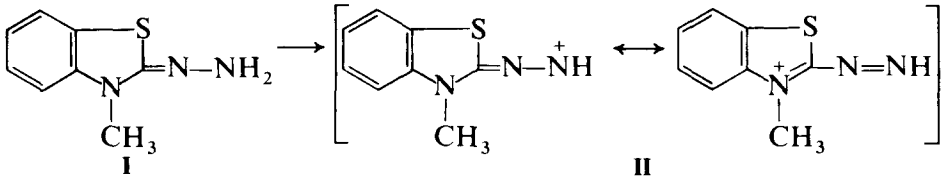
Parameter	*TBHQ*	*BHA*	*GA*
Beer's law limits (μg/ml)	1·0–8·0	0·5–9·0	1·0–15·0
Molar absorptivity (litre/mole/cm)	$1{\cdot}11 \times 10^4$	$1{\cdot}8 \times 10^4$	$1{\cdot}02 \times 10^4$
Regression equation[a]	0·001 7	−0·001 7	0·008 2
	+ 0·048 9C	+ 0·100 1C	+ 0·059 5C
Correlation coefficient	0·999 7	0·999 9	0·999 8
Optimum photometric range (μg/ml)	2·5–8·0	2·0–8·9	3·0–14·0
Sandell's sensitivity (μg/cm²/0·001 absorbance unit)	0·02	0·01	0·02
Per cent RSD	1·89	1·51	2·14
Per cent range of error at 95% level	1·97	1·59	2·24

[a] Found in this work; it must be determined independently by users of the method.

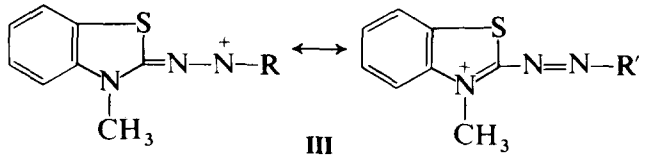
sunflower and coconut oils and analyzing by the proposed and reported methods (Viplava Prasad *et al.*, 1985). A very good recovery ranging from 96·9% to 98·7% was obtained and the results are comparable with the reported method (Table 3).

A tenfold excess of the substances, namely, BHT, citric acid, tartaric acid and methyl silicone, present did not interfere in the determination of TBHQ.

TBHQ, BHA and GA contain one or more phenolic hydroxyl groups and so react with MBTH in the presence of Ce (IV) to form coloured oxidative coupling products similar to that of MBTH and phenols (Gasparic *et al.*, 1977). Under the reaction conditions MBTH (**I**) loses two electrons and one proton on oxidation, forming the electrophilic intermediate (**II**) which has been postulated to be the active coupling species. The intermediate (**II**) undergoes electrophilic substitution with GA, BHA or TBHQ (*p*-, or *o*- if *p* is blocked) to form the coloured species (**III**). The nature of the coloured species formed may be represented as in the structure below.



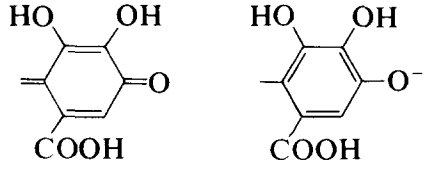
I **II**



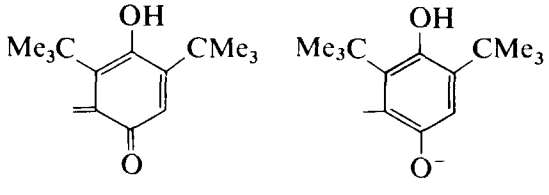
III

R **R′**

For GA



TBHQ



BHA

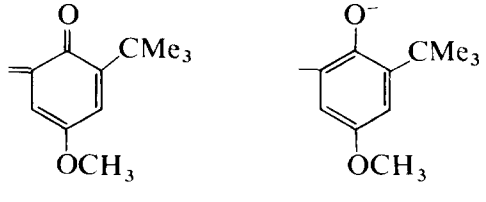


TABLE 3
Recovery of Antioxidants Added to Edible Oils

Name of the oil	*Antioxidant added (mg)*	*Recovery (%)*	
		Proposed method	*Reported method*
Groundnut oil	GA, 10	98·1	97·1
Groundnut oil	BHA, 10	97·4	97·2
Groundnut oil	TBHQ, 10	98·5	—
Sunflower oil	BHA, 10	96·9	95·8
Coconut oil	TBHQ, 10	98·7	—

So far, no spectrophotometric method has been reported for the determination of TBHQ. The proposed methods for BHA and GA are more sensitive than the methods reported earlier. The proposed methods are simple, accurate, sensitive and useful in the routine analysis of antioxidants in oils and fats.

ACKNOWLEDGEMENTS

The authors thank the authorities of Andhra University for providing the research facilities and are grateful to the CSIR, New Delhi, for the award of a Junior Research Fellowship (to T. E. Divakar).

REFERENCES

Anglin, C., Mahan, J. H. & Chapman, R. A. (1956). Determination of antioxidants in edible fats. *J. Agr. Food Chem.*, **4**, 1018.

Austin, J. J. (1954). Analysis of BHA in paper and paper board. *J. Am. Oil Chem. Soc.*, **31**, 424.

Bhatia, I. S., Madan, T. R. & Nagpal, M. L. (1971). Studies on the estimation of gallic acid and glucose from their mixtures. *Sci. Cult.*, **37**, 490.

Cassidy, W. & Fischer, A. J. (1960). Determination of gallates in edible fats. *Analyst*, **85**, 295.

Conroy, H. W. (1959). Report on antioxidants in oils, fats and waxes. *J. Assoc. Off. Agric. Chem.*, **42**, 358.

Gasparic, J., Svobodova, D. & Pespisileva, M. (1977). Investigation of the colour reaction of phenols with the MBTH reagent. *Mikrochimica Acta (Wein)*, **1**, 241.

Heidrich, P. L. & Conroy, H. W. (1962). Antioxidants in oils, fats and waxes. *J. Assoc. Off. Agric. Chem.*, **45**, 244.

Kahan, S. (1954). Determination of nordihydroguaiaretic acid and butylated hydroxyanisole. *J. Assoc. Off. Agric. Chem.*, **37**, 828.

Mahan, J. H. & Chapman, R. A. (1951). Estimation of antioxidants in lard and shortenings. *Anal. Chem.*, **23**, 1116.

Pozo, A. del & Salazar, R. (1962). Ferrous salts in the determination of alkyl gallates. *Pharm. Acta Helv.*, **37**, 460.

Sastry, C. S. P., Ekambareswara Rao, K. & Prasad, U. V. (1982). Spectrophotometric determination of phenols using metol and sodium periodate. *Talanta*, **29**, 917.

Schwien, W. G. & Conroy, H. W. (1965). Qualitative analysis of propyl gallate, nordihydroguaiaretic acid, butylated hydroxy anisole and butylated hydroxy toluene in fats and oils. *J. Assoc. Off. Agric. Chem.*, **48**, 489.

Sloman, K. G., Remagnoli, R. J. & Cavagnol, J. C. (1962). Trace analysis of BHA & BHT in food products. *J. Assoc. Off. Agric. Chem.*, **45**, 76.

Viplava Prasad, U., Ekambareswara Rao, K. & Sastry, C. S. P. (1985). Spectrophotometric determination of some antioxidants with potassium permanganate and metol. *Food Chemistry*, **17**, 209.

Vos, H. J., Wessels, H. & Six, C. W. T. (1957). Quantitative determination of the antioxidants propyl, octyl and dodecyl gallate in oils and fats. *Analyst*, **82**, 362.

U. Viplava Prasad, T. E. Divakar, K. Hariprasad & C. S. P. Sastry
Food and Drug Laboratories, School of Chemistry,
Andhra University, Waltair 530 003, India

(Received 26 September 1986; revised version received 31 October 1986; accepted 25 November 1986)