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Spectrophotometric Determination of Some Antioxidants with Potassium Permanganate and Metol

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ABSTRACT

New spectrophotometric methods for the assay of some antioxidants have been developed using potassium permanganate and metol (p-N-methyl amino phenol). Metol is oxidised at pH 3·0 with potassium permanganate and coupled with antioxidants to give λ_{max} at 560 nm for propyl gallate and gallic acid and 510 nm for butylated hydroxy anisole. The method is simple, sensitive, reproducible and accurate within ± 1% and applicable to the assay of antioxidants (gallic acid, propyl gallate and butylated hydroxy anisole) in oils and fats.

INTRODUCTION

Antioxidants (propyl gallate, gallic acid and butylated hydroxy anisole) are permitted to be added to edible oils and fats in a concentration of 0·025%, either individually or in combination, as stated in the Prevention of Food Adulteration Act, India (1954, Rules 1955, as amended up to 1984). Several spectrophotometric methods 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 (Mohan & 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

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reported the determination of antioxidants and phenols with sodium metaperiodate and amino phenols (Sastry *et al.*, 1982). We have now, for the first time, developed a simple, rapid, sensitive and accurate method of spectrophotometric determination of some antioxidants (PG, GA and BHA) at the microgram level with potasssium permanganate in the presence of aminophenol. This method is more sensitive than that described by Sastry *et al.* (1982), as well as being useful in purity assays and the estimation of these antioxidants in oils and fats.

MATERIALS AND METHOD

Spectral absorbance measurements were made with a Systronics model 105 (MKI) spectrophotometer. A Systronics pH meter was used for all pH measurements.

All the solutions were prepared in double distilled water using GR or CP grade chemicals. Metol (2% BDH, AnalaR), potassium permanganate (0·01M), PG (1 mg/ml BDH, AnalaR), and GA (1 mg/ml BDH, AnalaR) were prepared in distilled water. BHA (1 mg/ml BDH, AnalaR) was dissolved initially in the minimum volume of alcohol and made up to the mark with double distilled water.

Potassium hydrogen phthalate and hydrochloric acid buffer solution (pH 3·0) were prepared as described by Lurie (1975).

Preparation of standard curve

To a series of 15-ml volumetric flasks were added, in the following order, 9 ml of buffer solution, 2 ml of metol solution, 1·5 ml of potassium permanganate solution and 1–3 ml of antioxidant solution and water (diluted to the mark). Absorbance was measured at appropriate wavelengths (λ_{max}) after attaining colour stability.

Method for determining 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 neutralized with 1 g of calcium carbonate, then diluted with water to 100 ml in a standard flask and filtered through dry paper. The filtrate was used for propyl gallate or

gallic acid determinations and their contents were computed from the standard curve.

For the determination of butylated hydroxy anisole in oils or the simultaneous determination of BHA and PG, if both were present, a separation procedure was followed, as suggested by Schwien & Conroy (1965) and the general procedure was then applied.

RESULTS AND DISCUSSION

The absorption maxima of the reaction products, the time taken for maximum colour development, the colour stability time and the pH and experimental conditions are given in Table 1. The order of addition

TABLE 1
Experimental Conditions for Determination of Antioxidants

Antioxidant	*pH*	*Metol (ml)*	*MnO_4 solution (ml)*	*Time for maximum colour development (min)*	*Colour stability time (min)*	*λ_{max} (nm)*
Propyl gallate	3·0 (2·0–3·5)	2·0	1·5	3	13	560
Gallic acid	3·0	2·0	1·5	Immediate	30	550
Butylated hydroxy anisole	3·0	2·0	1·0	105	100	510

of the reagents should be buffer, metol, oxidant and compound under estimation. Change of the order of addition causes a decrease in absorbance. Beer's law limits, molar absorptivity for the proposed method and that reported by Sastry *et al.* (1982) and relative standard deviations are given in Table 2.

Comparison of the values of the recovery experiments of antioxidants in various oils with those from methods proposed by Bhatia *et al.* (1971), Conroy (1959) and Sastry *et al.* (1982), reveal good recovery and accuracy (Table 3). By comparison of molar absorptivity values it can be seen that the proposed method is more sensitive than the methods reported earlier.

The following substances—BHA (1:1), BHT (1:2), phenol (1:3), resorcinol (1:2), ascorbic acid (1:4), benzoic acid (1:40), citric acid (1:20),

TABLE 2
Optical Characteristics, Precision and Accuracy

Antioxidants	*Beer's law limits* ($\mu g/15\,ml$)	*Molar absorptivity at* λ_{max} (*litres* $mol^{-1}\,cm^{-1}$)		*Relative standard deviation* (%)[a]
		Proposed method	*Reported method*	
Propyl gallate	50–400	$5{\cdot}82 \times 10^3$	$5{\cdot}8 \times 10^3$	0·2
Gallic acid	25–225	$7{\cdot}52 \times 10^3$	$5{\cdot}0 \times 10^3$	0·3
Butylated hydroxy anisole	150–700	$2{\cdot}92 \times 10^3$	$8{\cdot}8 \times 10^2$	0·2

[a] Absorbance obtained from eight determinations.

tryptophan (1:4), cystein (1:4), glucose (1:40), sucrose (1:40) and sodium chloride (1:40)—did not interfere in the determination of PG.

The proposed method is very sensitive and is reasonably accurate. If interfering substances are present in food samples, suitable separation techniques must necessarily be applied before individual determinations are carried out.

The colour developed may be due to charge–transfer complex formation involving electron transfer between the metol (or its intermediate oxidative product) and the antioxidant (or its oxidative product).

TABLE 3
Recovery of Added Antioxidants from Edible Oils and Fats

Sample	*Antioxidant added* (μg)	*Proposed method*	*Recovery* (%)[b]	
			Reported method	
			A	*B*
Coconut oil	PG, 10	96·5	95·7	96·1
Groundnut oil	PG, 10	97·2	96·6	96·8
Sunflower oil	PG, 10	98·9	97·8	98·1
Cotton seed oil (hydrogenated)	PG, 10	96·2	95·9	95·6
Groundnut oil	GA, 10	97·1[c]	96·1	95·5
Sunflower oil	BHA, 10	97·2	96·5	96·1
Sunflower oil	PG, 5[a]	97·3	97·5	97·1
	BHA, 5[a]	96·4	96·3	95·8

[a] Separated initially by the Schwien & Conroy (1965) method.
[b] Average of three determinations.
[c] Method of Bhatia *et al.* (1971).
A: Method of Conroy (1959). B: Method of Sastry *et al.* (1982).

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