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Electrochemical ultra-micro sensors for the determination of synthetic and natural antioxidants in edible vegetable oils

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ABSTRACT

We describe the application of square wave voltammetry at ultramicroelectrodes for the determination of natural antioxidants (α, δ, and γ tocopherols), and tert-butyl hydroxytoluene in edible vegetable oils.

Tocopherol determinations were performed in benzene/ethanol (1:2) + 0.1 mol L^{-1} H_2SO_4 + oil samples at a carbon fiber disk ultramicroelectrode, and tert-butyl hydroxytoluene was determined in acetonitrile (ACN) + 0.1 mol L^{-1} $(C_4H_9)_4NF_6P$ at a Pt band ultramicroelectrode after performing its extraction from the oil sample with ACN.

Recovery percentages determined by the standard additions method were in the range from 92% to 102%, with variation coefficients between 0.5% and 4%.

Antioxidant concentrations calculated by this methodology were in good agreement with those values declared by the manufacturers.

1. Introduction

Seed oils are part of the human diet, and their production has increased in recent years due to the tendency of gradually replace animal fat by that of vegetable origin. Such changes arise from healthier lifestyles, which involve the consumption of foods rich in beneficial compounds for human health [1].

Some compounds such as polyphenols, flavonoids, and vitamin E have antioxidant activity and provide protection to cell membranes, preventing their oxidation by free radicals and their subsequent degradation, and provide protection against age-related diseases, cardiovascular disorders, or Alzheimer [2–4]. Considering that the human body does not synthesize these natural antioxidants, they must be incorporated through the diet. Some foods rich in these essential compounds are vegetable oils [3]. Vitamin E presents in vegetable oils is particularly important not only for its nutritional value but also because it helps to prevent oxidation of lipids, resulting in the formation of undesirable products which deteriorate oils [5–7]. Synthetic antioxidants have a similar function. Thus, tert-butyl hydroxyanisole (BHA), tert-butyl hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG) are added to vegetable oils in amounts allowed by international law [8]. However, these synthetic antioxidants widely used in the food industry could be responsible for liver damage and carcinogenesis [9–12].

Vitamins E, A, D, and K are fat-soluble. Vitamin E consists of four tocopherols and four tocotrienols, which differ by the saturation of their side chains. Thus, tocopherols have a saturated chain and tocotrienols an unsaturated chain with three double bonds at carbons 3, 7 and 11 [13]. Within each group, the isomers differ in the number and position of methyl groups on the aromatic ring, and are called as α, β, γ and δ.

Various methods have been used for the determination of synthetic and natural antioxidants in edible oils [8], being the most used HPLC chromatography [13–17].

Moreover, studies have been conducted in recent years to the development of analytical techniques to determine the total content of tocopherols in vegetable oils as well as the differentiation and the determination of their isomers [3,18,19]. Electroanalytical techniques have also proved to be a convenient alternative to determine antioxidants in edible oils [20–24].

In this work, we propose a simple electroanalytical method to determine tocopherols, and BHT in edible vegetable oils based on

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the application of square wave voltammetry (SWV) at carbon fiber disk and Pt band ultramicroelectrodes (UME), and discuss results obtained by cyclic voltammetry (CV) at a conventional glassy carbon (GC) electrode.

2. Materials and methods

BHT, tocopherols, and $(C_4H_9)_4NF_6P$ (TBAHFP) were purchased from SIGMA Chemical Company, USA. Ethanol (EtOH), and H_2SO_4 were Merck p.a. Benzene (Bz), acetonitrile (ACN), and water were Sintorgan, HPLC grade. ACN was kept over 3 Å molecular sieves, and then used without further purification. Other reagents were used as received.

Edible oils were purchased in local supermarkets. Two reaction media were used for the quantification of antioxidants. Therefore, oils were dissolved in a mixture of Bz/EtOH (1:2) + 0.1 mol L^{-1} H_2SO_4. This solution was then used to perform the determination of tocopherols. On the other hand, BHT was extracted with ACN from oils following a procedure previously described by us [24]. Then, its determination was carried out in ACN + 0.1 mol L^{-1} TBAHFP.

A two-compartment pyrex cell using a conventional three-electrode configuration was used to perform SWV and CV experiments, which was coupled to an AutoLab PGSTAT 12 potentiostat, controlled by the GPES 4.9 electrochemical software. The characteristic parameters of SW voltammograms were square wave amplitude, $\Delta E_{SW} = 0.050$ V, staircase step height, $\Delta E_s = 0.005$ V, and frequency, $f = 25$ Hz. The scan rate (v) in CV was varied from 0.025 to 0.200 V s^{-1}.

Working electrodes were a carbon fiber disk UME (BAS Electroanalytical System, USA, diameter, $\emptyset = 11$ μm), a Pt band UME constructed in our laboratory as described in literature [25], and a GC disk (from BAS, $\emptyset = 3$ mm). The pretreatment of UME was previously described [20,26]. The GC electrode was polished with 0.3 and then 0.05 μm wet alumina powder (from Fischer), copiously rinsed with water, and sonicated in a water bath for 3 min. Then, the electrode was transferred to an electrochemical cell containing the corresponding supporting electrolyte, and cycled 10 times between 0 and 1 V. This pre-treatment produced an electrochemical activation of its surface and allowed to obtain reproducible responses. The reference electrode was an aqueous saturated calomel electrode (SCE), and the counter electrode was a Pt foil of large area ($A \approx 2$ cm^2).

All solutions were deoxygenated by bubbling N_2 prior to measurements. The standard additions method was used to determine recovery percentages from oil samples spiked with antioxidants. The temperature was 25.0 ± 0.5 °C.

3. Results and discussion

3.1. Qualitative determination in olive and corn oils

Fig. 1 shows cyclic voltammograms recorded for BHT in ACN + 0.1 mol L^{-1} TBAHFP at a GC electrode at different v. An irreversible oxidation peak is clearly defined in the potential range from 0.95 to 1.45 V. Successive scans showed that voltammetric signals are highly reproducible. A plot of peak currents (I_p) as a function of $v^{1/2}$ was linear (inset Fig. 1), showing a diffusion control for the electrode process [27]. Similar results were found for the other antioxidants studied by CV (results not shown).

Then, we developed an electrochemical method for the determination of antioxidants in edible oils. We use an olive oil which has no synthetic antioxidants, and two corn oils, one of which contains BHT, and the other one does not contain any.

We first studied SWV responses in two reaction media using the corresponding commercial reagents. Thus, net peak potentials

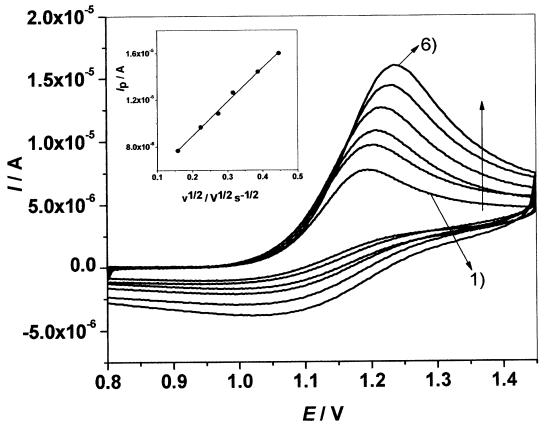

Fig. 1. Cyclic voltammograms recorded for BHT in ACN + 0.1 mol L^{-1} TBAHFP at different scan rates. Working electrode: GC $c^*_{BHT} = 3.2 \times 10^{-4}$ mol L^{-1}. The scan rate was varied from 0.025 to 0.200 V s^{-1} (1–6). Inset: plot of I_p as a function of $v^{1/2}$.

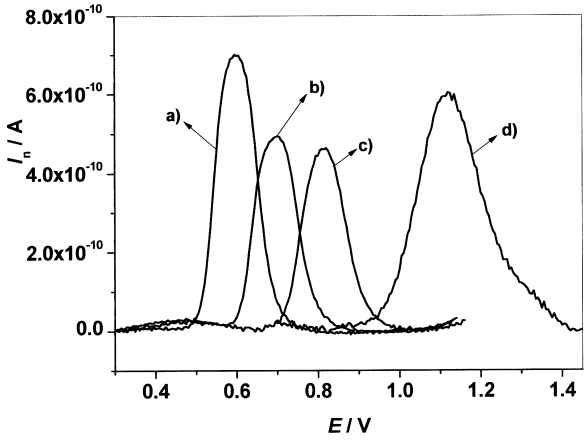

Fig. 2. Square wave voltammograms recorded for: (a) α, (b) γ, (c) δ tocopherols and (d) BHT at a CF disk UME ($\emptyset = 11$ μm) in Bz/EtOH (1:2) + 0.1 M H_2SO_4. Concentrations: 6.9×10^{-4} M, 2.6×10^{-4} M, 2.7×10^{-4} M and 3.8×10^{-4} M, respectively. $\Delta E_{SW} = 0.050$ V, $\Delta E_s = 0.005$ V, $f = 25$ Hz.

($E_{p,n}$) of α, γ, and δ tocopherols, and BHT were 0.59, 0.70, 0.81, and 1.1 V, respectively, in Bz/EtOH (1:2) + 0.1 mol L^{-1} H_2SO_4 at a carbon fiber disk UME (Fig. 2). On the other hand, $E_{p,n}$ of α, γ, and δ tocopherols, and BHT in ACN + 0.1 mol L^{-1} TBAHFP at a Pt band UME were 0.72, 0.82, 0.92, and 1.3 V, respectively (Fig. 3). We do

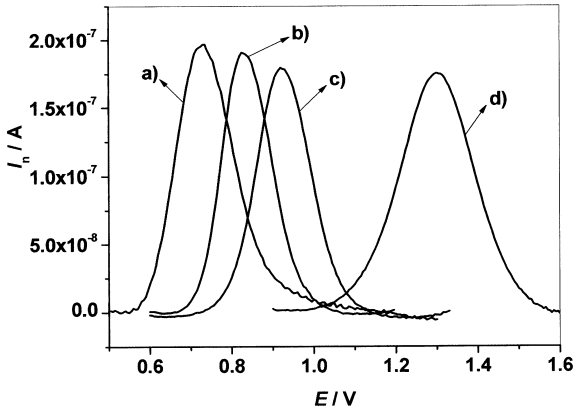

Fig. 3. Square wave voltammograms recorded for: (a) α, (b) γ, (c) δ tocopherols and (d) BHT at a Pt band UME in ACN + 0.1 M TBAHFP. Concentrations: 5.6×10^{-5} M, 1.3×10^{-4} M, 8.4×10^{-5} M and 9.3×10^{-5} M, respectively. Square wave parameters are the same as in Fig. 2.