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Analytical Methods

Simultaneous voltammetric determination of phenolic antioxidants in food using a boron-doped diamond electrode

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ABSTRACT

A method for the simultaneous determination of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food was developed using square-wave voltammetry (SWV). The determination of these phenolic antioxidants was carried out using a cathodically pre-treated boron-doped diamond electrode (BDD) and an aqueous-ethanolic (30% ethanol, v/v) 10 mmol L^{-1} KNO_3 solution ($pH_{cond.}$ 1.5) as supporting electrolyte. In the SWV measurements using the BDD electrode, the oxidation peak potentials of BHA and BHT present in binary mixtures were separated by about 0.3 V. The attained detection limits for the simultaneous determination of BHA and BHT (0.14 and 0.25 μmol L^{-1}, respectively) are lower than the ones by voltammetric techniques reported in the literature. The proposed method was successfully applied in the simultaneous determination of BHA and BHT in food products, with results similar to those obtained using a high-performance liquid chromatography method, at a 95% confidence level.

1. Introduction

Antioxidants (natural and synthetic) play a significant role in retarding lipid oxidation reactions in food products. Thus, currently there are around 30 types of synthetic antioxidants whose addition to food directly or indirectly is allowed. The phenolic compounds BHA and BHT are among the primary synthetic antioxidants widely used to interrupt the chain of free radicals involved in the autoxidation that constitutes the most common form of deterioration of fats used in the food industry. They have been used both alone and in mixtures in oils, margarine, and mayonnaise (Delgado-Zamarreno, Gonzalez-Maza, Sanchez-Perez, & Martinez, 2007; Diaz, Cabanillas, Franco, Salinas, & Vire, 1998), but their use is not a problem-less solution. Since BHA and BHT are suspected of being responsible for liver damage and carcinogenesis in laboratory animals, their potential harmful effects on health have been extensively discussed and studied. Therefore, in several countries the use of these additives is subject to regulations, which define specific approved antioxidants, establish permitted use levels, and include labelling requirements. However, there are differences among the individual countries, i.e., antioxidants permitted in one country may be prohibited in another. Internationally, the JECFA (Joint FAO/WHO Expert Committee on Food Additives) periodically considers food additives, including synthetic phenolic antioxidants (SPAs), on the basis of all available scientific data, to establish acceptable daily intake levels and specifications of identity and purity for them (FAO/WHO, 1995; Guan, Chu, Fu, Wu, & Ye, 2006). In the European Union, for example, the amount of synthetic antioxidants in food is limited to 0.01% (100 mg kg^{-1}) for each antioxidant, if used individually, and to 0.02% as total fraction, if the antioxidants are used in mixtures (Delgado-Zamarreno et al., 2007). In Brazil, the use of these antioxidants is controlled by The National Health Surveillance Agency (ANVISA), which limits the amount to 200 mg kg^{-1}, for BHA, and to 100 mg kg^{-1}, for BHT (ANVISA, 2005). Thus, the determination of SPAs in foods is necessary to ensure the fulfilment of legal requirements as well as quality-control procedures in the food industry.

Many methods for determining BHA and BHT individually or simultaneously have been recently reported, based on spectrophotometry (Capitan-Vallvey, Valencia, & Nicolas, 2004), liquid and gas chromatography (Guo, Xie, Yan, Wan, & Wu, 2006; Perrin & Meyer, 2002; Saad et al., 2007), micellar electrokinetic chromatography (Delgado-Zamarreno et al., 2007; Guan et al., 2006), and flow injection and HPLC with amperometric detection (Luque, Rios, & Valcarcel, 1999; Riber, de la Fuente, Vazquez, Tascon, & Batanero, 2000; Ruiz, Garcia-Moreno, Barbas, & Pingarron, 1999). But they are prone to many drawbacks, such as expensiveness, complicated and lengthy procedures, and unsuitability for field use.

Electrochemical techniques, such as the voltammetric ones, are a promising alternative to classical approaches due to their relatively low operational cost, good miniaturization potential, and rapid and sensitive detection procedures, which are suitable for faster analyses. Some methods for determining BHA and BHT by

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voltammetric techniques were already reported (Agüí, Reviejo, Yanezsedeno, & Pingarron, 1995; Ceballos, 2006; Ceballos & Fernandez, 2000a; Ceballos & Fernandez, 2000b; De la Fuente, Acuna, Vazquez, Tascon, & Batanero, 1999; Diaz et al., 1998; Kumar & Narayanan, 2008; Ni, Wang, & Kokot, 2000; Raymundo, Paula, Franco, & Fett, 2007). Diaz et al. (1998) studied the voltammetric behaviour of propyl gallate (PG), BHA, and BHT at a glassy-carbon (GC) electrode (static and rotating) in an acetonitrile–water medium; they used a chemometric procedure for the determination of these antioxidants in different spiked samples of packet soup. Ni et al. (2000) studied the voltammetric behaviour of BHA, BHT, PG, and *tert*-butylhydroquinone, at a GC electrode in a 0.1 mol L^{-1} perchloric acid solution containing 1% methanol, using chemometric approaches such as classical least squares, principal-component regression, and partial least squares. Linear calibration plots were obtained in the concentration ranges 2.8–83 μmol L^{-1}, for BHA, and 2.8–36 μmol L^{-1}, for BHT, with detection limits of 1.0 and 0.68 μmol L^{-1}, respectively. The method was applied to determine the four antioxidants in a set of synthetic mixtures as well as in several commercial food samples. Ceballos and Fernandez (2000b) used SWV with carbon-disk ultramicroelectrodes to determine BHA and BHT in vegetable oils. The determinations were carried out directly in benzene/ethanol/H_2SO_4 solutions or in acetonitrile after an extractive procedure, with better results in the latter case. Agüí et al. (1995) used cylindrical carbon-fibre microelectrodes in the simultaneous determination of BHA and BHT by SWV, obtaining detection limits of 4.0 μmol L^{-1}, for BHA, and 0.37 μmol L^{-1}, for BHT; however, the supporting electrolyte used contained acetonitrile, a high-cost reagent.

Thin films of BDD have emerged as excellent electrode materials for several electrochemical applications, especially electroanalytical ones, mainly due to properties such as: a wide potential window in aqueous solutions (up to 3 V), low background currents, long term stability, and low sensitivity to dissolved oxygen (Hupert et al., 2003; Panizza & Cerisola, 2005). The properties of BDD are commonly affected by the quantity and kind of the doping agent, morphologic factors and defects in the film, presence of impurities (sp^2 carbon), crystallographic orientation, and surface terminations (hydrogen or oxygen) that may be markedly determined by electrochemical pre-treatments (Salazar-Banda et al., 2006; Suffredini et al., 2004). Suffredini et al. (2004) called to attention that a cathodic pre-treatment of a BDD electrode dramatically increased the electroanalytical detection limit for chlorophenols, indicating that the analytical performance of BDD electrodes greatly depends on their surface termination, i.e., whether they are hydrogen or oxygen terminated. Recently, in our research group cathodically pre-treated BDD electrodes were used for the determination of aspartame and cyclamate in dietary products, individually (Medeiros, de Carvalho, Rocha-Filho, & Fatibello-Filho, 2007; Medeiros, de Carvalho, Rocha-Filho, & Fatibello-Filho, 2008b) or simultaneously (Medeiros, de Carvalho, Rocha-Filho, & Fatibello-Filho, 2008a), acetylsalicylic acid (Sartori, Medeiros, Rocha-Filho, & Fatibello-Filho, 2009), paracetamol and caffeine (Lourenção, Medeiros, Rocha-Filho, Mazo, & Fatibello-Fiho, 2009) or sulfamethoxazole and trimethoprim (Andrade, Rocha-Filho, Cass, & Fatibello-Filho, 2009) simultaneously, and sildenafil citrate – Viagra® (Batista, Sartori, Medeiros, Rocha-Filho, & Fatibello-Filho, 2010) in pharmaceutical formulations.

In this paper, we report on the coupling of voltammetric techniques with the unique properties of the BDD electrode for the development and optimisation of a method for the simultaneous determination of BHA and BHT in several food products. The practical use of the method is demonstrated by determining the concentration of BHA and BHT in commercial margarine and mayonnaise samples and by comparing the obtained results with those from a high-performance liquid chromatography (HPLC) method.

2. Materials and methods

2.1. Apparatus

The cyclic (CV), differential pulse (DPV), and square-wave (SWV) voltammetric experiments at a stationary BDD electrode were performed using an Autolab PGSTAT-30 (Ecochemie) potentiostat/galvanostat controlled with the GPES 4.0 software. A three-electrode cell system was also used: a BDD working electrode, a Pt-wire auxiliary electrode, and an Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode to which all electrode potentials hereinafter are referred.

The boron-doped (8000 ppm) diamond (0.72 cm^2 exposed area) film on a silicon wafer was obtained from the Centre Suisse de Electronique et de Microtechnique SA (CSEM), Neuchatêl, Switzerland (Salazar-Banda et al., 2006). Prior to use, the BDD electrode was cathodically or anodically pre-treated in a 0.5 M H_2SO_4 solution by applying -1.0 A cm^{-2} or 1.0 A cm^{-2}, respectively, during 120 s. A GC electrode (0.2 cm^2) was also used for comparative purposes. Prior to use, this electrode was pre-treated by sequential polishing with alumina (1 and 0.05 μm)/water slurries on felt pads, followed by rinsing with ultra-pure water.

The BHA and BHT determinations by HPLC were carried out using an LC-10 AT Shimadzu system, with an ultraviolet–vis detector (SPD-M10-AVP) set at 290 and 278 nm. A Shim-Pack CLC-ODS (6.0 mm × 250 mm, 5 μm) chromatographic column was used. The mobile phase was an acetonitrile/methanol mixture (50/50, v/v) at a flow rate of 1.0 mL min^{-1}, while the injection volume was 30 μL (Perrin & Meyer, 2002).

2.2. Reagents, supporting electrolyte and standards

All reagents were of analytical grade: BHA and BHT (Sigma), KNO_3 (Aldrich), and ethanol (Quemis, Brazil). An aqueous-ethanolic (30% ethanol, v/v) 10 mmol L^{-1} KNO_3 solution (pH_{cond} 1.5 adjusted with 1.0 mol L^{-1} HNO_3) was used as supporting electrolyte. Standard 1.0 mmol L^{-1} BHA and BHT solutions were prepared in this supporting electrolyte. All solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore®) with a resistivity greater than 18 MΩ cm.

2.3. Measurement procedures

After optimising the experimental parameters for the proposed methods, the analytical curves were constructed by adding small and equal volumes of the concentrated standard solutions of the two analytes to the supporting electrolyte in order to have the following concentrations: 0.6, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1}. The detection limit was calculated as three times the standard deviation for the blank solution divided by the slope of the analytical curve.

2.4. Influence of voltammetric techniques in the determination of the antioxidants

The electrochemical behaviour of the antioxidants was investigated using three different voltammetric techniques. CV was used for preliminary studies, such as the choosing of supporting electrolyte and pH. DPV and SWV were used for investigating the determination of the antioxidants and finding the best conditions.

2.5. Treatment of commercial food samples

A procedure similar to that proposed by Luque et al. (1999) and Raymundo et al. (2007) was followed for the determination of BHA