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Electroanalytical determination of total phenolic compounds by square-wave voltammetry using a poly(vinylpyrrolidone)-modified carbon-paste electrode

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ABSTRACT

A carbon paste electrode (CPE) modified with poly(vinylpyrrolidone) (PVP) was employed as an electrochemical sensor for the determination of total phenolic compounds (TPCs) in vegetables by square-wave voltammetry (SWV). The calibration curve obtained using kaempferol as a model for TPCs showed two linear ranges from 0.05 to 0.50 μmol L^{-1} (R^2 = 0.980) and from 0.50 to 6.0 μmol L^{-1} (R^2 = 0.998). With the use of the most sensitive range, the limits of detection and quantification were 40 nmol L^{-1} and 160 nmol L^{-1}, respectively. The following sequence was determined for the content of TPCs in the vegetables analysed: spinach > cabbage > broccoli > chicory. The accuracy of the results provided by the proposed sensor was evaluated by comparison with the values obtained applying the Folin-Ciocalteau (FC) methodology.

1. Introduction

Phenolic compounds are related to healthy dietary habits [1], since they are commonly found in fruits and vegetables. They are often present in grapes, apples and onions and in beverages such as fruit juice, soft drinks, wine and tea. As a consequence, the intake of foods rich in phenolic compounds has been recommended. The compounds reportedly have different beneficial effects on human health, since they can have anti-thrombotic, anti-bacterial, anti-allergic and anti-inflammatory properties [2,3]. Since 1980, several studies have also shown that lower risk of chronic diseases is correlated with a diet containing fruits and vegetables rich in phenolic compounds [4,5]. In addition, many studies have indicated that the consumption of phenolic compounds reduces the occurrence of cancer [6]. Phenolic compounds are strong antioxidant reactants necessary for cell functioning. The mechanism of the antioxidant activity is mainly influenced by the number of hydroxyl groups and their position on the molecule ring [7]. Kaempferol (5,7,4′ trihydroxy-flavonol, Fig. 1) is an example of a phenolic compound with various natural sources, including apples, grapes, *Ginkgo biloba*, onions, leeks, citrus fruits and red wine [8]. Several clinical studies have shown that kaempferol and some glycosides of kaempferol also have a wide range of pharmacological application as an antioxidant, anti-inflammatory, anticancer, antimicrobial, cardioprotective, neuroprotective, anti-diabetic, anti-osteoporotic, anxiolytic, analgesic and anti-allergic agent [8]. For these reasons, kaempferol is an excellent model molecule for the determination of total phenolic compounds (TPCs).

Concerning the relevance of phenolic compounds, several methods have been employed for their determination. Separation techniques such as chromatography [9,10] and capillary electrophoresis [11,12] have been used in order to study the properties of phenolic compounds and for their quantification. Optical methods [10,13] have also been widely used. In this context, the Folin-Ciocalteau (FC) methodology [13] is one of the most commonly used procedures for the determination of TPCs in food samples and plant extracts. However, low specificity is observed since the FC components react with other non-phenolic substances, resulting in an overestimation of the TPC content [14]. Various simpler and faster procedures employing electrochemical sensors have also been developed with appropriate selectivity and sensitivity for the determination of phenolic compounds [15–17]. In order to maximize the analytical response, the application of modified

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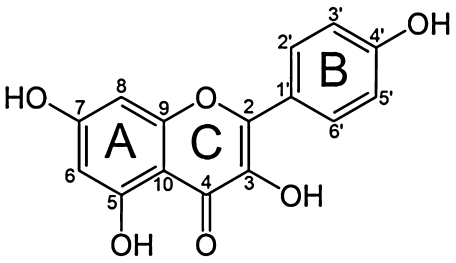

Fig. 1. Chemical structure of kaempferol.

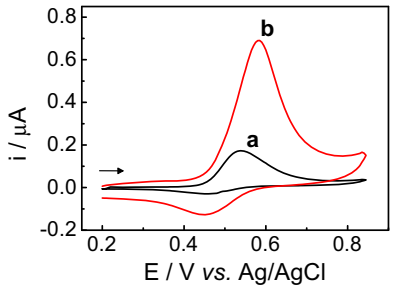

Fig. 2. Cyclic voltammograms obtained using the CPE (a) and CPE/PVP sensor (b) for 10 μmol L^{-1} kaempferol in B–R buffer (pH 3.0), ν = 100 mV s^{-1}.

electrodes with specific reactants has been reported [18]. For example, the well-known ability of the polymer poly(vinylpyrrolidone) (PVP) to extract phenolic compounds from fruit juices and plant extracts [19,20] has been exploited for the construction of electroanalytical sensors for single phenolic compounds [21–23].

The aim of this study was to apply a PVP-modified CPE for the quantification of total phenolic compounds (TPCs) instead of the determination of specific phenolic compounds. Kaempferol was used as the model molecule for the TPCs, and its content was determined in spinach, broccoli, cabbage and chicory samples by square-wave voltammetry. The accuracy of the analysis was evaluated by comparison with the results obtained applying the Folin-Ciocalteau methodology.

2. Experimental

2.1. Reagents, solutions and samples

All reagents used in this study were purchased from Sigma–Aldrich. They were of analytical grade and used without further purification. The solutions were prepared with water purified using a Milli-Q system manufactured by Millipore (Bedford, MA, USA) with a resistivity of 18.2 MΩ cm^{-1}. A stock solution of kaempferol was prepared at a concentration of 0.1 mmol L^{-1} in a mixture of water:ethanol (30:70 v/v). Britton–Robinson (B–R) and phosphate buffers were prepared at an initial concentration of 0.1 mol L^{-1} and tested as the supporting electrolyte. The pH was adjusted with 0.5 mol L^{-1} HCl or NaOH solution. Buffers and stock solutions of kaempferol were kept at 5 °C for a maximum of 90 days. The PVP, with a molecular weight of 1,300,000 g mol^{-1}, was kindly provided by the Study Group on Polymeric Materials (POLIMAT-UFSC). For the determination of TPCs four different vegetables were used: spinach, broccoli, cabbage and chicory, all purchased in a supermarket in the city of Florianópolis, SC, Brazil. The vegetables were washed with distilled water and dried at a temperature of 25.0 ± 0.5 °C. The extraction of TPCs from these matrices was carried out by soaking 5 g of fresh plant material (leaves, stems and flowers) with 100 mL of a solution of ethanol and water (70:30, v/v) for a period of 10 min. A further 100 mL of ethanol were then added and the mixture was sonicated for 10 min. Finally, the ethanolic extracts were filtered using a filter paper of medium porosity (25 μm) and stored at 5 °C for 48 h. For the determination of TPCs, 100 μL of the ethanolic extracts were added to the electrochemical cell containing the supporting electrolyte. The TPC content was expressed as milligrams of kaempferol per gram of fresh plant material (mg g^{-1}). Solutions of myricetin, luteolin, caffeic acid, ascorbic acid, rutin and quercetin, all at a concentration of 5.0 μmol L^{-1}, were electrochemically analysed to evaluate if these molecules are oxidized at the same potential as kaempferol. In this study, they cannot be considered interferents, but components of the plant material which can contribute to the content of total phenolic compounds.

2.2. Instruments and electrodes

The voltammetric measurements were carried out on a potentiostat/galvanostat model PalmSens (Palm Instruments BV, The Netherlands) interfaced to a computer with the PSTrace software (version 2.5.2) for the acquisition and processing of data. A three-electrode electrochemical cell containing the CPE/PVP as the working electrode (A = 0.314 mm^2), an Ag/AgCl electrode (3.0 mol L^{-1} KCl) as the reference and a Pt plate as the auxiliary electrode was used. All pH measurements were carried out using a pH meter model HI 2221 (HANNA Instruments Inc., Woonsocket, USA). The CPE/PVP was prepared by manual soaking of 10 mg (5% w/w) PVP and 160 mg (80% w/w) graphite powder for 10 min to obtain a uniform dispersion of the polymer in the graphite powder. Next, 30 mg (15% m/m) of mineral oil were added and macerated for 20 min to obtain the paste. The modified paste was introduced into a plastic syringe with a volume of 1.0 mL and a copper wire was inserted to obtain the electrical contact. To ensure an active and fresh surface, the modified CPE was gently manually abraded on a sheet of paper between experiments. The as prepared CPE/PVP sensor can be used until the complete depletion of the paste inserted in the syringe. For comparison purposes, electrodes with different amounts of PVP were prepared, as well as an unmodified CPE. For the determination of TPCs by the Folin-Ciocalteau method [24], a monochromatic Micronal spectrophotometer model B572 (Micronal SA, São Paulo, Brazil) and a glass cell with an optical path of 1.0 cm were used.

2.3. Electrochemical measurements

Electrochemical measurements, namely cyclic voltammetry (CV) and square-wave voltammetry (SWV), were carried out in 10 mL of buffer solution (Britton–Robinson (B–R) or phosphate). The parameters of the SWV (ΔEs – scan increment, a – amplitude and f – frequency) were optimized in the following ranges: ΔEs = 1–10 mV, a = 10–100 mV and f = 10–100 Hz. For the construction of the calibration curve, successive additions of a stock solution of kaempferol were performed using a micropipette. After each addition, the solution was stirred in order to homogenize its composition. CV and SWV voltammograms were then recorded for the unstirred solution.

3. Results and discussion

3.1. Voltammetric behaviour of kaempferol using the CPE and CPE/PVP sensor

Fig. 2 shows the CV results obtained for 10 μmol L^{-1} kaempferol in B–R buffer solution (pH 3.0) recorded at between +0.2 and +0.8 V. For the CPE (Fig. 2, curve a) a well-defined oxidation peak at +0.54 V was observed, while the reduction peak was detected at +0.44 V. For the CPE/PVP sensor (Fig. 2, curve b) the same peaks