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Original Article

Determination of carnosic acid in *Rosmarinus officinalis* L. using square wave voltammetry and electrochemical behavior

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ABSTRACT

A new, fast, sensitive and simple voltammetric method is established for the direct determination of carnosic acid (CA). And the electroreduction of carnosic acid (CA) was studied using electrochemical methods. The number of electrons transferred in electrode mechanisms were calculated for reversible and adsorption-controlled electrochemical reduction of CA at 17 mV versus Ag/AgCl at pH 7.0 in Britton–Robinson buffer (BR) on a hanging mercury drop electrode. Square-wave voltammetry was developed and validated for direct determination of CA. Square-wave parameters were optimized as accumulation potential = 0.0 mV, accumulation time = 5 s, frequency = 50 Hz, pulse amplitude = 50 mV, and staircase step potential = 5 mV. The developed method displays three linear responses from 2 to 9 μM, 10 to 30 and 40 to 90 μM for carnosic acid with a correlation coefficient of 0.996, 0.999 and 0.999. The detection limits were found to be 1.5 μM, 4.0 μM and 40.1 μM, respectively. The interference effect of most common organic and inorganic species was investigated. Proposed method was successfully applied for determination of CA in natural extract of rosemary and the average content was determined as 11.9 ± 1.0 (μg CA/1 g rosemary). The results were in agreement with that obtained by HPLC-UV comparison method. The developed method can be widely used in routine quality control of herbal materials as well as other in foods, medicinal, pharmaceutical and environmental analysis.

1. Introduction

Synthetic antioxidants are commonly used in the food industry to avoid or delay the oxidative deterioration, but in more recent years, due to cause adverse health problems, there has been a growing interest in the use natural sources of antioxidants. The addition of antioxidants to foods in order to prevent rancidity has long been common practice to increase

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the shelf-life of good-quality food product [1]. Immensely plants, such as spices, fruits, especially vegetables, contain important protective agents for human health that have antioxidant properties [2,3].

Rosemary (*Rosmarinus officinalis*) is well-known for its antioxidant properties and in recent years their extracts have been used as antioxidants in food industry [4,5]. The most effective antioxidant in rosemary has been found to be a diterpene species, carnosic acid [6]. The determination of antioxidant compounds contained in the extract can be used to evaluate the quality of commercial extracts and could be important for industries. Therefore, the purpose of this study was to establish a new voltammetric method for the determination of low levels of carnosic acid (CA) in real systems, such as plant extracts, oils and fats, meat and meat products.

There are only a few analytical techniques for the quantitative determination of CA in different samples and most of them are based on chromatographic methods. The some of chromatographic detection methods, including high-performance liquid chromatography (HPLC) with electrochemical detection [7–9] or high-performance liquid chromatography (HPLC) with UV-diode array (DAD) [10–12] and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS) [13] have been reported for determination of CA. These methods gave reproducible results but unfortunately are laborious, expensive, time-consuming and require sophisticated instrumentation for routine analysis. Therefore, a fast, simple, low cost, accurate, precise and sensitive method is very important especially for analysis of plant extracts and food additives which used as antioxidants in the food industry [14].

Electroanalytical techniques such as cyclic voltammetry (CV), square-wave voltammetry (SWV), have some advantages when compared to chromatography, e.g., their low cost, analysis without extraction or other pretreatments, and speed. These methods also make it possible to evaluate the redox characteristics, to propose the plausible mechanism pathways, to evaluate the adsorption–diffusion parameters of molecules and these parameters may have importance for their distribution, pharmacological [15], toxicological [16] and pharmacokinetic properties [17]. Voltammetric techniques are generally used for the quantitative determination of electroactive and electro inactive species.

Up to now, we found that only one paper is available for amperometric determination of antioxidant activity in herbs. Cosio et al. [18] described a amperometrically method for the determination of antioxidant activity in herb methanolic extracts using a flow injection (FI) system with an electrochemical detector. Herb methanolic extracts were analyzed with the proposed method and the results were expressed as mg of trolox equivalents/g of dried weight. However, in this study, carnosic acid have been reported as the compound having the highest antioxidant activity.

To our present knowledge, no information about the voltammetric or electroreduction behavior of CA has so far been reported in the literature. Therefore, for the first time, this paper describes an electrochemical approach to CA. The aim of this work was to develop a new validated square-wave voltammetric assay method for the direct determination of CA on HMDE (hanging mercury dropping electrode). The developed method was applied for the determination of CA in natural extract of rosemary and the obtained results were compared using HPLC-UV as a second method. In addition, to obtain valuable information about the electroreduction mechanism of CA at HMDE, the cyclic voltammetry (CV) technique was applied.

2. Materials and methods

2.1. Apparatus

A IviumStat model electrochemical analyzer (Potentiostat/Galvanostat, Netherlands) was used for square wave voltammetry (SWV) and cyclic voltammetry (CV) measurements. A three electrode system was used, consisting of a platinum counter electrode, an Ag/AgCl (3 M NaCl) reference electrode and a working hanging mercury drop electrode (HMDE) as a working electrode. pH values were measured using a Mettler Toledo pH/Conductivity Meter pH meter with combined glass electrode was used to measure pH of all the solutions.

2.2. Reagents

Carnosic acid (CA) was purchased from Aldrich (Germany) and a primary solution of 1×10^{-3} M CA was prepared daily in ethyl alcohol–water (1:1, v/v) solvent before every use in order to avoid aging of the solution. Working solutions were prepared by diluting the stock solution with distilled water and storing in the dark at 4 °C. All chemicals used for the supporting electrolyte, solvents and other reagents were of analytical reagent grade (Merck, Darmstadt, Germany). Britton–Robinson buffer (BR buffer) solutions were prepared from a stock solution containing 0.04 M phosphoric, boric and acetic acids (Sigma–Aldrich, Germany) by adding 2 M NaOH to obtain pH values ranging from 2 to 12.

2.3. Sample collection and preparation

According to the scientific literature [6,19], 99.8% ethanol (v/v) was selected as a solvent for the production of rosemary extracts. Rosemary extracts were obtained in September from fresh leaves of a wild rosemary plant by the following extraction protocol: After 20 g of air-dried leaves was placed in a glass long-necked flask, 250 mL of ethanol was added to macerate. And then the sample was boiled at 80 °C for 40 min. The solvent was removed with a rotary evaporator and sample was treated with hexane in the extraction flask. Hexane removes the insoluble residue of rosemary more effectively. And then, the extract was boiled at 80 °C for 20 min in 150 mL of water. The extract was analyzed for quantification of CA by using electrochemical method.

2.4. Voltammetric procedure

The voltammograms of CA were recorded in phosphate and B–R buffers within the pH range of 2.0–12.0. A certain volume of 10.0 mL of one of the buffers used was transferred to the voltammetric cell. After, the electrodes were put in the solutions through which pure nitrogen gas was passed for 15 min before obtaining the voltammograms. The square-wave

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