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Electrochemical evaluation and adsorptive stripping voltammetric determination of capsaicin or dihydrocapsaicin on a disposable pencil graphite electrode

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ABSTRACT

Capsaicin and dihydrocapsaicin are the two most abundant capsaicinoids in peppers, which are responsible for about 90% of the spiciness. A detailed study of the electrochemical properties of these compounds at pencil graphite (PG) electrode was carried out in the pH range 1.0–12.0 in aqueous solutions. The compounds underwent irreversible oxidation at PG electrode, which was an adsorption-controlled process with two protons and two electrons. The voltammetric data indicated that their oxidation proceeded *via* an ECE mechanism. Using the square-wave adsorptive stripping voltammetry with accumulation at a fixed potential of –0.1 V for 120 s, both of them yielded a well-defined voltammetric response at +0.31 V (vs. Ag/AgCl) in Britton-Robinson buffer, pH 9.0. Capsaicin and dihydrocapsaicin could be determined with detection limits of 1.12 ng mL^{-1} (3.7×10^{-9} M) and 0.28 ng mL^{-1} (9.1×10^{-10} M), respectively. The practical applicability of this methodology was tested in commercial Turkish pepper products. The concentration of total capsaicinoids was determined using capsaicin as standard.

1. Introduction

Hot or spicy peppers and chilies, herbaceous plant belonging to the genus *Capsicum* (family: Solanaceae), are the most popular food additives that are widely utilised in many parts of the world [1]. In the southeastern Anatolian region of Turkey peppers represent a culinary tradition and cultural identity.

The spicy pungent taste of most varieties of hot peppers is due to a group of closely related lipophilic alkaloids called capsaicinoids. The common structure of capsaicinoids is composed of a vanillylamide and a hydrophobic C_9–C_{11} branched-chain fatty acids with the degree of unsaturation. Capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide) are the two most active components of capsaicinoids, and their molecules differ only in the saturation of the acyl group. The capsaicin comprises 33–59% of the natural capsaicinoids found in peppers, followed by dihydrocapsaicin (30–51%), located mainly within the white ribs (palcenta) and seeds of the fruit. These two compounds are also about twice as potent to the spiciness and nerves as the minor capsaicinoids nordihydrocapsaicin (7–15%) and the reminder (less than 5%), which is homodihydrocapsaicin and homocapsaicin. The concentration of capsaicinoids in fresh red pepper varieties, especially in paprika, ranges from 0.001 to 0.01%, and in strong chili varietes the concentration ranges from 0.1 to <1% by weight [1–4].

In addition to food additive uses in our diet, the other biological properties and medical applications of capsaicin make this compound very popular. It is currently available as various topical pharmaceutical forms (ointments, high-dose dermal patches, creams, large bandages) for a number of diverse clinical conditions, such as relief the pain of peripheral neuropathy, symptomatic treatment of arthritis, muscle and joint pains, other types of neurogenic pains such as Herpes zoster. It is used in the treatment of obesity and ulcer in the forms of oral herbal supplements. It is also the active ingredient in riot control and personal defense pepper spray chemical agents. Furthermore, it was demonstrated that capsaicin induces apoptosis in various cancer cells. However, studies are available showing that capsaicin increases 2–5 fold the incidence of stomach, pharynx, esophagus and larynx cancer in countries where capsaicin is highly consumed [4–6]. Because of the increasing demand by consumers for hot and spicy foods and also the increasing use in medicine and pharmacy, it has become important to establish a sensitive, accurate and simple technique for detection of capsaicin and its analogues in food products and pharmaceuticals.

Total capsaicinoid content of heat-producing chemicals is one of the main parameters that determine its commercial quality, and it is directly related to the heat (pungency) of the level of a pepper.

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This level has been measured in Scoville heat units (SHU) by an organoleptic method (Scoville Organoleptic Test) [7]. In addition to traditional Scoville method, several analytical techniques for determining capsaicin and/or the related capsaicinoids in food, pharmaceutical and clinical samples were described using high-performance liquid chromatography (HPLC) equipped with ultraviolet, diode-array, fluorescence, electrochemical or mass/tandem mass spectrometric detection [8–21], gas chomatography–mass spectrometry [22,23], capillary electrophoresis [24], micellar electrokinetic capillary chromatography [25], colorimetry [26], spectrophotometry [27], chemometry based on spectrophotometry [28] or thin layer chromatography-in situ densitometry [29]. Pungency is also measured by a method which uses HPLC technique. This method yields results, not in Scoville units, but in American Spice Trade Association (ASTA) pungency units [30]. Nowadays, HPLC method has replaced Scoville method because of being more reproducible and accurate [31].

Of the above mentioned methods, the most recent and widely used are gas and high-performance liquid chromatography coupled to more selective mass spectrophotometry, which offer sufficient sensitivity and high resolution, however they suffer from disadvantages such as complicated procedure, long response time and requirement of expensive instruments and/or tedious derivation steps to increase the volatility of capsaicinoids.

From the electroanalytical point of view, literature survey revealed only three voltammetric studies made on capsaicin so far. All of them are quite new and involve the use of modified electrodes. The first investigation performed by Compton et al. [32] was concerned with studying the electroactivity and determination of capsaicin using adsorptive stripping voltammetry at carbon nanotube-based electrochemical sensors. Its applicability was evaluated in a variety of hot pepper sauces. Previous work of our research group was demonstrated the effectiveness of boron doped diamond electrode in presence of sodium dodecylsulfate for the electrochemical oxidation of capsaicin. Adsorptive stripping voltammetry was proposed for its determination in two samples of commercial pepper products [33]. Last attempt was made to study the electrooxidation of capsaicin at a carbon paste electrode modified with amino-functionalized mesoporous silica. Adsorptive stripping voltammetry was applied to determine capsaicin in hot pepper samples [34]. On the other hand, to the best of our knowledge, no literature data were found on the electrochemical behaviors of dihydrocapsaicin and other capsaicinoids.

The single-use disposable electrodes may overcome the regeneration drawback of the solid electrodes. In this manner, pencil leads can be seen as important electrode materials which present high electrochemical reactivity, good mechanical stability, low cost, low technology, eases of renewal, and other interesting qualities [35,36].

In continuation to our earlier reports [37–40] on the electroanalytical application of pencil leads, and with the limited electrochemical data on capsaicinoids, the present study is intended to develop a new rapid and sensitive electroanalytical procedure for two members of capsaicinoid family, capsaicin or dihydrocapsaicin, at a pencil graphite electrode using adsorptive stripping voltammetry that could be adopted in two different commercial pepper products, such as flakes of Turkish red pepper and Urfa crushed red pepper (also known as Isot pepper).

2. Experimental

2.1. Caution

It should be used gloves and mask when handling of very strong irritant agents, capsaicin and dihydrocapsaicin, and their concentrated solutions.

2.2. Apparatus

Cyclic voltammetric (CV), linear sweep voltammetric (LSV) and square-wave adsorptive stripping voltammetric (SW-AdSV) measurements were performed by μAutolab type III electrochemical analyzer with GPES 4.9 Version conversion software (EcoChemie, The Netherlands). For SW voltammograms the raw data were treated using the Savicky and Golay filter and a moving average baseline correction (peak width = 0.01 V) of the GPES software. Electrochemical experiments were carried out by a conventional three-electrode configuration. Pencil graphite (PG) electrode was used as working electrode. A platinum wire and an Ag/AgCl electrode (3 M NaCl) (Model RE-1, BAS, USA) were used as auxiliary and reference electrode, respectively. The pH values of solutions were measured using a WTW inoLab pH 720 m with a combined electrode (glass-reference electrodes).

PG electrodes were Tombo (Japan) leads with a total length of 60 mm and a diameter of 0.5 mm, which were purchased from a local bookstore. A Rotring pencil Model T 0.5 (Germany) was used as a holder for PG electrode. Electrical contact with the PG electrode was obtained by soldering a metallic wire to the metallic part of the holder. A total of 10 mm of lead was immersed in solution per measurement. The electrochemical pretreatment of PG was performed by applying a potential of +1.40 V for 30 s in aqueous media containing the blank supporting electrolyte without stirring, in order to increase the effective surface area of the electrode and to introduce the oxygen containing functional groups on the electrode surface by the oxidation of graphite layers [41].

2.3. Chemicals

All chemicals were of analytical-reagent grade and used as received without any further purification. The stock solutions ($1\ \mathrm{mg\ mL^{-1}}$) of capsaicin and dihydrocapsaicin (Sigma), which are the two standards of capsaicinoids commercially available, were prepared in ethanol due to their poor solubility in water. The stock solutions were stored in the dark and under refrigeration when not in use. Working solutions of these compounds were prepared daily by diluting the stock solution with a selected supporting electrolyte. Four different supporting electrolytes, namely perchloric acid (0.1 M), acetate buffer (0.1 M, pH 4.8), Britton-Robinson buffer (BR, 0.1 M, pH 9), and phosphate buffer (0.1 M, pH 2.5, 7.4 and 12.0) solutions were used. The content of organic solvent in the supporting electrolyte did not exceed 1% (v/v) for SW-AdSV measurements, which did not influence electrochemical determination of these capsaicinoids. All other chemicals were of analytical-reagent grade and used as received without any further purification. Aqueous solutions were prepared with deionised water further purified via a Milli-Q unit (Millipore).

2.4. Analytical procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: The required aliquot of the capsaicinoid working solutions was placed in a voltammetric cell containing BR buffer as a selected supporting electrolyte (unless otherwise stated) at a desired pH (e.g. pH 9.0). The previously treated electrode was placed in the cell, and the solution was stirred at 200 rpm at a chosen accumulation potential (e.g. –0.1 V) throughout the selected accumulation period (e.g. 120 s). Following the pre-concentration period, the stirring was stopped, and after a 10 s had elapsed, a sweep was carried out towards positive potentials over the range +0.1 to +1.1 V (for acidic media) and –0.1 to +1.1 V (for alkaline media) using the SWV technique. Each measurement was performed using a new pencil surface in a