Scientific paper

Study of Capsaicin Content in Various Parts of Pepper Fruit by Liquid Chromatography with Electrochemical Detection

Veronika Supalkova, a,b Helena Stavelikova, c Sona Krizkova, b Vojtech Adam, b Ales Horna, d Ladislav Havel, a Pavel Ryant, e Petr Babula, f Rene Kizek b,*

a Department of Plant Biology, b Department of Chemistry and Biochemistry, and c Department of Plant Nutrition, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic. Tel.: +420(5)45133350, Fax.: +420(5)45212044, E-mail: kizek@sci.muni.cz.

c Department of Gene Bank in Olomouc, Research Institute of Crop Production, Slechtitelu 11, CZ-783 71 Olomouc-Holice, Czech Republic

d Department of Food Engineering and Chemistry, Faculty of Technology, Tomas Bata University, T.G. Masaryka 275, CZ-762 72 Zlin, Czech Republic

f Department of Natural Products, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic

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Abstract

The burning taste of pepper is induced by six chemically related compounds derived from phenylalkylamide alkaloid (capsaicinoids) group. Capsaicin and its derivative dihydrocapsaicin have the strongest burning effects from them. The aim of this work was to determine capsaicin content in different fruit parts (ovary, lower flesh, upper flesh and seeds). For these purposes, we optimized high performance liquid chromatography with electrochemical detection. The most suitable conditions for capsaicin determination were as follows: working electrode potential of 750 mV, mobile phase of acetate buffer (pH 4) and methanol in ratio 40:60 (v/v, %). At these conditions we were able to detect picomoles of capsaicin per injection. Finally, we utilized this technique to determine capsaicin in various cultivars of peppers. The highest content of capsaicin (227 mg per 100 g of fresh weight) was found in ‘Takanotsume’ cultivar.

Keywords: capsaicin, cyclic voltammetry, adsorptive transfer stripping technique, pepper

1. Introduction

Pepper (Capsicum, Solanaceae) is a plant of South- and middle- American origin.1–4 It was imported into Europe by Spaniards and became quickly a favourite component of our diet both in the vegetable and spice form.4–6 The burning taste of pepper is induced by six chemically related compounds derived from phenylalkylamide alkaloid (capsaicinoids) group.7,8 Capsaicin and its derivative dihydrocapsaicin have the strongest burning effects among them (Figure 1).

![Figure 1. Chemical structure of capsaicin.](image-url)
Capsaicin was discovered in 1816, when it was first isolated from peppers.\(^9\) After that it has been found out that this compound is responsible for the burning effect on mucous membranes and increases the secretion of digestive fluids.\(^6,10\) It is assumed that capsaicin was evolved as a plant protection against herbivores. Habanero (Capsicum chinense) is thought to be the most burning pepper species. Due to biological properties of this compound, it has been widely used, e.g. it is used for relief of pain and sprays for personal protection.\(^11–17\) Recently, it has been shown that capsaicin inhibits the growth of androgen-independent, p53 mutant prostate cancer cells.\(^18–20\)

The aim of this work was to determine capsaicin content in different fruit parts (ovary, lower flesh, upper flesh and seeds) of different pepper varieties. For these purposes, we optimized a liquid chromatographic method with electrochemical detection.

## 2. Material and Methods

### 2.1. Chemicals

Capsaicin and other chemical of ACS purity were purchased from Sigma Aldrich (St. Louis, USA) unless noted otherwise. The stock solution of capsaicin 1 mg mL\(^{-1}\) was prepared in ACS methanol (Sigma-Aldrich, USA) and stored in darkness at −20 °C. The working standard solutions were prepared daily by diluting the stock solution.

### 2.2. pH Measurements

The pH was measured by using the WTW inoLab Level 3 (Weilheim, Germany) instrument, controlled by personal computer with software (MultiLab Pilot; Weilheim, Germany). The pH electrode (SenTix-H, pH 0–14/3M KCl) was calibrated with WTW buffers (Weilheim, Germany).

### 2.3. Biological Samples

The samples of pepper were obtained from Research Institute of Crop Production, Department of Gene Bank in Olomouc, Czech Republic. All pepper fruits were collected in 2006. Each pepper fruit collected was divided into four parts: ovary, seeds, upper and bottom flesh (Figure 2). The tissue (100 mg) was weighted, disintegrated in liquid nitrogen and then homogenized with 1 mL of methanol for 15 min. The homogenate was vortexed for 10 min using Vortex (Genie, USA) and centrifuged for 20 min at 4 °C, 14,000 g. (Eppendorf 5402, USA). The electrochemical analysis of the supernatant followed.

### 2.4. Electrochemical Measurements

Electrochemical measurements were performed using an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with a VA-Stand 663 (Metrohm, Zurich, Switzerland), a standard cell with three electrodes. The electrode system consisted of a carbon-paste working electrode, an Ag/AgCl/3 mol L\(^{-1}\) KCl reference electrode, and a platinum wire counter electrode. Acetate buffer (0.1 mol L\(^{-1}\) CH\(_3\)COOH + 0.1 mol L\(^{-1}\) CH\(_3\)COONa, pH 4.0) was used as the supporting electrolyte. Adsorptive transfer stripping cyclic voltammetry (AdTS CV) was performed using the following parameters: initial potential = 0.1 V, end potential = 1.3 V, amplitude = 25 mV, step potential = 5 mV, and frequency = 200 Hz. All experiments were carried out at 25 °C. The raw data were treated using the Savitzky and Golay filter (level 2) and a moving average baseline correction (peak width = 0.05 mV) of the GPES software.

### 2.5. Preparation of Carbon Paste Electrode

Carbon paste (about 0.5 g) was made of 70% graphite powder (Sigma-Aldrich) and 30% mineral oil (Sigma-Aldrich; free of DNase, RNase, and protease) according to Kizek et al.\(^{21}\) and Masarik et al.\(^{22}\) This paste was housed in a Teflon body having a 2.5-mm-diameter disk surface. Prior to measurements, the electrode surface was renewed by polishing with a soft filter paper. Then, the surface was ready for measurement; sample volume was 5 µL.

### 2.6. Flow Injection Analysis and Liquid Chromatography with Electrochemical Detection

Flow injection analysis with electrochemical detection (FIA-ED) and/or high performance liquid chromatography with electrochemical detection (HPLC-ED) system consisted of a solvent delivery pump operating in range of 0.001–9.999 mL min\(^{-1}\) (Model 583 ESA Inc., Chelmsford, MA, USA), a guard cell (Model 5020 ESA, USA), a reaction coil (1 m) and/or a chromatographic column (Polaris C18-A, 150 × 4.6 mm, 5 µm particle size, Varian, Inc., USA), and an electrochemical detector. The electrochemical detector includes a low-volume flow-through analytical cells (Model 5040, ESA, USA), which is consisted of glassy carbon working electrode, palla-
A glassy carbon electrode was polished mechanically by 0.1 µm of alumina (ESA Inc., USA) and sonicated at the laboratory temperature for 5 min using a Sonorex Digital 10 P Sonicator (Bandelin, Berlin, Germany) at 40 W.

3. Results and Discussion

3.1. Detection of Capsaicin Using Cyclic Voltammetry

Primarily we aimed at investigation of the basic electrochemical behaviour and properties of pure capsaicin by means of cyclic voltammetry carried out on a stationary electrochemical analyzer. We found out that the capsaicin was electroactive and gave an electrochemical response at approximately 0.6 V measured in the presence of 0.2 mol L\(^{-1}\) acetate buffer (pH 4). These parameters were consequently used in the flow system to determine capsaicin. The characterisation and optimisation of capsaicin determination in the flow system was carried out by using flow injection analysis coupled with an electrochemical detector.

3.2. Flow Injection Analysis with Electrochemical Detection

We attempted to optimize the FIA-ED technique to determine capsaicin prior to analysis of real samples. Thus, the effect of different potentials at the glassy carbon working electrodes within the range from 100 to 850 mV on capsaicin signal was investigated. According to previous research, capsaicin and its derivatives can be oxidized in the range from 600 to 900 mV.\(^{14,23,24}\) As the most suitable working electrode potential, 750 mV was chosen (Figure 3).

Under these conditions, we studied the influence of mobile phase composition on the capsaicin signal (Figure 3, inset). The signal of capsaicin increased with increasing methanol content up to 40% and then decreased rapidly. This phenomenon is associated with negative effect of organic solvents on electrochemical detection of a compound of interest.\(^{25-27}\) It clearly follows from the results obtained that the optimal mobile phase consists of acetate buffer (pH 4) and methanol in the ratio 40 : 60 (v/v, %).

Under the most suitable conditions for capsaicin detection, we constructed the calibration curve (Figure 4).

3.3. High Performance Liquid Chromatography with Electrochemical Detection

There is considerable literature regarding chromatographic determination of capsaicin in connection with various detectors.\(^{28-35}\) HPLC-ED has not been utilized for the determination of capsaicin yet. After the optimization of electrochemical detection, the appropriate chromatographic conditions were selected. A typical chromatogram of capsaicin standard is shown in Figure 5. We observed a well separated signal of capsaicin at 20.5 min. The peak at 18.8 min could be associated to dihydrocapsaicin, which is the most commonly impurity of commercially available capsaicin standard. The obtained chromatographic signals on electrochemical detector were well defined, symmetric and increasing linearly with concentration (Figure 5, inset). The equation of the calibration curve was \(y = 14.968x + 194.99\) with coefficient of \(R^2 = 0.9948\). The detection limit of our technique was 5 pmol of capsaicin per injection (5 µL) or 305 ng mL\(^{-1}\).

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Figure 3. Hydrodynamic voltammogram of capsaicin (10 µg mL\(^{-1}\)), mobile phase flow rate 0.4 mL min\(^{-1}\); inset: the dependence of capsaicin signal on the methanol content in the mobile phase.

Figure 4. Dependence of capsaicin signal height on its concentration; inset: FIA ED signals capsaicin at 31.3, 62.5 and 125 µg mL\(^{-1}\). Detection potential 750 mV, mobile phase of acetate buffer (pH 4) and methanol in ratio 40:60 (v/v, %), flow rate 0.5 mL min\(^{-1}\).
content can be found in seeds. These results are in a good agreement with the common knowledge of pepper consumers regarding the pungency of different parts of pepper fruit.

3.4. Determination of Capsaicin in Real Samples

This optimized method was used to analyze the difference in capsaicin content in various cultivars of pepper from the Collection of Gene Bank in Olomouc. It clearly follows from the results obtained (Table 1) that capsaicin is not evenly distributed in pepper fruit. In general, the highest capsaicin concentrations are found in the ovary and in the lower flesh and the lowest capsaicin

<table>
<thead>
<tr>
<th>Variety</th>
<th>Ovary</th>
<th>Bottom flesh</th>
<th>Upper flesh</th>
<th>Seeds</th>
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<tr>
<td>Takanotsume</td>
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<td>122</td>
<td>21</td>
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<td>2.7</td>
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<td>Jubila</td>
<td>1.38</td>
<td>1.33</td>
<td>1.08</td>
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</table>

6. References

Povzetek

Pekoči okus paprike izvira iz šestih kemijsko sorodnih spojih, ki izvirajo iz skupine fenilalkilamidnih alkaloidov (kapsaicinoid). Kapsaicin in njegov derivat dihidrokapsaicin dajeta najbolj pekoč občutek. Cilj dela je bil določitev vsebnosti kapsaicina v plodovih različnih kultivarjev. Največja vsebnost kapsaicina v plodovih različnih kultivarjev je bila v številki 227 mg na 100 g sveže mase.

Supalkova et al.: Study of Capsaicin Content ...