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Estimation of Scoville index of hot chili peppers using flow injection analysis with electrochemical detection

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ABSTRACT

Method for the determination of Scoville index of pepper pungency using flow injection analysis with an amperometric dual electrode detection on thin-layer glassy carbon electrode is described, with applied combination of electrode potentials +1.0 V and 0.0 V. The sample is prepared by 15 min extraction of 0.2 g of dried powdered peppers of pepper by 10 mL of acetonitrile, followed by 15 times dilution by carrier solution, i.e. acetonitrile: acetate buffer pH 4 (95:5, *v*/v). The dual electrode detection offers accurate results for pepper samples of medium to high pungency. The suggested method can therefore offer efficient tool for the routine pepper pungency determination.

1. Introduction

Capsaicinoids are vanillylamides of fatty acids present in some pepper varieties. Due to their structure, they activate in body vanilloid receptors, which have a primary role in the thermoregulation and also in the excessive heat sensation [1]. The resulting feeling of hotness is well known to anyone, who tasted such a pepper in food; nevertheless, the same effect in a more pronounced form is used as a non-lethal weapon [2]. The period of hot sensation can be followed by a prolonged period of receptors inhibition, which is utilized in pain-relieving procedures in medicine [3]. Other pharmacological properties, connected with antioxidant power of capsaicinoids, are also reported; in this case, the pungency is often undesired [4].

Content of capsaicinoids in peppers varies not only in dependence to the pepper variety [5,6], but also in dependence to part of the fruit [7], its ripeness [8], conditions of growing [6] etc. The strongest effect is caused by the two main members of this class, prevailing capsaicin and less abundant dihydrocapsaicin; lower content and effect of the other capsaicinoids make them less important. Pepper hotness was originally quantified by sensoric Scoville test [9]. The test, initially introduced in 1912, is based on the maceration of grounded capsicum, left over-night in alcohol. After shaking and filtration, this alcoholic solution is added to sweetened water in definite proportions until a distinct but weak pungency is still perceptible on the tongue. This imprecise and demanding test was abandoned long time ago and replaced by chromatographic determination [10], although results are still expressed as Scoville heat units (SHU) [11–13]. HPLC determination, usually combined with diode-array detection (DAD) [6,14,15] or mass spectrometry [16], or gas chromatography (GC) with mass spectrometric detection [17] is undoubtedly the most suitable and most frequent method for determination of SHU in the laboratory application. These methods require specialised analytical laboratory with appropriate equipment, instrumentation, and personnel. Nevertheless, the variability of the capsaicin content caused the request of a method, which would allow the pepper producers to estimate SHU themselves, in an operative way and with their own equipment. This application can sustain lower accuracy, if it is compensated by inexpensive instrumentation, simple sample preparation, and straightforward data evaluation.

Numerous voltammetric methods were developed for the determination of capsaicin, particularly recently. Some of these employ carbon-based electrodes [18,19], but mostly they use complex electrode composition, including polymeric films or nanoparticles of carbon or metals [20–24], which make their accessibility for everyday measurement difficult. Moreover, operating these electrodes and data evaluation can be demanding for a non-experienced user.

Combination of flow injection analysis with electrochemical detection (FIA-ED) seems to be a promising method, utilizing the natural selectivity of the electrochemical methods and in the same time taking advantage of the high content of capsaicinoids in the plant material.

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The combination of electrochemical measurement of capsaicin in flow was tested for the medical application [25]; the other two works are aimed to pepper fruits and they utilize the combination of HPLC with either amperometric [26] or coulometric [27] detection.

The above-mentioned selectivity of the electrochemical determination can further be enhanced by the utilization of the thin-layer dual electrode cell. This setting in serial anodic arrangement employs a pair of electrodes, where the upstream one is kept on a high potential and it serves as the generator of the oxidized form of analytes for the downstream, detector electrode, which is kept on a low potential and detects only the products of the upstream reaction. As a result, compounds with low or no electrochemical reversibility do not interfere during the detection [28,29]. Capsaicin oxidation proceeds in two steps, where the second one is reversible, which suggests the suitability of the technique; nevertheless, speed of the reaction might influence the response of dual electrode detection [30]. Number of the detector constructions based on this principle were developed with various electrode materials and settings [28,30,31]; in our case, we chose commercially available solution with glassy carbon as the electrode material.

In this work, we focused on the development of simple and rapid method for Scoville heat index estimation using FIA-ED, with the confirmation of the obtained results by the comparison with the HPLC-DAD results. Moreover, it was investigated, whether the selectivity and applicability of the method is increased by the utilization of the dual electrode detection.

2. Experimental

2.1. Apparatus

Agilent 1260 Infinity Binary LC system was used for the flow measurements; for HPLC, it was equipped with column LiChroCart 125-4 Purospher STAR, RP-18e (5 µm). Flow rate of 1 mL min^{-1}, injection volume of 5 µL, and detection wavelength 280 nm was set. Electrochemical measurements were performed using Autolab PGSTAT128N analyzer (Metrohm, Netherlands). For the electrochemical detection in both HPLC and FIA, thin-layer cell with dual working electrode from glassy carbon of diameter 3 mm (ALS, Japan) and gasket thickness of 50 µm was used in serial arrangement. As the reference electrode, Ag/AgCl (3 M NaCl) was used; all the potential values are referred to this electrode.

2.2. Procedures

Fruits of 23 hot pepper varieties (Table 3) in ripe state were provided by Xundgarten (St. Jakob/Leifers, Italy). The peppers were heat-dried at 65 °C and grounded by vibrational mill. In the final assay, the extraction of capsaicinoids was performed by stirring of 0.2 g of pepper powder with 10 mL of acetonitrile for 15 min under room temperature. The aliquots were then filtered using 0.45 µm membrane filters directly into the 2 mL HPLC vials. For flow injection measurements extracts were diluted 1:15 with carrier solution. Three extracts were prepared for every variety. Brasilian Pumpkin variety was used as their representative during the method optimization due to the expected middle values of both capsaicinoids and interfering compounds content.

Chromatographic conditions were adopted from [7] and modified; mobile phase consisted of acetonitrile and aqueous buffer (70:30, *v/v*). For FIA, mixture of acetonitrile and aqueous buffer in proportion 95:5 (*v/v*) was used. Quantification of both capsaicin and dihydrocapsaicin was based on the capsaicin standard (Sigma-Aldrich). For calculation of Scoville index, equivalence of 16 ppm of capsaicin/dihydrocapsaicin for 1 SHU was used [32]. Each measurement was made in triplicate, unless specified otherwise.

3. Results and discussion

3.1. Sample preparation optimization

As can be assumed from previous works, a number of solvents can be used for extraction of capsaicinoids from peppers with high efficiency [33,34]. Preliminary experiments were initially conducted to choose and optimize the most suitable solvent system to extract capsaicinoids. Aside from ethanol, used in the original Scoville test, acetonitrile and methanol were selected for this experiment due to the compatibility with electrochemical detection together with reported high yields and simple applicability. Besides DAD, electrochemical detection on single electrode (E_{DET} = +1.0 V) was applied; results of DAD were used for the calculation of capsaicinoids yields, while relative response of capsaicinoids and undesired electrochemically active compounds was observed by ED.

For all three solvents, proportion of pepper powder/solvent varied from 1 to 0.1 g/10 mL and extraction was carried out for 60 min. Resulting capsaicinoids content in extracts were consistent for all tested conditions, suggesting complete extraction. However, both alcohols, particularly methanol, were able to extract also a great concentration of other compounds, and although the selectivity of electrochemical detection was much higher than that of DAD, their presence would cause major interference in FIA (Fig. 1). Therefore, acetonitrile was selected as the most suitable extraction solvent.

As the last step, time of extraction was varied in the range from 5 to 60 min, without any significant difference in results. Due to the fact that the efficiency of the extraction is not substantially influenced by the optimized conditions, also other factors can be considered, such as sample consumption and simple workflow. Thus, the optimal conditions, selected for further measurements, are: extraction of 0.2 g of pepper powder into 10 mL of acetonitrile, lasting for 15 min.

3.2. Method optimization

While HPLC-DAD conditions for capsaicinoids determination are known and relatively straightforward, as it does not require buffered

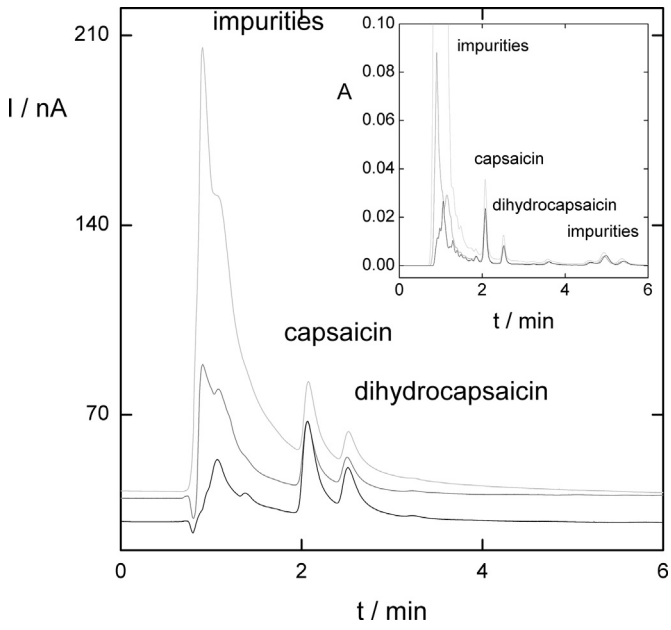

Fig. 1. Chromatograms of pepper extracts to ethanol (grey), methanol (light grey) and acetonitrile (black) obtained by ED (main graph) and DAD (inset). Extracted 0.5 g of pepper powder by 10 mL of the respective solvent for 60 min, column LiChroCart 125-4 Purospher STAR 125-4, RP 18E (5 µm), mobile phase acetate buffer pH 4: acetonitrile (30:70, *v/v*), λ_{DET} = 280 nm, E_{DET} = +1.0 V.