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Analytical Methods

Determination of resveratrol in grains, hulls and leaves of common and tartary buckwheat by HPLC with electrochemical detection at carbon paste electrode

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ABSTRACT

A reverse-phase high-performance liquid chromatographic method for the determination of *trans*-resveratrol with spectrophotometric detection (306 nm) and amperometric detection at carbon paste electrode (E = + 1,2 V) was developed and tested on real samples of grains, hulls and leaves of six varieties of common buckwheat (*Fagopyrum esculentum* Möench) and two varieties of tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). Optimal conditions for the determination of *trans*-resveratrol were as follows: column Kromasil C-18 (7 μm), 125 × 4 mm; mobile phase acetonitrile: diluted BR buffer pH 7 (50:50, 30:70 for grains and hulls and 20:80 for leaves); flow rate 1 ml min^{-1}. Under these conditions, the limit of detection of *trans*-resveratrol (L_D) was 3.5×10^{-8} mol l^{-1} (R^2 = 0.9986) for electrochemical detection and 3.2×10^{-8} mol l^{-1} (R^2 = 0.9993) for spectrophotometric detection.

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1. Introduction

Trans-reveratrol (*trans*-3,5,4′-trihydroxystilbene) is a polyphenol phytoalexin produced by plants in response to exogenous stimuli like UV light, ozone exposition, mechanical damage or fungal infection (Šmidrkal et al., 2001). Resveratrol exists in two isomeric forms *trans*-resveratrol and *cis*-resveratrol. The *trans*-isomer is the more stable form, with *trans* to *cis* isomerisation facilitated by UV light and high pH, while *cis* to *trans* conversion is facilitated by visible light, high temperature, or low pH (Trela & Waterhouse, 1996). Both isomers can be present in variable amounts in plants, but amount of *trans*-resveratrol usually predominates. Resveratrol was first detected in the roots of white hellebore (*Veratrum grandiflorum*) in 1940 (Shakibaei, Harikumar, & Aggarwal, 2009). Up to now it was found in more than 72 plants, which are often components of a human diet, for example in wine grapes (Fan, Zhang, Jinag, Yan, & Bai, 2008), with the result of average concentrations of 2.57 mg l^{-1} in red wine (Souto et al., 2001) and average concentration of 0.36 mg l^{-1} in white wine (López-Hernández, Paseiro-Losada, Sanches-Silva, & Lage-Yusty, 2007), in peanuts (Sobolev, Horn, Potter, Deyrup, & Gloer, 2006), cabbage, beetroot, broccoli, cranberries, blueberries (Rimando & Cody, 2005), etc. At present, a large body of evidence from *in vitro* and animal studies indicates that resveratrol may be beneficial to many aspects of human health. The remarkable interest in resveratrol is mainly due to its observed anti-cancer activities (Harikumar & Aggarwal, 2008), cardio-protective properties (coronary artery protection cumulating in the so called "French paradox") (Pirola & Fröjdo, 2008), anti-inflammatory, inhibition of platelet aggregation, antioxidation, neuroprotective (Sönmez, Sönmez, Erbil, Tekmen, & Baykara, 2007), antidiabetic and phytoestrogenic properties (Sharma, Anjaneyulu, Kulkarni, & Chopra, 2006) and anti-ageing properties. The quantitative determination of *trans*-resveratrol is mainly done by HPLC with UV/VIS (Piñeiro, Palma, & Barroso, 2006), MS (Stecher, Huck, Popp, & Bonn, 2001) and electrochemical detection (McMurtrey, Minn, Pobanz, & Schultz, 1994), by GC/MS (Luan, Li, & Zhang, 2000) or capillary zone electrophoresis (Gu, Chu, ÓDwyer, & Zeece, 2000). The HPLC with electrochemical detection is sufficiently sensitive and cost-effective alternative method to HPLC with UV/VIS or MS detection or capillary zone electrophoresis. In some matrices, electrochemical detection is superior to spectrophotometric one because of a large number of substances which are not electrochemically active and thus do not interfere with the detection of electrochemically active analyte. For HPLC-ED determination of resveratrol a glassy carbon electrode has been previously used (McMurtrey et al., 1994) at +0.5 V in 0.05 mol l^{-1}

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$NH_4H_2PO_4$ in 25% (v/v) aqueous acetonitrile. A carbon paste electrode used in this work for detection of resveratrol has (especially in more complicated matrices such as plant extracts) several advantages, namely the ease of renewal of the electrode passivated surface and lower background current. Resveratrol was identified in buckwheat amongst several other flavonoids (Qian, Mayer, & Kuhn, 1999). Buckwheat is pseudocereal but its grains belong to cereals because of their similar use. There are two buckwheat (*Fagopyrum*) species used for food around the world. Common buckwheat (*Fagopyrum esculentum* Möench) originates from Southwest China, while tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is grown and used in the mountainous regions of northern India, Bhutan and Nepal (Christa & Soral-Śmietana, 2008). Flavonoid content in tartary buckwheat is higher than that of common buckwheat. Historically, buckwheat was a very important crop in Europe. In many regions, buckwheat was very popular and it was included in many daily meals. But its importance in the course of years decreased, yet recently, it has been observed to increase because of the health-promoting properties of its grains.

The aim of this work was to develop an HPLC method with UV spectrophotometric detection and amperometric detection on carbon paste electrode (CPE) for the determination of trace amounts of *trans*-resveratrol in samples of common and tartary buckwheat. The well-known advantages of CPEs (Zima, Švancara, Barek, & Vytřas, 2009; Švancara, Vytřas, Barek, & Zima, 2001) are broad potential window, low background current, possibility of chemical or biological modification of the carbon paste and ease of renewal of working surface of the carbon paste electrode (Barek, Muck, Wang, & Zima, 2004). To the best of our knowledge CPEs have not yet been used as amperometric detector in HPLC-ED determination of resveratrol. Their main advantage is ease of preparation and ease of regeneration of the working electrode surface. CPE based detectors present less expensive, comparatively sensitive and more selective alternative to spectrophotometric detection.

2. Materials and methods

2.1. Instruments

The HPLC system consisted of the high-pressure piston pump HPP 5001 (Laboratorní přístroje Praha, Prague, Czech Republic), injection valve D with 20 µl sample loop (Ecom, Prague, Czech Republic), spectrophotometric detector Sapphire 800 UV/VIS (Ecom, Prague, Czech Republic), electrochemical detector CHI 802B (CH Instruments, Austin TX, USA) with three-electrode system consisting of reference silver/silver chloride electrode RAE 113 (Monokrystaly, Turnov, Czech Republic) filled with 3 M KCl, working CPE made of a Teflon body (active part 3 mm in diameter) (Vytřas & Švancara, 1994) and platinum wire auxiliary electrode. Column Kromasil C-18 (7 µm), 125 × 4 mm (Prochrome, Mumbai, India) and precolumn Gemini C-18, 4 × 3 mm (Phenomenex, Torrance CA, USA) were used. The amperometric detector, employing electrochemical oxidation of phenolic hydroxy groups, was placed behind the UV/VIS detector operating at 306 nm (or 286 nm for *cis*-resveratrol). CPE based electrochemical detector was working in a wall-jet configuration. The system was operated by Clarity 2.3.0 programme (DataApex, Prague, Czech Republic) and CHI 6.26 programme (CH Instruments, Austin TX, USA) working in the Windows XP system (Microsoft, Redmond WA, USA). The mobile phase was acetonitrile: Britton-Robinson buffer pH 7, 10 times diluted by deionised water (50:50, 30:70 and 20:80 v/v), the flow rate was 1 ml min^{-1}. For the preparation of the concentrated ethanolic samples of buckwheat seeds a vacuum evaporator Büchi B-480, R-114 (Büchi, Flawil, Switzerland) was used. For preparation of samples of leaves a freeze dryer (Martin Christ, Osterode, Germany) was used. An ultrasonic bath PS02000A (Powersonic, San Diego CA, USA) was used to facilitate the dissolution of the analytes. The pH of the solutions was measured with a pH meter Jenway 4330 (Jenway, Felsted, UK) with a combined glass electrode. Spectrophotometer Agilent 8453 (Agilent, Santa Clara CA, USA) was used to study the stability of the stock solution of *trans*-resveratrol. All experiments were carried out at a laboratory temperature.

2.2. Materials

Trans-resveratrol was purchased from Sigma–Aldrich (Saint Louis MO, USA). Its stock solution (1×10^{-3} mol l^{-1}) was prepared by dissolving an accurately weighed amount of the substance in p.a. methanol (Lach-Ner, Neratovice, Czech Republic) and stored in dark at 4 °C. Solutions of lower concentrations were prepared by dilution of the stock solution with methanol. Stock solution of *cis*-resveratrol was prepared from solution of *trans*-resveratrol (1×10^{-4} mol l^{-1}) by UV-irradiation for 48 h on daylight. Optimum conditions were determined by our spectrophotometric measurements of the isomerisation on daylight for a total period of 48 h (conversion 85%) which were based on previous findings in the literature (Trela & Waterhouse, 1996). A spectrophotometric study of the stability of the *trans*-resveratrol stock solution was measured in solution 1×10^{-3} mol l^{-1} in 1 mm silica cuvette for two absorption maxima (306 and 217 nm) and it demonstrated that it was stable for at least 1 year, if it is stored in dark at 4 °C. Britton-Robinson (BR) buffers were prepared in a usual way, by mixing 0.04 mol l^{-1} phosphoric acid, 0.04 mol l^{-1} acetic acid and 0.04 mol l^{-1} boric acid with an appropriate amount of 0.2 mol l^{-1} sodium hydroxide. Ethanol was used for extraction of resveratrol from buckwheat. All the chemicals used were of analytical reagent grade (Lachema, Brno, Czech Republic). The mobile phase for HPLC contained acetonitrile for HPLC (Merck, Darmstadt, Germany) and aqueous BR buffer diluted 10 times. Carbon paste consisted of 250 mg of spherical micro particles of glassy carbon with a diameter of 0.4–12 µm (Alpha Aesar, Ward Hill MA, USA) and 90 µl of mineral oil (Fluka Biochemica, Buchs, Switzerland). All aqueous solutions were prepared using deionised water obtained from a MilliQ Plus system (Millipore, Molsheim, France).

2.3. Samples of buckwheat

Six samples of common buckwheat and two samples of tartary buckwheat (Table 1) were supplied by Crop Research Institute, Department of Gene Bank (Prague, Czech Republic).

2.4. Extraction of buckwheat

At first, we tried a simple extraction 17 g of grinded grains in 50 ml of ethanol (ratio 1:3) in the refrigerator overnight in the dark (Kolouchová, Melzoch, Šmidrkal, & Filip, 2005). Then we tried another method (Qian et al., 1999) for extraction of antioxidant compounds from buckwheat flour (4 g of buckwheat flour with 50 ml of ethanol extracted under reflux for 1 h and concentrated under reduced pressure to 5 ml). But the extracts from the two above mentioned extractions contained resveratrol very near to the limit od detection. For these reasons, we modified published method (Qian et al., 1999) to extract resveratrol from buckwheat grains, hulls and leaves. Grains were separated from hulls except of two samples of tartary buckwheat (with tartary buckwheat the separation of hulls from grains is impossible) and grains and hulls were grinded by kitchen mixer. The samples of leaves were a mixture of leaves picked before and during flowering, which was immediately frozen to the temperature below −18 °C, lyophilised on freeze dryer (Martin Christ, Osterode, Germany) during

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