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Short communication

Rapid sucrose monitoring in green coffee samples using multienzymatic biosensor

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ABSTRACT

Amperometric biosensor utilizing FAD-dependent glucose dehydrogenase (FAD-GDH) for a specific sucrose monitoring in green coffee is described. FAD-GDH was co-immobilized with invertase and mutarotase on a thin-layer gold planar electrode using chitosan. The biosensor showed a wide linearity (from 10 to 1200 μM), low detection limit (8.4 μM), fast response time (50 s), and appeared to be O2 independent. In addition the biosensors exhibited a good operational (3 days) and storage (1 year) stability. Finally, the results achieved from the biosensor measurements of sucrose in 17 samples of green coffee (Coffea arabica, C. canephora and C. liberica) were compared with those obtained by the standard HPLC method. The good correlation among results of real samples, satisfactory analytical performance and simple use of the presented biosensor make it suitable for application in coffee industry.

1. Introduction

Coffee is the most commercialized food product and most widely consumed beverage in the world. In 2010, coffee production reached 8.1 million tons worldwide which represents more than 500 billion cups. The cup quality is affected primarily by the composition of green coffee being influenced with agricultural practices, environmental factors, variety and maturity (Farah, 2012). Sucrose is one of the major constituent of green coffee and is responsible for coffee flavour and quality (Dessaleng, Labuschagne, Osthoff, & Herselman, 2007). It is an important precursor of taste and aroma developed during the roasting process. Besides Borém et al. (2016) recently found that the level of sucrose is a good discriminant marker for the beverage quality. For instance, the higher sucrose content is one of the reasons for the superior aroma and overall flavour of Arabica coffee in comparison to Robusta one. In fact, Arabica contains from about 6 to 11% and Robusta from 3 to 7% of sucrose in green beans (Ky et al. 2001; Campa et al., 2004; Knopp, Bytof, & Selmar, 2006; Farah, 2012).

Several methods have been used for determination of sucrose in green coffee, including high performance liquid chromatography (HPLC; O'Driscoll, 2014; Borém et al., 2016), anion-exchange chromatography coupled to pulsed amperometric detection (Ky et al., 2001), enzymatic spectrophotometric method (Alcázar, Jurado, Martín, Pablos, & González, 2005), near infrared spectroscopy (Aluka et al., 2016; Santos et al., 2016). However, these methods require expensive laboratory equipment and educated personnel. Moreover, the HPLC analyses are time-consuming. Biosensors can represent an alternative method to overcome these drawbacks (Monošík, Stredansky, Tkáč, & Šturdík, 2012a). They exhibit rapid response, high selectivity, cost effectiveness, and they provide an option to perform analysis in situ due to their ability to be miniaturized. Various enzymatic compositions and detection principles were described for the construction of sucrose biosensors. Sole invertase (INV) was used for the thermometric (Thavarungkul, Suppapitnarm, Kanatharana, & Mattiasson, 1999) or fluorescent (Bagal-Kestwal, Kestwal, & Chiang, 2015) biosensors. The combination of INV, glucose oxidase (GOX) and mutarotase (MUT) was employed for conductometric (Soldatkin et al., 2013; Pyeshkova et al., 2015) and amperometric (Surareungchai, Worasing, Sritongkum, Tanticharoen, & Kirtikara, 1999; Gouda, Thakur, & Karanth, 2001; Majer-Baranyi, Adányi, & Váradi, 2008). The simultaneous use of INV and fructose dehydrogenase (FDH) was presented, too. However, FDH is relatively expensive enzyme and the GOX based biosensors are susceptible to oxygen concentration in the measuring media, which can lead to a decrease in the signal, and underestimation of measured

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values in cases where artificial mediators are used (Tang et al., 2001). Recently we have proposed the implementation of FAD-dependent glucose dehydrogenase (FAD-GDH) in the biosensor for glucose analyses in various beverages (Monošík, Stredansky, Lušpai, Magdolen, & Šturdík, 2012b). This commercially available convenient enzyme exhibits no dependency on oxygen and a high stability.

The aim of the present study was to develop a sucrose biosensor based on the combination of three enzymes (INV, MUT, FAD-GDH) suitable for the rapid and selective sucrose analysis in green coffee and compatible with the portable analytical device Omnilab currently serving beverage producers as an alternative to classic analytical methods.

2. Experimental

2.1. Materials

Glucose dehydrogenase FAD-dependent (GDH-FAD, 1160 U mg^{-1} solid) was purchased from Sekisui Diagnostic (Tokyo, Japan), and is reported to have been isolated for *Aspergillus* sp., invertase and mutarotase from Sorachim (Lausanne, Switzerland). Meldola blue, Azure A, Azure C, methylene blue, thionine, N-methylphenazonium methyl sulfate, sucrose, trehalose and chitosan from shrimp shells (85% deacetylated) were supplied by Sigma-Aldrich (St. Louis, USA). Potassium phosphate monobasic and potassium phosphate dibasic were purchased from Riedel-de Haen (Seelze, Germany). Water deionized by a Millipore Milli-Q purification system was used. All chemicals used were of analytical grade. Gold planar electrodes with diameter of 1.6 mm equipped with Ag/AgCl reference electrode (diameter 2 mm, screen-printed) deposited on the planar glass-epoxy-laminate substrate were obtained from Biorealis (Bratislava, Slovakia).

Nine different samples of green *Coffea arabica* L. beans (geographical origin: El Salvador, India, Ethiopia, Brazil, Indonesia, Tanzania, Colombia), five different samples of green *C. canephora* Pierre ex Froehner var. robusta beans (geographical origin: Indonesia, Ivory Coast, Vietnam, Tanzania, Cameroon) and three different samples of green *C. liberica* Bull ex Hiern beans (geographical origin: Indonesia) from commercial lots were used. *C. arabica* sample from El Salvador was a Low Caffeine Bourbon (BLC) cultivar.

2.2. Apparatus

Electrochemical measurements were performed with electrochemical analyzers Autolab M101 (Methrom Autolab, Netherlands) and Omnilab from Biorealis (Bratislava, Slovakia).

Reference HPLC assays were run on Waters 600E HPLC System (Waters, Milford, USA) equipped with the refractometer detector (model PU 4026, Philips, Eindhoven, Netherlands).

2.3. Preparation of biosensors

The planar gold electrodes were cleaned with Milli-Q water and ethanol. The immobilization of the enzymes on the electrode surface was carried out by their sandwiching between (1% w/w) chitosan layers. Each layer was deposited after the previous one was dried. All enzymes were dissolved in Milli-Q water before procedure. The prepared biosensors were stored at room temperature in a desiccator until use. The details on the quantities of enzymes are given in Section 3.

2.4. Preparation of green coffee samples

Green coffee beans were ground to a fine powder using a mixer mill Retsch MM400 (Retsch GmbH., Germany). Then 2 g of each sample were deposited into a 100-mL flask, mixed with 40 mL of deionized water, heated up to the boiling point agitated and left slowly until laboratory temperature. The extracts were subsequently filtered through a fine paper.

2.5. Amperometric measurements

Electrochemical measurements were performed with electrochemical analyzers Autolab M101 (Methrom Autolab, Netherlands) and Omnilab from Biorealis (Bratislava, Slovakia). Chronoamperometry was performed by applying selected constant potential (vs. Ag/AgCl) after inserting the biosensor in volume of a measuring solution either 1 mL in microtube or 10 mL in beaker under stirring at laboratory temperature. Values from −300 mV to +300 mV were tested for the optimization of working potential. The pH values of a 0.1 M phosphate buffer solution (PBS) were optimized from pH 5.0 to 8.0. Similarly, the suitable concentrations of electrochemical mediators (from 0.1 to 2 mM) in the working media were also investigated. The biosensors were stored after measurements in 0.1 M PBS of pH 6.0 at laboratory temperature (up to 10 h) or at 4 °C (for longer operational stability studies). The biosensors were kept dry in a desiccator at laboratory temperature for the storage stability studies.

2.6. HPLC analysis

Reference HPLC assays of sucrose were run on Waters 600E HPLC System (Waters, Milford, USA) equipped with the refractometer detector (model PU 4026, Philips, Eindhoven, Netherlands). The analytical conditions were as follows: column Polymer IEX in H^+ form 250 mm × 8 mm, 8 µm in diameter (Watrex, Bratislava, Slovakia); column temperature 80 °C and pressure 300 Psi; mobile phase Milli-Q water; flow rate 1.0 mL min^{-1}. Data were collected and processed by Clarity chromatography station DataApex (Prague, Czech Republic). Samples were diluted in a mobile phase and filtered through 0.22 µm Chromafil AO filters, Macherey-Nagel (Dűren, Germany) prior to analysis. Sugars were identified by comparison with retention times and coelution of authentic standard solutions.

3. Results and discussion

The principle of the presented biosensor is illustrated in Fig. 1. It is based on the amperometric detection of reduced electron acceptor, further referred to as mediator (Med), which is generated during the course of the GDH-FAD-catalyzed oxidation of β-D-glucose formed from sucrose by the co-immobilized INV and MUT. The GDH-FAD enzyme was previously employed in the development of glucose specific biosensor and its specificity is reported in the work by (Monošík et al., 2012b). From this study, the high specificity for β-D-glucose of GDH-FAD enzyme was proved against other sugars, alcohols, and acids. The reduced mediator is oxidized on the electrode surface and the resulting current proportional to the analyte concentration is measured. Gülce, Çelebi, Özyörük, and Yildiz (1995) reported that phosphate ions used in the medium at a high concentration catalyse the conversion of α-glucose to β-glucose, eliminating the need for MUT. When we applied the high level of phosphates instead of MUT the biosensor response became sluggish. Another possible principal problem of the used enzyme cascade comes from the fact that glucose presented in real samples could cause an interference, but its content in green coffee is negligible in comparison with sucrose (Knopp et al., 2006; Smrke, Kroslakova, Gloess, & Yeretzian, 2015). Besides small amounts of glucose in coffee samples did not influence the results obtained by the sucrose biosensor because differential measurements were applied and the signal obtained by the biosensor without invertase (measuring only glucose) was subtracted from the signal of the sucrose biosensor (measuring sucrose + glucose).

3.1. Optimization of biocatalytic layer

The quantities of enzymes on the electrode surfaces were optimized from 0.5 to 15 U. The optimal amounts of 6.0 U of FAD-GDH, 1.75 U of MUT, and 2.5 U of INV were found for immobilization on the electrode.