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Original Research Article

On the electrochemical biosensing of phenolic compounds in wines

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ABSTRACT

A simple electrochemical sensor based on polyphenol oxidases was proposed for determination of the total phenol content in wine. Application of two oxidases: tyrosinase and laccase (separately) was examined and the results were compared. An analytical characteristic of proposed sensor towards gallic acid was evaluated. The influence of sample matrix components on the sensor response was studied according to Plackett–Burman experimental design. The potential interferents ethanol, tartaric acid, sorbate, sulfate(IV), putrescine, Fe(III) and glucose, which are usually encountered in wines, were taken into account in the examination. Because of the significant matrix effect found, analyses of wine samples towards polyphenol contents were carried out using standard addition method and expressed as gallic acid equivalents. For comparative quantification of phenolic compounds well-established Folin–Ciocalteu spectrophotometric method was applied. Significant inter-method differences were observed in electrochemical behaviour of standard substance – gallic acid – and phenolic constituents of real wine samples in the presence of tartaric acid.

1. Introduction

The important groups of biologically active compounds occurring in wines are phenols and polyphenols. They are responsible for the wine appearance (colour), taste: astringency and bitterness, mouth-feel, fragrance and other tactile sensation defined as structure or body of wine (Waterhouse and Wrolstad, 2001; Soleas et al., 1997). Phenolic compound may originate from grapes (skins and seeds), vine stems, and products of yeast metabolism or from wood cooperage (Soleas et al., 1997). The composition of phenols and their concentration depend on grape variety, geographical origin, soil type, collection system and grape processing technology (Russo et al., 2008). The methods of vinification and applied technological procedures (maceration, fermentation, clarification, aging, etc.) can significantly modify both the concentration and composition of phenolic compounds and, therefore, also the colour intensity and hue of red vines (Balik et al., 2008). Wine clarification influences strongly the content of polyphenols in wine. The range of changes and main losses of individual phenolic compounds differ depending on the type of the clarifying agent and the chemical structure of phenols. Red wine, produced from whole grapes, with skin and seeds, contains high levels of phenols, while white wine, made by pressing off the skins and seeds immediately after harvesting contains them in small quantities only.

Nutritional importance of phenolic constituents is related to their antioxidant power; they are known as anti-carcinogenic and anti-inflammatory substances, when they are regularly consumed. Therefore it is important to estimate the antioxidant capacity of wines related to phenol content. However, there is no consensus of opinion on the method which should be applied (Gil and Rebelo, 2010). Spectrophotometric Folin-Ciocalteu method has been widely used for that purpose (Waterhouse and Wrolstad, 2001; Gil ad Rebelo, 2009). Recently biosensors based on polyphenol oxidases were employed for evaluation of phenol content in wines. Gil and Rebelo (2010) reported the application of a laccase-based biosensor with caffeic acid as a model substrate for determination of the antioxidant compounds in wines. Fusco et al. (2010) used a biosensor with laccase for determination of polyphenol index in

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wine. Studies on the interference of metabisulfite, a food and beverage preservatives, on laccase-based biosensing and on Folin–Ciocalteu analysis of polyphenols were performed too (Gil and Rebelo, 2009). Lanzellotto et al. (2014) constructed a nanostructured laccase biosensor based on fullerene and gold nanoparticles for detection of phenolic compounds in wine. A disposable laccase-tyrosinase based biosensor was employed for the detection of phenolic compounds in must and wine (Montreali et al., 2010). Tyrosinase and laccase voltammetric biosensors were also used as a part of BioElectronic Tongue, which allowed not only for determination of total polyphenol content but also for discrimination of individual constituents (Cetó et al., 2014). Pusch et al. (2013) applied another enzyme, peroxidase, immobilized in a matrix of Pt–Pd bimetallic alloy nanoparticles dispersed in ionic liquid for fabrication of a biosensor used for monitoring polyphenol content in wine.

In our previous work we proposed a new simple sensor, based on tyrosinase, for determination of phenolic compounds in water (Adamski et al., 2010). The developed sensor showed high sensitivity towards phenols and satisfying detection and quantification limits (10^{-8} mol/L).

The aim of this study was to test the usefulness of the proposed approach in determination of phenolic compounds in wine samples. For this purpose tyrosinase and laccase, separately, were applied. The content of phenols was expressed as gallic acid equivalents. Analytical characteristics of the sensor were estimated and its function was verified in a synthetic solution of the analyte. The effect of sample matrix composition on the response of the sensor was studied according to Plackett–Burman design. The potential interferents ethanol, tartaric acid, sorbate, sulfate(IV), putrescine (as representative of biogenic amines that are present in wine (Marcobal et al., 2005)), Fe(III) and glucose, which are usually encountered in wines, were taken into account in the examination. For a comparative quantification of phenolic compounds the well-established Folin–Ciocalteu (F–C) spectrophotometric method was applied (Waterhouse and Wrolstad, 2001; Gil and Rebelo, 2009).

2. Materials and methods

2.1. Chemicals

Tyrosinase (TYR) from mushroom (polyphenol oxidase, E.C. 1.14.18.1, 5370 units/mg) was purchased from Sigma–Aldrich (St. Louis, MO, USA). According to the manufacturer, one unit caused a 0.001 increase in absorbance at 280 nm/min at pH 6.5 and 25 °C in a 3-mL reaction mix containing L-tyrosine. Laccase solution (LAC, unknown concentration), *Cerrena unicolor*, was obtained from the Biochemistry Department, Maria Curie Sklodowska University, Lublin, Poland. Gallic acid (GA), catechol and potassium sorbate (>99.9%) were also purchased from Sigma–Aldrich; phenol, $Na_2HPO_4{\cdot}2H_2O$, ethanol (99.9%) and Folin–Ciocalteu reagent were obtained from Merck; KH_2PO_4, Na_2CO_3, $NaHCO_3$, methanol, tartaric acid, $C_4H_8O_6$, sodium sulfate(IV), $Na_2SO_3{\cdot}7H_2O$, iron(III) chloride, $FeCl_3{\cdot}6H_2O$ and glucose were purchased from POCh (Gliwice, Poland); putrescine was obtained from Fluka (St. Louis, MO, USA). All reagents were of analytical reagent grade purity. Solutions were prepared using double-distilled water.

2.2. Apparatus

Digital multimeter BM857 (Brymen, New Taipei City, Taiwan) together with BS85x Data Logging System V5.1.0.4 (2) software was used for chronoamperometric measurements. Glassy carbon (carbon vitreous foil 2 mm; Goodfellow, Cambridge, UK) working electrode was shaped to the form of a disc (d = 1.0 cm), glued to a poly(methyl methacrylate) block using epoxy resin, and used after polishing with 0.3 μm alumina (Buehler Micropolish, Lake Bluff, IL, USA). Copper wire and conducting glue were employed to provide electrical contact to the back of the electrode. Ag/AgCl electrode was prepared by anodizing silver wire in an HCl solution. To increase the area of the electrode the wire was made in the shape of a coil. CP-501 pH-meter (Elmetron, Zabrze, Poland) with combined glass electrode (ERH-11 type; HYDROMED, Gliwice, Poland) was used for pH measurements.

2.3. Sensor construction

The sensor was constructed according to the procedure described before (Adamski et al., 2010). In short, a small droplet of the solution of analyte and enzyme was placed on the surface of a circular glassy carbon electrode. Glass tubing ending with a glass frit was positioned over the GC electrode (GCE) in such a manner that the investigated solution formed a thin (0.5 mm) layer between the surface of the GC electrode and the surface of the glass frit. A high-area Ag/AgCl electrode was placed in the glass tubing with saturated KCl solution. Passing small current through the Ag/AgCl electrode did not cause its polarization, so that the electrode served simultaneously as a reference electrode and as a counter electrode. The GCE working electrode and the Ag/AgCl electrode were connected through a resistor and current flowing in the circuit was measured as a potential drop on the resistor. The resistance (R = 100 Ω) was chosen in a way to make the potential drop on the resistor negligible (in our case usually less than 10 mV). To increase the sensitivity of the sensor, current was integrated for 10 min and the value correlated with the analyte concentration was the charge, not the instantaneous current.

2.4. Measuring procedures

2.4.1. Chronoamperometric measurements

The experimental conditions used were selected on the basis of earlier studies (Adamski et al., 2010). Analyte (gallic acid, GA) was dissolved in phosphate buffer (pH 7, concentration 0.05 mol/L) to obtain a predetermined concentration. In the case of tyrosinase 1 μL of 5 mg/mL solution of enzyme was mixed with 100 μL of analyte solution. In the case of laccase 5 μL of enzyme solution were mixed with 95 μL of analyte solution. The mixture of the analyte solution and enzyme solution was then shaken for 1 min. Then the reaction mixture was poured into the crevice between the GCE and the reference electrode. Chronoamperometric response was recorded within 10 min and numerically integrated. Calibration curves were constructed as the $Q_{corr} = f(c_{phenol})$ dependence, where Q_{corr} was the charge corrected for the blank signal. Between measurements the electrode surface was cleaned with double distilled water and methanol.

Analyses of wine samples (red and white type) were carried out using standard addition method (SAM). Wine samples were analyzed according to the same protocol as described above after 25-, 50- or 100 fold dilution with phosphate buffer (pH 7, 0.05 mol/L), depending on their polyphenols content and the enzyme solution used. Due to the fact that white wines contain less phenolic compounds the range of gallic acid concentration used in construction of calibration plot for white wines was narrower (typically 0–136 mg GA/L) compared to red ones (typically 0–272 mg GA/L).

2.4.2. Spectrophotometric Folin–Ciocalteu method

Spectrophotometric analysis of the total phenolic content by means of Folin–Ciocalteu method (F–C) was performed according to Waterhouse and Wrolstad (2001). Because of the matrix effects