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The advantages of disposable screen-printed biosensors in a bioelectronic tongue for the analysis of grapes

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ABSTRACT

Disposable screen-printed sensors have been modified with enzymes and used to form a bioelectronic tongue dedicated to the discrimination between different grape varieties. The multisensory system combined serigraphied electrodes modified with carbon, platinum, gold, graphene, Prussian blue and nickel oxide nanoparticles (M-SPE) covered with glucose oxidase (M-GOX-SPE) or tyrosinase (M-Tyr-SPE).

The M-GOX-SPE and M-Tyr-SPE sensors produced a variety of responses due to the different behavior of the electron mediators of the six screen-printed materials used for the electro-catalysis of the glucose and phenols by means of glucose oxidase and tyrosinase.

This variety of responses, together with the capability of the sensors to detect glucose or phenols, allowed the bioelectronic tongue developed here to discriminate between the juices obtained from different varieties of grape.

Partial least-squares (PLS-1) multivariate calibration of electrochemical data has been successfully applied to the simultaneous determination of glucose and polyphenols in musts.

The discrimination capability shown by this array of cheap and single-use sensors was similar to that found in other complex bioelectronic tongues. The lower price, ease of use and portability of the modified screen-printed electrode system makes the bioelectronic tongue developed here an alternative tool that can be used in situ in the vineyard block.

1. Introduction

The use of accurate techniques for the analysis of grapes is an important need in the wine sector. Glucose and polyphenols are among the analytes that need to be monitored and measured in order to guarantee the maturity of the grapes and the quality of the final product. In the laboratory, the assessment of grape maturity is performed by analyzing representative samples by standard analytical techniques such as spectroscopy, chromatography (to measure phenols) or refractometry (to measure the sugar content in °Brix) (Blouin & Guimberteau, 2000). In the last few years, a variety of sensors dedicated to the grape analysis have been developed. Most of them are fiber-optic fluorescence sensors that measure the levels of anthocyanins and chlorophyll (Ghozlen, Cerovic, Germain, Toutain, & Latouche, 2010), or near-infrared optical sensors that can assess the sugar content (°Brix) (Herrera, Guesalaga, & Agosin, 2003). Electrochemical glucose and polyphenol biosensors are a powerful alternative for monitoring such compounds due of their specificity, high sensitivity and short response time (Barroso, De los Santos Alvarez, Delerue-Matos, &

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Oliveira., 2011; Carralero Sanz, Mena, González-Cortés, Yáñez-Sedeño, & Pingarrón, 2005; Wang, 2008).

Typical enzymatic electrodes, based on glucose oxidase (or tyrosinase), undergo several electrochemical steps which produce a measurable current that is linearly related to the glucose (or polyphenol) content. The active sites of glucose oxidase and phenoloxidases are not accessible. In order to improve the electron transfer, electron mediators must be included in the sensor (Barroso et al., 2011; Chaubey & Malhotra, 2002; Ricci, Amine, Palleschi, & Moscone, 2003; Wang, 2008). Nanotechnology has opened up new opportunities to obtain more efficient electron mediators, such as metal nanoparticles or nanocarbons (i.e. carbon nanotubes or graphene) (Carralero et al., 2005; Rahman, Ahammad, Jin, Ahn, & Lee, 2010).

The analysis of complex mixtures such as wines will be improved by using the so-called electronic tongues, a new class of instruments that provide global information about the sample, instead of information about specific compounds. There have been a range of portable electronic tongues which have been produced based on a numerous core sensing technologies. including mass, optic, electrical or electrochemical transduction (Baldwin, Bai, Plotto, & Dea, 2011; Haddi et al., 2014; Riul, Dantas, Miyazaki, & Oliveira, 2010; Rodríguez-Méndez, Medina, De Saja, Apetrei, & Muñoz, 2012; Sghaier, Barhoumi, Maaref, Siadat, & Jaffrezic-Renault, 2009; Sliwinska, Wisniewska, Dymerski, Namiesnik, & Wardencki, 2014; Smyth & Cozzolino, 2013). Arrays of electrochemical sensors have been successfully used to discriminate between wines with different organoleptic characteristics (Apetrei et al., 2012; Cetó, Apetrei, Del Valle, & Rodríguez-Méndez, 2014; Cetó et al., 2012; Gay et al., 2010; Gil-Sánchez et al., 2011; Gutiérrez, Moreno-Barón, Pividori, Alegret, & Del Valle, 2010; Parra, Hernando, Rodríguez-Méndez, & De Saja, 2004; Rodríguez-Méndez et al., 2014; Zeravik, Hlavacek, Lacina, & Skládal, 2009) or grapes of different qualities (Gutiérrez et al., 2011; Moreno i Codinachs et al., 2008). It has also been established that arrays combining sensors and biosensors can be advantageous, as they bring together both the benefits of classical arrays of electrochemical sensors (which provide global information about the sample) with the specificity of the enzyme–substrate reaction typical of biosensors (Medina-Plaza, De Saja, & Rodríguez-Méndez, 2014; Medina-Plaza et al., 2014; Moreno i Codinachs et al., 2008).

In spite of the good results obtained with electronic and bioelectronic tongues, the use of liquid electrochemical cells and heavy potentiostats, the high cost of the sensors and the need for periodic calibration all restrict their use to the laboratory. It would be desirable to develop multisensor systems using disposable electrodes that could be used in the vineyard block.

During recent years, the screen-printing (thick film) technology, applied to sensor and biosensor construction, has been considerably improved. Screen-printed electrodes (SPEs) are in fact simple to prepare, rapid and versatile, and this technology also appears to be the most economical. Their low cost makes possible to use them as disposable electrodes. SPE containing a serigraphied pseudo-reference Ag/AgCl electrode, a counter electrode and a working electrode in the same device, can be prepared using a variety of modifiers that include metals, metal oxides, nanocarbons, nanoparticles, phthalocyanines, conducting polymers, or prussian blue, among many others (Metters, Kadara, & Banks, 2011). Moreover, enzymes can be immobilized on modified screen-printed electrodes to take advantage of the electron mediator properties of many of these materials for biosensing.

The aim of this work was to develop a bioelectronic tongue, based on an array of disposable screen-printed electrodes, dedicated to the analysis of grapes. For this purpose, six SPE sensors, modified with different materials (M-SPE) were selected, including (a) three classical electrode materials: graphite (C-SPE), platinum (Pt-SPE) and gold (Au-SPE) (b) two nanostructured electrocatalytic materials: graphene (GPH-SPE) and nickel oxide nanoparticles (NiONP-SPE) and (c) materials with well-known electron mediator properties and Prussian Blue (PB-SPE). Then, glucose oxidase (GOX) or Tyrosinase (Tyr) were immobilized on the surface of the M-SPE electrodes to obtain M-GOX-SPE and M-Tyr-SPE biosensors that could provide information about the glucose and phenol content respectively. After the optimization of the measurement conditions and the evaluation of the cross-selectivity of the sensors, the array was used to analyze musts prepared from different varieties of grapes. The discriminatory capability of the sensor array was investigated using Principal Component Analysis (PCA).

2. Material and methods

2.1. Chemicals

All chemicals and solvents were purchased from Sigma–Aldrich. Solvents were of reagent grade and used as supplied. Deionized water was obtained from a Millipore purifier, with a resistivity of 18.2 MΩ/cm.

Glucose oxidase (GOX), from *Aspergillus niger*, type VII (activity plus 0.001 kat/mg), and Tyrosinase (Tyr), from *Mushroom* (activity of 6.10^{-5} kat/mg), were purchased from Sigma Chemical Co. (USA).

2.2. Grape samples

Samples of five varieties of red grapes (Tempranillo, Garnacha, Cabernet-Sauvignon, Prieto Picudo and Mencía) were harvested in September 2013. For each variety, berries were collected in the vineyards of the 'Bodega Cooperativa de Cigales' and of the 'Instituto Tecnologico Agrario de Castilla y León', both located in the Valladolid area of Castilla y León (Spain). To obtain the musts, 200 berries were introduced in a plastic bag and crushed for 1 min manually. For each variety of grape, this process was carried out by septuplicate, giving a total of 35 samples. The musts were analyzed after separation from the seeds and the peel. The Oenological Center of Castilla y León carried out the chemical analysis of the grapes following international regulations (OIV, 2013). Parameters analyzed included the chemical indicators of the sugar content (°Brix) and of the polyphenolic content (Total Polyphenol Index: TPI). Total acidity (expressed as g/L of tartaric acid) and pH were also analyzed, due to the influence of the pH and the ionic strength in the enzymatic activity. The results are collected in Table 1.

2.3. Screen-printed electrodes and biosensors

Six screen-printed electrodes modified with different materials (M-SPE), purchased from Dropsens (www.dropsens.com), were used as substrates for the deposition of enzymes. Each sensor device contained a serigraphied pseudo-reference Ag/AgCl electrode (RE), a counter electrode (C or Pt) (CE) and a working electrode. As

Table 1
Results of the chemical analysis of grapes carried out by traditional chemical methods (seven replicas).

Grape	°Brix	pH	Total acidity (g/L)	Total polyphenol index
Prieto Picudo	23 ± 2	3.61 ± 0.08	4.8 ± 0.2	15 ± 3
Mencia	21 ± 2	4.18 ± 0.08	4.3 ± 0.2	13 ± 3
Tempranillo	24 ± 2	4.20 ± 0.08	4.1 ± 0.2	21 ± 4
Cabernet-Sauvignon	23 ± 2	3.65 ± 0.08	4.1 ± 0.2	16 ± 3
Garnacha	23 ± 2	3.39 ± 0.08	4.4 ± 0.2	15 ± 3