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Voltammetric e-tongue for the quantification of total polyphenol content in olive oils

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ABSTRACT

This work presents the application of a voltammetric electronic tongue made from an array of polypyrrole modified screen printed electrodes in the qualitative and quantitative analysis of phenolic compounds found in virgin olive oils. Virgin olive oil samples in the form of emulsions were analyzed by sensors using cyclic voltammetry. The cyclic voltammograms show peaks related to redox processes related to polypyrrole and phenolic compounds presents in the emulsions. The obtained responses were pre-processed employing kernel method in order to extract significant information from the voltammetric signals. These coefficients were used as matrix input in multivariate data analysis. Additionally, the kernel coefficients were used in multivariate calibration method by partial least squares, which accomplished the quantification of total polyphenol content. Training and test sample results were compared with the ones obtained with the spectrophotometric method. High significant correlation coefficient of 0.9976 and 0.9884 was accomplished in calibration and prediction, in the range from 111.75 to 482.42 mg × kg^{-1}. Qualitative discrimination of different phenolic compounds found in olive oils was also evaluated by principal component analysis and partial least squares discriminant analysis when these are added in olive oil emulsions.

1. Introduction

Olive oil is the main lipid source in the Mediterranean diet and is obtained from the fruit of the olive tree, *Olea europea* L. An extra virgin olive oil (EVOO) is obtained solely by mechanical or physical means under controlled conditions that do not lead to alteration in the oil (Harwood & Aparicio, 2000). EVOO chemical composition determines its intrinsic quality and could be influenced by agronomical and technological factors, such as olive variety (Rondanini, Castro, Searles, & Rousseaux, 2011), degree of maturation (Camposeo, Vivaldi, & Gattullo, 2013), and the technological process (Clodoveo, 2012; Guillaume, Ravetti, Ray, & Johnson, 2012) among others (Tsimidou, 2006).

The health benefits of EVOO concern the ability to prevent diseases that may be related to oxidative damages (heart diseases, different types of cancers etc.) (Giovannini & Masella, 2012; Iriti & Vitalini, 2012; Quiles, Ramírez-Tortosa, & Yaqoob, 2006) is the result of its specific composition including phenolic compounds, tocopherols, and carotenoids, well-known as antioxidants (Bakhouche et al., 2013; Boskou, Tsimidou, & Blekas, 2006). An EVOO contains at least 30 phenolic compounds that belong to the following classes: tyrosol, derivatives; derivatives of 4-hydroxybenzoic, 4-hydroxyphenylacetic, and 4-hydroxycinnamic acids; lignans, and flavonoids (Boskou et al., 2006).

The interest in developing analytical methods that have attained a sufficient level of reliability and can fully respond to the requirements of accuracy, precision and rapidity is still high.

Usually, the determination of total polyphenol content is carried out by spectrophotometric (Carrasco-Pancorbo et al., 2005; Herchi et al., 2011) or chromatographic (Dierkes et al., 2012; García-Villalba et al., 2010) methods. A relative small number of studies have been carried out aimed at developing sensors or biosensors able to work in non aqueous solvents (Campanella, Favero, Pastorino, & Tomassetti, 1999; Grassino, Milardović, Grabarić, & Grabarić, 2011). Numerous sensors and biosensors have been developed to determine phenolic compounds in aqueous solutions or emulsions (Apetrei, 2012; Pavinatto et al., 2011; Rodríguez-Méndez, Apetrei, & de Saja, 2008). However, the analysis of the total polyphenolic content of olive oil is characterized by particular features and needs due to the solubility of oils in organic solvents and insolubility in water.

In recent years there has been an emergent interest in the development of sensor systems, so called electronic tongue, for food and beverage applications. The e-tongue is a system that consists of an array of non-specific chemical sensors combined with appropriate data acquisition systems and chemometric tools. Different sensors and in the last years biosensors have been used for the construction of arrays for e-tongues, especially based on electrochemical transduction methods (Bulbarello, Cuenca, Schweikert, Mannino, & Scampicchio, 2012; Cetó, Céspedes, & Del Valle, 2012; del Valle, 2010; Escuder-Gilabert & Peris, 2010; Ghasemi-Varnamkhasti et al., 2012; Kirsanov et al., 2013; Kutyła-Olesiuk, Nowacka, Wesoły, & Ciosek, 2012; Lvova, Di Natale, &

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Paolesse, 2013; Riul, Dantas, Miyazaki, & Oliveira, 2010; Winquist, Olsson, & Eriksson, 2011). The data analysis has been usually carried out by means of principal component analysis (PCA) (Kirsanov et al., 2013), partial least square discriminant analysis (PLS-DA) (Kutyła-Olesiuk et al., 2012; Lvova et al., 2013), linear discriminant analysis (LDA) (Ghasemi-Varnamkhasti et al., 2012), Soft Independent Modeling of Class Analogies (SIMCA) (Gutiérrez et al., 2011), artificial neural networks (ANN) (Cetó et al., 2012; Ghasemi-Varnamkhasti et al., 2012) or support vector machines (SVM) (Liu, Wang, Wang, & Li, 2013). In the future, such systems could become alternatives or complementary tools for relative expensive and time-consuming procedures in food analysis, both chemical and sensory, as simple and fast devices for routine food quality control. At to the date, the e-tongue systems based either on the use of voltammetric sensors (Cetó et al., 2012) or potentiometric sensors (Gutiérrez et al., 2011) have been used for estimation of polyphenols in wines.

A small number of studies devoted to analyze olive oils using e-tongues have been reported in literature (Rodriguez-Mendez, Apetrei, & de Saja, 2009). Different strategies for olive oil analyses were employed such as carbon paste electrodes modified with EVOOs (Apetrei, Rodríguez-Méndez, & De Saja, 2005; Apetrei, Gutierez, Rodríguez-Méndez, & de Saja, 2007; Apetrei et al., 2010), extraction of polar fraction of EVOOs and analysis with chemically modified sensors (Rodríguez-Méndez et al., 2008), analysis of EVOO emulsions prepared with Triton X-100 with polypyrrole based sensors (Apetrei, 2012), amperometric detectors based on glassy carbon electrodes and dilution of sample with containing 2% acetic acid and 3.2% tetrabutylammoniumbromide (Cosio, Ballabio, Benedetti, & Gigliotti, 2007), and platinum microelectrode directly in edible oil (Oliveri, Baldo, Daniele, & Forina, 2009).

The objective of this work is to develop a novel method for qualitative and quantitative analysis of phenolic compounds found in extra virgin olive oils. The e-tongue system proposed here has been used to analyze the emulsions of EVOOs prepared by using an anionic surfactant, sodium dodecyl sulfate. The sensors array included six screen-printed electrodes modified with polypyrrole doped with different doping agents. The overall response of extra virgin olive oil emulsions has been registered by cyclic voltammetry. The discrimination and classification capability of the e-tongue has been evaluated by PCA, PLS-DA and SIMCA. Predictive models which accomplished the quantification of total polyphenol content have been constructed by means of partial least squares. Qualitative discrimination of different phenolic compounds added in olive oil emulsions was also evaluated.

2. Experimental

2.1. Reagent and solutions

All reagents used were analytical reagent grade and all solutions were prepared using ultrapure water from a Milli-Q system (Millipore, Simplicity®). Tyrosol (TY), vanillic acid (VA), p-coumaric (pCA), caffeic acid (CA), gallic acid (GA) and pyrrole were purchased from Sigma-Aldrich. Methanol, Folin–Ciocalteu's reagent, potassium hexacyanoferrate(II), potassium nitroprusside, phosphotungstic acid, sulfuric acid, sodium decanesulfonate, 9,10-anthraquinone-2-sulfonic acid sodium salt and sodium carbonate, all from Sigma-Aldrich, were used in this study also.

2.2. Apparatus

Voltammetric measurements were carried out on a Biologic Science Instruments SP 150 potentiostat/galvanostat using the EC-Lab Express software. An Elmasonic S10H ultrasonic bath was used for dissolving and homogenization of solutions. For the spectrophotometric studies an UV–vis spectrophotometer from Labomed Inc. connected to a PC (software UVWin) were used. A P-Selecta Ultrasons ultrasonic bath and a Griffin flask shaker were used for the preparation of emulsions.

2.3. Sensors and electrochemical cell

Screen-printed carbon electrodes (4 mm diameter, A = 12.56 mm^2) purchased from Dropsens (www.dropsens.com, model SP 150) were used as working electrode for polypyrrole deposition. A three-electrode configuration was used in all cases, a Princeton Applied Research Ag|AgCl/KCl 3 mol × L^{-1} and a Pt plate being used as reference electrode and counter electrode, respectively. After modification, polypyrrole modified SPE (Ppy-SPE) was used for virgin olive oil emulsion analysis. In these experiments the reference and the counter electrode integrated in the device were used (counter electrode — platinum, reference electrode — silver).

Cyclic voltammograms were registered from −1.3 to +0.5 V (the scan started at 0 V) at a sweep rate of 0.05 V × s^{-1} (except otherwise indicated).

The polypyrrole films were electrochemically synthesized from an aqueous solution containing 0.1 mol × L^{-1} pyrrole and 0.1 mol × L^{-1} doping agent (except phosphotungstic acid and 9,10-anthraquinone-2-sulfonic acid sodium salt where the concentration used were 0.01 M), by chronoamperometry at a fixed potential of 0.8 V.

The doping agents employed for the fabrication of polypyrrole sensors were potassium hexacyanoferrate(II) (FCN), potassium nitroprusside (NP), phosphotungstic acid (PWA), sulfuric acid (H_2SO_4), sodium decanesulfonate (DSA), and 9,10-anthraquinone-2-sulfonic acid sodium salt (AQS).

The conditions of electropolymerization used in the development of polypyrrole sensors were presented in Table 1.

Once prepared, the modified polymeric electrodes were extracted from the synthesis solution and washed thoroughly.

2.4. Olive oil samples

A total set of 18 extra virgin olive oil samples from Greece, Spain and Italy were acquired from local supermarkets and analyzed. Samples were chosen in order to obtain a set with different total polyphenol content (Table 2).

Total polyphenol content was ranging from 111.75 to 482.42 mg × kg^{-1}, from small values to high values typically reported for EVOOs (Baiano et al., 2009).

2.5. Determination of total phenolic content

Total phenolic content was determined spectrophotometrically following the Folin–Ciocalteu spectrophotometric method described by Herchi et al. (2011) with slight modifications. A 1 g virgin olive oil sample was weighed, dissolved in 10 mL hexane and transferred to a separatory funnel. Then, 20 mL of a methanol–water mixture (80:10 v/v) was added. After 3 min of shaking, the methanol–water layer was separated. The extraction process was repeated trice and the methanol–water phases were combined. The methanol–water extract was driven to dryness in a rotary evaporator under vacuum at 40 °C. The dry residue was then dissolved in 1 mL of methanol. The

Table 1
The conditions of electropolymerization employed in the development of polypyrrole sensors.

Doping agent	Electrochemical technique	Conditions
DSA	Chronoamperometry	0.8 V; 480 s
FCN		0.8 V; 240 s
NP		0.8 V; 360 s
AQS		0.8 V; 720 s
H_2SO_4		0.8 V; 240 s
PWA		0.8 V; 720 s