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Antioxidant potential of white grape pomaces: Phenolic composition and antioxidant capacity measured by spectrophotometric and cyclic voltammetry methods

M. José Jara-Palacios [a], Dolores Hernanz [b], M. Luisa Escudero-Gilete [a], Francisco J. Heredia [a,*]

[a] Food Colour& Quality Laboratory, Dept. Nutrition & Food Science, Universidad de Sevilla, Facultad de Farmacia, 41012 Sevilla, Spain
[b] Department of Analytical Chemistry, Universidad de Sevilla, Facultad de Farmacia, 41012 Sevilla, Spain

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ABSTRACT

Antioxidant potential of white grape pomaces from nine different varieties has been evaluated and compared to Zalema variety. The detailed phenolic composition was measured by RRLC/MS, the total phenolic content (TPC) by Folin–Ciocalteu method, and the antioxidant activity by ABTS assay and cyclic voltammetry (CV). Grape pomaces exhibited different quantitative phenolic profiles and different antioxidant activities, with significant differences ($p < 0.05$). Parellada, Zalema, Sauvignon blanc and Moscatel showed the highest values of TPC and ABTS. The total flavanol, flavonol and phenolic acid contents were significantly correlated to the electrochemical parameter anodic peak current ($I_{p,a}$). Finally, a stepwise linear discriminant analysis (SLDA) was carried out, and Zalema variety was differentiated from other varieties based on the total flavonol content, mainly quercetin-3-*O*-glucoside.

1. Introduction

Grapes are one of the major fruit crops and about 80% of the harvest is used by the winemaking industry. Winemaking is a seasonal activity, which leads to the generation of large quantities of wastes during a short period every year (grape harvesting), especially in high production regions. Accumulation of these wastes is a serious environmental problem and its removal is necessary. Traditionally, winemaking byproducts have been sent to distilleries for obtaining ethanol, or to be used as fertilizers or biomass but these activities are usually carried out by external companies representing economic costs for the wine industry. Therefore, alternative solutions for the exploitation and valorization of those byproducts are very interesting because it would involve economic, social and environmental advantages (Devesa-Rey et al., 2011; Lavelli, Sri Harsha, Torri, & Zeppa, 2014; Pedroza, Carmona, Pardo, Salinas, & Zalacain, 2012).

Byproducts from winemaking, such as grape pomace, have received much attention because they contain large amounts of phenolic compounds, which have antioxidant properties and benefits on human health (Jayaprakasha, Selvi, & Sakariah, 2003; Rockenbach et al., 2011). Seeds, skins and stems of grape pomace exhibit different qualitative and quantitative phenolic profiles and different antioxidant activities (Jara-Palacios et al., 2014; Rodríguez Montealegre, Romero Peces, Chacón Vozmediano, Martínez Gascueña, & García Romero, 2006).

White grape must is not usually fermented with the solid parts of the grape and then higher proportions of phenolic compounds remain in the grape pomace from white grapes than from red grapes. On the other hand, the phenolic composition of grape pomace depends on the variety of grape and, is influenced by agroclimatic factors. There are reports emphasizing the influence of the grape variety, agricultural practices, agroclimatic factors and type of soil on the chemical composition of the grapes (Rodríguez Montealegre et al., 2006; Ruberto et al., 2007).

Different techniques have been used for the separation, identification and quantification of phenolic compounds, being the high performance liquid chromatography (HPLC) the most commonly used (Fontana, Antoniolli, & Bottini, 2013). However, the advantages of the rapid resolution liquid chromatography (RRLC), which shows high resolution and sensitivity, and short retention times, are increasing the use of this technique.

Antioxidant activity has been widely measured by several *in vitro* methods such as spectrophotometric methods (ABTS, FRAP, DPPH and ORAC assays) (Floegel, Kim, Chung, Koo, & Chun, 2011) and electroanalytical methods, such as cyclic voltammetry (CV). CV has been successfully applied to the total antioxidant capacity measurement in plant extracts, wines, and juices (Chevion, Chevion, Chock, & Beecher, 1999; Kilmartin & Hsu, 2003; Kilmartin, Zou, & Waterhouse, 2002;

* Corresponding author. Tel.: +34 954556495; fax: +34 954556110.
E-mail address: heredia@us.es (F.J. Heredia).

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Makhotkina & Kilmartin, 2012; Sousa, da Rocha, Cardoso, Silva, & Zanoni, 2004) and it has become an alternative to traditional spectrophotometric techniques (Sánchez Arribas, Martínez-Fernández, & Chicharro, 2012; Tufan, Baki, Güçlü, Ozyürek, & Apak, 2014).

Spain is a geographical area with the typical climatological conditions of warm climate. Many white grape varieties grow in different areas and are used to the 'production of wines with differences in sensory characteristics and phenolic composition, for example, Spanish autochthonous white varieties such as Zalema, Verdejo, Airén, Moscatel, Montepila, Pedro Ximénez, Baladí, Parellada, and originating in other regions such as Sauvignon blanc.

Zalema is a white grape variety grown exclusively in southwestern Spain. Previous studies about the phenolic composition and antioxidant activity of winemaking byproducts from Zalema have reported the possibility of applications based on reusing these byproducts (Jara-Palacios, Hernanz, et al., 2014; Jara-Palacios et al., 2013; Jara-Palacios et al., 2014). In this sense, the aim of this work was to evaluate the differences, in the antioxidant potential, between nine white grape pomaces and to compare them to Zalema variety.

2. Materials and methods

2.1. Standards and reagents

Formic acid, acetic acid, HPLC-grade acetonitrile, methanol, and Folin–Ciocalteu reagent were obtained from Panreac (Barcelona, Spain). ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were purchased from Fluka (Madrid, Spain).

Gallic acid, (+)-catechin, (−)-epicatechin, quercetin, kaempferol, ferulic acid, caffeic acid, *p*-coumaric acid, sodium carbonate, sodium acetate, potassium persulfate and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (Madrid, Spain). Quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside were obtained from Extrasynthese (Lyon, France).

2.2. Samples

The pomaces from white grape varieties grown in "Montilla-Moriles" Designation of Origin (Cordoba, south-eastern Spain), with the typical climatological conditions of warm climate regions, were supplied by "Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA)" experimental vineyard (Cabra, Spain), when the grapes were at technological ripeness (12°–13° Baumé). Nine varieties, Zalema (Z), Pedro Ximénez (PX), Moscatel (MG), Baladí (B), Parellada (P), Sauvignon blanc (SB), Montepila (M), Airén (A) and Verdejo (V) were evaluated. All varieties were grown in the same warm climate vineyard in order to evaluate the differences only due to the grape variety and not influenced by different climates.

2.3. Sample preparation and extraction

A sample of 100 g of clusters (including grapes and stems) was manually pressed to mix the skins, the seeds and the stems. The obtained must was discarded, and the resulting solid sample (grape pomace) was weighed and freeze-dried.

The dry pomaces were extracted with 75% methanol according to the methodology described by Jara-Palacios, Hernanz, et al. (2014). The extractions were carried out in triplicate and the obtained extracts were used for analysis.

2.4. Total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay (Singlenton & Rossi, 1965) with some modifications (Jara-Palacios, Hernanz, et al., 2014). Gallic acid was employed as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/100 g of dry pomace (DP)).

2.5. Individual phenolic compounds

The individual phenolic compounds were determined by RRLC/MS following the method described by (Jara-Palacios, Hernanz, et al., 2014). Phenolic compounds were identified by their retention time, UV–vis spectra and mass spectra, as well as by comparison with our data library and standards when available. The corresponding calibration curves were made up of ten standards: catechin, epicatechin, gallic acid, caffeic acid, ferulic acid, *p*-coumaric acid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside. Procyanidins were quantified with the calibration curve of catechin. Caftaric, fertaric and coutaric acids were quantified using the calibration curves of caffeic, ferulic and coumaric acids, respectively. Quercetin and isorhamnetin derivatives were quantified as quercetin-3-*O*-glucoside and kaempferol derivates as kaempferol-3-*O*-glucoside.

Each extract was injected three times ($n = 9$) to quantify each compound, and the results were expressed as mg phenolic compound/100 g of DP. Total flavanols, total flavonols and total phenolic acids were also estimated by summing the content of each individual phenolic compound identified by RRLC.

2.6. ABTS/persulfate assay

The $ABTS^{\bullet+}$ radical was produced by the oxidation of 7 mM ABTS with potassium persulfate (2.45 mM) in water (Re et al., 1999). The mixture was allowed to stand in the dark at room temperature for 16 h before use, and then the $ABTS^{\bullet+}$ solution was diluted with PBS at pH 7.4 to give an absorbance of 0.7 ± 0.02 at 734 nm. The extracts (50 μL) of pomace were mixed with 2 mL of the $ABTS^{\bullet+}$ diluted solution, vortexed for 10 s, and the absorbance measured at 734 nm after 4 min of reaction at 30 °C. Different dilutions of each extract were assayed and the results were obtained by interpolating the absorbance on a calibration curve obtained with Trolox (30–1000 μM). Three independent experiments were performed in triplicate for each of the assayed extracts and the results were expressed as Trolox-equivalent antioxidant capacity (TEAC; millimoles of Trolox with the same antioxidant capacity as 100 g DP).

2.7. Electrochemical assays

A potentiostat/galvanostat (AUTOLAB model PGSTAT 302 N) controlled by a General Purpose Electrochemical System (GPES) software (Metrohm Autolab B.V., Utrecht, The Netherlands) was used for all electrochemical measurements.

1 mL of the extracts was diluted with 0.1 M sodium acetate–acetic acid buffer at pH 3.6 (Rebelo, Rego, Ferreira, & Oliveira, 2013). The diluted sample was transferred into a glass water-jacketed electrochemical cell (EG&G, Princeton, NJ) connected to a circulator that held the sample temperature at 25.0 ± 0.5 °C. Prior to the measurements, the electrolyte solutions were de-aerated with an inert gas (N_2) for 10 min. All measurements were carried out at room temperature using a conventional three-electrode system consisting of a glassy carbon working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode. The cyclic voltammogram scans were made from 0.0 to 1.0 V at a scanning rate of 5 mV/s.

The electrochemical parameters extracted from the cyclic voltammetry curves were the anodic current area (Q), the anodic peak current ($I_{p,a}$), and the anodic peak potential ($E_{p,a}$) of the main peaks in the cyclic voltammograms. Q^{I} represents the integrated area of the cyclic voltammogram for scans taken from 0.12 to 0.32 V, Q^{II} from 0.35 to 0.55 V, and Q^{III} from 0.65 to 0.85 V. All of the cyclic voltammograms were recorded in duplicate.