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

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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 a different quantitative phenolic profile 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 flavanols, flavonols and phenolic acids 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 to the total flavonols content, mainly quercetin-3-*O*-glucoside.

Keywords

Antioxidant potential, grape pomace, phenolic compounds, cyclic voltammetry (CV), rapid resolution liquid chromatography (RRLC).

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; Pedroza, Carmona, Pardo, Salinas, & Zalacain, 2012; Lavelli, Sri Harsha, Torri, & Zeppa, 2014).

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 a different qualitative and quantitative phenolic profile and different antioxidant activities (Rodríguez Montealegre, Romero Peces, Chacón Vozmediano, Martínez Gascueña, & García Romero, 2006; Jara-Palacios et al., 2014a).

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, Zou, & Waterhouse, 2002; Kilmartin, & Hsu, 2003; Sousa, da Rocha, Cardoso, Silva, Zanoni, 2004; Makhotkina, & Kilmartin, 2012) 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 wine 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 et al., 2013; Jara-Palacios et al., 2014a; Jara-Palacios et al., 2014b). 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-ethylbenzothiazolne-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 persulphate 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 grapes 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 et al. (2014a). The extractions were carried out in triplicate and the obtained extracts were used for analysis.

2.4. Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay (Singlenton, & Rossi, 1965) with some modifications (Jara-Palacios et al., 2014a). 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 et al., 2014a). 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/persulphate assay

The $ABTS^{\bullet+}$ radical was produced by the oxidation of 7 mM ABTS with potassium persulphate (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•+ 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 ºC. 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 voltammograms 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.

2.8. Statistical analysis

One way analysis of variance (ANOVA) was applied to determine whether significant differences ($p<0.05$) exist among the different grape pomace vatieties. In addition, simple and multiple correlations between the contents of phenolic compounds and the antioxidant activity, measured by ABTS method and CV, were studied. In all cases, statistically significant level was considered at $p<0.05$.

Pattern recognition techniques (PR), like stepwise linear discriminant analysis (SLDA), were applied on experimental standardized data in order to classify different grape pomace varieties.

These statistical analyses of the data were performed using the Statsoft Statistica® V 8.0 software (StatSoft, 2007).

3. Results and discussion

3.1. Phenolic composition

The average TPC for pomaces of white grape varieties ranged from 455 mg/100 g DP (Baladí variety) to 3113 mg/100 g DP (Parellada variety) (Table 1). The comparison among the nine studied grape varieties reveals that Parellada, Zalema, Sauvignon blanc and Moscatel varieties exhibited higher TPC (3113, 2513, 2305 and 2194 mg/100 g DP, respectively) than the other varieties. These values are in accordance with results published by Anastasiadi, Pratsinis, Kletsas, Skaltsounis, and Haroutounian (2010), which studied the TPC in skin and seeds from white grape varieties (values ranged 65-3300 mg/100 g DP).

Thirteen flavanols (catechin, epicatechin, procyanidins B1, B2, B3, B4, B7 and B2-3-*O*-gallate, two trimers, two tetramers and one galloylled procyanidin), ten flavonols (five quercetin, three kaempferol and two isorhamnetin derivatives) and five phenolic acids ((a) benzoic acids: gallic acid; (b) hydroxycinnamoyl derivatives: caftaric, fertaric, and cis- and trans-coutaric acids) were identified and quantified.

In all varieties, flavanols were the most abundant phenolic compounds with concentrations from 874 mg/100 g DP (Moscatel variety) to 315 mg/100 g DP (Montepila variety). These concentrations were statistically different ($p<0.05$) among the studied varieties. Flavonols were the second phenolic group, whose concentrations ranged from 146 mg/100 g DP (Zalema variety) to 33 mg/100 g DP (Sauvignon blanc variety). Zalema was the variety with highest flavonol concentrations, showing significant differences ($p<0.05$) with the other studied varieties. Some authors have reported the possibility of using flavonol profile as chemical markers for authenticity and differentiation of white grape cultivars (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2010; Andrade, Mendes, Falco, Valentao, & Seabra, 2001). The third phenolic group, in quantitative terms, was phenolic acids groups, its

levels ranged from 35 mg/100 g DP in Pedro Ximénez variety to 11 mg/100 g DP in Verdejo variety (Table 1).

Figure 1 shows the relative proportion of the different phenolic groups in the grape pomace of each variety. It is well known that in grape pomace, flavanols are mainly provided by the seeds, flavonols by the skins and phenolic acids by the stems (Jara-Palacios et al., 2014a).

Considering individual phenolic compounds, the quantitative data are summarized in Table 2. The chromatographic analysis of grape pomace samples revealed that the nine varieties did not differ in their qualitative phenolic profile. However, the ANOVA test revealed that they have a different quantitative profile.

Catechin was the most abundant flavanol in all studied varieties, being Moscatel variety which presented the highest concentration (264 mg/100 g DP) followed by Parellada, Sauvignon blanc and Airén varieties (201, 184 and 179 mg/100 g DP, respectively). In contrast, its isomeric epicatechin was detected in lower concentrations in all varieties, ranging from 86 to 19 mg/100 g DP for Moscatel and Verdejo, respectively.

The dimeric procyanidins B1, B2, B3, B4 and B7 were identified. Procyanidin B1 was the most abundant and was detected in large amount in Parellada, Moscatel and Airén varieties (135, 123 and 111 mg/100 g DP, respectively). Two trimeric procyanidins were also identified and quantified in the samples. Parellada, Moscatel and Zalema varieties presented the highest amounts of these compounds; however Verdejo and Pedro Ximénez showed the lowest concentrations. In addition, two tetrameric procyanidins were detected at very high concentrations in Parellada, Moscatel, Airén, Zalema and Sauvignon blanc varieties (Table 2). Two galloyled procyanidins were quantified, and procyanidin B2-3-*O*-gallate was very abundant, with concentrations between 137 mg/100 g DP for Moscatel variety and 30 mg/100 g DP for Baladí variety.

Regarding flavonols, the glycosides of quercetin, kaempferol and isorhamnetin were quantified, being quercetin-3-*O*-glucuronide and quercetin-3-*O*-glucoside the most abundant in all grape pomaces varieties. Quercetin-3-*O*-glucuronide is more abundant than quercetin-3-*O*-glucoside in Moscatel, Parellada, Sauvignon blanc and Airén varieties, while quercetin-3-*O*-glucoside concentration is higher in Zalema, Pedro Ximénez, Baladí, Montepila and Verdejo varieties. Zalema variety presented the highest concentrations of quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucuronide, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside (4, 52, 8 and 56 mg/100 g DP, respectively). Quercetin-3-*O*-glucuronide was also abundant in Baladí and Airén varieties (46 and 40 mg/100 g DP, respectively) followed by Pedro Ximénez and Moscatel varieties (32 mg/100 g DP), while the lowest values were found in Montepila and Verdejo varieties (14 and 12 mg/100 g DP, respectively). Quercetin-3-*O*-glucoside ranged between 56 and 12 mg/100 g DP, being most abundant in Zalema and Baladí varieties. Kaempferol-3-*O*-glucoside and kaempferol-3-*O*-galactoside were the most abundant kaempferol glycosides in Zalema variety (19 and 5 mg/100 g DP, respectively), followed by Pedro Ximénez variety (14 and 3 mg/100 g DP, respectively) and Moscatel variety (12 and 3 mg/100 g DP, respectively). Some of these varieties have been analyzed by other authors and mentioned flavonols were found (Rodríguez Montealegre et al., 2006; Castillo-Muñoz et al., 2010; Jara-Palacios et al., 2014a; Gordillo et al., 2014).

The main phenolic acid was caftaric acid, which had highest concentrations in Baladí variety (28 mg/100 g DP) followed by Airén, Pedro Ximénez and Zalema varieties (25, 22 and 20 mg/100 g DP, respectively), and the lowest values in Sauvignon blanc, Montepila and Verdejo varieties (8, 7 and 7 mg/100 g DP, respectively). Other cynnamoyl derivates (fertaricacid, and *trans*- and *cis*-coutaric acids), and gallic acid were detected at levels lower than 10 mg/100 g DP.

3.2. Antioxidant activity measured by ABTS assay

The antioxidant activity of grape pomaces extract from the nine varieties was measured by the ABTS assay and the results are shown in Table 1. The ABTS assay showed large significantly difference in antioxidant profile of varieties. Parellada, Zalema and Sauvignon blanc varieties, followed by Moscatel, had the highest ABTS values (59, 57, 54 and 31 millimoles TE/100 g DP, respectively). As can be seen, these varieties with the most antioxidant activity had highest TPC values (Table 1).

It is well known that antioxidant activity is related to phenolic content, and then a regression analysis was carried out to correlate the results. A high and significant correlation coefficient (R = 0.818) was found between the antioxidant activity and the TPC in all samples, which is in accordance with others authors (Guendez, Kallithraka, Makris, & Kefalas, 2005; Anastasiadi et al., 2010).

Moreover, three multiple regression analyses were carried out to determine the relative importance of phenolic compounds on the antioxidant activity. Then, the regression analysis between antioxidant activity (dependent variable) and flavanols, flavonols or phenolic acids contents (independent variables) was performed to assess the influence of these phenolic compounds. Correlations with flavanols and flavonols had higher regression coefficients (R = 0.990 and 0.934, respectively) than phenolic acids (R = 0.647). All phenolic compounds, except procyanidins B1 and B4 for flavanols, kaempferol-3-*O*-glucuronide and quercetin pentoside for flavonols, and *cis*-coutaric and gallic acids for phenolic acids, had significant influence ($p<0.05$).

3.3. Antioxidant activity measured by cyclic voltammetry

CV was used to study the electrochemical behavior of grape pomaces. Table 3 shows the electrochemical parameters extracted from the cyclic voltammetry curves of the extracts. These parameters describe the process of oxidation and characterize the

phenolic compounds as reducing agents. Some significant differences between the parameters indicate a different voltammetric profile of phenolic compounds.

The cyclic voltammogram of 25-fold diluted extract of Zalema, for scan from 0 to 1 V, is shown in Figure 2, as a typical voltammogram of an extract of grape pomace. The cyclic voltammograms gave a set of anodic (positive) and cathodic (negative current) peaks. Three different anodic peaks can be observed in the voltammogram: peak I at 0.22 V, peak II at 0.42 V, and peak III at 0.77 V. On the reverse scan, only one peak is depicted, at approximately 0.40 V. This peak was found to be related to peak I on a reversible electrode reaction, which is typical of ortho-diphenol compounds (Kilmartin, Zou, & Waterhouse, 2001). According to Rebelo et al. (2013), the absence of more cathodic peaks shows that most of the oxidation products are not reduced on the glassy carbon electrode and then quantitative data was only determined from the anodic peaks. Some authors have reported the presence of three anodic peaks and one cathodic peak in wine and grape juice samples, with similar $E_{p,a}$ values (Kilmartin et al., 2001; Makhotkina et al., 2012; Rebelo et al., 2013).

Extracts of all varieties revealed the presence of these three anodic peaks (Figure 3), which showed different electrochemical parameters. An ANOVA tests was made to establish significant differences between varieties in relation to the parameters Q, $E_{p,a}$, and $I_{p,a}$. As can be observed, the anodic current area for peak I (Q^{I}) ranged between 0.26-0.28 V, for peak II (Q^{II}) between 0.30-0.37 V and for peak III (Q^{III}) between 0.24-0.33 V, and significant differences between varieties were found regarding Q^{II} and Q^{III}. The area under the curve, as well as the $I_{p,a}$ were used a measure of the concentration of total phenols (Kilmartin et al., 2001). In this sense, univariate linear regression was applied to explore relationships between the Q and $I_{p,a}$ parameters, and strong and

significant correlations (R = 0.95, $p<0.05$) were found, therefore next results will be discussed based on the parameter I_{pa}.

Three multiple regression analyses were carried out to check the more influential phenolic groups (total flavanols, flavonols and phenolic acids contents as independent variables) on the different anodic peaks currents ($I^{I}_{p,a}$, $I^{II}_{p,a}$ or $I^{III}_{p,a}$ as dependent variable). $I^{I}_{p,a}$ showed a good multiple correlation coefficient (R = 0.70), being phenolic acids (β = 0.82) and flavonols (β = -1.1) more influential than flavanols (β = -0.36). Results of multiple regression analysis considering $I^{II}_{p,a}$ (R = 0.70) indicated that flavanols (β= 0.39) had significant influence ($p<0.05$) while phenolic acids (β = 0.46) and flavonols were not significant (β = - 0.49). The third regression had a high correlation (R = 0.82) having flavanols (β= 0.65), flavonols (β = 1.31) and phenolic acids (β = - 1.1) with significant influence ($p<0.05$).

According to Kilmartin et al. (2001), Makhotkina et al. (2012), and Rebelo et al. (2013), peak I is ascribed to phenolic compounds containing a flavonoid structure with a catechol or a galloyl group (i.e., ortho-diphenol and triphenol groups) at B-ring, like catechin, epicatechin, quercetin and quercetin-3-*O*-rutinoside, which were found in grape pomace extracts of the nine varieties.

In regard to peak I, $E_{p,a}$ was 220 mV for all extracts, while $I^{I}_{p,a}$ varied between varieties, being Zalema extracts ($I^{I}_{p,a}$ = 1.34 µA) significantly different to $I^{I}_{p,a}$ of all other varieties. As described in literature, $I_{p,a}$ increases with increasing concentrations of phenolics although the relationship is not always linear (Kilmartin et al., 2002). Regarding peak II, Sauvignon blanc extract showed the highest $I^{II}_{p,a}$ (2.41 µA), followed by Moscatel, Airén and Parellada extract ($I^{II}_{p,a}$ = 2.28, 2.24 and 2.23µA, respectively). The $E^{I}_{p,a}$ parameter varied depending on the variety, ranging between 422 and 430 mV. This second oxidation peak, which was the most intense anodic peak, may correspond to the

irreversible oxidation of the -OH group at position 3 on the C-ring (Cosio, Buratti, Mannino, & Beneditti, 2006; Janeiro & Brett, 2004). Makhotkina et al., (2012) reported that flavonols that are derivatives of quercetin are able to produce the peak II at approximately 0.5 V.

The third voltammetric peak could be due to phenolic acids such as *p*-coumaric acid, or flavanols, such as catechin (Kilmartin et al., 2001; Rebelo et al., 2013). Zalema and Moscatel extracts had the highest values of $I^{III}_{p,a}$ (1.95 µA) while Airén and Parellada extracts showed the lowest values ($I^{III}_{p,a}$ = 1.36 and 1.35 µA, respectively).

Finally, a non-linear correlation between cyclic voltammetric parameters and results of antioxidant activity by ABTS assay or TPC by Folin-Ciocalteu method was obtained. This is in accordance to other authors and it could indicate that the CV technique not provides only phenolic information (Rebelo et al., 2013).

3.4. Stepwise linear discriminant analysis

To ascertain whether it was possible to discriminate between different varieties of grape pomaces as a function of TPC, ABTS, $I^{I}_{p,a}$, $I^{II}_{p,a}$, $I^{III}_{p,a}$ values and total flavanols, flavonols and phenolic acids contents (independent variables), a SLDA was carried out. All variables were found statistically significant ($p<0.05$). Two classification functions were obtained, which yielded a good separation (100% correct classification) among samples (Figure 4). The discriminant function 1 was mainly related to total flavonols content, ABTS and TPC values (with negative sign), and total flavanols content and $I^{III}_{p,a}$ values (positive sign), whereas the discriminant function 2 was mainly linked to $I^{I}_{p,a}$ and $I^{II}_{p,a}$ (positive sign), and total flavanols (negative sign). As can be seen in Figure 4, the discriminant function 1 mostly discriminates between Zalema and Moscatel varieties, and the discriminant function 2 between Parellada and Sauvgnon blanc, and Zalema and Moscatel varieties.

4. Conclusions

Grape pomaces of nine varieties exhibited different quantitative phenolic profile and different antioxidant activities measured by the ABTS assay and CV, showing statistically significant differences ($p<0.05$) among varieties. In all varieties, flavanols were the main phenolic group, being catechin and procyanidin B1 the most abundant compounds. Moscatel and Parellada varieties showed highest levels of flavanols, and phenolic acids levels were highest in Pedro Ximénez and Baladí varieties. Zalema variety showed the highest concentration in flavonols, showing significant differences with the other varieties.

The antioxidant activity measured by the ABTS assay showed a good correlation to the TPC and particularly to the flavanols and flavonols contents, however a linear correlation was not found between CV results and ABTS or TPC data. The electrochemical parameters $I^{I}_{p,a}$, $I^{II}_{p,a}$ and $I^{III}_{p,a}$ were significantly different among varieties, being $I^{I}_{p,a}$ mainly ascribed to flavonols and phenolic acids, $I^{II}_{p,a}$ to flavanols, and $I^{III}_{p,a}$ to the three mentioned phenolic groups. Eight variables allowed classify correctly 100% of the grape pomace samples. Total flavanols content was the variable which had the most influence in the discrimination of Zalema variety, mainly due to quercetin-3-*O*-glucoside concentration; therefore, as reported by some authors, flavonolic profile could be used to differentiate the white grape pomace of this variety.

Results suggest that the electrochemical response of phenolic compounds in grape pomace extracts could be used as a measurement of the antioxidant potential.

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Figure captions

Figure 1. Relative proportion of the three phenolic groups in the grape pomaces from the nine grape varieties.

Figure 2. Representative cyclic voltammogram of a grape pomace extract of the Zalema grape variety.

Figure 3. Cyclic voltammograms of grape pomace extracts from the nine varieties.

Figure 4. Scatterplot of the grape pomaces samples in the plane defined by the canonical function when phenolic composition and antioxidant activity are considered for discrimination.

Abbreviations

Dry pomace (DP)

Zalema (Z)

Pedro Ximénez (PX)

Moscatel (MG)

Baladí (B)

Parellada (P)

Sauvignon blanc (SB)

Montepila (M)

Airén (A)

Verdejo (V)

Total phenolic content (TPC)

Cyclic voltammetry (CV)

Rapid resolution liquid chromatography (RRLC)

Mass spectrometry (MS)

Anodic current area (Q)

Anodic peak current ($I_{p,a}$)

Anodic peak potential ($E_{p,a}$)

One way analysis of variance (ANOVA)

Stepwise linear discriminant analysis (SLDA)

Pattern recognition techniques (PR)

Trolox-equivalent antioxidant capacity (TEAC)

Trolox-equivalent (TE)

Oxygen radical absorbance capacity (ORAC)

1,1-diphenyl-2-picrylhydrazyl (DPPH)

2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

Ferric reducing antioxidant power (FRAP)

Phosphate buffered saline (PBS)

High performance liquid chromatography (HPLC)

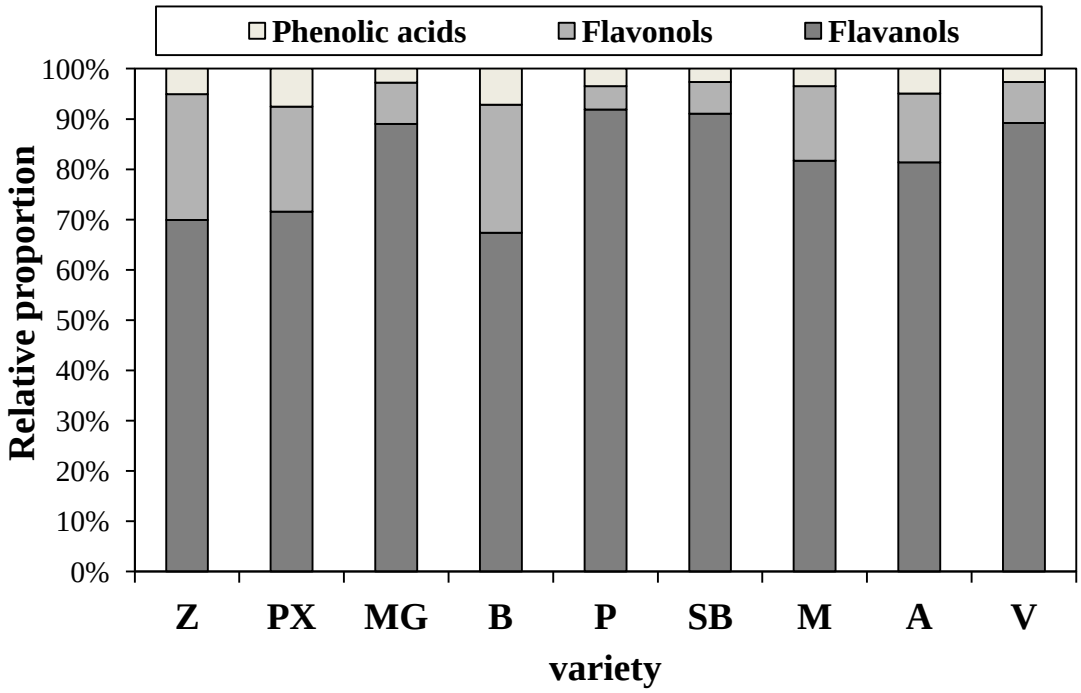

Figure 1

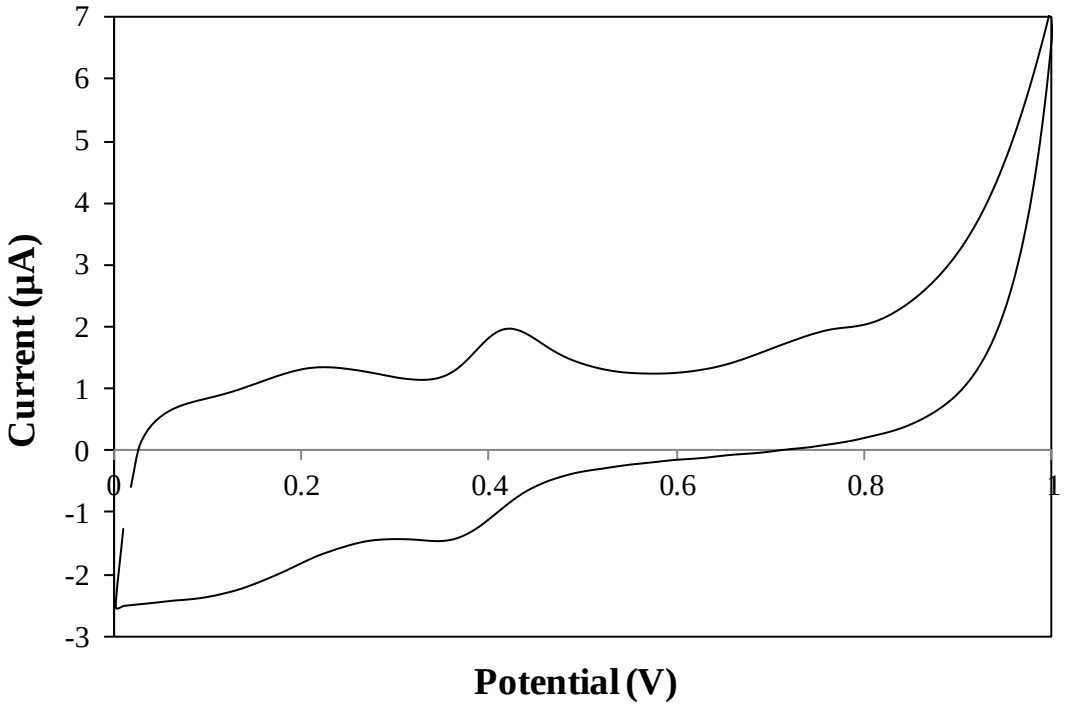

Figure 2

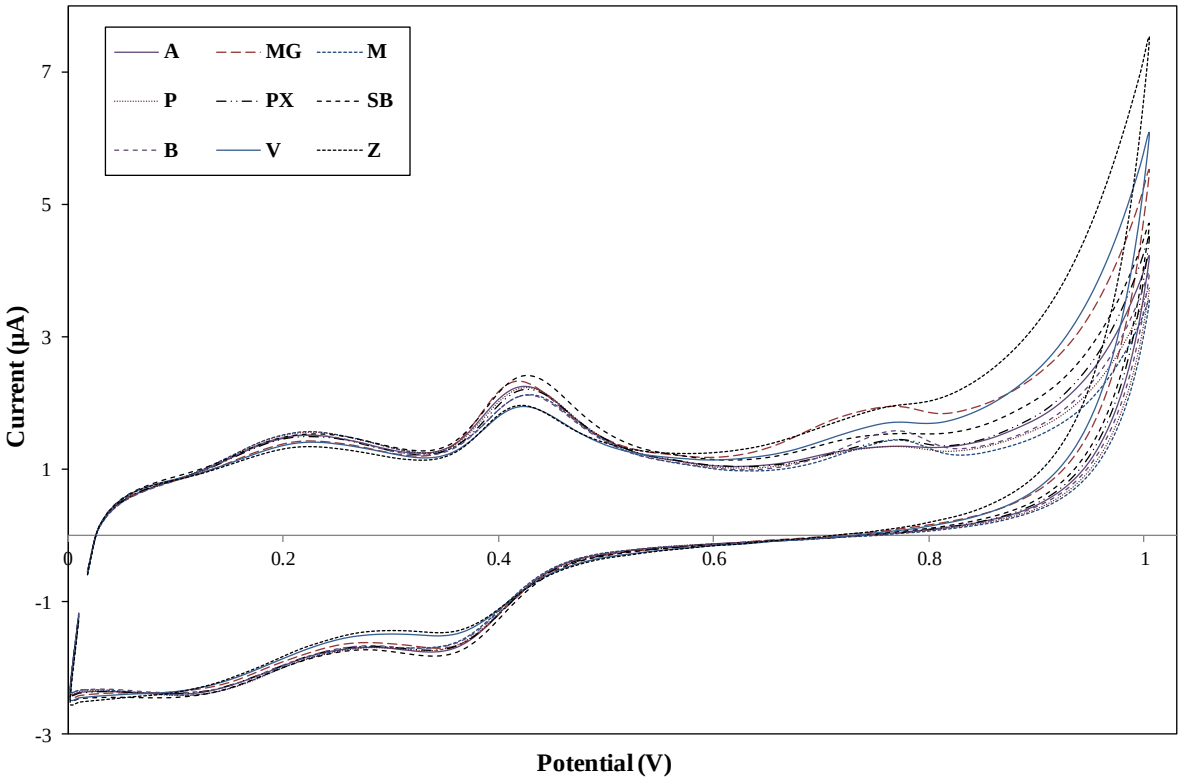

Figure 3

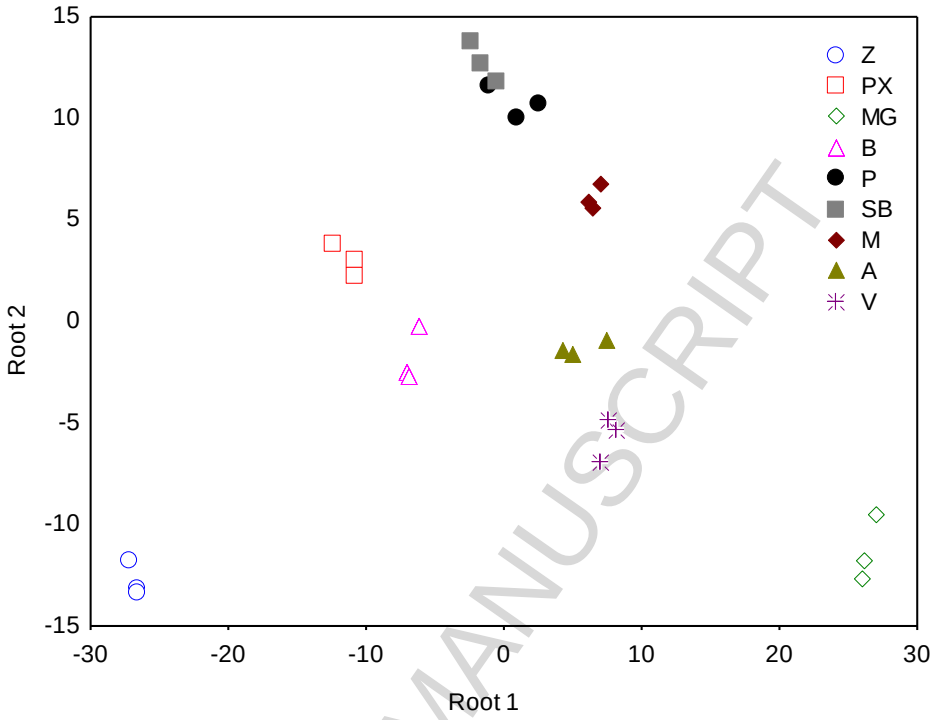

Figure 4

Table 1. Total flavanols, flavonols and phenolic acids contents, total phenolic content, and antioxidant activity for the grape pomaces

Variety	∑Flavanols[1]	∑Flavonols[2]	∑Phenolic acids[3]	TPC[4]	ABTS[5]
Z	$406.05^{a} \pm 20.50$	$145.54^{a} \pm 9.14$	$29.35^{a,d} \pm 1.65$	$2513.28^{a,c} \pm 139.52$	$56.77^{a} \pm 2.65$
PX	$328.24^{a} \pm 13.86$	$95.63^{b} \pm 4.91$	$34.65^{b,d} \pm 1.71$	$714.73^{b,d} \pm 90.70$	$26.69^{b,c} \pm 1.32$
MG	$874.26^{b} \pm 44.65$	$80.44^{b} \pm 3.59$	$27.14^{a} \pm 1.02$	$2193.74^{a} \pm 216.14$	$31.42^{b} \pm 0.17$
B	$325.39^{a} \pm 16.33$	$123.17^{c} \pm 12.57$	$34.63^{b,d} \pm 2.99$	$454.85^{b} \pm 12.69$	$28.62^{b,c} \pm 3.06$
P	$777.02^{b} \pm 67.56$	$39.42^{d,e} \pm 5.73$	$29.12^{a} \pm 1.80$	$3113.29^{c} \pm 167.95$	$59.42^{a} \pm 5.39$
SB	$609.82^{c} \pm 29.34$	$42.41^{d,e} \pm 1.38$	$17.63^{c} \pm 0.72$	$2304.75^{a} \pm 120.24$	$53.85^{a} \pm 1.11$
M	$314.65^{a} \pm 11.02$	$57.13^{e} \pm 3.55$	$13.34^{c,e} \pm 0.80$	$539.99^{b,d} \pm 71.16$	$29.66^{b,c} \pm 2.09$
A	$568.06^{c} \pm 18.72$	$95.79^{b} \pm 4.29$	$34.25^{d} \pm 1.78$	$1153.59^{b,d} \pm 36.21$	$25.30^{b,c} \pm 3.97$
V	$357.21^{a} \pm 55.44$	$32.79^{d} \pm 3.49$	$10.56^{e} \pm 0.96$	$1277.65^{d} \pm 192.31$	$22.50^{c} \pm 1.78$

Each value represents mean (n=9) ± SD. Different letters in the same column indicate significant differences by ANOVA test ($p<0.05$).

[1]Sum of flavanols (mg phenolic compound/100 g DP)

[2]Sum of flavonols (mg phenolic compound/100 g DP)

[3]Sum of phenolic acids (mg phenolic compound/100 g DP)

[4]mg GAE/100 g DP

[5]mmols millimoles TE/100 g DP

Table 2. Concentrations of individual phenolic compounds identified by the RRLC/MS analysis in the grape pomaces of the different varieties

	Variety								
	Z	PX	MG	B	P	SB	M	A	V
Flavanols									
Catechin	69.86^{a} ± 3.73	66.15^{a} ± 2.87	263.54^{b} ± 17.68	64.13^{a} ± 5.68	201.38^{c} ± 20.21	184.47^{c} ± 9.80	65.03^{a} ± 2.26	179.39^{c} ± 5.42	86.88^{a} ± 1.12
Epicatechin	33.10^{a} ± 1.84	20.98^{b} ± 0.65	86.47^{c} ± 5.63	22.99^{b} ± 0.74	50.28^{d} ± 3.35	57.32^{d} ± 2.38	38.59^{a} ± 0.36	34.86^{a} ± 0.77	19.00^{b} ± 2.07
Pc B1	66.72^{a} ± 2.59	54.71^{a,f} ± 2.57	122.95^{b,e} ± 6.66	54.05^{a,f} ± 3.10	134.61^{b} ± 9.01	93.38^{c} ± 5.24	36.42^{d} ± 2.69	110.85^{e} ± 4.70	47.29^{d,f} ± 5.39
Pc B2	12.55^{a} ± 0.48	10.13^{b} ± 0.41	18.81^{c} ± 0.84	8.26^{b} ± 0.44	24.41^{d} ± 1.67	17.43^{c,e} ± 0.96	9.44^{b} ± 0.43	12.75^{a} ± 0.38	15.25^{e} ± 0.92
Pc B3	26.19^{a} ± 1.20	29.48^{a,d} ± 1.45	34.30^{b,e} ± 0.95	26.86^{a} ± 1.79	34.20^{b,e} ± 2.34	28.62^{a,d} ± 0.49	19.84^{c} ± 1.19	31.71^{d,e} ± 1.17	11.98^{f} ± 1.16
Pc B4	32.28^{a} ± 1.56	22.38^{b} ± 1.53	31.96^{a} ± 1.65	23.47^{b} ± 1.61	51.52^{c} ± 1.79	32.50^{a} ± 1.81	23.69^{b} ± 0.41	27.42^{a,b} ± 1.06	22.34^{b} ± 4.00
Pc B7	7.81^{a,e} ± 1.46	7.23^{a,d} ± 0.21	5.11^{b,d,f} ± 0.39	7.77^{a,e} ± 0.48	0.00^{c} ± 0.00	6.14^{d,f} ± 0.19	4.48^{b,f} ± 0.15	9.00^{e} ± 0.27	5.34^{f} ± 0.46
Pc trimer 1	16.02^{a} ± 0.70	11.02^{b,d} ± 0.43	16.04^{a} ± 0.62	9.85^{b,d} ± 0.28	20.11^{c} ± 1.20	14.16^{a,d} ± 0.56	9.69^{b,d} ± 0.36	10.67^{b,d} ± 0.29	11.87^{d} ± 2.23
Pc trimer 2	33.03^{a} ± 1.39	21.19^{b} ± 0.75	34.43^{a} ± 1.50	23.87^{b,d} ± 1.19	44.97^{c} ± 3.12	30.37^{a,d} ± 1.93	27.65^{d} ± 0.40	26.45^{d} ± 0.95	19.72^{b} ± 2.78
Pc tetramer 1	38.27^{a,d} ± 1.79	29.91^{a,e} ± 1.84	85.25^{b} ± 4.33	35.17^{a,e} ± 5.20	70.23^{c} ± 5.17	48.14^{d} ± 2.68	25.22^{e} ± 1.50	53.17^{d} ± 2.85	30.72^{a,e} ± 4.85
Pc tetramer 2	13.27^{a,b} ± 1.86	10.44^{a} ± 0.35	14.39^{a,b} ± 0.25	11.26^{a} ± 0.51	26.67^{b} ± 15.26	11.70^{a} ± 0.45	8.20^{a} ± 0.28	13.56^{a,b} ± 1.05	9.40^{a} ± 0.31
Galloylled Pc	11.38^{a,d} ± 0.31	10.33^{a,d,e} ± 0.34	23.62^{b} ± 1.26	7.39^{a} ± 0.14	16.41^{c} ± 0.94	18.56^{c} ± 0.65	8.92^{a,d} ± 0.53	11.88^{d} ± 0.31	22.94^{e} ± 3.96
Pc B2-3-O-gall	45.57^{a,e} ± 3.03	34.30^{a} ± 1.59	137.29^{b} ± 6.96	30.34^{a} ± 1.84	102.24^{c} ± 7.80	66.97^{d,e} ± 3.73	37.49^{a,e} ± 1.38	46.35^{a,e} ± 2.06	54.45^{e} ± 9.83
Flavonols									
Q-3-*O*-rutin	3.95^{a} ± 0.46	2.02^{d,c} ± 0.26	3.13^{a,c} ± 0.23	3.66^{a} ± 1.97	1.95^{a,c} ± 0.24	2.31^{a,c} ± 0.61	2.46^{a,c} ± 0.23	6.40^{b} ± 0.37	1.30^{c} ± 0.22

Q-3-*O*-glucu	52.25^{a} ± 0.60	31.98^{b} ± 0.29	31.72^{b} ± 0.25	45.90^{a,d} ± 2.57	15.99^{c,e} ± 0.44	19.66^{c} ± 0.09	14.12^{c,e} ± 0.17	40.40^{d} ± 0.33	11.72^{e} ± 0.23
Q-3-*O*-gal	7.92^{a} ± 0.09	4.38^{b,f,g} ± 0.04	2.90^{c,g} ± 0.02	6.38^{d} ± 0.53	2.01^{c,e} ± 0.06	1.08^{e} ± 0.01	4.26^{f} ± 0.05	3.75^{g} ± 0.03	1.22^{e} ± 0.03
Q-3-*O*-gluc	55.75^{a} ± 0.64	38.31^{b} ± 0.35	25.42^{c} ± 0.20	54.97^{a} ± 4.29	14.52^{d} ± 0.40	12.23^{d} ± 0.06	27.49^{c} ± 0.32	32.39^{b,c} ± 0.27	12.05^{d} ± 0.23
Q pentoside	0.22^{a,c} ± 0.01	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.30^{a,c} ± 0.00	0.37^{a,c} ± 0.00	0.29^{a,c} ± 0.00	0.25^{a} ± 0.00	0.89^{b} ± 0.00	0.40^{c} ± 0.01
K-3-*O*-gal	4.74 ± 0.06	3.38 ± 0.04	2.91 ± 0.02	2.33 ± 0.49	0.59 ± 0.04	0.78 ± 0.00	1.37 ± 0.02	1.99 ± 0.02	0.40 ± 0.02
K-3-*O*-glucu	0.29^{a} ± 0.01	0.48^{a} ± 0.01	0.37^{a} ± 0.01	0.33^{a} ± 0.00	0.54^{a} ± 0.04	0.35^{a} ± 0.00	0.28^{a} ± 0.00	1.31^{b} ± 0.05	0.26^{a} ± 0.00
K-3-*O*-gluc	18.91^{a} ± 0.24	13.97^{a,b} ± 0.17	12.00^{b,d} ± 0.10	7.86^{b,c,d} ± 1.19	1.84^{c,d} ± 0.14	4.14^{c,d} ± 0.00	6.20^{d} ± 0.07	6.24^{d} ± 0.04	3.12^{c,d} ± 0.04
I-3-*O*-gluc	1.08^{a,d} ± 0.02	0.91^{a,d} ± 0.01	0.93^{a,d} ± 0.01	0.56^{a,b} ± 0.03	0.70^{a,b} ± 0.03	0.65^{a,b} ± 0.00	0.32^{b} ± 0.03	1.81^{c} ± 0.09	1.43^{c,d} ± 0.04
I-3-*O*-glucu	0.43^{a} ± 0.01	0.19 ± 0.00	1.06 ± 0.02	0.88 ± 0.00	0.90 ± 0.00	0.91 ± 0.01	0.39 ± 0.00	0.61 ± 0.01	0.88 ± 0.03
Phenolic acids									
Gallic acid	4.14^{a} ± 0.20	4.26^{a} ± 0.28	10.09^{b} ± 0.85	2.34^{c} ± 0.21	7.42^{d} ± 0.31	6.77^{d} ± 0.36	3.96^{a} ± 0.13	3.64^{a} ± 0.18	1.78^{a} ± 0.18
Caftaric acid	19.97^{a} ± 1.52	21.82^{a,e} ± 1.30	13.56^{b} ± 0.95	27.72^{c,e} ± 2.73	13.58^{b} ± 1.68	7.71^{d} ± 0.40	6.82^{d} ± 0.67	24.59^{e} ± 0.92	6.56^{d} ± 0.80
Fertaric acid	0.55^{a,b,d,e} ± 0.02	0.56^{a,b,d} ± 0.02	0.57^{a} ± 0.01	0.59^{a} ± 0.03	0.51^{b,c,d,e} ± 0.01	0.59^{a} ± 0.02	0.48^{c,d,e} ± 0.01	0.51^{d,e} ± 0.01	0.50^{e} ± 0.04
c-Coutaric acid	1.13^{a,e} ± 0.02	1.66^{b} ± 0.04	0.93^{c} ± 0.01	1.09^{a,c} ± 0.10	1.54^{b} ± 0.08	0.94^{c} ± 0.09	0.71^{d} ± 0.04	1.28^{e} ± 0.05	0.75^{d} ± 0.06
t-Coutaric acid	3.55^{a,d} ± 0.26	6.34^{b} ± 0.39	1.98^{c} ± 0.06	2.89^{a} ± 0.37	6.07^{b} ± 0.27	1.62^{c,e} ± 0.28	1.38^{c,e} ± 0.09	4.23^{d} ± 0.21	0.97^{e} ± 0.07

Each value represents mean (n=27) ± SD. Different letters in the same row indicate significant differences by ANOVA test (p <0.05). Results are expressed in mg /100 g DP
Pc, procyanidin; gall, gallate; Q, quercetin; K: kaempferol; I: isorhamnetin; rutin, rutinoside; glucu, glucuronide; gal, galactoside; gluc, glucoside; *c*, *cis*; *t*, *trans*

Table 3. Electrochemical parameters of three anodic peaks extracted from the cyclic voltammetry curves of the grape pomaces.

	Peak I			Peak II			Peak III		
	Q^{I}	$E^{I}_{p,a}$	$I^{I}_{p,a}$	Q^{II}	$E^{II}_{p,a}$	$I^{II}_{p,a}$	Q^{III}	E^{III}_{pa}	$I^{III}_{p,a}$
Z	0.26^{a}	0.22	1.34^{a}	$0.30^{a,d}$	0.42	1.97^{a}	$0.28^{a,b}$	0.76	1.95^{a}
PX	0.27^{a}	0.22	1.50^{b}	0.34^{b}	0.42	$2.20^{b,c}$	$0.25^{a,b}$	0.77	$1.45^{b,c}$
MG	0.26^{a}	0.22	$1.43^{c,f}$	$0.35^{b,c}$	0.42	2.28^{b}	0.33^{b}	0.76	1.95^{a}
B	0.27^{a}	0.22	1.52^{b}	$0.33^{b,d}$	0.43	2.12^{c}	$0.25^{a,b}$	0.77	$1.58^{b,d}$
P	0.28^{a}	0.22	$1.56^{d,g}$	0.34^{b}	0.42	$2.23^{b,c}$	0.24^{a}	0.77	1.35^{c}
SB	0.28^{a}	0.22	$1.53^{b,d}$	0.37^{c}	0.43	2.41^{d}	$0.27^{a,b}$	0.76	1.54^{b}
M	0.28^{a}	0.22	$1.57^{e,g}$	0.33^{b}	0.43	2.13^{c}	0.24^{a}	0.77	$1.45^{b,c}$
A	0.27^{a}	0.22	1.52^{b}	0.34^{b}	0.42	2.24^{b}	$0.24^{a,b}$	0.77	1.36^{c}
V	0.26^{a}	0.22	1.41^{f}	0.31^{d}	0.42	1.95^{a}	$0.29^{a,b}$	0.76	1.73^{c}

Each value represents mean (n=6) ± SD. Different letters in the same column indicate significant differences by ANOVA test (p<0.05). $E_{p,a}$ is expressed as V and $I_{p,a}$ as µA.

Highlights

1. Antioxidant potential of white grape pomaces from nine varieties has been evaluated
2. Cyclic voltammetry was used to measure the antioxidant activity
3. A RRLC method has been used to determine the phenolic composition of grape pomaces
4. Samples exhibited different phenolic profiles and antioxidant activities
5. Zalema variety was well differentiated from other varieties