CYCLIC VOLTAMMETRY TO EVALUATE THE ANTIOXIDANT POTENTIAL IN WINEMAKING BY-PRODUCTS

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

[a]Food Colour & Quality Lab., Dept. Nutrition & Food Science, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.

[b]Department of Analytical Chemistry, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.

**** Corresponding author:***

Dolores Hernanz Vila

Department of Analytical Chemistry, Universidad de Sevilla. Facultad de Farmacia, 41012 Sevilla, Spain.

Tel.: +34 954553804 Fax: +34 954556749

e-mail: vila@us.es

Abstract

Grape pomace is composed of seeds, skins and stems that are an important source of phenolic substances, which have antioxidant properties and potential benefits to human health. Cyclic voltammetry (CV) has been used to measure the total antioxidant potential of different winemaking by-products. The electrochemical behavior of pomace, seeds, skins and stems was measured by CV and lipid peroxidation inhibition by thiobarbituric acid reactive substances (TBARS) method. Differences for the electrochemical parameter were found between the by-products, pomace and seeds, which presented the greatest voltammetric peak area. Furthermore, the by-products induced inhibition of lipid peroxidation in rat liver homogenates. Pomace and seeds showed higher capacity to inhibit lipid peroxidation than stems and skins, which could be because these by-products are richer in flavanols. Simple regression analyses showed that voltammetric parameters are highly correlated to the values obtained for lipid peroxidation inhibition. CV is a promising technique to estimate the total antioxidant potential of phenolic extract from winemaking by-products.

Keywords

Antioxidant potential, by-products, phenolic compounds, cyclic voltammetry, lipid peroxidation.

INTRODUCTION

Oxidative stress takes place when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense systems [1]. ROS, such as superoxide anion radical ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$), are constantly generated in living organisms by endogenous (metabolism, inflammatory reactions) or exogenous sources (environmental factors) [2]. They attack proteins, DNA and lipids generating important tissue damage. Specifically, lipid peroxidation affects unsaturated fatty acids in membrane phospholipids and cholesterol causing irreversible cellular and tissue damage associated with several pathologies and health conditions, like artherosclerosis, cancer and aging [3, 4].

Antioxidants delay the oxidation of proteins, DNA and lipids due to their antioxidant properties [4]. Phenolic compounds (flavonoids and non-flavonoids) from winemaking by-products are recognized as important antioxidants and have been related with benefits on human health [5, 6]. These by-products are considered as a natural source of antioxidants that may be used as functional compounds; therefore, the recovery of phenolic compounds from winemaking by-products has received much attention in the past years and nowadays, and industries are finding alternative solutions for the exploitation and valorization of those by-products [7].

Different spectrophotometric methods have been used to determine the *in vitro* antioxidant activity, such as ABTS (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl) and ORAC (Oxygen radical absorbance capacity), that measure the ability of antioxidants to scavenge a radical, FRAP (Ferric reducing antioxidant power), CUPRAC (Cupric reducing antioxidant capacity) and CERAC (Cerium reducing antioxidant capacity), that measure the capacity of antioxidant to

reduce metals, and TBARS (Thiobarbituric acid reactive substances), which is used to measure the lipid peroxidation inhibition [8].

On the other hand, electroanalytical methods are also used to measure the antioxidant activity [9, 10]. Cyclic voltammetry (CV) is a simple, fast and inexpensive electrochemical technique that could become an alternative to traditional spectrophotometric techniques to measure the antioxidant activity. CV has been successfully used to determine the antioxidant capacity of blood plasma, vegetable oils, milk, orange juice, wines and winemaking by-products and to correlate the analytical response to the phenolic composition [11-17].

The application of cyclic voltammetry to wine analysis dates back to 1988 [18]. In that work, CV was used to evaluate the suitability of using an electrochemical detection method coupled to HPLC, to detect and identify flavonoids and procyanidins in wine extracts. However, the first application of CV to characterize antioxidant properties in red and white wines was reported in 2001 [15]. It was shown that CV provides a qualitative and quantitative assessment of wine phenolics based on their reducing strength, and charge passed to 500 mV (vs Ag/AgCl). They also demonstrated the coherence of the cyclic voltammetric response with the phenolic information provided by HPLC, Folin-Ciocalteau assay and absorbance at 280 nm on white and red wines [16].

Cyclic voltammetry appears as a simple method to estimate the total antioxidant capacity, as previously reported [19]. This paper indicates that total antioxidant potential comprises both parameters peak anodic current and peak anodic potential (E_{pa} and I_{pa}, respectively). E_{pa} describe the antioxidant property and I_{pa} is directly proportional to the concentration. I_{pa} is highly correlated to the area under the curve and is used as a measure of the concentration of total phenols [17-20]. Several *in vitro* methods have been employed to measure the antioxidant activity, however they only evaluate a particular mechanism (for

example, ABTS method measures scavenging activity against certain types of free radicals) and not the total antioxidant activity [8]. Thus, previous studies reported a non-correlation between cyclic voltammetric parameters and results of spectrophotometric methods such as ABTS assay [17, 20].

In this sense, the aim of this work was to check if the cyclic voltammetry could be used as a measurement of the total antioxidant potential of different winemaking by-products, and to correlate the results with those obtained from the TBARS method, using livers as *in vitro* biological system. Firstly, samples were tested using cyclic voltammetry to evaluate their electrochemical behavior. Secondly, samples were analyzed using the TBARS assay in order to determine their ability to inhibit the lipid peroxidation. Finally, correlations between results were also stablished.

MATERIALS AND METHODS

Samples and reagents

White winemaking by-products from Zalema grapes (*Vitis vinifera* L.) used in this study were pomace, and skins, seeds, and stems separated from pomace. They were obtained from a winery located in Condado de Huelva Designation of Origin (southwestern Spain). Ten samples of grape pomace were taken every week from the beginning of the harvest (30 August) until the ending (30 September), after the grapes were pressed for winemaking. Seeds, skins and stems were manually separated from the grape pomace samples and all samples were further freeze-dried.

Methanol, Folin-Ciocalteu reagent, trichloroacetic acid and ethylenediaminetetraacetic acid were obtained from Panreac (Barcelona, Spain). Gallic acid, catechin, quercetin, caffeic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium carbonate, sodium chloride, tris(hidroxymethyl)amino-methane, phenylmethylsulfonyl

fluoride and cumene hydroperoxide were purchased from Sigma-Aldrich (Madrid, Spain). 2-thiobarbituric acid was obtained from Merck (Madrid, Spain).

Sample preparation and extraction

The by-products samples (pomace and seeds, skins and stems) were extracted with 75% methanol according to the methodology described previously [21]. The sample (5 g) was homogenized in 25 mL of the solvent, kept under shaking for 1 h in a shaking apparatus (VWR Incubating minishaker) and further centrifuged at 4190*g* for 15 min; the supernatant was collected and the residue submitted to the same process twice, and the supernatants were combined. The phenolic extracts thus obtained were used for CV analysis and for the determination of total phenolic content (TPC) and lipid peroxidation inhibition by spectrophotometric methods.

Determination of total phenolic content

TPC was determined using the Folin-Ciocalteu assay with some modifications [21]. Gallic acid was employed as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/g of dry matter (DM)).

Electrochemical assays

A potentiostat/galvanostat, AUTOLAB model PGSTAT 302 N (Metrohm-Eco Chemie, Netherlands) controlled by a General Purpose Electrochemical System (GPES) software, was used for all electrochemical measurements. One mL of the extracts was diluted with 25 mL phosphate buffer with 65% (w/v) 50 mM disodium hydrogen phosphate and 35 % (w/v) 50 mM sodium dihydrogen phosphate at pH 7.0 (Kilmartin et al., 2001). The dilute 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, working electrode was polished in alumina/water suspension, rinsed with Milli-Q water and sonicated for 2 min. Samples solutions were

de-aerated with an inert gas (N_2) for 10 min, and after a few minutes running scan was taken.".

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 0.5 V at a scanning rate of 5 mV/s.

The electrochemical parameters extracted from the cyclic voltammetry curve were the anodic current area (Q_{500}) that represents the integrated area of the cyclic voltammogram for scans taken from 0 to 0.5 V, the peak anodic current and potential ($I_{p,a}$ and $E_{p,a}$, respectively), the peak cathodic current and potential ($I_{p,c}$ and $E_{p,c}$, respectively), the potential mid-way between the anodic and cathodic peaks (E_{mid}) calculated from ½ ($E_{p,c}$ + $E_{p,a}$), $I_{p,a}/I_{p,c}$, and $E_{p,a}$- $E_{p/2}$. These parameters were taken from the cyclic voltammograms after substracting cyclic voltammogram data of the blank (1 mL of 75% methanol diluted with 25 mL phosphate buffer). All of the cyclic voltammograms were recorded in triplicate.

Catechin was employed as a calibration standard and Q_{500} values were also expressed as catechin equivalents (mg CAT/g DM) (Appendix).

Determination of lipid peroxidation inhibition

The lipid peroxidation inhibition was determined by the TBARS assay [22]. Livers of rats, obtained as described by Rosillo et al. [23], were weighed and homogenized in 20 mM Tris-HCl buffer (pH 7.5). The homogenate was centrifuged for 10 min at 3000*g* obtaining a supernatant that was used for the TBARS assay.

The reaction mixture, prepared on ice, contained rat liver (200 µL), Tris-HCl buffer (675 µL), phenolic extracts (100 µL), cumene hydroperoxide (20 mM, 25 µL) in a reaction volume of 1 mL. Total oxidation samples contained all reagents except phenolic extracts.

The mixture was incubated at 37 ºC for 1 h and the reaction was stopped by adding 10 % trichloroacetic acid at 4ºC to precipitate the proteins. The mixture was centrifuged at 3000*g* for 10 min and 1 mL of 2-thiobarbituric acid was added to the supernatant, which was incubated at 100 ºC for 1 h. The TBARS were measured by determining absorbance at 535 nm. Results are expressed as percentage of inhibition of lipid peroxidation (% inhibition) and as catechin-equivalent antioxidant capacity (CAC: mg of catechin with the same antioxidant capacity as 1 g of DM; mg CAT/g) (Appendix).

Statistics analysis

Statistica v.8.0 software [24] was used for the statistical treatment of the data. One-way analysis of variance (ANOVA) (Tukey *post hoc* test) was applied to determine whether significant differences exist among the by-products (in relation to total phenolic content, antioxidant potential and lipid peroxidation inhibition). Correlations between electrochemical parameters and lipid peroxidation inhibition were studied by simple regression (Pearson's correlation). In all cases, statistically significant level was considered at $p < 0.05$.

Unsupervised pattern recognition methods are widely applied in order to observe trends in the data indicating relationships between samples and/or between variables. The unsupervised method used for data by electrochemical response was principal components analysis (PCA). Moreover, in order to obtain information about the relationship between the voltammetric variability and phenolic composition, a Pearson's correlation analyses between the principal component and the phenolic content were carried out.

RESULTS AND DISCUSSION

The quantitative data of TPC are summarized in Table 1. Regarding to the TPC, significant differences ($p<0.05$) were found among samples depending on the type of

winemaking by-product. Seeds presented the highest TPC (48 mg/g DM,) followed by stems and skins (36 and 34 mg/g DM, respectively). Pomace had a similar TPC level to seeds (44 mg/g DM).

Electrochemical behavior of by-products

The oxidation potentials of by-products extracts were obtained by cyclic voltammetry. Figure 1 shows cyclic voltammograms of 25-fold diluted by-products extracts (pomace and its parts: seeds, skins and stems). As can be observed, the cyclic voltammograms gave an anodic (positive) peak, which correspond to the total oxidation potential of by-products [15]. The electrochemical parameters extracted from the cyclic voltammetry curves of the by-products extracts are reported in Table 2. Differences between the parameters indicate a different voltammetric profile of by-products.

In regard to $E_{p,a}$, the anodic peak was observed at 244 mV in pomace, and its parts skins and stems, and 252 mV in seeds. On the other hand, significant differences ($p<0.05$) were found in the case of $I_{p,a}$ between pomace and seeds, with higher values than skins and stems (1.44, 1.33, 0.84 and 0.73 µA, respectively). According to the literature [15, 17, 20, 25], this peak could correspond to the reversible oxidation of the 3´, 4´-dihydroxyl moiety of phenolic compounds containing a flavonoid structure with a catechol or a galloyl group (i.e., ortho-diphenol and triphenol groups) at B-ring, like flavanols and flavonols, which are abundant in byproducts winemaking. This oxidation is pH- and concentration-depend and include a two-electron (2e)-two-proton (2H) oxidation reaction mechanism. The voltammetric determination is based on this anodic peak due to its reversibility, good reproducibility and linear dependence on current density of phenolic compounds [26]. Moreover, I_{pa} was expected to be proportional to the concentration of the antioxidants.

The area under the curve (Q_{500}), that is, the integral charge passed by 500 mV was used as measure of the concentration of total phenolic compounds. A major difference between

by-products was the appearance of Q_{500} in the voltammograms, the Q_{500} values were significantly lower for the skins and stems (2.36 and 2.17, respectively) than for pomace and seeds (3.74 and 3.29, respectively).

A cathodic peak was found on the reverse scan, which is related to anodic peak on a reversible electrode reaction. As seen in Table 2, there were also differences between by-products in the cathodic peak, both in the $I_{p,c}$ (163, 172, 139 and 147 mV for pomace and its parts seeds, skins and stems, respectively), and $E_{p,c}$ (1.27, 1.18, 0.78 and 0.67 μA for pomace and seeds, skins and stems, respectively).

Estimates of the formal potential are given by the potential mid-way between the anodic and cathodic peaks (E_{mid}), and by the potential midway between the half peak potential ($E_{p/2}$) and the anodic peak potential, which are particularly useful in ranking the relative reducing strength when some of the antioxidant are irreversibly oxidized. The largest values of E_{mid} were for the pomace and seeds, 203 and 212 mV, respectively.

A measure of reversibility is provided by the ratio of the cathodic to anodic peak current ($I_{p,c}/I_{p,a}$), which is closer to 1 with better reversibility for skins and stems. The reversibility is not related to the stability of the phenolic compounds, but does provide an additional parameter that can help identify the phenolic compounds present in a complex mixture such as wine [16].

To ascertain whether it was possible to classify between solid parts of grape (seeds, skins and stems) and pomace, as a function of the electrochemical profile, principal component (PCA) was carried out using the anodic data. In the space defined by the principal component analysis of the data (Figure 2), almost all the anodic voltammetric variability was explained by the first principal component (PC1, 99.6%). It is noteworthy that the main differences in PC 1 scores are related with the different by-products using in this study. A clear separation was found for pomace and seeds vs stems and skins. Within the

aforesaid two groups, a clear separation was found for the pomace and seeds group. Moreover, differences among samples for each subgroup were also evident for pomace and, in a lesser extent, for skins, stems and seeds. In the case of seeds, the evolution of samples was mainly found in PC 2, which only describes 0.2 % of the voltammetric variability.

The voltammetric regions close to 0.20 V and 0.49 V showed important contributions to the loadings which may be assigned to the oxidation of phenolic compounds containing a flavonoid structure with a catechol or a galloyl group at B-ring [20] and also may correspond to the irreversible oxidation of the -OH group at position 3 on the C-ring [27, 28].

Furthermore, in order to obtain more information about the chemical basis of the different electrochemical characteristics of by-products, attention was paid to the relationship between principal component 1 (PC1) and total phenolic content. The scores of this PC1 showed low but significant correlation (0.64, $p<0.05$) with total phenolic compounds. Therefore, this variability could be related with the differences in the amount of phenolic compounds.

Inhibition of lipid peroxidation

The extracts of pomace and each of its parts, seeds, skins and stems, induced inhibition of lipid peroxidation in rat liver homogenates exposed to oxidation with cumene hydroperoxide. A significant increase ($p<0.01$) in inhibition, relative to control, was observed after treatments with all by-products (Table 1). Pomace showed the greatest capacity to inhibit lipid peroxidation followed by seeds, stems and skins (90, 64, 49 and 48%, respectively). Pearson's correlation was applied to data to explore relationships between the lipid peroxidation inhibition and the TPC, and a significant correlation (R = 0.6, $p<0.05$) was found.

Our data agree with previous studies indicating that phenolic compounds are capable of inhibiting the lipid peroxidation in rat livers [29, 30]. In a study published by De Beer et al. [30], wines inhibited lipid peroxidation ranging from 33% to 59% for white wines and from 58 to 74% for red wines.

In previous works, flavanol contents exhibited the strongest correlations with antioxidant activity and lipid peroxidation inhibition [31, 32]. Our results indicate that pomace and seeds have the highest ability for inhibiting lipid peroxidation than stems and skins, which could be because these by-products are richer in flavanols than stems and skins [21, 32].

Voltammetric and TBARS data relationship

Finally, Pearson's correlation were applied to explore relationships between the voltammetric parameters (Q_{500} and $I_{p,a}$) and the results of TBARS assay. Significant and high linear correlations were found between values of lipid peroxidation inhibition and $I_{p,a}$ and Q_{500} ($p<0.05$; R = 0.81 and R = 0.83, respectively).

CONCLUSIONS

Cyclic voltammetry provides a reliable and good estimation of the total antioxidant potential of winemaking by-products, which showed different electrochemical behavior mainly due to the differences in the amount of phenolic compounds. The winemaking by-products greatly inhibited the lipid peroxidation, depending on the type of by-product and phenolic contents from each one.

Correlation analysis between electrochemical data and values of lipid peroxidation inhibition indicate a good correlation. Therefore, results suggest that cyclic voltammetry could be a promising technique to estimate the ability of phenolic extracts from winemaking by-products to inhibit lipid peroxidation in an *in vitro* biological system. Cyclic voltammetry, although it is not a substitute for biological analysis, it is an attractive alternative due to its speed measurement.

Acknowledgements

The authors acknowledge the collaboration of Consejo Regulador Condado de Huelva D.O. for supplying samples and the assistance of the technical staff of Biology Service (SGI, Universidad de Sevilla). Authors also thank the Ministerio de Economia y Competitividad, Gobierno de España, for financial support (Project AGL2014-58486-C2-2-R). M.J.J.P. thanks VPPI-Universidad de Sevilla for a postdoctoral grant.

References

[1] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of ageing, Nature, 408 (2000) 239-247.

[2] B. Halliwell, Antioxidants in human health and disease, Annu. Rev. Nut. 16 (1996) 33-50.

[3] C. López-Alarcón, A. Denicola, Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays, Anal. Chim. Acta 763 (2013) 1-10.

[4] F. Shahidi, P. Ambigaipalan, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review, J. Funct. Foods 18 (2015) 820-897.

[5] M.J. Jara-Palacios, D. Hernanz, T. Cifuentes-Gómez, M.L. Escudero-Gilete, F.J. Heredia, J.P.E. Spencer, Assessment of white grape pomace from winemaking as source of bioactive compounds, and its antiproliferative activity, Food Chem. 183 (2015) 78-82.

[6] F.J. Barba, A. Zhu, M. Koubaa, A.S. Sant'Ana, V. Orlien, Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review, Trends Food Sci. Tech. 49 (2016) 96-109.

[7] A.R. Fontana, A. Antoniolli, R.J. Bottini, Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics, J. Agr. Food Chem. 61 (2013) 8987-9003.

[8] F.Shahidi, Y. Zhong, Measurement of antioxidant activity, J. Funct. Foods 18 (2015) 757-781

[9] Y. Yang, J. Zhou, H. Zhang, P. Gai, X. Zhang, J. Chen, Electrochemical evaluation of total antioxidant capacities in fruit juice based on the guanine/graphene nanoribbon/glassy carbon electrode, Talanta 106 (2013) 206-211.

[10] S.M. Gomes, M.E. Ghica, I.A. Rodrigues, E. de Souza Gil, A.M. Oliveira-Brett, Flavonoids electrochemical detection in fruit extracts and total antioxidant capacity evaluation, Talanta 154 (2016) 284-291.

[11] S. Chevion, M.A. Roberts, M. Chevion, The use of cyclic voltammetry for the evaluation of antioxidant capacity, Free Radic. Biol.Med. 28 (2000) 860-870.

[12] C. Ceballos, H. Fernandez, Synthetic antioxidants determination in lard and vegetable oils by the use of voltammetric methods on disk ultramicroelectrodes, Food Res. Int. 33 (2000) 357-365.

[13] J. Chen, L. Gorton, B. Åkesson, Electrochemical studies on antioxidants in bovine milk, Anal. Chim. Acta, 474 (2002) 137-146.

[14] W.R. Sousa, C. da Rocha, C.L. Cardoso, D.H.S. Silva, M.V.B. Zanoni, Determination of the relative contribution of phenolic antioxidants in orange juice by voltammetric methods, J. Food Comp. Anal. 17 (2004) 619-633.

[15] P.A. Kilmartin, H. Zou, A.L. Waterhouse, A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics, J. Agr. Food Chem. 49 (2001) 1957-1965.

[16] P.A., Kilmartin, H. Zou, A.L. Waterhouse, Correlation of wine phenolic composition versus cyclic voltammetry response, Am. J. Enol. Vitic. 53, (2002) 294-302.

[17] M.J. Jara-Palacios, D. Hernanz, M.L. Escudero-Gilete, F.J. Heredia, Antioxidant potential of white grape pomaces: Phenolic composition and antioxidant capacity measured by spectrophotometric and cyclic voltammetry methods, Food Res. Int. 66 (2014) 150-157.

[18] S.M. Lunte, K.D. Blankenship, S.A. Read, Detection and identification of procyanidins and flavanols in wine by dual-electrode liquid chromatography-electrochemistry, Analyst 113 (1988) 99-102.

[19] B.K. Głod, I. Kiersztyn, P. Piszcz, Total antioxidant potential assay with cyclic voltammetry and/or differential pulse voltammetry measurements, J. Electroanal. Chem. 719 (2014) 24-29.

[20] M.J. Rebelo, R. Rego, M. Ferreira, M.C. Oliveira, Comparative study of the antioxidant capacity and polyphenol content of Douro wines by chemical and electrochemical methods, Food Chem. 141 (2013) 566-573.

[21] M.J. Jara-Palacios, D. Hernanz, S. González-Manzano, C. Santos-Buelga, M.L. Escudero-Gilete, F.J. Heredia, Detailed phenolic composition of white grape byproducts by RRLC/MS and measurement of the antioxidant activity, Talanta125 (2014) 51-57.

[22] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351-358.

[23] M.A. Rosillo, M. Sánchez-Hidalgo, A. Cárdeno, M. Aparicio-Soto, S. Sánchez-Fidalgo, I. Villegas, C.A. de la Lastra, Dietary supplementation of an ellagic acid enriched pomegranate extract attenuates chronic colonic inflammation in rats, Pharmacol. Res. 66 (2012) 235-42.

[24] StatSoft Inc. STATISTICA (data analysis software system), v 8.0. (Tulsa, OK) (2007).

[25] O. Makhotkina, P.A. Kilmartin, The phenolic composition of Sauvignon blanc juice profiled by cyclic voltammetry, Electrochim. Acta 83 (2012) 188-195.

[26] M. Šeruga, I. Novak, L. Jakobek, Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods, Food Chem. 124 (2011) 1208-1216.

[27] M.S. Cosio, S. Buratti, S. Mannino, S. Benedetti, Use of an electrochemical method to evaluate the antioxidant activity of herb extracts from the Labiatae family, Food Chem. 97 (2006) 725-731.

[28] P. Janeiro, A.M.O. Brett, Catechin electrochemical oxidation mechanisms, Anal. Chim. Acta 518 (2004) 109-115.

[29] S. Gonçalves, D. Gomes, P. Costa, A. Romano, The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants, Ind. Crops Prod. 43, (2013) 465- 471.

[30] A.E. Irondi, G. Oboh, S.O. Agboola, A.A. Boligon, M.L. Athayde, Phenolics extract of *Tetrapleura tetraptera*fruit inhibits xanthine oxidase and Fe2+-induced lipid peroxidation in the kidney, liver, and lungs tissues of rats *in vitro*, Food Sci.Human Well. 5 (2016) 17-23.

[31] D. De Beer, E. Joubert, W.C.A. Gelderblom, M. Manley, Antioxidant activity of South African red and white cultivar wines and selected phenolic compounds: In vitro inhibition of microsomal lipid peroxidation, Food Chem. 90 (2005) 569‑577.

[32] M.J. Jara-Palacios, B. Gordillo, M.L. González-Miret, D. Hernanz, M.L. Escudero-Gilete, F.J. Heredia, Comparative study of the enological potential of different

winemaking byproducts: Implications in the antioxidant activity and color expression of red wine anthocyanins in a model solution. J. Agr. Food Chem, 62 (2014) 6975-6983.

Figure captions

Figure 1. Cyclic voltammogram of pomace, seeds, skins and stems from the Zalema grape variety

Figure 2. Scatter plots of scores for principal component 1 and principal component 2 when voltammetric data for pomace, seeds, skins and stems were used as independent variables in principal component analysis.

Abbreviations

Cyclic voltammetry (CV)

Dry pomace (DP)

Principal component analysis (PCA)

Principal component (PC)

Total phenolic content (TPC)

Figure 1.

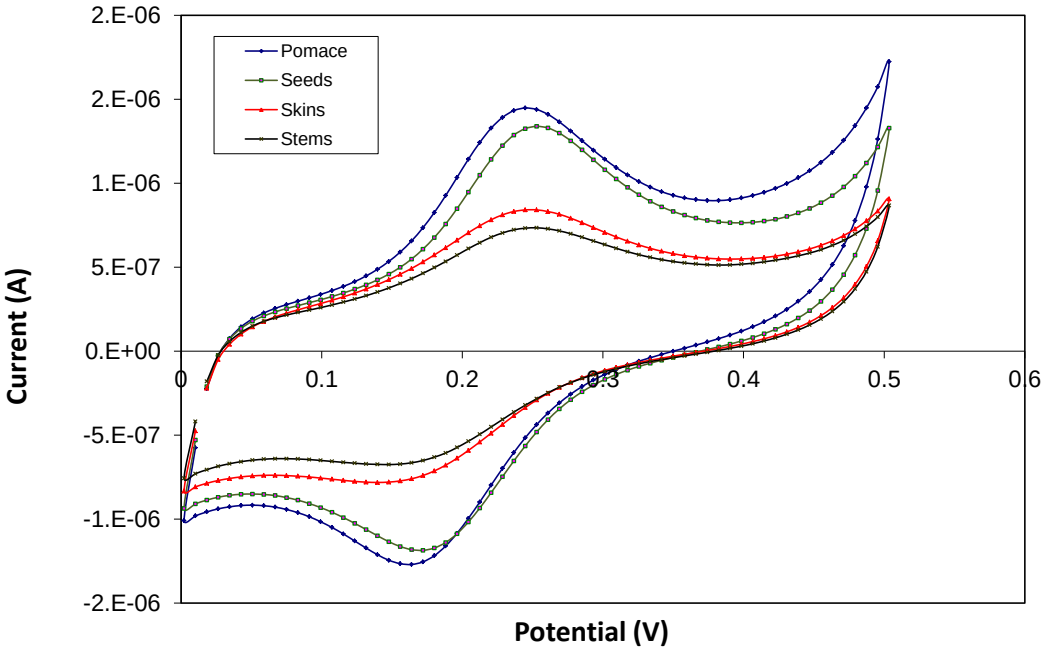
Pomace
Seeds
Skins
Stems
2.E-06
2.E-06
1.E-06
5.E-07
0.E+00
-5.E-07
-1.E-06
-2.E-06
0
0.1
0.2
0.3
0.4
0.5
0.6
Current (A)
Potential (V)

Figure 2.

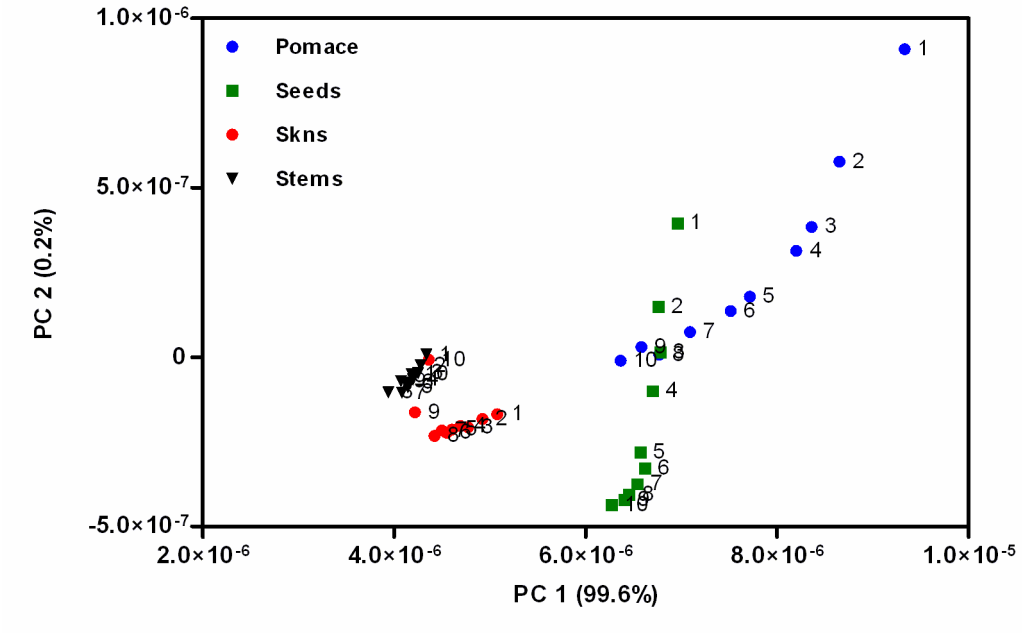
Pomace
Seeds
Skns
Stems
PC 2 (0.2%)
PC 1 (99.6%)
1.0×10-6
5.0×10-7
0
-5.0×10-7
2.0×10-6
4.0×10-6
6.0×10-6
8.0×10-6
1.0×10-5

Table 1. Total phenolic content (TPC) and TBARS values of by-products from *Vitis vinifera* L. cv. Zalema

Analysis/By-product	Pomace	Seeds	Skins	Stems
TPC (mg/g)	44.49 ± 4.15^{a}	48.13 ± 7.26^{a}	34.27 ± 9.60^{b}	35.73 ± 5.69^{b}
TBARS (%)	90.30 ± 6.36^{a}	64.15 ± 11.9^{b}	47.57 ± 8.74^{c}	49.41 ± 9.35^{c}

Different letters in the same row indicate significant differences by ANOVA test ($p< 0.05$).

Table 2. Electrochemical parameters extracted from the cyclic voltammetry curves of by-products from *Vitis vinifera* L. cv. Zalema

	$E_{p,a}$ (mV)	$E_{p,c}$ (mV)	$I_{p,a}$ (μA)	$I_{p,c}$ (μA)	E_{mid} (mV) (E°´)	$E_{p,a}$-$E_{p/2}$	$I_{p,c}/I_{p,a}$	Q_{500}
Pomace	244	163	1.44 ± 0.15^{d}	1.27 ± 0.12	203	73	0.88	3.745^{c}
Seeds	252	172	1.33 ± 0.05^{c}	1.18 ± 0.03	212	72	0.88	3.299^{b}
Skins	244	139	0.84 ± 0.08^{b}	0.78 ± 0.07	191	97	0.93	2.364^{a}
Stems	244	147	0.73 ± 0.03^{a}	0.67 ± 0.03	195	97	0.92	2.159^{a}

Different letters in the same column indicate significant differences by ANOVA test ($p< 0.05$).