Chapter 5

Applications of Voltammetric Analysis to Wine Products

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Additional information is available at the end of the chapter

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Abstract

Wine contains polyphenols that are responsible for its quality. Moreover, phenolic compounds have antioxidant properties and benefits on human health. Cyclic voltammetry (CV) was the first electrochemical method used for polyphenols characterization and determination of polyphenols content in wine products. Electrochemical behaviour of standard solutions of phenolic compounds has been investigated and evaluated the importance of the phenolic concentration and pH. The electrochemical parameters extracted from the voltammograms have been correlated with the antioxidant potential in wine products. In addition, CV allowed establishing differences in the antioxidant activity of wines with different addition of grape seeds. In winemaking by-products, different I_{pa} and Q_{500} values were found depending on the state of maturation of the grape pomace. On the other hand, the total flavonoids and phenolic acids contents were significantly correlated to the electrochemical parameters. Differences for the electrochemical parameters were found between by-products, being pomace and seeds which presented the greatest values of Q_{500}. Simple regression analyses showed that voltammetric parameters are correlated to their values of lipid peroxidation inhibition by thiobarbituric acid reactive substances method. Our results open the possibility of CV as a promising technique to estimate the global antioxidant potential of wine products rich in phenolic compounds.

Keywords: antioxidant activity, phenolic composition, electrochemical parameters, wine, wine by-products

1. Introduction

Grape juice is rich in phenolic compounds and plays a very important role in winemaking, mainly due to its content in pigments and tannins. Several hundred phenolic compounds have been identified in grapes, and they are transferred from grapes to wines during vinification. These compounds accumulate rapidly during berry maturation and they are very important for grape (and wine) character because they include red pigments, astringent flavours and browning substrates [1, 2].

The total phenol content of wine is less than that present in the grape because traditional methods of destemming, crushing and fermenting usually give extraction rates of no more than 60% [3].

The major phenolic compounds found in wine are either members of the diphenylpropanoids (flavonoids) or phenylpropanoids (nonflavonoids). Flavonoids, including anthocyanins, flavonols and flavan-3-ols (catechin, epicatechin, and their procyanidin polymers), are the most important phenolics for wine quality [4]. Flavonoids are derived primarily from the seeds, skins and stems of the grape. Anthocyanins and flavonols are extracted mainly from skins, and catechins and leucoanthocyanins reside mainly in seeds and stems. Increasing skin contact time and fermentation temperature, and the degree of berry disruption increase the flavonoid content of a wine [5].

Nonflavonoids are structurally simpler, but their origin in wine is more diverse. In wines not aged in oak, the primary nonflavonoids are derivatives of hydroxycinnamic and hydroxybenzoic acids [1]. They are stored primarily in cell vacuoles of skin and pulp, and are easily extracted on crushing. The most numerous and variable are hydroxycinnamic acid derivatives. They occur principally as esters with tartaric acid (for example, caftaric, coutaric and fertaric acids, the tartaric acid esters of caffeic, p-coumaric and ferulic acids, respectively), but may also be associated with sugars, various alcohols or other organic acids. The esters also slowly hydrolyse during fermentation [6]. The most common nonflavonoid in grapes is caftaric acid, one of the primary substrates for polyphenol oxidase. It often plays an important role in oxidative browning of must [7, 8].

Particularly in wine red, flavonoids and some phenolic acids (colourless phenols) are involved in the chemical stabilization of anthocyanin pigments by means of non-covalent interactions through intermolecular co-pigmentation reactions [8, 9]. Studies were carried out in model solution and focused on the application of objective colour measurements. These studies have demonstrated that co-pigmentation causes the stabilization of the coloured forms of the anthocyanins and consequently enhance their colour of wine [10, 11].

In the last years, the pre-fermentative cold maceration, also known as cold soaking or cryomaceration, is being increasingly used by enologists worldwide in order to improve some important quality characteristics of wines such as colour and aroma [12–16]. This technique consists in maintaining the crushed grapes at low temperatures (5–10°C) for a variable period (from one to several weeks), and thus the beginning of the fermentation process is delayed. During this period, the extraction of polyphenols from the skins to the

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must takes place in the absence of ethanol. With reference to the phenolic compounds, these compounds contribute to colour stability since they can act as oxidation substrates in white wines [17–19]. Controlling skin contact conditions is vital to obtain high-quality white wines [20]. Skin contact may greatly increase both the total hydroxycinnamate and flavanol concentration [21].

Wine, especially red wine, is a very rich source of polyphenols, such as flavanols (catechin, epicatechin, etc.), flavonols (quercetin, rutin, myricetin, etc.), anthocyanins (the most abundant is malvidin-3-O-glucoside), oligomeric and polymeric proanthocyanidins, phenolic acids (gallic acid, caffeic acid, p-coumaric acid, etc.), stilbenes (trans-resveratrol) and many others polyphenols.

Winemaking generates a high amount of by-products that cause environmental and economic problems, which could be minimized by the exploitation and valorization of those products, such as their use in pharmaceutical and food industries. Grape pomace, consisting of seeds, skins and stems, is the main winemaking by-product and is a rich source in phenolic compounds with interest by their biological and antioxidant properties [22, 23].

Many of these phenolic compounds have been reported to have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties [24, 25]. These biological properties are attributed mainly to their powerful antioxidant and antiradical activity.

1.1. Antioxidant activity

Oxidative stress takes place when there is an imbalance between the production of reactive species and the antioxidant defence systems, that is, there is a disturbance in the pro-oxidant-antioxidant balance in favour of the oxidant species, leading to potential damage [26–29]. The oxidative damage that takes place is defined as biomolecular damage caused by the attack of reactive species on the cells and tissues of the living organisms [30].

These reactive species are oxidant agents and/or convertible into free radicals easily. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most common reactive species. ROS, such as superoxide anion radical ($O_2^{\bullet -}$), 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) [30].

The action mechanism of the free radical is based on the attack to the target molecule to remove a hydrogen atom, or an electron, and so the unpaired electron of the radical one turns into a more stable electrons par. In this process, the target molecule oxidizes. The principal target molecules are DNA, proteins and lipids. Antioxidants are compounds or systems that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

The antioxidant capacity is defined as the ability of compound (or mixture of compounds) to inhibit the oxidative degradation of various compounds. Antioxidant functions imply

lowering oxidative stress, DNA mutations, malignant transformations, as well as other parameters of cell damage.

The human organism has an antioxidant defence system to neutralize the excessive levels of ROS and RNS and protect to the cells from oxidative damage. This defence system can be from endogenous (enzymatic and non-enzymatic) or exogenous origin. The enzymatic systems, especially superoxide dismutases (SOD), catalases (CAT) and glutathione peroxidases (GPX), are recognized as being highly efficient in ROS detoxification [31–33]. The main small molecule non-enzymatic antioxidants present in the human organism are bilirubin, estrogenic sex hormones, uric acid, ascorbic acid, coenzyme Q, melanin, melatonin, α-tocopherol and lipoic acid [34, 35]. The diet provides to the body with basic nutrients (proteins, vitamins, minerals) and phytochemical substances that have antioxidant activity and help endogenous defence systems.

Fruits and vegetables are accepted as good sources of natural antioxidants, which provide protection against free radicals and have been associated with lower incidence and mortality rates of cancer and heart diseases in addition to a number of other health benefits [36–38]. Higher plants and their constituents provide a rich source of natural antioxidants, such as carotenoids, tocopherols and polyphenols that are found abundantly in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas and oils. In addition, by-products from the food and agricultural industries have been explored for their potential use as antioxidants. For example, seeds, skins and stem of pomace from winemaking, hulls, shells and skins of nuts and cereals, citrus peels and seeds have been found to possess antioxidant activity [39–42].

There are several methods for evaluating antioxidant activity, either *in vitro* or *in vivo*. The *in vitro* assays can be classified into chemical methods (spectrophotometric methods and electrochemical techniques) and biological methods (cellular systems).

The most used spectrophotometric methods are 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC), which measure the ability of antioxidants to scavenge a radical; ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) and cerium reducing antioxidant capacity (CERAC), which measure the capacity of antioxidant to reduce metals; and thiobarbituric acid reactive substances (TBARS), which is used to measure the lipid peroxidation inhibition [43]. These spectrometric methods are mainly used in the analysis of antioxidant properties. However, these methods are dependent on many parameters, such as temperature, time of the analysis, character of a compound or mixture of compounds (extracts), concentration of antioxidants and pro-oxidants and many other substances. In addition, they are based on different action mechanisms so the antioxidant activity value of the samples differs according to the used test [44, 45]. The measurement of antioxidant activity cannot be evaluated satisfactorily using a simple antioxidant test due to the many variables influencing the results [46].

Total phenolic content (TPC) is another parameter used for evaluation of antioxidant extracts. The Folin-Ciocalteu assay is the well-known method for determination of TPC. This method

is used to analyses phenolic components in wine, and it became a routine analysis for antioxidant assessment of food and plant extracts [43].

On the other hand, electrochemical methods for evaluating antioxidant activity have emerged in the past decade, among which cyclic voltammetry (CV) has attracted much attention as an alternative method to conventional chemical assays. Cyclic voltammetry measures electron-donation capability (redox potential) of antioxidants, which respond to a voltammetric scan according to their redox potential [43].

1.2. 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 already been applied to evaluate of antioxidant capacity in blood plasma [47], plant extracts [48], vegetable oils [49], milk [50] and orange juice [51].

CV has been successfully used to determine the phenolic content of wines and to correlate the analytical response to the antioxidant capacity of these wines [52–54]. 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). Other studies also demonstrated the coherence of the cyclic voltammetric response with the information provided by HPLC, Folin-Ciocalteu assays and absorbance at 280 nm on white and red wines [53]. CV does not allow identifying individual antioxidants present in the sample; however, the technique provides the sum of total antioxidants [55]. According to Kilmartin et al. [52], Makhotkina and Kilmartin [56] and Rebelo et al. [57], each voltammetric peak is ascribed to different groups of phenolic compounds.

In addition, CV was also used to investigate the influence of sulphur dioxide, glutathione and ascorbic acid on polyphenol oxidation processes relevant to wine oxidation [58] and to correlate analytical response to sensory characteristics such as astringency [59].

2. Experimental part

2.1. Cyclic voltammetry measurement

The experimental configuration for recording cyclic voltammograms consists of an electrochemical cell that has a tree electrodes, counter or auxiliary electrode, reference electrode and working electrode, all immersed in a liquid and connected to a potentiostat. The potentiostat AUTOLAB model PGSTAT 302 N (Metrohm-Eco Chemie, Netherlands), controlled by a General Purpose Electrochemical System (GPES) software and conventional three-electrode system consisting of a glassy carbon working electrode, platinum auxiliary electrode and Ag/AgCl reference electrode, was used for all electrochemical measurements.

All measurements were carried out at room temperature using a conventional three-electrode system. Prior to the measurements, the working electrode was polished in alumina/water

suspension, rinsed with Milli-Q water and sonicated for 2 min. The electrolyte solution 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. The solution was de-aerated with an inert gas (N_2) for 10 min, and after a 1 min running scan was taken. The cyclic voltammogram scans were made from 0.0 to 0.5 V at a scanning rate of 5 mV/s for winemaking by-products and 0.0 to 1.0 V for wine solutions.

The electrochemical parameters extracted from the cyclic voltammetry curve were 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 $E_{p,a} - E_p/2$. The anodic current area (Q), which represents the total integrated area of the cyclic voltammogram for scans taken from 0 to 1 V (QT) or from 0 to 0.5 V (Q_{500}), was also extracted. In addition, QI, QII and QIII were also calculated; these parameters represent the area corresponding to peaks I, II and III, respectively (**Figure 1**).

These parameters were taken from the cyclic voltammograms after subtracting 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.

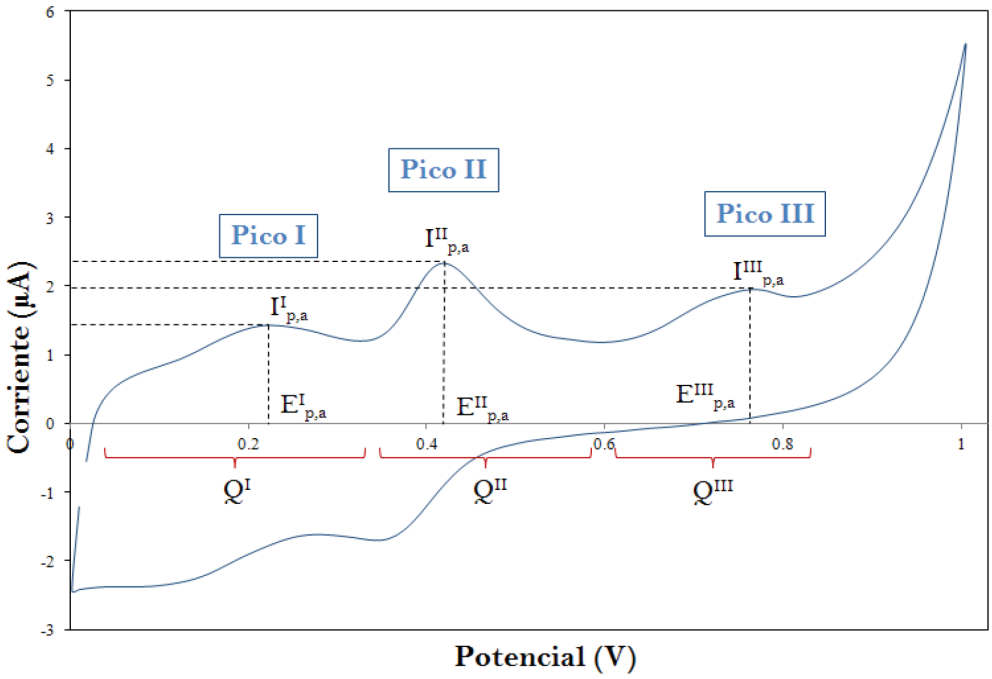

Figure 1. Representative cyclic voltammogram of a grape pomace.

2.2. Applications of cyclic voltammetry

2.2.1. Electrochemical properties of phenolic compounds

In this study, CV was used to monitor the electrochemical behaviour of four phenolic compounds representing the main phenolic groups found in wines and winemaking by-products, namely gallic acid (hydroxibenzoic acid), caffeic acid (hydroxycinnamic acid), catechin (flavanol) and quercetin (flavonol). These compounds have in common a catechol moiety believed to be the electrochemically active group [52]. All the compounds measured acted as powerful antioxidants and were oxidized on a glassy carbon electrode. The electrochemical measurements were taken at different pH (3.6 and 7), concentrations (100 and 500 mg/L) and potential scans (0.5 and 1 V). **Table 1** shows the electrochemical parameters ($I_{p,a}$, $E_{p,a}$, Q) extracted from the cyclic voltammetry curves of the compounds. In a previous paper, Q and $I_{p,a}$ parameters showed a strong and significant correlations ($R = 0.95$, $p < 0.05$), and therefore results are discussed based on parameter $I_{p,a}$.

The pH influence was very important on electrochemical behaviour of these compounds. As can be seen in **Figure 2**, cyclic voltammograms of phenolic compounds, adjusted to pH 7, showed one well-defined anodic peak between 0.2 and 0.3 V. However, two anodic peaks were exhibited to pH 3.6, between 0.1 and 0.3 V, and between 0.3 and 0.5 V. Considering cyclic voltammograms to pH 7, catechin and quercetin showed higher values of $I_{p,a}$ (1.32 μA) than gallic and caffeic acids (1.20 and 1.10 μA, respectively). The anodic peak potential ($E_{p,a}$) ranged between 0.25 V for quercetin and 0.27 for caffeic acid.

			Peak I			Peak II		
	pH	**Concentration (mg/L)**	E_{pa} **(V)**	I_{pa} **(μA)**	Q	E_{pa} **(V)**	I_{pa} **(μA)**	Q
Gallic acid	3	100	0.21	1.76	0.346	0.43	4.24	0.489
	3	500	0.22	1.93	0.354	0.43	5.97	0.659
	7	100	0.26	1.20	-	-	-	-
Caffeic acid	3	100	0.22	1.94	0.371	0.46	3.86	0.446
	3	500	0.21	1.74	0.330	0.46	5.25	0.569
	7	100	0.27	1.10	-	-	-	-
Catechin	3	100	0.21	1.72	0.341	0.46	3.29	0.415
	3	500	0.24	1.61	0.306	0.41	2.59	0.333
	7	100	0.26	1.32	-	-	-	-
Quercetin	3	100	0.22	1.88	0.363	0.45	2.52	0.340
	3	500	0.22	1.73	0.328	0.42	2.49	0.329
	7	100	0.25	1.32	-	-	-	-

Table 1. Electrochemical parameters of anodic peaks extracted from the cyclic voltammetry curves of the phenolic compounds.

Regarding to cyclic voltammograms to pH 3.6, values of $I_{p,a}$, $E_{p,a}$ and Q depended on the peak (I or II) and the concentration (100 or 500 mg/L). As can be observed in **Table 1**, peak II had higher values of $I_{p,a}$ than peak I; gallic acid showed the highest values at 100 and 500 mg/L (4.24 and 5.97 μA, respectively).

It is known that pH is the most significant factor determining the antioxidant activity of phenolic compounds. Yakovleva et al. [48] observed that with increasing pH values the anodic peak voltage decreased, which was caused by the decrease in the degree of antioxidant protonation and the resulting shift of the charge of the molecule to negative values.

The phenolic concentration is also an important factor for values of electrochemical parameters. As previously described, $I_{p,a}$ increases with increasing concentrations of phenolics although the relationship is not always linear [53]. $I_{p,a}$ increases with the concentration for gallic acid (4.24 and 5.97 μA for 100 and 500 mg/L, respectively) and caffeic acid (3.86 and 5.25 μA for 100 and 500 mg/L, respectively); however, this increase did not occur for catechin and quercetin (**Table 1**).

Cyclic voltammograms of gallic acid and catechin at 1 V are shown in **Figure 3**. The main peak for both compounds was at 0.43 V, and $I_{p,a}$ was 6.98 and 2.95 μA for gallic acid and catechin, respectively. The potential range is a significant factor since the flexibility of adjusting the electrode potential to higher values allows a progressively wider range of phenolic compounds to be monitored [60].

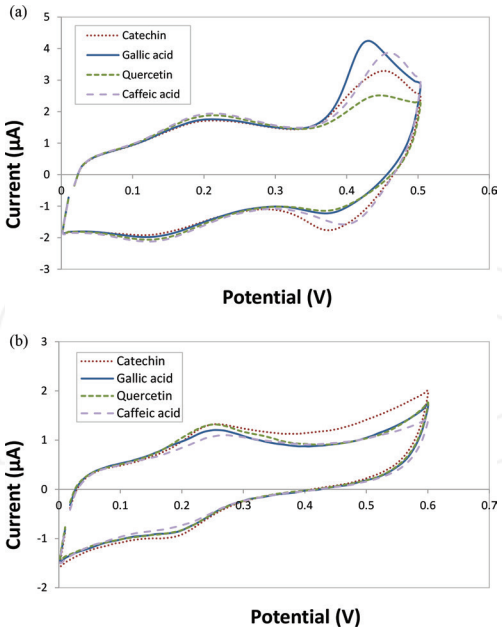

Figure 2. Cyclic voltammograms of phenolic compounds adjusted to pH 3.6 (a) and pH 7 (b).

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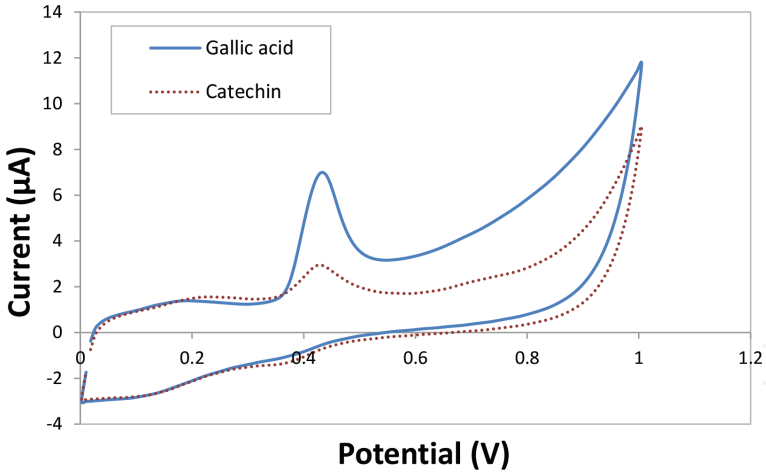

Figure 3. Cyclic voltammograms of gallic acid and catechin at 1 V.

2.2.2. Electrochemical properties of wine products

Before measures by CV, the samples containing phenolic compounds must be prepared correctly. It is important to control factors such as phenolic concentration and pH of the solution, because, as previously mentioned, these factors influence the electrochemical properties.

In order to obtain the suitable phenolic concentration and pH, samples are diluted with the corresponding buffer: 0.1 M sodium acetate-acetic acid buffer for pH 3.6, and 5% (w/v) 50 mM disodium hydrogen phosphate and 35% (w/v) 50 mM sodium dihydrogen phosphate for pH 7 [39, 61].

For this study, several dilutions (1/50, 1/25, 1/17, 1/12.5) of grape pomace extract were prepared in phosphate buffer (pH 7). **Table 2** shows the electrochemical parameters for diluted grape pomace extract determined from CV. These dilutions provided well-defined voltammetric peaks which had peak currents ($I_{p,a}$ and $I_{p,c}$) that changed linearly with wine dilution. $I_{p,a}$ ranged between 2.15 and 2.62 µA for most diluted (1/50) and most concentrated (1/12.5) solutions, respectively. As can be observed in **Figure 4**, the anodic current area was highest for solution most concentrated (Q_{500} = 5.67), which has more amount of phenolic compounds.

In order to explore the relationship between the concentration of grape pomace extract (according to dilution) and the electrochemical parameters ($I_{p,a}$ and Q_{500}), simple correlation analysis was realized. Significant and high linear correlations were found for $I_{p,a}$ and Q_{500} with concentration (**Figure 5**).

Each type of sample requires a different dilution to obtain well-defined voltammetric peaks and anodic peaks charge directly proportional to the volume fraction of the samples. Kilmartin [60] indicated that white wines require around 10-fold dilution and red wines up to 400-fold.

Rebelo et al. [57] reported that red wine samples required a 50-fold dilution to reach a range in which the anodic peak charge was directly proportional to the final volume of the wines. In our previous studies [39, 61, 62], a 25-fold dilution was required for winemaking by-products and wine samples.

Dilution	$E_{p,a}$ (mV)	$E_{p,c}$ (mV)	$I_{p,a}$ (μA)	$I_{p,c}$ (μA)	$E_{mid\ (mV)\ (E^{o'})}$	$E_{p,a}$–$E_{p/2}$	$I_{p,c}/I_{p,a}$	$\Delta E > 59$	Q_{500}
1/50	236	171	2.15	1.72	203	65	0.80	65	5.02
1/25	236	163	2.41	1.86	200	65	0.77	73	5.27
1/17	236	163	2.59	2.00	200	56	0.77	73	5.59
1/12.5	244	155	2.62	2.00	200	64	0.76	89	5.67

Table 2. Electrochemical parameters for diluted pomace extracts determined from cyclic voltammetry.

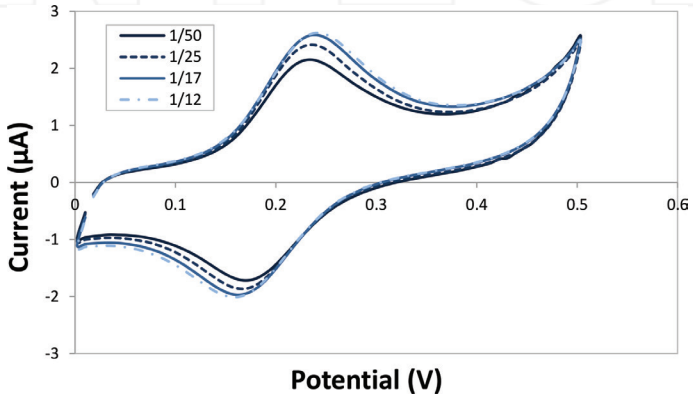

Figure 4. Cyclic voltammograms of diluted pomace extracts.

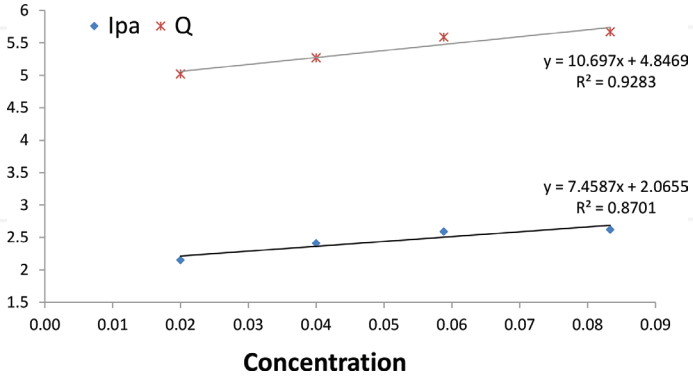

Figure 5. Correlations between the concentration of grape pomace extract and the electrochemical parameters ($I_{p,a}$ and Q_{500}).

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2.2.2.1. Winemaking by-products

CV was used for determining the total antioxidant activity of phenolic compounds present in winemaking by-products.

Firstly, extracts from grape pomaces (including seeds, skins and stems) at different states of maturation (early, technological and late harvest: EH, TH, LH, respectively) were measured at pH 7 and 0.5 V, and a one well-defined anodic peak was showed at 0.24 V in the three cyclic voltammograms (**Figure 6**). Differences were found in I_{pa} depending on the state of maturation (**Table 3**). $I_{p,a}$ was highest for grape pomace at EH, followed by TH and LH (1.62, 1.50 and 1.28 μA, respectively). In addition, the anodic current area (Q_{500}) was extracted from the cyclic voltammetry curves (**Table 3**). This electrochemical parameter represents the integrated area of the cyclic voltammogram for scans taken from 0 to 0.5 V. Values of Q_{500} were in accordance with $I_{p,a}$, thus, grape pomace at early harvest, with the highest I_{pa}, showed the highest Q_{500} (0.42) followed in decreasing order by those at TH and LH (0.38 and 0.33, respectively). As shown in **Table 3**, these results were also in accordance with the total phenolic content. $E_{p,a}$ value was 0.24 V in all states of maturation.

Seeds, skins and stems from grape pomace were also measured separately at three states of maturation. **Figures 7–9** show the cyclic voltammograms for seeds, skins and stems, respectively. As can be observed, differences in voltammograms depending on the state of maturation were found. For skins and stems, $I_{p,a}$ decreased from EH to LH; however, for seeds the evolution of this parameter was different. Considering Q_{500}, differences depending on the state of maturation were found for seeds, skins and stems, and the evolution was the same as for $I_{p,a}$ (**Table 3**).

These data indicate that CV provides a reliable and good estimation of the state of maturation of winemaking by-products, because different electrochemical behaviour is shown depending on the state of maturation.

CV is also used to evaluate the differences in the antioxidant potential between grape pomaces from different variety. In our previous study [39], the electrochemical behaviour of grape pomaces

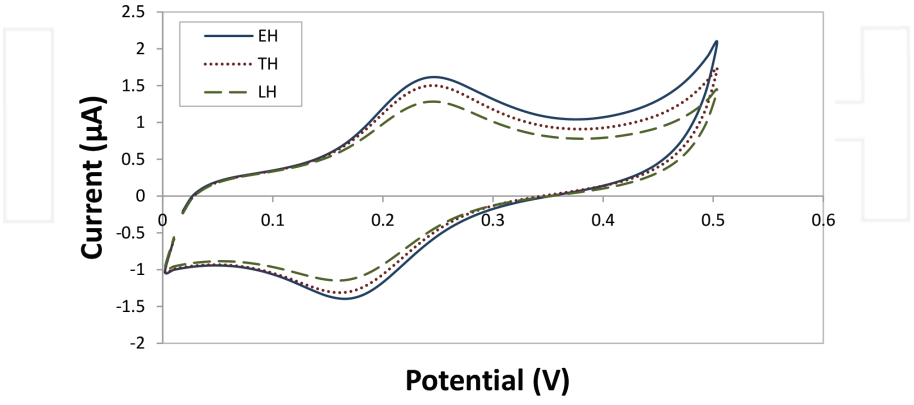

Figure 6. Cyclic voltammograms of grape pomace at different states of maturation (EH, TH, LH).

	Electrochemical parameters			
	State of maturation	$E_{p,a}$	$I_{p,a}$	Q_{500}
Pomace	EH	0.24	1.62	0.42
	TH	0.24	1.50	0.38
	LH	0.24	1.28	0.33
Seeds	EH	0.25	1.36	0.34
	TH	0.25	1.38	0.33
	LH	0.25	1.32	0.32
Skins	EH	0.25	0.82	0.23
	TH	0.25	0.78	0.22
	LH	0.25	0.75	0.21
Stems	EH	0.25	0.79	0.23
	TH	0.25	0.77	0.22
	LH	0.25	0.73	0.21

Table 3. Electrochemical parameters of anodic peak extracted from the cyclic voltammetry curves of winemaking by-products at different state of maturation.

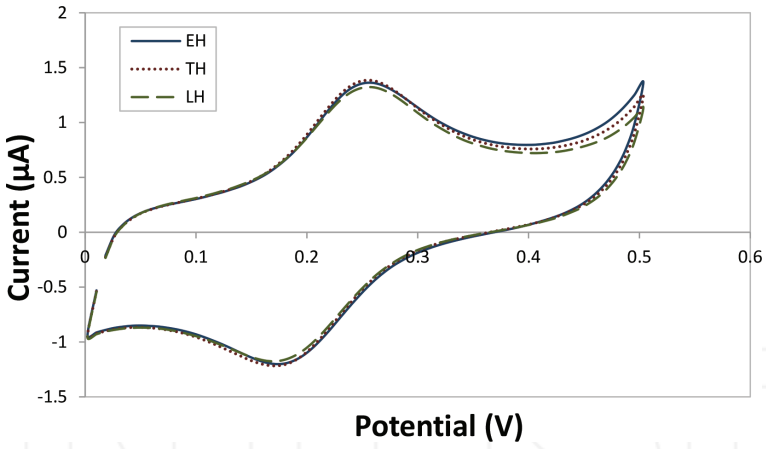

Figure 7. Cyclic voltammograms of seeds at different states of maturation (EH, TH, LH).

from nine different varieties of white grapes was studied. The cyclic voltammogram scans were made from 0 to 1 V at pH 3.6 and three different anodic peaks were observed in the voltammogram. The electrochemical parameter $I_{p,a}$ for peaks I, II and III was significantly different among varieties. Peak I was correlated mainly to phenolic acids and flavonols, peak II to flavanols and peak III to three phenolic groups. Results suggested 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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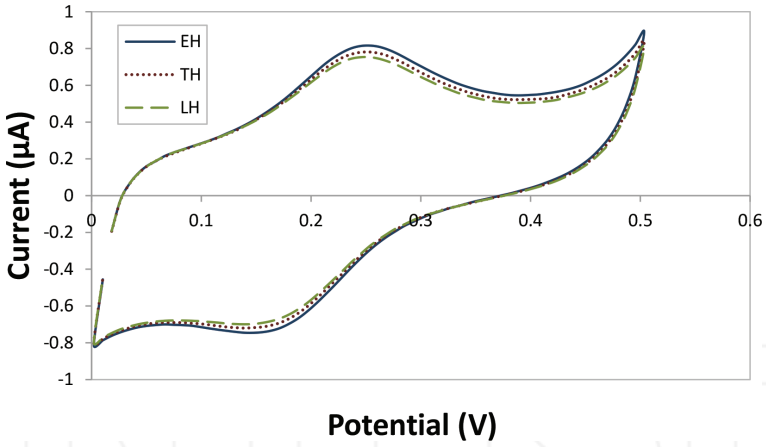

Figure 8. Cyclic voltammograms of skins at different states of maturation (EH, TH, LH).

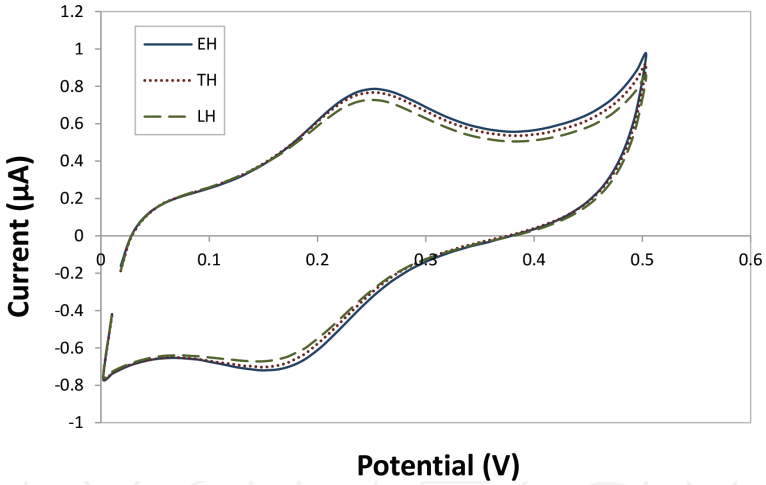

Figure 9. Cyclic voltammograms of stems at different states of maturation (EH, TH, LH).

On the other hand, CV was used as a measurement of the total antioxidant activity of different winemaking by-products: grape pomace, seeds, skins and stems [61]. The cyclic voltammograms scans were made from 0 to 0.5 V at pH 7 and the main anodic peak was examined by extracting the electrochemical parameters. Regarding $I_{p,a}$, significant differences ($p < 0.05$) were found between pomace and seeds, with higher values than skins and stems (1.44, 1.33, 0.84 and 0.73 µA, respectively). Q_{500} was used as a measure of the concentration of the total phenolic compounds and values of this parameter 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). In this study, a principal component analysis allowed to classify between seeds, skins, stems and pomace, as a function of the electrochemical profile. Finally in this study,

relationships between the voltammetric parameters (Q_{500} and $I_{p,a}$) and the results of inhibition of lipid peroxidation of winemaking by-products were explored. Results suggested that CV could be a good technique to estimate the ability of winemaking by-products to inhibit lipid peroxidation in an *in vitro* biological system.

2.2.2.2. Wine

Finally, CV can be used for the characterization of phenolic compounds in wine on the basis that practically all polyphenolic molecules present in wine are electrochemically active. CV was the first electrochemical method used for characterization of phenolic compounds and determination of the total phenolic content in wines [63].

CV can be utilized for direct evaluation of antioxidant activity in real samples of red wine and white wine [54, 64], and for the quantification of antioxidants on a carbon electrode in wine samples [53, 58].

In a previous work [62], we use CV to determine the electrochemical behaviour of red wines at the beginning and the end of vinification in order to study their antioxidant activity. In the cited study, three types of experimental vinification processes were performed with mixtures of Syrah grapes and addition of Pedro Ximénez seeds (simple and double dose). Grape seeds are a natural source of phenolic compounds, particularly flavanols, and their addition could improve the biological properties of wines. CV allowed establishing differences according to the area under the curve (QT, QI, QII and QIII) between control wines (without seeds) and wines with addition of seeds. Electrochemical results indicated that wines with double dose of seeds had better antioxidant activity than control wines.

3. Conclusion

Electrochemical technique, specifically cyclic voltammetry, has been used to estimate the total antioxidant potential of phenolic extract from wine products. This contribution accentuates the role of electrochemical techniques for the determination of antioxidant activity in samples.

The electrochemical behaviour of standard solutions of the main phenolic groups found in wines has been investigated and the influence of the sample matrix has been evaluated. In winemaking by-products, CV provides a reliable and good estimation of the state of maturation and the electrochemical parameters were significantly correlated to the total flavanols, flavonols and phenolic acid contents. Moreover, a good correlated was obtained between voltammetric parameters and values of lipid peroxidation inhibition *in vitro* biological system, measured by TBARS procedure. Additionally, CV allowed establishing differences in the antioxidant activity of wines with different addition of grape seeds.

Cyclic voltammetry proved to be a useful technique to estimate the antioxidant potential of wine products.

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