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Original Research Article

Contribution of metals, sulfur-dioxide and phenolic compounds to the antioxidant capacity of Carménère wines

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ABSTRACT

In this research, we determined the chemical composition of 37 *Vitis vinifera* cv. Carménère wines and established their antioxidant capacity (AC). A set of 26 measurements of chemical species potentially related to AC, including phenolic compounds, free and combined sulfites and iron, copper and manganese were obtained for each wine. The AC was estimated by DPPH$^{\bullet}$ and ORAC-FL methods. Statistical analyses showed good correlations between chemical profiles and AC values (DPPH $\rho^2 = 0.90$ and ORAC-FL $\rho^2 = 0.87$). The main chemical markers contributing to AC were the fraction of color given by free and copigmented anthocyanins (39.1%) for DPPH and gallic acid (30.7%) for ORAC-FL. These are a good indication of the complexity of the wine matrix, and the wide variety of substances possibly contributing to AC.

1. Introduction

Wine, especially red, contains a wide array of antioxidant species (e.g. phenolic compounds), some of which have been linked with the wine's capacity to withstand aeration and aging (Laurie and Clark, 2010). Although phenolics seem to be the main contributors to the antioxidant capacity (AC) in wines (Laurie and Clark, 2010; Li et al., 2009) – among them, flavanols, flavonols and anthocyanins are the most abundant subclasses, in concentrations ranging from 100–200 mg L^{-1} for flavanols, 53–200 mg L^{-1} for flavonols, and 90–400 mg L^{-1} for anthocyanins, for a broad range of red wines (Waterhouse, 2002) – a larger pool of compounds such as transition metals and sulfites are also known to influence the radical quenching ability of wine (Laggnera et al., 2005). Moreover, the interaction between these compounds might increase or reduce AC in wines (Danilewicz et al., 2008).

In red wines, flavonoids are the predominant phenolic species (Neveu et al., 2010; Waterhouse, 2002), so they could be expected to be one of the main contributors to AC. However, the chemical structure and the degree of condensation of wine phenolics are also relevant in determining AC (Danilewicz et al., 2008). For instance, as the wine ages, some of the larger polymeric phenolics could precipitate, while others could break into smaller units resulting in additional reactive sites (Villamor et al., 2009) and unusual variations in AC.

In addition to the phenolic content, free metal ions such as Cu^{2+} or Fe^{2+} can be relevant because they catalyze Fenton-type reactions (Danilewicz, 2003), and therefore decrease AC. On the other hand, sulfites may produce the inverse opposite effect (Laurie and Waterhouse, 2006; Waterhouse and Laurie, 2006; Elias et al., 2009). The most abundant transition metals in wines are iron and copper, with concentrations ranging between 2.8–16 and 0.11–3.6 mg L^{-1}, respectively (Laurie et al., 2010; Pohl, 2007).

Recently, Elias et al. (2009) offered further evidence regarding an oxidative mechanism in wines in which the presence of metals is key. According to their proposal, oxygen is converted to a hydroperoxyl radical by a reduced metal (i.e. Fe^{2+}), which then directly oxidizes a catechol to its semiquinone radical, producing also hydrogen peroxide, a form of oxygen that is incapable of directly oxidizing significant amounts of ethanol. However, if this peroxide reacts with copper or iron (catalysts in Fenton-type reactions), the highly reactive hydroxyl radical could be formed

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(Danilewicz, 2003; Laurie and Waterhouse, 2006). Another action of the metals in wine is the formation of complexes with polyphenols (Esparza et al., 2004; Hynes and Ó Coinceanainn, 2001).

Sulfites, on the other hand (bisulfite in particular), can react directly with hydrogen peroxide, thus decreasing the formation of oxygen radicals and increasing AC. Likewise, sulfites and thiols can also react with quinones, which in effect reduce browning as well as the AC (Laurie et al., 2012).

Among the many available assays for estimating AC, some of the most common are based on either hydrogen atom transfer (HAT) or electron transfer (ET). Due to the complexity of the wine matrices and the intricate nature of the reactions involving radicals, different methods of quantification of AC should be used, as they may even yield opposite results (Ou et al., 2001; Huang et al., 2005).

This study was carried out in order to obtain a range of compositional values representative of the Carménère variety of wine. Even though a few studies have reported some compositional features of this variety (Pszczólkowski, 2004; Fernández et al., 2007; Obreque-Slier et al., 2010), none of them have focused on the relative contribution of specific chemical species that could affect AC. Only varietal Carménère wines were employed in this research as an opportunity to further characterize the chemical composition of this recently rediscovered red grape variety in Chile. Furthermore, the influence of the vintage on the relative contribution of the chemical composition on antioxidant capacity is presented.

Consequently, this study explored AC in Carménère wines using two different methodologies: oxygen radical absorbance capacity (ORAC-FL) and α, α-diphenyl-β-picrylhydrazyl free radical scavenging (DPPH); we also determined the relative influence of selected compositional features on the AC values obtained. Specifically, this study offered a detailed compositional profiling of Carménère wines, which is rarely available in the literature as this variety has only been recently rediscovered.

2. Materials and methods

2.1. Wine samples

Commercial varietal red wines of the Carménère variety ($n = 37$), from 2008 to 2010 vintages, were obtained directly from Chilean wineries located throughout a geographic span of almost 700 km north to south (Fig. 1). One of the samples was obtained in Elqui valley (Lat. 29°58′S, Long. 71°4′W. Altitude 198 m), 14 of them were gathered between Maipo and Colchagua valley (Lat/long between 33°45′S/70°42′W and 34°36″S/71°21′W.; altitude between 472 and 158 m), and the remaining 22 were obtained between the Curicó and Maule valleys (Lat/long between 34°44′S/70°59′W and 35°56′S and 72°19′W.; altitude between 325 and 154 m).

The sample aliquots for analysis were obtained by puncturing the corks of each bottle with a syringe needle and sealing them afterwards to avoid over-aerating the wine between analyses. The sample aliquots needed for each analysis were taken at the time of each measurement in order to reflect the chemical composition of the wines as accurately as possible.

2.2. Chemicals

AAPH (2,2-azobis (2-methylpropionamidine) dihydrochloride), fluorescein (as disodium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH, Folin-Ciocalteu reagent, hydrochloric acid, triethanolamine, bovine serum album, ferric chloride hexahydrate, sodium dodecyl sulfate, potassium metabisulfite, maleic acid, and sodium carbonate, gallic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, *trans*-resveratrol, myricetin, (−)-epicatechin and kaempferol also were obtained from Sigma (St. Louis, MO, USA). Milli-Q water was used for dilutions in all measurements.

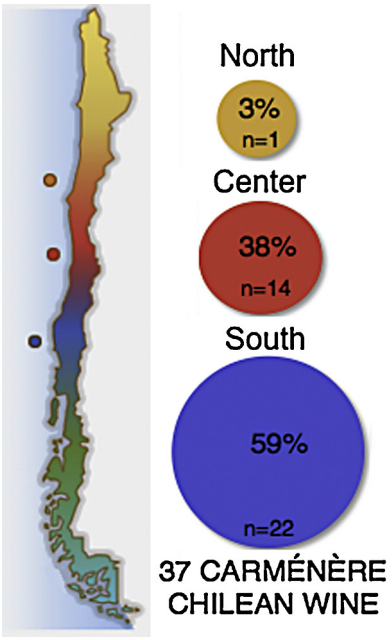

Fig. 1. Distribution map of samples by origin (by north, central and south zone of Chile). Diagram not to scale. Color dots are reference points of a wider geographical area. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.3. Compositional analyses

2.3.1. Polyphenol compound analysis

The measurement of total phenolics was performed using the Folin-Ciocalteu method (Slinkard and Singleton, 1977), measuring the absorbance at 725 nm by means of a Shimadzu UV-160 (UV–Visible recording) spectrophotometer, and estimating the concentration of phenolics based on a standard curve of gallic acid (0–500 mg L^{-1}). Anthocyanins, polymeric pigments, tannic and non-tannic phenols were determined with the Harbertson–Adams assay (Harbertson et al., 2003). This method also yielded the contributions of small and large polymeric pigments (SPP and LPP, respectively). The contribution of phenolics to red wine color, given by free anthocyanins, co-pigmented anthocyanins, and polymeric pigments, was calculated separately following the method proposed by Boulton (Downey et al., 2006; Harbertson et al., 2003; Boulton, 2001).

Measurements of gallic, caffeic, and coumaric acids, as well as catechin, epicatechin, myricetin, kaempferol, and resveratrol were carried out by HPLC-DAD. The extractions from the wine samples were performed exactly as was described by Peña-Neira et al. (2000). Detection was performed in the UV region (210–360 nm). Identification of individual compounds was based on retention times of original standards and spectral data. Standard calibration curve was established by plotting the area of peaks against different concentrations (from 0.5 to 50 mg L^{-1}) for the 8 compounds. Correlation coefficients were between 0.9989 and 0.9999. Quantitation limits were between 0.017 and 0.150 mg L^{-1} for the 8 compounds. The polyphenols measures in wine samples were performed in triplicate, and the highest relative standard deviation observed was less than 5%.

2.3.2. Metal analysis

The concentrations of iron, copper and manganese were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using a Varian Liberty II series spectrometer (Agilent, Santa Clara, CA, USA). The detection wavelengths were 248.3 nm for iron, 324.8 nm for copper and 257.6 nm for manganese. Limits of detection and quantitation were 0.034 and 0.115 $\mu g\ L^{-1}$ for iron, 2×10^{-4} and 7×10^{-4} $\mu g\ L^{-1}$ for copper and 0.0021 and 0.007 $\mu g\ L^{-1}$ for manganese, respectively (Esparza et al., 2004). All of these measurements were performed in triplicate, and the highest relative standard deviation observed was less than 5%.

2.3.3. Free and total sulfite analysis

Free and total sulfites were measured using the standard OIV-MA-AS323-04A method for wine analysis (O.I.V, 2011). All of these measurements were performed in triplicate, and the highest relative standard deviation observed was less than 5%.

2.4. Antioxidant activity analyses

2.4.1. DPPH• scavenging capacity assay

DPPH• (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity assay was monitored spectrophotometrically (Moon and Shibamoto, 2009; Huang et al., 2005). Wine was diluted 1:10, with a 10% ethanol solution. Subsequently, 6 aliquots of diluted wine with increasing volumes were added to 2.5 mL of a DPPH• solution. Previously, the kinetics of the reaction of the diluted wine with DPPH was recorded, to determine the time in which the reaction stabilizes. After 15 min of reaction, changes in absorbance at 516 nm were registered for the 6 wine dilutions. The IC_{50} values obtained from these curves expressed the antioxidant capacity of the wine samples. These values account for the volume (in μL) of sample required to scavenge 50% of the DPPH• radicals. Since higher IC_{50} values imply that larger sample volumes are required to scavenge oxidizing radicals, the results from this technique are inversely proportional to the antioxidant capacity (i.e. higher DPPH values imply lower AC). It is important to note that these results provide information according to the stoichiometry of antioxidants, whereas other methods used in conjunction with DPPH can deliver information according to the reactivity of antioxidants. All AC measurements were performed in triplicate.

2.4.2. Oxygen radical absorbance capacity

ORAC-FL was evaluated following on a Synergy HT multi-detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, Vermont, USA), using 96-well polystyrene white microplates purchased from Nalge Nunc International (Roskilde, Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The oxygen radical absorbance capacity was determined as described by Ou et al. (2001), with slight modifications. Samples of 10 μL of wine were diluted in 1 mL of a 10% (v/v) ethanol solution in water. Six aliquots of this dilution with volumes ranging from 30 to 100 μL were mixed with a 75 mM sodium phosphate buffer (pH 7.4) to reach a final volume of 300 μL. Subsequently, 25 μL of each of the 6 mixtures were placed in cells of a 96-well, along with 150 μL of a 40 nM solution of fluorescein in phosphate buffer. The mixture was preincubated for 15 min at 37 °C, before the AAPH solution (25 μL; 18 mM, final concentration) was rapidly added. Blanks were prepared similarly, using the 10% ethanol solution without wine. The fluorescence was recorded every 1 min for 90 min and was quantified by integration of the area under the curve (AUC). The slope for each wine sample was normalized using the slope obtained with Trolox (μmol Trolox equivalents/μL wine). All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample.

2.5. Statistical analysis

Statistical analyses were performed using the software R (R Core Team, 2013, Vienna, Austria). The data were checked for normality using the Shapiro–Wilk test. Equal variances among groups of samples were checked using the Brown–Forsythe correction for Levene's test (Noguchi et al., 2012). Groups of measurements whose values did not pass normality and equal variance tests were analyzed using the non-parametric Kruskal–Wallis test. A threshold p-value of 0.05 was used to treat differences as statistically significant. For chemical markers found to be significantly different, pairwise comparisons using the Bonferroni–Holm method were performed to identify differences among groups. If the samples did not follow assumptions of normality, Wilcoxon non-parametric tests were employed instead.

Correlations between the two measures of AC with compositional parameters were assessed for all 37 wine samples. Linear regressions were used for these analyses. Correlations between the measurements of AC and compositional data were obtained by multiple linear regressions, calculated by the least squares method. Relevant factors were discriminated by backward selection, which consisted in first fitting a model having all factors incorporated, and then proceeding by sequential elimination of those without statistical significance (linear regression coefficients with $p > 0.05$). The validity of the chosen set of parameters was confirmed by selecting the model with the minimum Bayesian Information Criterion (BIC) (Venables and Ripley, 2002). A data point was regarded as an outlier if its Cook's distance, a statistical measurement of the point's influence in the overall regression, was above 0.5.

3. Results and discussion

3.1. Chemical composition

Chemical compositions of the varietal Carménère wines are reported in Table 1. Wines from the 2010 vintage showed a few compositional features that were significantly different from those from 2008 and 2009 vintages as shown in Table 2. The p-values from pairwise comparisons by vintage year of selected chemical parameters showed that the vintage year significantly influenced AC. Compared with older vintages, namely the wines from the 2010 harvest, had significantly different AC than those from 2009 and 2008, with the sole exception than DPPH between 2010 and 2008 ($p = 0.06$). Overall, wines from the 2010 vintage appeared to have lower AC, regardless of the method used. These results were somehow expected as the wines from the 2010 vintage showed lower phenolic content than those from prior years, these results could be explained by edaphoclimatic and cultural practices with respect to environmental impact up to 2010. Most of the data showed distributions that did not fit the assumptions of normality and equal variance required for ANOVA tests. Therefore, most of the statistical validation was performed using a non-parametric substitute. Table 1 summarizes the results of 26 compositional variables along with results from previously published research (Neveu et al., 2010; Laurie et al., 2010; Skogerson et al., 2007; Pohl, 2007; Soyollkham et al., 2011; Rastija et al., 2009; Fanzone et al., 2010; Gambelli and Santaroni, 2004; Peña-Neira et al., 2000; Gerogiannaki-Christopoulou et al., 2006).

Total phenolics, i.e. anthocyanins and tannins, were within the ranges already published for red wines. The concentration of small polymeric pigments (SPP) was in the upper range of the reported values (Skogerson et al., 2007).

Table 1
Average (± standard deviation) values of selected chemical parameters in Carménère wines, grouped by their vintage year and compared with results from the literature.

Variable	2008 ($n=10$)	2009 ($n=8$)	2010 ($n=19$)	Literature	Ref.[a]
Total polyphenols	2374 [1874–3591]	2808 [2230–2886]	1886 [1569–3536]	2150 ± 650	Neveu et al. (2010)
Anthocyanins	69 [32–143]	89 [27–177]	285 [210–397]	0–1000	Skogerson et al. (2007)
Tanins	110 [63–161]	117 [37–197]	65 [14–241]	8.1–769	Skogerson et al. (2007)
Color given by:					
Polymeric pigments	4.80 AU [3.68–7.11]	6.69 AU [3.19–8.95]	3.58 AU [2.72–5.24]		
Free anthocyanins	0.97 AU [0.22–1.69]	2.02 AU [0.86–2.91]	3.08 AU [2.50–3.86]		
Copigmented anthocyanins	0.66 AU [0.34–1.37]	0.81 AU [0.21–1.35]	0.76 AU [0.28–1.48]		
Fraction of polymeric pigments (fPP)	0.77 [0.56–0.85]	0.70 [0.47–0.79]	0.49 [0.39–0.57]		
Fraction of free anthocyanins (fFA)	0.14 [0.04–0.23]	0.19 [0.09–0.39]	f = 0.40 [0.35–0.47]		
Fraction of copigmented anthocyanins (fCA)	0.11 [0.04–0.21]	0.07 [0.03–0.14]	0.12 [0.04–0.21]		
Large polymeric pigments (LPP)	2.8 [0.8–4.1]	2.9 [1.0–4.5]	0.8 [0.1–2.4]	0.5–3.1	Skogerson et al. (2007)
Small polymeric pigments (SPP)	3.3 [2.5–3.9]	4.8 [2.6–7.8]	3.6 [2.8–4.9]	0.0–4.2	Skogerson et al. (2007)
Flavonols	8.8 [6.1–13.5]	11.5 [10.1–12.7]	9.9 [6.7–11.8]		
Gallic acid	21.9 [9.2–29.3]	20.1 [13.6–31.8]	11.6 [7.7–20.6]	36 (0–126)	[b]
Caffeic acid	3.5 [0.6–6.0]	3.8 [1.5–9.3]	1.3 [0.6–4.6]	19 (0–77)	[b]
Coumaric acid	4.6 [0.1–10.7]	3.2 [1.3–8.4]	0.6 [0.4–4.6]	5.5 (0–40)	[b]
Catechin	14.1 [11.5–17.0]	15.2 [13.1–24.5]	14.3 [8.7–22.2]	68 (13.8–390)	
Epicatechin	2.2 [0.6–5.0]	2.9 [1.2–4.8]	6.5 [4.2–15.0]	38 (0–165)	[b]
Kaempferol	1.3 [0.4–2.3]	2.1 [1.2–3.6]	1.4 [0.5–2.5]	2.3 (0–3.6)	[b]
Myricetin	3.0 [0.5–5.8]	3.2 [0.04–7.3]	3.5 [2.1–4.9]	8.3 (0–18)	[b]
Resveratrol	1.5 [0.5–9.1]	3.3 [2.2–7.8]	1.5 [0.7–2.1]	2.7 (0–28)	[b]
Copper	0.17 [0.10–0.35]	0.13 [0.08–0.17]	0.08 [0.01–0.12]	0–3.1	Pohl (2007)
Iron	2.0 [1.6–3.8]	3.1 [1.5–4.0]	0.8 [0.09–2.4]	3.3 ± 1.1	Laurie et al. (2010)
Manganese	0.9 [0.2–1.4]	1.2 [0.7–2.5]	0.7 [0.2–2.4]	0–5.5	Pohl (2007)
Sulfites, free	6.5 [2.4–12.8]	2.4 [1.6–4.0]	5.2 [1.6–8.8]		
Sulfites, combined	24.0 [15.6–39.2]	17.2 [8.8–23.2]	35.6 [16.4–57.2]		
Sulfites, total	31.2 [25.6–45.2]	19.0 [12.0–25.6]	40.8 [18.0–66.0]		

Values are expressed in mg L^{-1} except those marked by *AU* (absorbance units) and *f* (fraction of the total absorbance measured at 520 nm). Tanins are expressed as mg L^{-1} of catechin equivalents, while anthocyanins are in mg L^{-1} of malvidin-3-glucoside. The pound symbols (#) indicate variables whose averages for the 2010 vintage were significantly different ($p < 0.05$) from those of the 2008 and 2009 vintages, with these two taken as separate groups.
Measured values expressed as medians and range [minimum–maximum].
[a] Reported values from the literature are expressed range, or average (minimum–maximum).
[b] Soyollkham et al. (2011), Rastija et al. (2009), Fanzone et al. (2010), Gambelli and Santaroni (2004), Peña-Neira et al. (2000) and Gerogiannaki-Christopoulou et al. (2006).

Furthermore, the concentration range for low-molecular mass phenolics was very broad, as already reported for wines from other countries.

Gallic acid was the most abundant phenolic compound (mean 18.03 mg L^{-1}) in Carmenere wines; the highest level (21.6 mg L^{-1}) was found for the 2009 vintage ($n = 8$), while the lowest level (12.2 mg L^{-1}) was found for the 2010 vintage ($n = 19$). Previously published data for other red wines varieties, such as Austrian Zweigelt, Argentinian malbec and a croatian grape variety showed slightly lower concentrations of gallic acid, ranging from 12.7 to 18.0 mg L^{-1}(Soyollkham et al., 2011;Rastija et al., 2009; Fanzone et al., 2010)

Italian red wines from Puglia and Molise regions showed an average concentration 3 times higher than the Carménère wines (Gambelli and Santaroni, 2004); finally, selected Spanish red wines of different geographical origin (Peña-Neira et al., 2000) contained 9 fold less gallic acid than the Carménère wines.

Catechin was the second most abundant phenolic compound (mean 14.83 mg L^{-1}); the difference between years vintage was almost negligible. Austrian and Czech (Soyollkham et al., 2011) and Argentinian (Fanzone et al., 2010) red wines had an average concentration 2-times higher than the Carménère wines and, again, Spanish (Peña-Neira et al., 2000) and Croatian (Rastija et al., 2009) wine were 5–14 times, respectively, lower than the Carménère wines.

Caffeic and coumaric acid showed mean concentrations of 3.18 and 3.21 mg L^{-1}, respectively; in both cases the lowest level (1.7 and 0.99 mg L^{-1}, respectively) was found from the 2010 vintage,

Table 2
p-Values from pairwise comparisons by vintage year of selected chemical parameters (shown by a pound sign in Table 1).

Variable	2008–2009 ($n=18$)	2008–2010 ($n=29$)	2009–2010 ($n=27$)
Total polyphenols (NP)		0.003	7×10^{-4}
Anthocyanins	2×10^{-4}	$<10^{-6}$	2×10^{-5}
Tannins (NP)		0.014	0.013
Fraction of polymeric pigments (fPP)		$<10^{-6}$	$<10^{-6}$
Fraction of free anthocyanins (fFA)	0.010	$<10^{-6}$	$<10^{-6}$
Large polymeric pigments (LPP)		4×10^{-5}	9×10^{-6}
Gallic acid		1×10^{-4}	6×10^{-5}
Caffeic acid (NP)		0.019	0.002
Coumaric acid		0.002	1×10^{-4}
Copper (NP)		3×10^{-5}	0.002
Iron (NP)		8×10^{-5}	2×10^{-4}

Significance was measured using the Holm–Bonferroni method for normally distributed samples and by Wilcoxon tests in the case of non-parametric (NP) distributions. Blanks indicate non-significant values ($p > 0.05$).

while the highest levels (4.45 and 4.58 mg L^{-1}) was found from the 2009 and 2008 vintages, respectively. The results for these 2 compounds were slightly higher than those found in other countries, except for Italy.

The results of kaemferol in Carménère wines were 4 times higher than for Croatian wines. Myricetin results were similar to concentrations found in Italian wines and 2 times higher than Croatian wines.

The average resveratrol concentrations in Carménère wines was 2.81 mg L^{-1}; the highest level (4.11 mg L^{-1}) was found in the 2009 vintage and the lowest level (1.53 mg L^{-1}) in the 2010 vintage. Exceptionally, Austrian and Czech (Soyollkham et al., 2011), Italian (Gambelli and Santaroni, 2004), Croatian (Rastija et al., 2009), and Greek (Gerogiannaki-Christopoulou et al., 2006) wines were 1.5–3 times lower than the resveratrol concentration in Carménère wines.

With regards to transition metals, these were within the ranges reported elsewhere (Laurie et al., 2010; Pohl, 2007). Regardless of this observation, the role of Mn in this type of reaction is still unknown. We could have obtained clear information about the action of metals, had a metal speciation analysis been performed, and the capacity of the metal complexation with polyphenols present in wine been considered. Another important point to consider is metal redox chemistry; for example, manganese involves two electron transfers, unlike iron and copper for which only one electron transfer is involved.

The concentrations of sulfites were low compared with those reported in the literature (Jacobs, 1976), but this can vary widely depending on pH, temperature and oxygen exposure.

3.2. Antioxidant activity

Significant variation in antioxidant activity among vintages was observed, both with ORAC-FL ($p < 10^{-6}$) or DPPH ($p = 0.009$), calculated using the non-parametric Kruskal–Wallis method. Compared with older vintages, wines from the 2010 harvest had significantly lower AC with the exception of DPPH for 2008 ($p = 0.06$) (a lower DPPH value implies a higher AC). These results were expected as the wines from the 2010 vintage showed lower phenolic content than those from prior years. In terms of absolute values, ORAC-FL measurements were in the range of 3.60–8.73 μmol equivalent Trolox μL^{-1} wine, with an average of 5.99 μmol equivalent Trolox μL^{-1}. These values were lower than those reported in previous studies (Kondrashov et al., 2009) for Cabernet Sauvignon (7.8–16.6 μmol mL^{-1}) and Merlot wines (7.5–11.2 μmol mL^{-1}). The mean DPPH for the sampled Carménère wines was 7.70 μL, ranging from 4.8 to 11.0 μL. The units chosen to represent this form of AC (μL of wine required to bleach 50% of the target molecule) (Prior et al., 2005) are not comparable with other published results in wine. These results offer information based on the stoichiometry of antioxidants as ORAC-FL, whereas other methods, including DPPH, refer to the reactivity of antioxidants present within a sample.

3.3. Relationships between compositional features and AC measurements

3.3.1. Total phenolics

The phenolic content obtained by Folin-Ciocalteu was plotted against the two AC measurements (Fig. 2). Linear regressions were calculated for values below 3000 mg L^{-1}, where the behavior of the data was linear. Samples with higher polyphenol concentrations showed interferences and/or saturation of the readings, especially with DPPH. The latter can occur as increasing numbers of large antioxidant molecules interfere with the access of each other to the DPPH, thus hindering the reaction site. The linear regression

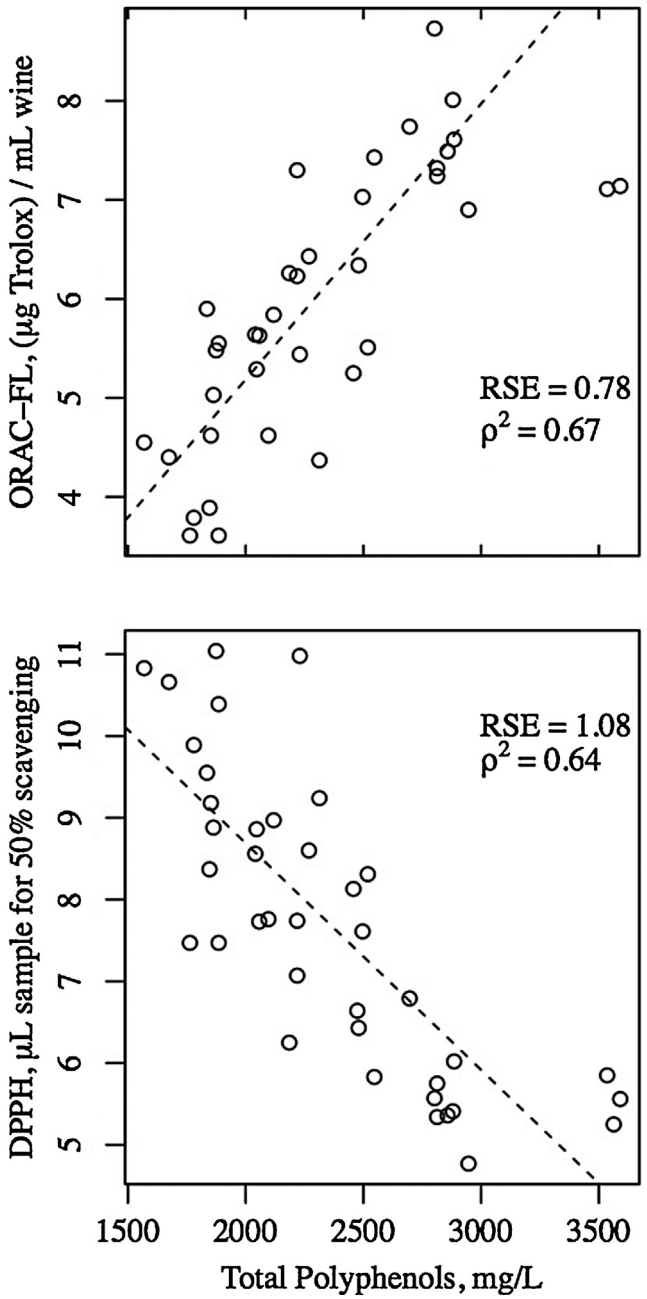

Fig. 2. Plot of the antioxidant capacity versus Folin-Ciocalteu measurements for 37 Carménère wines. Dotted lines represent the best regression curves for Folin-C concentrations below 3000 mg L^{-1} (regarded as outliers).

between TP (total phenolics by Folin-Ciocalteu) and the two AC methods indicated a moderate but significant correlation with ORAC-FL ($\rho^2 = 0.67$, RSE = 0.78) and DPPH ($\rho^2 = 0.64$, RSE = 1.08). These results were expected, as the Folin-Ciocalteu method gives information about polyphenols amount present in wine, whereas ORAC-FL and DPPH give information about of the overall antioxidant capacity displayed by all of the antioxidant substances present in the wines, not all of which are phenolics. Other reports have found significant similar correlations between AC and phenolic content (Fernández-Pachón et al., 2004; Vrcek et al., 2011; Lucena et al., 2010).

3.3.2. Transition metals and sulfite

Correlations between metals and sulfite with the two AC methods were lower than 0.2, except for Fe and ORAC-FL

($\rho^2 = 0.51$), indicating that only this variable might be a significant contributor to the AC.

3.3.3. Other phenolic and metallic composition

Given that the wine matrix is a complex system, measurements of other specific phenolic compounds besides TP, along with metallic compositions were performed in order to better explain the variability of AC according to compositional parameters. We considered that a multiple linear regression model was necessary to determine the joint contribution of the different studied markers for the two representations of AC; linear regressions were calculated from the sets of chemical markers that produced the best linear fit. Fig. 3 displays the corresponding average contributions of each regression term to AC, defined as the product of the linear coefficients and the mean value of the markers. In other words, for a regression of the type $y = a_1 \cdot x_1 + a_2 \cdot x_2$, where a_i is the linear coefficient for the chemical marker i obtained by fitting, the contribution for i is given by $a_i \cdot \bar{X}_i$, where $\bar{X}_i$ is the average value of i. Using this methodology, similar results for DPPH ($\rho^2 = 0.90$, $p < 10^{-6}$) and ORAC-FL ($\rho^2 = 0.87$, $p < 10^{-6}$) were achieved, using different combinations of chemical markers.

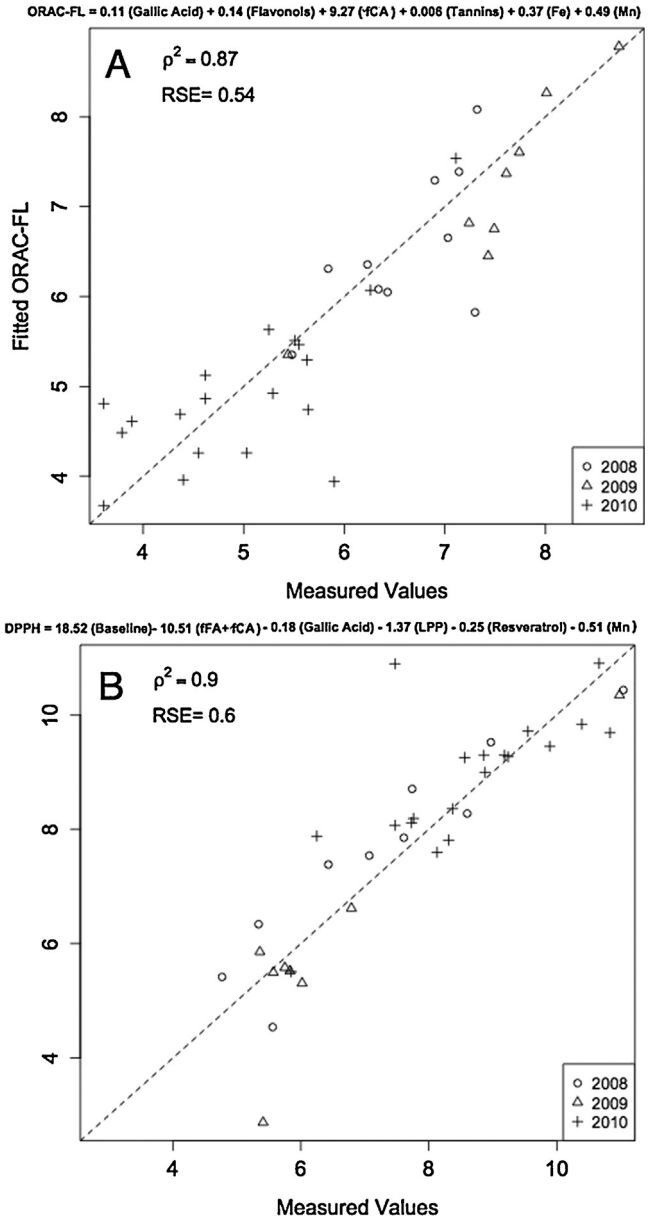

Fig. 3. Graphical representation of the contribution of the chemical markers measured to the average value of antioxidant capacity (ORAC-FL (A) and DPPH (B)), defined as the product of the linear coefficients and the mean value of the explaining variables.

The main chemical markers contributing to ORAC-FL were gallic acid and flavonols. With an average ORAC-FL value of 5.99 μmol equivalent Trolox μL^{-1} wine, these two variables contributed with 30.7% and 23.6%, respectively. López-Alarcón and Lissi (2006) observed that, from among 8 of the most commonly found polyphenols in wine, gallic acid appears to be the most reactive, which might explain its preponderant role in defining ORAC values. Other measurements or substances such as copigmented anthocyanins, tannin iron and manganese also contribute, but to a lesser extent (16.3%, 9.6%, 11.0 and 8.9% respectively), with metal ions apparently increasing AC. Although counter-intuitive, this can be explained by the fact that the ICP-OES measurements encompassed both bound and free metals, instead of only free ions catalyzing Fenton-type reactions.

As DPPH gives decreasing values with increasing AC, it is expected to have a baseline value given by the intercept of the linear fit (17.8 μL in this case), which represents the DPPH value when no antioxidants (or pro-oxidants) are available in a wine. The fraction of color given by free and copigmented anthocyanins ($f_{FA} + f_{CA}$) had a strong correlation with the antioxidant effect contributing 39.1% of the total. Similarly, gallic acid and LPP are relevant contributors in decreasing DPPH, with 27.3% and 23.4%, respectively. The relative contribution of resveratrol and manganese is much lower, with 5.0% and 5.1%, respectively.

These results show that both colored samples and LPP have a positive correlation with antioxidant capacity when using the DPPH method. Since this method is more specific for lypophilic antioxidants or non-protic matrices, high AC contribution of LPP was expected (as they primarily consist in non-polar molecules) (Prior et al., 2005). Color was provided by Polymeric pigments, as well as copigmented and free anthocyanins, which have large molecules that are well detected by this method. This suggests that DPPH does not preferentially react with small linear polymer chains, which are represented by SPP, but only with those given by LPP.

Rivero-Pérez et al. (2008) previously demonstrated anthocyanin contributions to AC. In that study, the authors evaluated antioxidant capacity of anthocyanin extracts from red wine by several methods including electron transfer (ET) and hydrogen atom transfer (HAT). The former method showed that the free anthocyanin fraction is mainly responsible for AC. On the contrary, the HAT method did not show a clear contribution of any particular fraction.

Those results are consistent with the results obtained in the research outlined in this paper. Through the DPPH (ET method) we show that the free and copigmented anthocyanins contribute to the AC in red wines; moreover, we show that they are the main contributors of AC with 39.1% of the total AC. However, the AC of simple anthocyanin evaluated by the HAT method had a minor impact on the overall AC of red wines (results consistent with the decrease in AC detected by ORAC-FL).

Regressions between AC and individual chemical compounds have also been reviewed in the literature, with mixed results. Guendez et al. (2005) measured the concentration of 8 low-molecular phenolics, but from grape seed extracts of Greek wines.

4. Conclusions

Overall, this paper provides a description of the compositional and antioxidant features of Carménère wines. Linear correlations

between compositional and radical-scavenging measurements were found. TP has a moderate but significant correlation with ORAC-FL (ρ^2 = 0.67) and DPPH (ρ^2 = 0.64). However, the relative sum of the selected chemical markers of the wine better explain the main contributors for antioxidant capacity in Carménère wines, obtaining good correlations, DPPH ρ^2 = 0.90 and ORAC-FL ρ^2 = 0.87. Particularly, free and copigmented anthocyanins and gallic acid were the main chemical markers with the greatest effect on the AC, but gallic acid turned out to be the chemical marker with the closest association with both AC methods. Finally, further insight on the action of transition metals should be investigated by performing a metal speciation analysis; the capacity of metal complexation with wine polyphenolics as well as the metal redox chemistry might also be considered.

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References

Boulton, R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. American Journal of Enology and Viticulture 52, 67–87.
Danilewicz, J.C., 2003. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: central role of iron and copper. American Journal of Enology and Viticulture 54, 73–85.
Danilewicz, J.C., Seccombe, J.T., Whelan, J., 2008. Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. American Journal of Enology and Viticulture 59, 128–136.
Downey, M., Skogerson, K., Mazza, M., 2006. Modified Adams Assay for Phenolics in Wine. URL (http://boulton.ucdavis.edu/uv-vis/adamsassay.htm).
Elias, R.J., Andersen, M.L., Skibsted, L.H., Waterhouse, A.L., 2009. Identification of free radical intermediates in oxidized wine using electron paramagnetic resonance spin trapping. Journal of Agricultural and Food Chemistry 57, 4359–4365.
Esparza, I., Salinas, I., Caballero, I., Santamaría, C., Calvo, I., García-Mina, J.M., Fernández, J.M., 2004. Evolution of metal and polyphenol content over a 1-year period of vinification: sample fractionation and correlation between metals and anthocyanins. Analytica Chimica Acta 524, 215–224.
Fanzone, M., Peña-Neira, A., Jofré, V., Assof, M., Zamora, F., 2010. Phenolic characterization of malbec wines from Mendoza province (Argentina). Journal of Agricultural and Food Chemistry 58, 2388–2397.
Fernández, K., Kennedy, J.A., Agosin, E., 2007. Characterization of *Vitis vinifera* L. Cv. Carménère grape and wine proanthocyanidins. Journal of Agricultural and Food Chemistry 55, 3675–3680.
Fernández-Pachón, M., Villano, D., García-Parrilla, M., Troncoso, A., 2004. Antioxidant activity of wines and relation with their polyphenolic composition. Analytica Chimica Acta 513, 113–118.
Gambelli, L., Santaroni, G.P., 2004. Polyphenols content in some Italian red wines of different geographical origins. Journal of Food Composition and Analysis 17, 613–618.
Gerogiannaki-Christopoulou, M., Athanasopoulos, P., Kyriakidis, N., Gerogiannaki, I.A., Spanos, M., 2006. *trans*-Resveratrol in wines from the major Greek red and white grape varieties. Food Control 17, 700–706.
Guendez, R., Kallithraka, S., Makris, D.P., Kefalas, P., 2005. Determination of low molecular weight polyphenolic constituents in grape (*Vitis vinifera* sp.) seed extracts: correlation with antiradical activity. Food Chemistry 89, 1.
Harbertson, J.F., Picciotto, E.A., Adams, D.O., 2003. Measurement of polymeric pigments in grape berry extract sand wines using a protein precipitation assay combined with bisulfite bleaching. American Journal of Enology and Viticulture 54, 301–306.
Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53, 1841–1856.
Hynes, M.J., Ó Coinceanainn, M., 2001. The kinetics and mechanisms of the reaction of iron(III) with gallic acid, gallic acid methyl ester and catechin. Journal of Inorganic Biochemistry 85, 131–142.
Jacobs, D.D., 1976. Effect of dissolved oxygen on free sulfur dioxide in red wines. American Journal of Enology and Viticulture 27, 42–43.
Kondrashov, A., Ševčík, R., Benáková, H., Koštířová, M., Štípek, S., 2009. The key role of grape variety for antioxidant capacity of red wines. e-SPEN, The European e-Journal of Clinical Nutrition and Metabolism 4, 41–46.
Laggnera, H., Hermann, M., Sturm, B., Gmeiner, B.M., Kapiotis, S., 2005. Sulfite facilitates LDL lipid oxidation by transition metal ions: a pro-oxidant in wine? FEBS Letters 579, 6486–6492.
Laurie, V.F., Clark, A.C., 2010. Wine oxidation. In: Decker, E., Elias, R., McClemens, E. (Eds.), Oxidation in Foods and Beverages and Antioxidant Applications, vol. 2: Management in Different Industry Sectors. Woodhead Publishing Limited, U.K., pp. 445–475.
Laurie, V.F., Villagra, E., Tapia, J., Sarkis, J.E.S., Hortellani, M.A., 2010. Analysis of major metallic elements in Chilean wines by atomic absorption spectroscopy. Ciencia e investigación agraria 37, 77–85.
Laurie, V.F., Waterhouse, A.L., 2006. Oxidation of glycerol in the presence of hydrogen peroxide and iron in model solutions and wine. Potential effects on wine color. Journal of Agricultural and Food Chemistry 54, 4668–4673.
Laurie, V.F., Zúñiga, M.C., Carrasco-Sánchez, V., Santos, L.S., Cañete, Á., Olea-Azar, C., Ugliano, M., Agosin, E., 2012. Reactivity of 3-sulfanyl-1-hexanol and catechol-containing phenolics in vitro. Food Chemistry 131, 1510–1516.
Li, Wang, X., Li, Y., Li, P., Wang, H., 2009. Polyphenolic compounds and antioxidant properties of selected China wines Hua. Food Chemistry 112, 454–460.
López-Alarcón, C., Lissi, E., 2006. A novel and simple ORAC methodology based on the interaction of Pyrogallol Red with peroxyl radicals. Free Radical Research 40, 979–985.
Lucena, A.P.S., Nascimento, R.J.B., Maciel, J.A.C., Tavares, J.X., Barbosa-Filho, J.M., Oliveira, E.J., 2010. Antioxidant activity and phenolics content of selected Brazilian wines. Journal of Food Composition and Analysis 23, 30–36.
Moon, J.K., Shibamoto, T., 2009. Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry 57, 1655–1666.
Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., Knox, C., Eisner, R., Cruz, J., Wishart, D., Scalbert, A., 2010. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. URL (http://www.phenol-explorer.eu/). Database 2010.
Noguchi, K., Wallace, W.L., Gel, Y.R., Gastwirth, J.L., Miao, W., 2012. lawstat: An R package for biostatistics, public policy, and law. R package version 2.3. URL (http://CRAN.R-project.org/package=lawstat). Date-Added 2012-07-26 11:58:19-0500, Date-Modified 2012-07-26 11:58:21-0500.
Obreque-Slier, E., Peña-Neira, Á., López-Solís, R., Zamora-Marín, F., Ricardo-da Silva, J.M., Laureano, O., 2010. Comparative study of the phenolic composition of seeds and skins from Carmeénère and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. Journal of Agricultural and Food Chemistry 58, 3591–3599.
O.I.V, 2011. Compendium of International Methods of Analysis of Wine and Musts. International Organization of Vine and Wine, Paris, France.
Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry 49, 4619–4626.
Peña-Neira, A., Hernández, T., García-Vallejo, C., Estrella, I., Suárez, J.A., 2000. A survey of phenolic compounds in Spanish wines of different geographical origin. European Food Research and Technology 210, 445–448.
Pohl, P., 2007. What do metals tell us about wine? TrAC Trends in Analytical Chemistry 26, 941–949.
Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53, 4290–4302.
Pszczólkowski, P., 2004. La invención del cv. Carménère (*Vitis vinifera* L) en Chile, desde la mirada de uno de sus actores. In Universum (Online), Talca, Chile 19, 150–165.
Rastija, V., Srečnik, G., Marica-Medić-Šarić, 2009. Polyphenolic composition of Croatian wines with different geographical origins. Food Chemistry 115, 54–60.
R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.
Rivero-Pérez, M.D., Muñiz, P., González-Sanjosé, M.L., 2008. Contribution of anthocyanin fraction to the antioxidant properties of wine. Food and Chemical Toxicology 46, 2815–2822.
Skogerson, K., Downey, M., Mazza, M., Boulton, R., 2007. Rapid determination of phenolic components in red wines from UV–Visible spectra and the method of partial least squares. American Journal of Enology and Viticulture 58, 318–325.
Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture 28, 49–55.
Soyollkham, B., Valášek, P., Fišera, M., Fic, V., Kubáň, V., Hoza, I., 2011. Total polyphenolic compounds contents (TPC), total antioxidant activities (TAA) and HPLC determination of individual polyphenolic compounds in selected Moravian and Austrian wines. Central European Journal of Chemistry 9, 677–687.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Springer, New York.
Villamor, R.R., Harbertson, J.F., Ross, C.F., 2009. Influence of tannin concentration, storage temperature, and time on chemical and sensory properties of Cabernet Sauvignon and Merlot wines. American Journal of Enology and Viticulture 60, 442–449.
Vrcek, I.V., Bojic, M., Zuntar, I., Mendas, G., Medic-Saric, M., 2011. Phenol content, antioxidant activity and metal composition of Croatian wines deriving from organically and conventionally grown grapes. Food Chemistry 124, 354–361.
Waterhouse, A.L., 2002. Wine phenolics. Annals of the New York Academy of Sciences 957, 21–36.
Waterhouse, A.L., Laurie, V.F., 2006. Oxidation of wine phenolics: a critical evaluation and hypotheses. American Journal of Enology and Viticulture 57, 306–313.