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RESEARCH NOTE

Influence of the growing region, quality classification and harvest year on the ORAC (Oxygen Radical Absorbance Capacity) index of Chilean Cabernet Sauvignon red wines

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Abstract

R. Bridi, S. Lobato, C. López-Alarcón, and E. Lissi. 2014. Influence of the growing region, quality classification and harvest year on the ORAC (Oxygen Radical Absorbance Capacity) index of Chilean Cabernet Sauvignon red wines. Cien. Inv. Agr. 41(3): 395-402. In the present work, we studied the effect of the growing region (IV, Metropolitan or VI - VII regions), quality classification ("Varietal", "Reserva", and "Gran Reserva") and harvest year (2000-2012) on the total phenolic content and antioxidant capacity of thirty-six Chilean Cabernet Sauvignon red wine samples. The Folin-Ciocalteu (FC) and (Oxygen Radical Absorbance Capacity) ORAC methods, employing fluorescein and pyrogallol red as probes, were applied to establish the properties of the wine samples. The obtained results show a strong dependence at of the three indexes on the grape growing region, with higher values obtained in wines from the IV region. These results can be related to the climatic characteristics of this Region, which include dry weather and large temperature differences (nearly 20 °C) between day and night.

Key words: Cabernet Sauvignon, Chilean red wines, ORAC, total phenolic content, growing region, quality classification, harvest year.

Introduction

During the 1990s, premium quality red wines from Chile entered international markets, mainly in the U.S. and Europe (Canziani and Scarel, 2008). The annual output of Chilean wines increased from 4.3 to 12.8 million hectoliters between 1997 and 2013, with the main production regions being the IV (Coquimbo), Metropolitan (Central), VI (Libertador Bernando O`Higgins), and VII (Maule) regions. These regions produce close to 95 percent of the total output of Chilean wines. Among the varieties produced, Cabernet Sauvignon is one of the most important, representing 35% of Chilean wine production, followed by Sauvignon Blanc (14.9%), Merlot (11.9 %), Carménère (8.9%), Chardonnay (8.7%) and Syrah (7.4%) (Le Journée Vinicole, 2013; SAG, 2013; Caceres-Mella et al., 2014).

Phenolic compounds play an important role in the quality of grapes and wines, greatly contributing to color, mouth feel and palatability. Moreover, polyphenols exert favorable effects on human health, which are at least partly associated with

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their antioxidant activity (Jaromír *et al.,* 2009; Jiang and Zhang, 2012). The total phenolic content of red wines and their antioxidant ability depend on factors such as the grape variety, quality classification, vineyard location, cultivation system, climate, soil type, harvest time, winemaking procedures, and storage conditions (Di Majo *et al.*, 2008; Jantschi *et al.*, 2013; Tenore *et al.*, 2013; Lissi *et al.*, 2014; Xiang *et al.*, 2014). Considerable attention has been directed towards understanding how vineyard management practices influence the phenolic content of red wines (Kennedy, 2008; Jaromír *et al.,* 2009). However, there has been no systematic study addressing the effects of the growing region, harvest year and quality classification on the *in vitro* antioxidant capacity of Chilean red wines. Therefore, the aim of the present study was to determine the influence of the abovementioned factors on the antioxidant activity (assessed through the ORAC, Oxygen Radical Absorbance Capacity, assay) and total phenolic content (assessed through the Folin-Ciocalteu method) of Chilean Cabernet Sauvignon wines from grapes cultivated in the IV, Metropolitan and VI - VII regions.

Materials and methods

Wine samples

Thirty-six samples of Cabernet Sauvignon wines produced in the IV, Metropolitan and VI - VII regions of Chile were selected. Samples obtained from local markets in Santiago, Chile, with different harvest years and quality classifications were analyzed during 2013. Aliquots of the wines were taken and immediately diluted in phosphate buffer (75 mM, pH 7.4). The characteristics of the analyzed wine samples are included in the supplementary material section (Table 1). It should be noted that, according to the Chilean Normative, in wines with a given DO (Denomination of Origin), up to 25% of the content can come from other cultivars and/or harvest years.

Chemicals

As a peroxyl radical source, 2,2'-Azo-bis(2-amidinopropane) dihydrochloride (AAPH) was used. Pyrogallol red (PGR), fluorescein (FL), Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), and AAPH were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, Folin-Ciocalteu's phenol reagent, di-sodium hydrogen phosphate dehydrate and sodium phosphate monobasic were supplied by Merck (Darmstadt, Germany). All compounds were employed as received.

Solutions

Stock solutions of PGR (0.1 mM) or FL (0.01 mM) were prepared daily in 75 mM phosphate buffer, pH 7.4. A reaction mixture containing AAPH (10 mM), with or without the samples being tested, was incubated in phosphate buffer (75 mM, pH 7.4) at 37°C. PGR consumption was evaluated based on the progressive decrease in absorbance measured at 540 nm using a Multilector Byotek Synergy HT. A similar procedure was carried out with FL (70 nM), but its consumption was assessed based on the decrease in the sample fluorescence intensity (excitation: 493 nm; emission: 515 nm).

Total phenolics

The total phenolic content of the wine samples was determined according to the Folin–Ciocalteu (FC) colorimetric method (Singleton and Rossi, 1965), using Trolox as a standard. Briefly, appropriate dilutions of the samples (1 mL) were added to the FC reagent (0.2 N, 5 mL). After 5 min, 4 mL of sodium carbonate (75 g L-1) was added. The mixtures were then incubated for 2 h at room temperature in the dark, and the absorbance was measured at 740 nm using an ultraviolet–visible Agilent 8453 spectrophotometer (Palo Alto, CA, USA). Quantification was carried out on the basis of the standard curve for Trolox, and the results were expressed as mM Trolox equivalents.

Table 1. Characteristics of the analyzed wine samples.

Sample	Quality classification	Harvest year	Region (DO)	Alcohol (GL)
(1)	Varietal	2011	Metropolitan (Central valley)	13.5°
(2)	Varietal	2012	Metropolitan (Central valley)	12.0°
(3)	Gran reserva	2007	IV Region (Elqui valley)	13.5°
(4)	Gran reserva	2008	IV Region (Elqui valley)	13.5°
(5)	Reserva	2009	IV Region (Elqui valley)	13.5°
(6)	Gran reserva	2007	IV Region (Elqui valley)	13.5°
(7)	Gran reserva	2008	IV Region (Elqui valley)	13.5°
(8)	Reserva	2009	IV Region (Elqui valley)	13.5°
(9)	Varietal	2011	Metropolitan (Central valley)	13.5°
(10)	Reserva	2010	Metropolitan (Maipo valley)	13.5°
(11)	Reserva	2009	Metropolitan (Maipo valley)	13.5°
(12)	Reserva	2010	Metropolitan (Maipo valley)	13.5°
(13)	Gran reserva	2009	Metropolitan (Maipo valley)	14.0°
(14)	Gran reserva	2010	Metropolitan (Maipo valley)	14.0°
(15)	Reserva	2011	Metropolitan (Maipo valley)	13.5°
(16)	Varietal	2011	VI – VII Region (Rapel valley)	13.5°
(17)	Varietal	2011	VI – VII Region (Colchagua valley)	13.5°
(18)	Varietal	2009	Metropolitan (Maipo valley)	14.5°
(19)	Varietal	2011	Metropolitan (Central valley)	13.5°
(20)	Varietal	2011	Metropolitan (Central valley)	12.5°
(21)	Varietal	2010	VI – VII Region (Maule valley)	12.5°
(22)	Varietal	2011	Metropolitan (Maipo valley)	14.0°
(23)	Varietal	2010	Metropolitan (Maipo valley)	14.0°
(24)	Varietal	2011	VI – VII Region	12.0°
(25)	Varietal	2012	VI – VII Region	12.0°
(26)	Reserva	2010	VI – VII Region (Aconcagua valley)	13.5°
(27)	Varietal	2011	Metropolitan (Central valley)	13.0°
(28)	Varietal	2012	Metropolitan (Central valley)	14.0°
(29)	Varietal	2010	Metropolitan (Maipo valley)	14.0°
(30)	Gran Reserva	2002	VI – VII Region (Curico valley)	13.5°
(31)	Varietal	2005	VI – VII Region (Colchagua valley)	14.5°
(32)	Reserva	2000	VI – VII Region (Rapel valley)	13.5°
(33)	Varietal	2012	VI – VII Region (Loncomilla valley)	13.0°
(34)	Gran Reserva	2008	Metropolitan (Maipo valley)	13.5°
(35)	Varietal	2012	Metropolitan (Central valley)	14.0°
(36)	Varietal	2012	Metropolitan (Central valley)	14.0°

ORAC determinations

The consumption of FL and PGR, associated with their incubation with AAPH, was estimated from fluorescence (F) and absorbance (A) measurements, respectively. The obtained (F/F_0) and (A/A^0) values were plotted as a function of time. Integration of the area under the curve (AUC) was performed up to the time at which (F/F_0) or (A/A_0) reached a value of 0.2. These areas were employed to

obtain ORAC values, according to Eqn. (1). All experiments were carried out in triplicate.

$$ORAC = \frac{(AUC - AUC_0)}{(AUC_{Trolox} - AUC_0)} f[Trolox] \quad \text{Eqn. (1)}$$

where AUC is the area under the curve in the presence of the red wine samples, integrated between time zero and the time corresponding to 80% of probe consumption; AUC0 is the area under the curve for the control; AUC_{Trolox} is the area under the curve for Trolox; f is the dilution factor of the sample, which was equal to the ratio between the total volume of the AAPH-PGR or AAPH-FL solution and the added sample volume; and [Trolox] is the millimolar concentration of Trolox (López-Alarcón and Lissi, 2006).

Statistical analyses

The data were analyzed via one-way analysis of variance (ANOVA), followed by the Tukey test when the F value was significant. All analyses were carried out using Origin software on a PC-compatible computer. A value of $P \leq 0.05$ was considered to be significant.

Results

We studied thirty-six Chilean Cabernet Sauvignon wines produced from grapes cultivated in the IV, Metropolitana, and VI - VII regions. Our analyses included evaluation of the total phenolic content (assessed via the Folin-Ciocalteu method) and antioxidant activity (assessed via the ORAC, Oxygen Radical Absorbance Capacity, assay), employing FL and PGR as probes (ORAC-FL and ORAC-PGR, respectively).

Figure 1 illustrates the dependence of the total phenolic content (FC index) on the harvest year of the samples. As shown this figure, the lowest and highest FC values were 33 and 51 mM Trolox equivalents, respectively (Table 2). These results (Figure 1) were characterized by a Gaussian-like distribution. The oldest sample, corresponding to the harvest year 2000, showed one of the lowest FC values. A clear increase of the total phenolic content was observed until ca. 2008, after which a decrease in the mean of FC values was observed. Similar non-linear relationships were observed for ORAC-FL and ORAC-PGR (data not shown).

Table 2. Total phenolic compounds (FC), ORAC-FL and ORAC-PGR in Cabernet Sauvignon wines. The results are expressed as mM Trolox equivalents.

Sample Number	Folin	ORAC-FL	ORAC-PGR	Sample number	Folin	ORAC-FL	ORAC-PGR
1	38 ± 1	74 ± 4	39 ± 2	19	39 ± 1	36 ± 1	16 ± 0
2	35 ± 1	39 ± 6	26 ± 2	20	33 ± 1	29 ± 0	9 ± 0
3	48 ± 1	97 ± 10	36 ± 2	21	35 ± 3	36 ± 0	20 ± 1
4	42 ± 0	75 ± 4	33 ± 1	22	33 ± 4	32 ± 0	18 ± 0
5	47 ± 1	79 ± 10	38 ± 1	23	35 ± 0	34 ± 1	19 ± 1
6	51 ± 1	66 ± 6	32 ± 2	24	40 ± 1	32 ± 0	16 ± 1
7	55 ± 1	58 ± 2	35 ± 2	25	36 ± 1	30 ± 0	14 ± 1
8	49 ± 2	69 ± 2	30 ± 2	26	38 ± 3	33 ± 1	18 ± 0
9	38 ± 1	23 ± 1	10 ± 0	27	36 ± 3	33 ± 0	17 ± 0
10	37 ± 5	34 ± 1	19 ± 1	28	41 ± 2	22 ± 1	11 ± 0
11	40 ± 1	34 ± 2	21 ± 1	29	47 ± 0	27 ± 1	14 ± 0
12	50 ± 2	34 ± 0	18 ± 0	30	35 ± 0	33 ± 1	5 ± 0
13	38 ± 5	37 ± 1	19 ± 0	31	40 ± 1	24 ± 1	12 ± 0
14	41 ± 2	33 ± 2	20 ± 0	32	33 ± 0	16 ± 0	8 ± 0
15	38 ± 5	34 ± 1	20 ± 0	33	42 ± 2	19 ± 1	10 ± 1
16	35 ± 5	35 ± 1	9 ± 0	34	45 ± 0	21 ± 0	12 ± 0
17	39 ± 1	34 ± 1	19 ± 0	35	47 ± 0	18 ± 0	12 ± 1
18	41 ± 0	36 ± 1	20 ± 1	36	39 ± 0	17 ± 0	9 ± 1

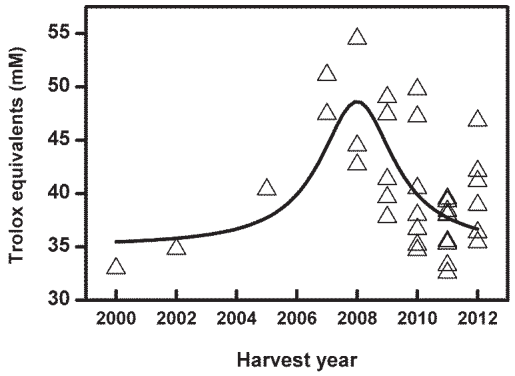

Figure 1. Dependence of the total phenolic content (FC index) on the harvest year of the samples. The results are expressed as mM Trolox equivalents.

Table 3. Average values of total phenolic compounds (FC), ORAC-FL and ORAC-PGR in wines from three quality classifications: "Varietal", "Reserva" and "Gran Reserva"; and from four regions of Chile: the IV region, Metropolitan region (MR) and VI – VII regions. The results are expressed as mM Trolox equivalents.

	Average values		
	Folin	ORAC-FL	ORAC-PGR
Region			
IV	49 ± 4[1]	75 ± 12[1]	34 ± 3[1]
MR	39 ± 4	32 ± 11	16 ± 5
VI - VII	39 ± 5	31 ± 5	14 ± 5
Quality classification			
"Varietal"	38 ± 4	31 ± 4	16 ± 7
"Reserva"	41 ± 7	36 ± 18	18 ± 8
"Grand Reserva"	45 ± 7[2]	55 ± 25[1]	25 ± 4[2]

[1]Difference compared with other groups (P≤0.05); [2]Difference compared with the "Varietal" group (P≤0.05). Tukey multiple range test.

The effect of the quality classification on FC, ORAC-FL and ORAC-PGR is shown in Figure 2. The analysis of these results indicated significant differences between the "Gran Reserva" wines and the other wine quality classifications (0.05 > P>0.005) regarding the ORAC-FL index. On the other hand, differences between ORAC-PGR and Folin were observed only when "Gran Reserva" and "Varietal" quality classifications were considered. The obtained data are summarized in Table 3.

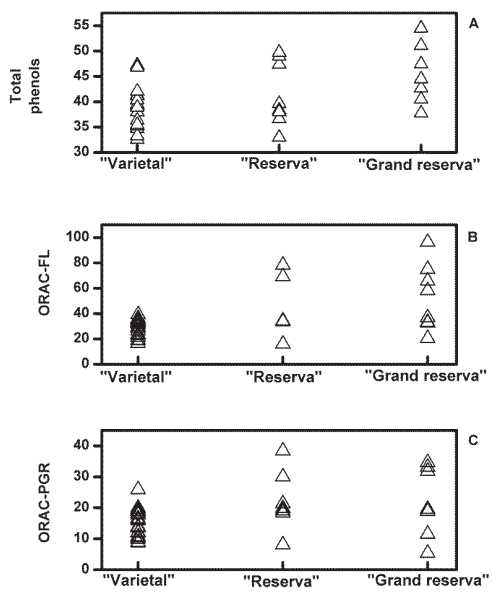

Figure 2. Total phenolic compounds (A), ORAC-FL (B) and ORAC-PGR (C) in Cabernet Sauvignon wines from three quality classifications: "Varietal", "Reserva" and "Gran Reserva". The results are expressed as mM Trolox equivalents.

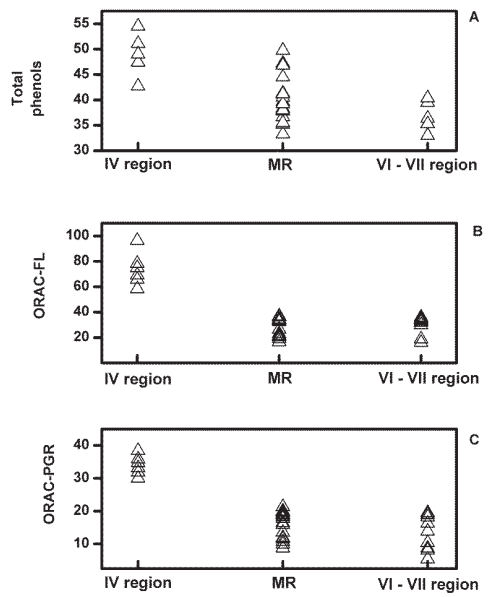

Figure 3. Total phenolic compounds (A), ORAC-FL (B) and ORAC-PGR (C) in Cabernet Sauvignon wines from four regions of Chile: the IV region, Metropolitan region (MR) and VI – VII regions. The results are expressed as mM Trolox equivalents.

Regarding the influence of the region, Figure 3 clearly shows that the three indexes considered (FC, ORAC-FL and ORAC-PGR) were higher in wines whose grapes had been cultivated in the IV region. In agreement with this finding, the statistical analyses indicated large differences ($P \leq 0.01$) between the mean values obtained in the IV region and those of the other regions (Table 3).

Discussion

Vineyard factors such as the grape variety, ripening, quality, climate, water, geographical origin, and soil characteristics affect the phenolic composition of grapes and wines (Teixeira *et al*., 2013). Therefore, it is expected that such factors may be reflected in the total phenolic content and the antioxidant capacity of wines (Burns *et al.,* 2001). The data provided in Table 3 show noticeable difference in the values of the three indexes considered, expressed in mM Trolox equivalents. This is an expected result given the main factors that determine the values of ORAC-FL and ORAC-PGR (López-Alarcón and Lissi, 2006). On the hand, the data presented in the Table 3 show that the differences between the wines were similar for the three indexes. This suggests that ORAC-type methodologies are valid tools for establishing these differences.

The results obtained indicate that:

- there is an optimal time (ca. four years after harvesting) for generating the maximal concentration of phenolic compounds in red wines;
- there is a small, but significant difference in the indexes for the different quality classifications, with an increase in Trolox equivalents being observed from the "Varietal" to "Grand Reserva" classifications;
- there is a large increase in Trolox equivalents in Cabernet Sauvignon red wines made with grapes grown in the IV region.

These results are similar to those of previously reported works showing an increase in total phenolic contents associated with bottle aging and the quality classification (Gómez-Plaza *et al*., 2000; Lissi *et al*., 2014). Nevertheless, it is important to note that other factors, such as climate conditions, maceration, fermentation and technological procedures, can influence the total phenolic content and antioxidant capacity of wines. In fact, these properties have been reported to be more dependent on viticulture and wine-making practices than on age (Roginsky *et al*., 2006; Lissi *et al*., 2014).

The most significant difference was observed between the wines from grapes grown in the IV region and the other regions. The wines considered in this study were obtained from the main wine-producing regions of Chile (the IV, Metropolitan, VI and VII regions). The VII region includes the Maule valley; the VI region includes the Rapel and Colchagua valleys; the Metropolitan region includes the Maipo and Central valleys; and the Elqui and Limarí valleys are the main wine-producing areas in the IV region. The climates of Metropolitan and VI-VII regions are similar (predominantly temperate-Mediterranean), and their valleys exhibit six months of dry season and a rainy winter. These factors explain the similarity of the results obtained in these regions (Figure 3). Converseyl, the climate of the IV region is different because it is located between a desert and a Mediterranean area. This region comprises the Limarí, Choapa and Elqui valleys, the last of which constitutes the northern border of the wine-producing region of Chile. In this region, the weather conditions for growing grapes are excellent, including windy, dry valleys with an annual rainfall of only 130 mm. Additionally, this region exhibits heavy soils that are rich in nutrients and able to store water. These findings are in accordance with studies showing a remarkable dependence of the polyphenolic composition of wines on their geographical origin (Worarat-phoka *et al*., 2007; Jiang and Zhang, 2012). In the present work, the particular increase in the

antioxidant content of wines from grapes grown in the IV region can be explained by the climatic characteristics of this region, which include dry weather and a temperature difference between day and night of nearly 20 °C. Our results are in agreement with results obtained in four regions of China, where positive correlations were found between sunlight exposure, water deficiency and large day-night time temperature differences and the total phenolic content of red wines (Gollop *et al*., 2002; Yamane *et al*., 2006; Jiang and Zhang, 2012).

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Resumen

R. Bridi, S. Lobato, C. López-Alarcón y E. Lissi. 2014. Influencia de la región de cultivo, tipo de guarda y el año de cosecha en el índice ORAC (*Oxygen Radical Absorbance Capacity)* de vinos tintos chilenos. Cien. Inv. Agr. 41(3):395-402. En el presente estudio se evaluó el contenido de compuestos fenólicos y la actividad antioxidante de treinta y seis Cabernet Sauvignon vinos tintos chilenos de diferentes zonas geográficas (IV, Metropolitana y VI - VII regiones), distintos tipos de guarda ("Varietal", "Reserva", and "Gran Reserva") y diferentes años de cosecha (2000-2012). El contenido total de fenoles fue determinado por el método de Folin-Ciocalteau (FC) y la actividad antioxidante fue evaluada por medio del ensayo ORAC (*Oxygen Radical Absorbance Capacity*) utilizando fluoresceína y rojo de pirogalol como moléculas blanco. Los resultados indicaran una fuerte influencia de la zona geográfica de cultivo en los tres índices estudiados, con valores superiores en los vinos procedentes de la IV región. Este resultado puede ser explicado en base a las características climáticas de esta región, la que se caracteriza por tiempo seco y diferencias de temperatura entre día y noche que superan los 20 grados.

Palabras clave: Año de cosecha, Cabernet Sauvignon, guarda, ORAC, compuestos fenólicos, región, vino tinto chileno.

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