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Location effects on the polyphenolic and polysaccharidic profiles and colour of Carignan grape variety wines from the Chilean Maule region

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ABSTRACT

This paper reports on a study of chemical characterization and colour parameters of cv. Carignan red wines from six locations and two production years of the Chilean Maule valley. The chemical study was performed on polyphenolic composition (benzoic acids, hydroxycinnamic acid derivatives, stilbenes, flavan-3-ols, flavonols and anthocyanins) and several fractions of proanthocyanidins and polysaccharides. Results revealed that although significantly ($p < 0.05$) different content of anthocyanins were observed according to the production year, it could be possible to establish fingerprints of the different locations of the Maule valley wines. Thus, wines from zones closer to the Andes Mountains had higher content of procyanidin B3 (Caliboro), polysaccharides and *cis*-resveratrol-glucoside (Loncomilla and Melozal), whereas the proximity to the Pacific Ocean provoked a unifying effect in chemical and colorimetric terms (Cauquenes, Sauzal and Huerta del Maule).

1. Introduction

In the past few years, there is a new tendency about valuing new-style vinifications, betting on minority and indigenous grapevines, potenting their use as complementary varieties with different sensorial characteristics to the renowned grape varieties. That fact could be the case of Carignan grape variety, with a natural lesser acidity compared with other varieties such as Cabernet Sauvignon or Carménère (Úbeda, del Barrio-Galán, Peña-Neira, Medel-Marabolí, & Durán-Guerrero, 2017). This characteristic could improve the chemical and microbiological stability, confer mouth freshness and deep violet-red colour to the resulting wines.

Some studies in terms of colour parameters and general chemical characteristics of French and Spanish Carignan red wines have been developed. Carignan wines from the north of Spain contained low anthocyanin total content in comparison with other grape varieties (Arozarena, Casp, Marín, & Navarro, 2000), although Edo-Roca, Nadal, Snchez-Ortiz, and Lampreave (2014) affirmed that a strong dependency on the plant vigour exists. Carignan wines cultivated in France also showed low values of total anthocyanins and polyphenols, with not very high amounts of (+)-catechin, (−)-epicatechin and hydroxycinnamic acids (Jensen, Demiray, Egebo, & Meyer, 2008). However, in other countries such as Turkey, the content of anthocyanins and phenols was intermediate compared to other grape varieties of the country (Orak, 2007). With regard to polysaccharides, Ducasse, Williams, Meudec, Cheynier, and Doco (2010), Doco, Quellec, Moutounet, and Pellerin (1999) and Doco, Williams, Meudec, Cheynier, and Sommerer (2015) developed an accurately identification of oligosaccharides and polysaccharides in Carignan red wines cultivated in France. However, in spite of the importance in terms of winemaking, very scarce studies about Carignan grape variety have been published in the main vinicultural countries.

Chile is a long and narrow country, whose territory presents a tremendous diversity of landscapes. The central region, with a Mediterranean climate, is the traditional wine region of the country. In 2016, Chile was the fourth largest exporter of wines in the world, and the eighth largest producer (Organización Internacional de la Viña y el Vino, OIV, http://www.oiv.int/). Over thirty grape varieties in production (72% of world red varieties) are grown in Chile, such as Carménère, Cabernet Sauvignon and Syrah, among others.

Most of Chile's premium wine regions are dependent on irrigation to sustain vineyards, getting the necessary water from melting snow caps in the Andes. However, nowadays, the strategic plan of Wines of Chile 2020 includes an item to promote dry-farmed and old-vine wines. In this sense, Carignan vines are mainly cultivated in the dry-farmed or Secano Costero in the Maule Valley (350 km to the south of Santiago de Chile), with vineyards with > 60 year old vine-age. Although each year Carignan is increasingly used in the blends of different commercial

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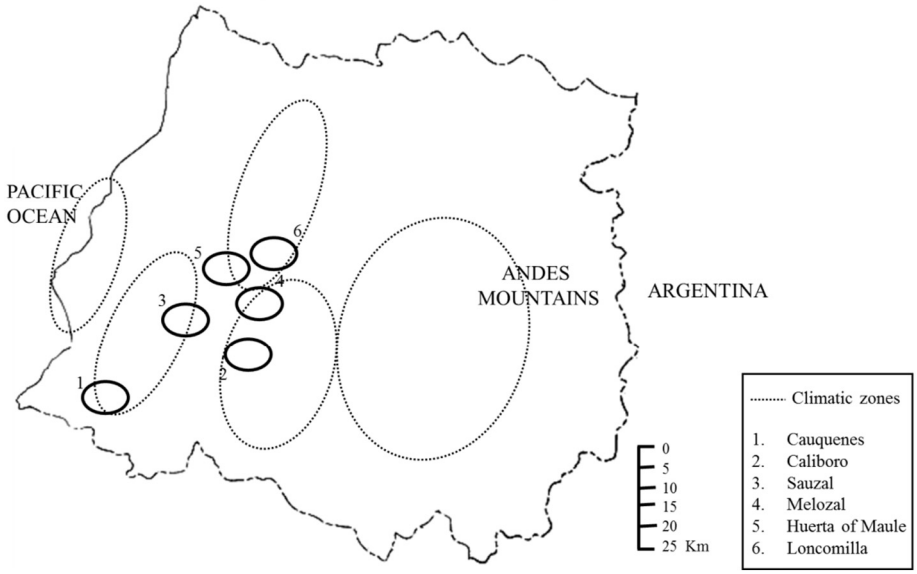

Fig. 1. Climatic zones of the Chilean Maule region and locations studied (Cauquenes, Caliboro, Sauzal, Melozal, Huerta del Maule and Loncomilla).

wines in Chile and in the world, very scarce previous scientific studies to typify Carignan wines has been developed, especially from the Maule region that concentrates more than the 70% of the total Carignan vineyard area (≈857 ha) in Chile (SAG, Servicio Agrícola y Ganadero, http://www.sag.gob.cl/).Only Martínez-Gil, Gutiérrez-Gamboa, Garde-Cerdán, Pérez-Álvarez, and Moreno-Simunovic (2018), Úbeda et al. (2017) and Gutiérrez-Gamboa et al. (2018) have been very recently published about phenolic, volatile and amino acid composition of different locations of the Chilean Maule Valley.

Climate conditions and geographical sites influence on the final quality of the wines, in terms of sugar content, aromas and colour (Van Leeuwen et al., 2004). In this way, the wines from the Chilean Maule Valley, with a heterogeneous orography, are greatly influenced by the proximity to the Andes Mountains and the Pacific Ocean. In fact, Montes, Perez-Quezada, Peña-Neira, and Tonietto (2012) divided the Maule Valley into five climatic zones (Fig. 1) employing three climate indexes (Huglin Index, the Cold Night Index and the Drought Index). These indexes estimate the potential climate of a particular place to ensure the maturation of different grape varieties (Huglin & Schneider, 1998). Moreover, they also suppose a climate classification system for wine regions which permits the grouping of regions according to their similarities (Montes et al., 2012; Tonietto & Carbonneau, 2004).

To the best of our knowledge, this is the first attempt to deeply and jointly characterise Chilean Carignan wines from the Maule Valley from a colour and chemical (phenolic, proanthocyanins, anthocyanin and polysaccharides) points of view. Besides, in the light of the influence of the climatic conditions on the wine quality, different locations and years of production of Carignan red wines have been taken into account. This work would not only suppose a diversification of the oenological market that could permit to elaborate young monovarietal wines, coupages or even with a short aging, but also contribute to the social and economic development of a Chilean vinicultural area poorly developed as the Maule valley.

2. Material and methods

2.1. Chemical and solvents

Methylcellulose (1500 cP viscosity at 20 g/L) and standards of caffeic acid, *p*-coumaric acid, (+)-catechin and (−)-epicatechin (purity > 98%), gallic acid and caftaric acid (purity > 97%), quercetin (purity > 95%) and malvidin-3-glucoside (purity > 90%) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Polyethylene membranes of 0.22 μm pore size were acquired from EMD Millipore (Billerica, MA, USA). Merck (Darmstadt, Germany) supplied sodium sulphate (anhydrous), vanillin (990 g/L), ethyl acetate, potassium metabisulfite, diethyl ether, sodium hydroxide, hydrochloric acid, sulfuric acid, high-performance liquid chromatography (HPLC)-grade acetonitrile, acetic acid, formic acid and methanol. All reagents were of analytical grade or higher. Sep-Pack Plus Environmental C_{18} cartridges (900 mg) and Sep-PackPlus Short C_{18} cartridges (400 mg) were obtained from Waters (Milford, MA, USA). Phosphate buffer (pH 7) was acquired from Mallinckrodt Baker (Phillipsburg, NJ, USA). Nitrogen gas was supplied by Indura SA (Santiago, Chile).

2.2. Red wines samples

Twenty-eight commercial monovarietal cv. Carignan red wines corresponding to two vintages (2012 and 2014) and different areas of the Maule Valley (in the VII region of Chile) were analyzed. Vines were > 50 years old, without irrigation, ungrafted and growing in good phytosanitary conditions. All the cellars followed traditional winemaking methods, in which maceration was developed until the alcoholic fermentation finished (three days at 10 °C), punched down twice a day until breaking the cap fully. Fermentation progress was tracked monitoring the relative density. Sulphur dioxide was used for all the assays.

The samples were collected from wine cellars from six different areas: Caliboro (8) (35°49′S; 71°54′W), Melozal (8) (35°42′S; 71°48′W); Cauquenes (2) (35°58′S; 72°21′W), Huerta of Maule (2) (35°40′S; 71°57′W), Loncomilla (4) (35°34′S; 71°45′W), and Sauzal (4) (35°45′S; 72°07′W) (Fig. 1). Once supplied, wines were stored at 10 °C until their analysis.

2.3. Spectrophotometric measurement

Wine conventional analytical data were obtained by O.I.V. official methods. Absorbance measurements were made with a Hewlett-Packard UV–Vis 1700 Pharmaspec spectrophotometer (Shimadzu, Kyoto, Japan).

Colour measurements were determined using the whole visible spectrum (380–770 nm) at constant intervals ($\Delta\lambda$ = 2 nm), with 2-mm path length glass cells. Distilled water was used as reference. The CIELAB colour parameters (L^*, C^*_{ab} and h_{ab}) were determined according to Pérez-Magariño and González-Sanjosé (2003), following the

Commission Internationale de L'Eclairage's, CIE, recommendations (CIE, 2004): the CIE 1964 10° Standard Observer and the CIE Standard Illuminant D65.

The uniform space CIELAB are defined by different parameters: L^*, a psychometric index of lightness, correlate the lightness property according to which each colour in the grey scale, ranking from black ($L^* = 0$) to white ($L^* = 100$); the hue angle (h_{ab}) is the qualitative attribute, defined as red (0°/360°), yellow (90°), green (180°) or blue (270°); and chroma (C^*_{ab}) defines as the saturation of colour in comparison to a grey colour with the same lightness, also considered quantitative and qualitative attribute of colour, respectively.

2.4. Fractionation of proanthocyanidins using Sep-Pak C_{18} cartridges

Proanthocyanidins were fractionated according to their polymerization degree using Sep-Pak tC_{18} cartridges. 7 mL of wine sample was concentrated to dryness in a rotary evaporator at < 30 °C and the residue was dissolved in 20 mL of 67 mmol/L phosphate buffer (pH 7). After adjusting the pH to 7 under a nitrogen atmosphere, the sample was passed through two preconditioned neutral Sep-Pak tC_{18} cartridges connected in series (top, Sep-Pak Plus Environmental tC_{18} cartridge (900 mg); bottom, Sep-Pak Plus Short tC_{18} cartridge (400 mg)), according to the method described by Sun, Leandro, Ricardo Da Silva, and Spranger (1998) and briefly explained by Cáceres-Mella et al. (2014). For each fraction previously obtained (monomeric, oligomeric and polymeric fractions), flavan-3-ols were quantified using the modified vanillin assay described by Sun, Ricardo-da-Silva, and Spranger (1998). The absorbance at 500 nm was measured and methanol was used as a blank instead of vanillin.

2.5. HPLC-DAD phenolic and anthocyanin determination

Both anthocyanin and phenolic analyses were performed using an 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B photodiode array detector (DAD), a G1311A quaternary pump, a G1379A degasser and a G1329A autosampler.

For the anthocyanin analysis, water–formic acid (90:10) as solvent A and acetonitrile as solvent B were used. The injection volume was 150 µL. A reversed-phase LiChroCart C_{18} column (250 mm × 4.0 mm I.D., 5 µm; Merck, Darmstadt, Germany) was used. The chromatographic conditions were detailed by Fanzone et al. (2012). Briefly, the flow rate was 1.1 mL/min from 0 to 22 min and 1.5 mL/min from 22 to 35 min, as follows: 96–85% of A and 4–15% of B from 0 to 12 min, 85–85% of A and 15–15% of B from 12 to 22 min, 85–70% of A and 15–30% of B from 22 to 35 min. A final wash with 100% methanol and re-equilibration of the column was also used. UV– Vis spectra were recorded from 210 to 600 nm with a bandwidth of 2.0 nm. Prior direct injection, the samples were filtered through a 0.22-µm pore size membrane. All analyses were performed in triplicate. The wavelength of 520 nm was used for the quantification by comparing the areas and the retention times with the malvidin 3-glucoside standard.

50-mL aliquot of wine was extracted with diethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL) to concentrate the low-molecular-weight phenolic compounds, according to the method described by Peña-Neira, Cáceres, and Pastenes (2007). The organic fractions were combined, dehydrated with 2.5 g of anhydrous sodium sulphate and subsequently, evaporated to dryness under vacuum at 30 °C. The so obtained solid residue was dissolved in 2 mL of a methanol/water (1:1, v/v) solution and filtered through a 0.22-µm pore size membrane. 25 µL was injected and underwent chromatographic analysis. A reverse phase Nova-Pak C_{18} column (300 mm × 3.9 mm I.D.; 4 µm; Waters, Milford, MA) was used for HPLC-DAD analysis thermostatted at 20 °C. The calibration curves at 280 and 360 nm were produced by injecting the standard solutions under the same conditions. The proanthocyanidins and stilbene glycosides, for which no standards are available, were quantified using standard curves for (+)-catechin and *trans*-resveratrol, respectively. All analyses were performed in triplicate.

2.6. Polysaccharide analysis

High-performance size exclusion chromatography with refractive index detection (HPSEC-RID) was used to determine the molecular distributions and concentrations of polysaccharides. HPSECRID was performed using an Agilent 1260 Infinity Series liquid chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a G1362A refractive index detector (RID), a G1311B quaternary pump, a G1316A column oven with two Shodex columns, an OHpak SB-803 HQ and an SB-804 HQ connected in series (300 mm × 8 mm I.D., 6 µm and 10 µm, respectively; Showa Denko, Tokio, Japan), and a G1329A autosampler. The quantification of the polysaccharides fractions was carried out using dextrans and pectins (*Leuconostoc mesesteroides*) to prepare the calibration curves (Fanzone et al., 2012).

2.7. Statistical analysis

All statistical analyses were performed using Statistica v.8.0 software (Statistica, 2007). Analysis of variance (ANOVA) test was applied using the general linear model program to establish whether mean values of the sample data differed significantly from each other, with a significance level of 95% ($p < 0.05$). To obtain summarized and synthesized information from a large set of variables and to better understand the location effects, principal component analysis (PCA) was applied.

3. Results and discussion

The influence of the production zone and year of a set of wines from Maule Valley has been studied. Concretely, cv. Carignan wines from 2012 and 2014 were analyzed in Caliboro, Melozal, Cauquenes, Huerta del Maule, Loncomilla and Sauzal areas. Several chemical (phenolic composition, anthocyanins, proanthocyanidins and polysaccharides) and colour parameters were taken into account. In spite of the vast stretch of land of cv. Carignan in Chile, to our knowledge, this is the first report analyzing the chemical composition and colour characteristics of cv. Carignan grape variety from Maule Valley.

3.1. Climate conditions

According to the Köppen climatic classification (Kottek, Grieser, Beck, Rudolf, and Rubel, 2006), the Chilean Maule Valley has a “Csb” assignation, according to the classification of the main climates and precipitation and temperature conditions. Thus, “C” refers to “warm temperature”, “s” to “summer dry” and “b” to “warm summer”, constituting a typical warm-summer Mediterranean climate. In summary, this climate consists of the coldest month averaging above 0 °C (32 °F), all months with an average temperature below 22 °C (71.6 °F), and at least four months averaging above 10 °C (50 °F). In addition, this climate is characterized by at least three times as much precipitation in the wettest month of winter as in the driest month of summer, and driest month of summer receives < 30 mm (1.2 in).

According to the climate indexes established by Huglin and Schneider (1998), Loncomilla and Huerta del Maule locations had a mean Huglin index (HI) of 2400 units, an average value of Drought index (DI) of −222 mm and mean Cold Night Index (CI) of 9.7 °C. Followed by these growing zones, Sauzal and Cauquenes had a HI of 2223 units, with a DI of −240 mm and a CI of 9.6 °C. Caliboro and Melozal locations resulted with the lowest values of HI, DI and CI, with 2088 units, −145 mm and 8.8 °C, respectively.

3.2. Fractionation of proanthocyanidins

Table 1 displays the monomeric, oligomeric and polymeric

Table 1
Mean values and standard deviations ($n = 28$) for the pH, total acidity, colour parameters (L^*, C^*_{ab} and h_{ab}), and the fractions of monomeric, oligomeric and polymeric proanthocyanidins (PA) (mg/L) of Carignan red wines from different locations of Maule valley.

	Caliboro	Cauquenes	Huerta del Maule	Loncomilla	Melozal	Sauzal	*p*- location
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
pH	3.23 ± 0.08^{b}	3.28 ± 0.35^{b}	3.02 ± 0.07^{a}	3.13 ± 0.07^{b}	2.91 ± 0.13^{a}	3.19 ± 0.13^{b}	*
Total acidity	3.82 ± 0.14^{a}	3.81 ± 0.45^{a}	4.74 ± 0.15^{a}	3.85 ± 0.37^{a}	5.09 ± 0.51^{a}	5.65 ± 2.17^{a}	
L^*	37.08 ± 14.32	36.78 ± 7.95	35.20 ± 4.93	39.10 ± 7.18	39.09 ± 5.87	41.87 ± 8.59	
C^*_{ab}	55.08 ± 2.39	56.60 ± 3.89	59.76 ± 1.09	52.39 ± 13.22	60.17 ± 2.79	53.21 ± 9.43	
h_{ab}	14.18 ± 1.59	17.68 ± 1.57	14.86 ± 1.26	32.43 ± 34.64	12.09 ± 4.56	11.70 ± 1.81	
Monomeric PA	12.13 ± 4.93	6.86 ± 1.76	8.24 ± 1.57	8.59 ± 6.03	7.56 ± 2.29	6.91 ± 5.83	
Oligomeric PA	61.88 ± 27.56^{c}	21.92 ± 12.26^{ab}	47.34 ± 29.68^{bc}	57.79 ± 26.49^{c}	31.42 ± 15.06^{ab}	42.02 ± 24.58^{bc}	*
Polymeric PA	440.73 ± 320.25	333.28 ± 144.48	670.59 ± 447.26	782.51 ± 327.66	428.01 ± 265.41	466.28 ± 176.68	

Different superscripts in the same row denote significant ($p < 0.05$) differences according to Student-Newman-Keuls test. No superscripts denote any significant ($p < 0.05$) difference. Asterisks denote significant ($p < 0.05$) differences according to the location.

proanthocyanidin proportions in the wine samples.

According to Cáceres-Mella et al. (2014), the monomeric fraction consists only of (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate, whereas the oligomeric fraction is formed by proanthocyanidins of degree of polymerization ranging from 2 to 12–15. The polymeric fraction, instead, is composed of polymeric proanthocyanidins (> 12–15 units). The relative percentages of the proanthocyanidins fraction in Maule Carignan wines were as follows: flavan-3-ol polymers (85.6–92.4%), followed by flavan-3-ol oligomers (6.1–12.0%) and a lower percentage of flavan-3-ol monomers (1.0–2.4%), being the polymeric fraction predominant in comparison with the monomeric and oligomeric fractions. These results agreed with similar published studies for red wines (Fanzone et al., 2012; Granato, Katayama, & de Castro, 2011). The fractionation by molecular weight has been previously developed in other Chilean grape varieties, such as Cabernet Sauvignon, Carménère, Merlot and Cabernet Franc (Cáceres-Mella et al., 2014; del Barrio-Galán, Cáceres-Mella, Medel-Marabolí, & Peña-Neira, 2015), but this is the first time that this analysis is underwent in Carignan red wines.

3.3. Identification of polyphenolic composition

In this research, several types of polyphenolic compounds have been identified, like benzoic acids, hydroxycinnamic acid derivatives, stilbenes, flavan-3-ols, flavonols and anthocyanins (Table 2). The polyphenolic compounds identified were the expected, well-known, compounds usually present in wine (Cejudo-Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2011; Gordillo, Cejudo-Bastante, Rodríguez-Pulido, Lourdes González-Miret, & Heredia, 2013). *Cis*-resveratrol-glucoside was also identified, very scarcely reported in this grape variety (Lambert et al., 2013). Native grape anthocyanins were detected in Carignan red wines (Gordillo et al., 2014), including non-acylated, acetylated, *p*-coumaroylated and caffeoylated anthocyanins of the five expected anthocyanidins (delphinidin, cyaniding, petunidin, peonidin and malvidin) (Table 2).

3.4. Analysis of polysaccharides

Four fractions of polysaccharides were identified, quantified and classified according to the average molecular weights: fraction I, > 2000 kDa; fraction II, 200–300 kDa; fraction III, 60–80 kDa; fraction IV, ≥10 kDa. As seen in Table 3, the fractions II and III generally depicted the highest polysaccharide concentration regardless the location of the vineyard, being the high-molecular-weight fraction (fraction I) the one that presented the lower percentage of polysaccharides. In fact, similar number of fractions have been previously reported by del Barrio-Galán, Cáceres-Mella, et al. (2015) and del Barrio-Galán, Medel-Marabolí, and Peña-Neira (2015) in wines elaborated from Syrah and Cabernet Sauvignon grape varieties.

3.5. Effect of production year and location

It is highlighted that all wines were supplied by Chilean winemakers that employ similar winemaking practices of production and harvesting. Therefore, the differences observed on all the parameters are assumed to correspond to the location area and the harvest year.

To gain an insight into the "production year" effect, chromatic parameters and the total content of polyphenols, anthocyanins, proanthocyanidins and polysaccharides were subjected to ANOVA (Fig. 2). Production year did not remarkably affect compound family profiles except monomeric anthocyanins, having the 2012-vintage wines the lowest values in all growing areas. This fact could be due to the decrease of monomeric anthocyanins due to polymerization reactions occurring during wine storage (Gómez Gallego, Gómez García-Carpintero, Sánchez-Palomo, González Viñas, & Hermosín-Gutiérrez, 2013). Therefore, it could be affirmed that production year only affected the monomeric anthocyanins of Carignan red wines.

A post-hoc comparison Tukey's test was applied to the set of data according to the variable "location" (Tables 1-3). The pH and total acidity of wines from different areas of Maule Valley were similar and low, with the exception of wines from Melozal and Huerta del Maule, with significantly ($p < 0.05$) lower values of pH (Table 1). However, Cauquenes, Caliboro and Loncomilla red wines were considered as the less acidic wines. The colour of wines (the colorimetric characteristics L^*, C^*_{ab} and h_{ab}) was similar in all Carignan red wines regardless the production zone (Table 1).

With regard to the fractions of proanthocyanidins, it could be affirmed that Caliboro showed the highest values of monomeric and oligomeric proanthocyanidins, whereas Loncomilla showed the highest value of polymeric fraction (Table 1). These results were in concordance with the significantly ($p < 0.05$) higher amounts of (+)-catechin and procyanidin B3 of red wines elaborated in the Caliboro area, followed by red wines cultivated in Huerta del Maule and Loncomilla locations (Table 2). According to Huglin and Schneider (1998), the Carignan variety has an HI requirement of 2200 units to obtain a sugar content of ~18 to 20 Brix. This means that the grapes of Caliboro area could have a lesser degree of ripeness, fact that could explain the higher content of procyanidins and (+)-catechin as affirmed Pérez-Magariño and González-Sanjosé (2004). However, Cauquenes area, located in the south of the Maule valley and closer to the Pacific Ocean, provided to wines the lowest values of all fractions of proanthocyanidins.

With regard to the low-molecular-weight phenolic compounds, Table 2 reflects the content and ANOVA analysis of benzoic acids, hydroxycinnamic acid derivatives, stilbenes, flavonols and anthocyanins. It could be observed that the location area of the Maule valley region greatly influenced on the chemical composition. The most abundant concentration of benzoic acids (above all gallic acid) corresponded to wines elaborated in the Huerta del Maule area, whereas Cauquenes was found as the zone with the lowest amount. Among hydroxycinnamic

Table 2
Mean values of concentration (mg/L) and standard deviations (n = 28) of the individual polyphenolic compounds belonged to different chemical families identified by HPLC-DAD in Carignan red wines from different locations of Maule valley.

	Caliboro	Cauquenes	Huerta del Maule	Loncomilla	Melozal	Sauzal	*p*- location
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Benzoic acids							
Gallic acid	24.93 ± 9.72	15.51 ± 6.47	33.44 ± 15.88	25.02 ± 17.86	18.92 ± 12.76	18.52 ± 5.21	
Protocatechuic acid	1.85 ± 0.42	1.88 ± 0.68	1.77 ± 0.44	1.57 ± 0.48	1.75 ± 0.69	1.93 ± 0.23	
Vanillic acid	1.27 ± 0.03	1.68 ± 0.38	1.51 ± 0.15	1.47 ± 0.20	1.46 ± 0.23	1.59 ± 0.27	
Siringic acid	2.36 ± 0.19	3.62 ± 1.54	3.19 ± 0.51	3.75 ± 1.58	3.09 ± 0.84	3.15 ± 0.65	
Hydroxycinnamic acid derivatives							
Coutaric acid	7.89 ± 5.94	6.68 ± 3.03	7.04 ± 2.32	7.46 ± 4.59	5.77 ± 3.87	8.53 ± 6.27	
Caftaric acid	nd^{a}	4.69 ± 2.18^{b}	6.94 ± 3.12^{b}	5.92 ± 4.47^{b}	5.64 ± 2.53^{b}	4.75 ± 4.12^{b}	*
Caffeic acid	nd^{a}	2.36 ± 1.27^{bc}	2.73 ± 0.60^{bc}	5.39 ± 5.19^{c}	1.21 ± 0.75^{b}	3.18 ± 2.11^{bc}	*
Stilbenes							
c-Resveratrol-glc	1.26 ± 0.35^{b}	0.97 ± 0.36^{a}	0.74 ± 0.38^{a}	1.60 ± 0.39^{c}	0.63 ± 0.13^{a}	0.65 ± 0.25^{a}	*
Flavan-3-ols							
Procyanidin B3	4.38 ± 0.18^{c}	2.80 ± 1.23^{a}	3.29 ± 0.70^{a}	4.25 ± 0.30^{c}	1.96 ± 0.77^{a}	2.42 ± 0.42^{a}	*
(+)-Catechin	15.33 ± 4.65^{c}	6.80 ± 2.05^{ab}	12.07 ± 3.37^{a}	11.50 ± 7.24^{a}	7.75 ± 4.78^{ab}	7.89 ± 2.71^{a}	*
Flavonols							
Quercetin	9.43 ± 4.13	13.52 ± 3.66	10.63 ± 1.97	10.69 ± 3.41	9.76 ± 4.59	12.54 ± 6.48	
Monomeric anthocyanins							
Delphinidin-3-glc	24.95 ± 0.07	20.05 ± 0.21	18.35 ± 0.21	21.83 ± 0.06	15.61 ± 0.13	17.87 ± 0.03	
Cyanidin-3-glc	1.18 ± 0.00	0.79 ± 0.16	0.99 ± 0.04	0.99 ± 0.07	0.89 ± 0.01	1.04 ± 0.00	
Petunidin-3-glc	25.39 ± 0.22	22.80 ± 0.38	17.02 ± 0.30	23.89 ± 0.10	15.38 ± 0.10	19.99 ± 0.19	
Peonidin-3-glc	8.09 ± 0.00	7.63 ± 0.38	5.42 ± 0.05	6.88 ± 0.03	4.66 ± 0.28	4.17 ± 0.03	
Malvidin-3-glc	117.55 ± 1.45	102.44 ± 0.40	70.43 ± 0.40	110.22 ± 0.50	72.47 ± 0.33	72.95 ± 0.04	
Delphinidin-3-acet-glc	1.02 ± 0.00	0.47 ± 0.01	0.17 ± 0.01	0.89 ± 0.01	0.15 ± 0.05	0.91 ± 0.00	
Cyanidin-3-acet-glc	1.74 ± 0.05^{a}	4.11 ± 0.05^{bc}	3.30 ± 0.11^{b}	3.81 ± 0.03^{b}	3.17 ± 0.07^{b}	1.77 ± 0.03^{ab}	*
Petunidin-3-acet-glc	2.39 ± 0.17	2.49 ± 0.12	2.86 ± 0.07	2.47 ± 0.26	2.29 ± 0.39	1.70 ± 0.58	
Peonidin-3-acet-glc	1.26 ± 0.00	0.90 ± 0.05	0.84 ± 0.17	1.10 ± 0.07	0.36 ± 0.04	0.52 ± 0.02	
Malvidin-3-acet-glc	5.60 ± 0.35	5.65 ± 0.19	5.70 ± 0.01	7.61 ± 0.12	2.84 ± 0.17	3.06 ± 0.25	
t-Delphinidin-3-coum-glc	2.14 ± 0.17	2.71 ± 0.11	2.30 ± 0.03	3.22 ± 0.62	1.45 ± 0.05	2.21 ± 0.04	
t-Cyanidin-3-coum-glc	0.21 ± 0.03	0.29 ± 0.06	0.43 ± 0.03	0.44 ± 0.02	0.28 ± 0.02	0.58 ± 0.02	
t-Petunidin-3-coum-glc	1.52 ± 0.00	1.68 ± 0.04	1.35 ± 0.06	2.05 ± 0.03	1.23 ± 0.04	2.28 ± 0.00	
c-Malvidin-3-coum-glc	1.02 ± 0.00	0.45 ± 0.10	0.31 ± 0.03	0.52 ± 0.01	0.40 ± 0.09	0.49 ± 0.00	
t-Peonidin-3-coum-glc	0.67 ± 0.02	0.77 ± 0.01	0.57 ± 0.04	1.29 ± 0.01	0.55 ± 0.05	0.89 ± 0.16	
t-Malvidin-3-coum-glc	6.56 ± 0.12	8.51 ± 0.05	5.78 ± 0.02	10.27 ± 0.20	5.63 ± 0.06	5.91 ± 0.19	
Malvidin-3-caf-glc	0.03 ± 0.00	0.53 ± 0.08	0.26 ± 0.00	0.20 ± 0.00	0.69 ± 0.00	tr	

Different superscripts in the same row denote significant ($p < 0.05$) differences according to Student-Newman-Keuls test. No superscripts denote any significant ($p < 0.05$) difference. nd, not detected; *t*, *trans*; *c*, *cis*; glc, glucoside; acet, acetyl; coum, *p*-coumaroyl; caf, caffeoyl. Asterisks denote significant ($p < 0.05$) differences according to the location.

acid derivatives and stilbenes, Loncomilla also showed the significantly highest content of caffeic acid, *cis*-resveratrol-glucoside and procyanidin B3 (and oligomeric proanthocyanidins) (Tables 1 and 2). Caliboro also highlighted a huge concentration of the aforementioned compounds, together with (+)-catechin, in agreement with the highest values of monomeric and oligomeric proanthocyanidins previously described. This behaviour was also observed by Martínez-Gil et al. (2018), who affirmed that the synthesis of flavonols and hydroxycinnamic acid was favoured by cooler temperatures. Regarding anthocyanins, however, the location of the vineyard showed a low dispersion, in the light of the lack of significant ($p < 0.05$) differences. Contrary results were obtained by Martínez-Gil et al. (2018), who reported a relationship between the BEDD index and the anthocyanin content, in concordance with Spayd, Tarara, Mee, and Ferguson (2002) and Downey, Harvey, and Robinson (2004) who affirmed that higher acylation degree was attributed to an effect of light and high temperatures.

When polysaccharides were taken into account, Caliboro showed the highest amount of the first (FI) and second fraction (FII), together with Melozal in FI. However, Melozal showed the lowest values of FIII and FIV (Table 3). Taken into account that Caliboro and Melozal are ones of the closest regions to the Andes Mountains (Fig. 1), it is possible to affirm that the climatic zone could also influence on the polysaccharide composition.

Taken together, hydroxycinnamic acid derivatives, stilbenes, flavan-3-ols, oligomeric proanthocyanidins, and the two first fractions of polysaccharides were the parameters significantly ($p < 0.05$) more affected by the production area.

Based on the results obtained from ANOVA, non-supervised pattern recognition statistical analysis (Principal Component Analysis) was

Table 3
Mean values of concentration (mg/L) and standard deviations ($n = 28$) for the polysaccharides fractions (F) of Carignan red wines from different locations of Maule valley.

	Caliboro	Cauquenes	Huerta del Maule	Loncomilla	Melozal	Sauzal	*p*- location
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
[FI]	248.96 ± 14.46^{d}	71.03 ± 95.95^{a}	130.68 ± 99.06^{c}	78.18 ± 88.35^{a}	137.80 ± 92.74^{c}	11.39 ± 0.33^{a}	*
[FII]	582.51 ± 571.44^{b}	110.39 ± 61.73^{a}	199.99 ± 77.05^{a}	157.99 ± 61.91^{a}	206.39 ± 103.36^{a}	89.73 ± 31.05^{a}	*
[FIII]	161.60 ± 23.56	154.18 ± 67.05	219.54 ± 119.15^{a}	252.69 ± 148.77	123.11 ± 51.92	179.35 ± 63.33	
[FIV]	132.94 ± 62.32	136.98 ± 56.02	116.86 ± 9.51	150.81 ± 60.21	103.02 ± 28.29	181.43 ± 111.24	

Different superscripts in the same row denote significant ($p < 0.05$) differences according to Student-Newman-Keuls test. No superscripts denote any significant ($p < 0.05$) difference. Asterisks denote significant ($p < 0.05$) differences according to the location.

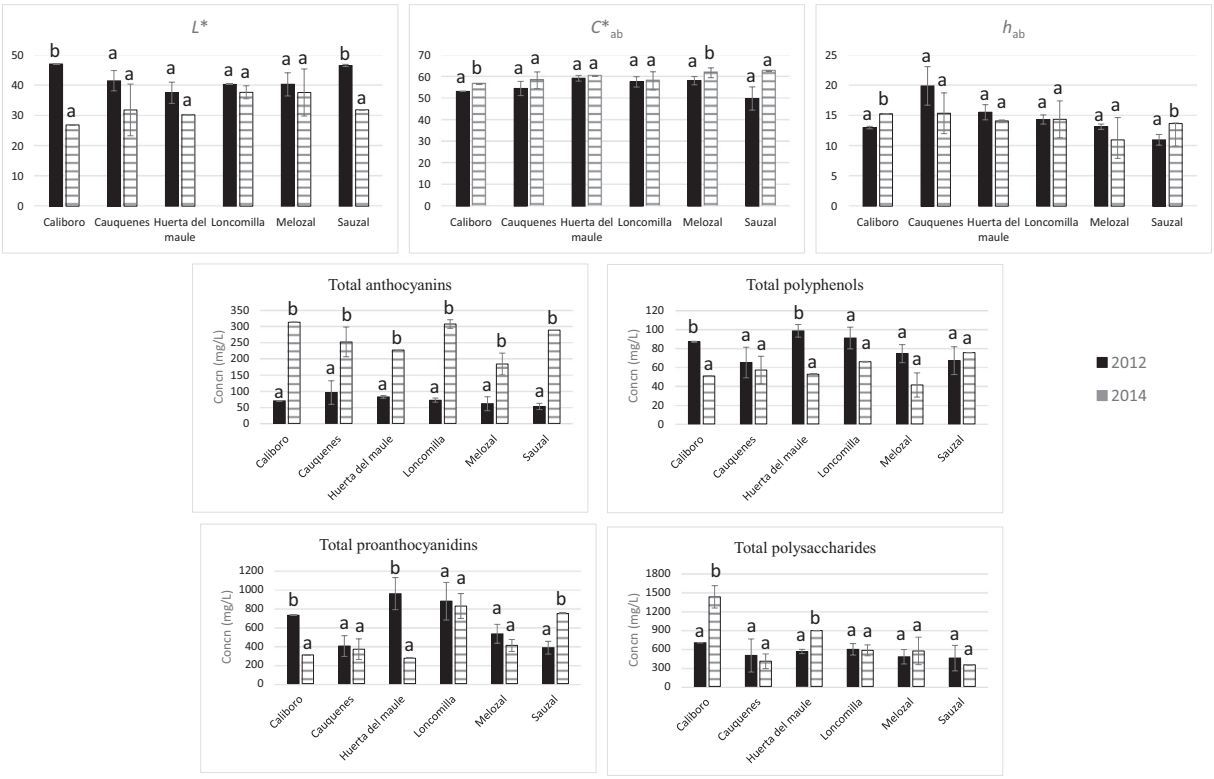

Fig. 2. Colour parameters (L^*, C^*_{ab} and h_{ab}) and concentration (mg/L) of total anthocyanins, polyphenols, proanthocyanidins and polysaccharides of Carignan red wines from different locations and production years of Maule valley. Different letters in each location showed significant differences ($p < 0.05$) by year according to Tukey test.

applied to the data of the parameters significantly affected by the location area. Three main significant principal components (PCs) were arisen according to Kaiser's criterion (eigenvalues > 1). With these factors, 78.4% of the total variance was explained. The first PC, PC1, which explained 32.5% of the total variance, mainly contains the two first fractions of polysaccharides (those with the higher average weigh) with a negative sign. In the case of PC2, which explained 26.1% of the total variance, *cis*-resveratrol-glucoside and procyanidin B3, both of them with a positive sign, are the main contributors.

Fig. 3 shows the samples to the plane defined by these two PCs, which explained 58.6% of the total variability. As can be seen, a separation by growing zone was achieved. Cv. Carignan wines derived from Caliboro area were clearly separated from the rest of locations, showing negative values for PC1. The other growing areas closer to the Andes Mountains (Melozal and Loncomilla) could be also differentiated. Both of them showed a positive sign in PC1, but Melozal and Loncomilla showed a negative and positive signs in PC2, respectively. However, the western areas (close to the Pacific Ocean) were intermingled. Caliboro area was characterized by providing wines with higher content of low-weight polysaccharides, whereas wines produced in Loncomilla and Melozal areas showed the highest and lowest content of *cis*-resveratrol-glucoside and procyanidin B3, respectively.

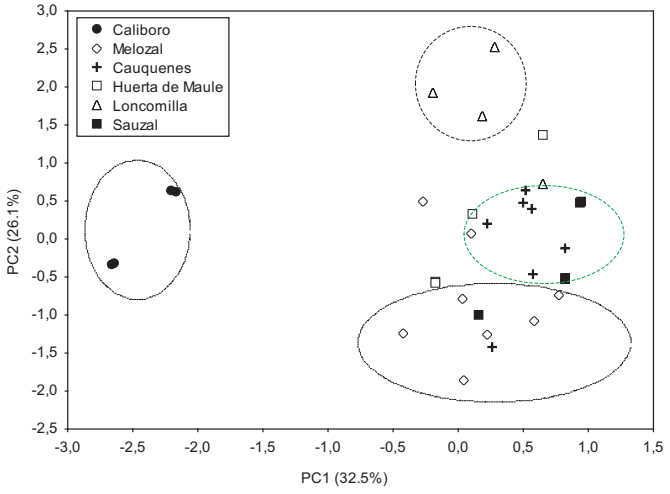

Fig. 3. Distribution of samples in the plane defined by the two first discriminate functions by location.

4. Conclusions

Several Chilean Carignan wines from different location areas and production years of the Maule Valley were studied for the first time on the basis of chemical composition and colour characteristics. The proximity to the ocean seemed to produce a unifying effect in chemical and colorimetric terms, while the closeness to The Andes Mountains produced Carignan red wines more different from each other, with high content of polysaccharides, *cis*-resveratrol-glucoside and procyanidin B3. In spite of the anthocyanin content significantly ($p < 0.05$) differed among harvesting year, it could be possible to determine the markers responsible for the different locations of Maule Valley. This could be an important advance forward valuing Carignan wines, above all from an economically poor region of Chile as Maule valley is.

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Conflict of interest

None.

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