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Phenolic and sensory profiles discriminate geographical indications for Malbec wines from different regions of Mendoza, Argentina

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ABSTRACT

Malbec wines from 27 parcels from the three most important winemaking regions of Mendoza, Argentina, were produced under standardized winemaking conditions, and analyzed for phenolic composition and by means of sensory descriptive analysis. Different methods of characterization and cluster analysis for each data matrix showed that some locations of Mendoza could be significantly separated from each other. The results of unsupervised statistical methods were compared using a test for similarities and divergences, also showing that different locations may be associated. The current report is the first one characterizing Malbec wines from the three major producing regions of Argentina using two different ways for locations classification. The effects of climate and geographical origin of Malbec grapes on the quality parameters of resulting wines were also evidenced. These results have enological and viticulture interest for the winemaking industry as the vineyard site selection for Malbec can considerably affect quality attributes.

1. Introduction

The Malbec variety is emblematic of Argentina's winemaking industry, being the most cultivated in the country with an area of 40,250 ha; where 86% of the production is located in the province of Mendoza (www.inv.gov.ar, 2016). Mendoza's climates and soils are quite variable within its territory, from cold Western areas close to the Andes mountains to warmer areas in the East. The prevailing climate is continental with annual precipitations between 170 and 350 mm, and an altitude gradient from 500 to 1600 m above sea level (m.a.s.l).

The environmental and human factors where grapes are grown, also known as "terroir", may impact the chemical composition and sensory attributes of wine, affecting its final quality. The French term terroir refers to the interaction of environmental (soil, climate, plant) and cultural (human) factors (Seguin, 1986). From the commercial point of view, the term terroir has become a communication tool to differentiate wine producing locations around the world (Hira & Swartz, 2014). Several studies showed that the origin of grapes is a factor that contributes to the consumer's decision in wine purchasing (Famularo, Bruwer, & Li, 2010), which are increasingly oriented towards high quality products. In this context, the identification of geographical origins exerts a doubtless commercial attraction, especially when typologies explicitly associated with high quality wines produced in different regions are among the criteria for pricing and guarantees of quality.

Phenolic compounds are relevant in red winemaking, being products of the secondary metabolism present in the berry skin vacuoles extracted during the winemaking process. They are classified as non-flavonoids and flavonoids. Phenolics have been proposed as chemical markers to establish cultivar authenticity and geographical origin of grapes (Makris, Kallithraka, & Mamalos, 2006). The non-flavonoids are phenolic acids and stilbenes, while the flavonoids are anthocyanins (red pigments), flavanols and flavonols (yellow pigments). Anthocyanins are the main compounds responsible for color in grapes and wines. Flavanols are found in different tissues of the vine (leaves and stems) and in the most solid parts of the berry (skin and seed), with polymers and oligomers known as pro-anthocyanidines or condensed tannins. These compounds are essential for wines sensory characteristics, such as color, astringency, and bitterness, and aging capacity; all of them are strongly related to quality perception of wines (Jaffré, Valentin, Dacremont, & Peyron, 2009).

Wine is a complex matrix containing volatile and non-volatile components that may interact with each other and these interactions can affect the perception of aromas, taste and mouthfeel. Therefore,

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sensory characteristics can be very different in wines with similar chemical characteristics. In this context, the importance of studying sensorial characteristics of wines and its association with their chemical composition is highlighted.

In previous studies it has been shown that the geographical location has a direct influence on the chemical and sensorial composition of wines, such as Cabernet Sauvignon from Australia (Robinson et al., 2012), China (Tao, Liu, & Li, 2009), France (Guinard & Cliff, 1987) and United States (Heymann & Noble, 1987), Chardonnay (Schlosser, Reynolds, King, & Cliff, 2005), and Sauvignon Blanc from New Zealand (Lund et al., 2009). Regarding Malbec, there are few studies that have evaluated sensory differences from diverse regions of Argentina and their association with chemical profiles. Goldner and Zamora (2007) performed the sensory characterization of Malbec wines from different large viticulture regions of Argentina, showing the main descriptors of each region. Other authors have compared regional differences in the sensory and volatile composition of Malbec wines, reporting larger separation between countries. (Heymann et al., 2015; King et al., 2014). This fact showed that wines of the same variety can be differentiated when the grapes are from different appellations of origin.

In terms of phenolic composition, previous studies performed with Malbec wines from Mendoza, reported total polyphenols ranged from 1900 to 3500 mg L^{-1}, total anthocyanins from 260 to 800 mg L^{-1}, and color intensity from 9 to 25, showing a wide variability for this cultivar (Fanzone, Peña-Neira, Jofre, Assof, & Zamora, 2010). However, the phenolic composition was assessed on wine samples from different wineries without standardized winemaking conditions, adding a source of variability to the results. Another study reported by Buscema and Boulton (2015), compared the phenolic profiles of 42 Malbec wines from Mendoza, Argentina and California, USA, made under the same winemaking conditions; however, the sensory profile of the wines was not evaluated.

As a result, there are few studies evaluating regional classifications through simultaneous phenolic and sensory profiling of wines. Moreover, the current study is the first one aimed at classifying Malbec wines from different regions by using such approach.

In the present study, Malbec wines coming from 27 parcels distributed in 13 sub-regions from 6 departments of the three most important regions of Mendoza (First Zone, East Zone and Uco Valley) were elaborated under standardized winemaking conditions and analyzed (sensory and phenolic profiles). The various parcels where grapes were obtained include zones with different environmental (climate and soil) conditions and represent the most important regions of Malbec wines production of Argentina in terms of quantity but specially on quality. A comparison of regions through measurement of anthocyanins and non-anthocyanins profiles complementary to sensory descriptive analysis is presented, aiming to compare the classification between different measurements ways by chemometric techniques.

2. Materials and methods

2.1. Selection of viticultural sites and winemaking procedure

Mendoza is politically divided into departments that are subdivided into districts, which are part of the geographical indications, like AVAs (American Viticultural Areas) in United States. This study includes grapes from the departments Luján de Cuyo, Maipú, Rivadavia, San Carlos, Tunuyán and Tupungato (Fig. S1), in which the major proportion of Malbec vineyards are located (82% of the total of Mendoza province). Twenty-seven parcels were selected in 13 districts belonging to the mentioned departments, where each parcel was selected to provide a representative sample of Malbec grapes in each department (see Table 1 for details). Each parcel has similar soil texture over its extension (that is, soil texture diverged amongst parcels of different districts), planted with Malbec vines obtained from mass selection, own-rooted, of more than 5 years old and managed with the same cultural practices (without leaf removal and cluster thinning).

The grapes were harvested between March, 14th and April, 21st, 2016. Table S1 show the chemical characteristics of must and wines. The winemaking was carried out at the Catena Institute of Wine pilot-winery using 800-L vessels in triplicate for each parcel. Four of twenty-seven parcels were vinified only in duplicate, due to the small size of the parcels. Grapes were de-stemmed, then crushed, and the resulting must was transferred to 800 L plastic vessels for fermentation. At the time of incubation, 50 mg L^{-1} of SO_2 (Enartis América Latina, Mendoza, Argentina) were added. After 24 h, 20 g L^{-1} Lavin EC-1118 (Lallemand Inc., Montréal, QC, Canada) active dry yeast were inoculated into the fermentation bins. One day after inoculation, 100 mg L^{-1} of $(NH_4)_3PO_4$ were added as the nitrogen source for the yeast. The fermentation temperature was 25 ± 2 °C, and was monitored every 12 h for density (°Brix) and temperature. After alcoholic fermentation and 10 days of maceration, 50 L of drained wine were removed. In no case was a wine press used. After 5 days of aging in the stainless steel tanks, 1 g L^{-1} of selected Lavin VP41 bacteria (Lallemand Inc., Montréal, QC, Canada) was inoculated to perform the malolactic fermentation, which was considered complete when the malic acid content was below 0.2 g L^{-1} as assessed by OenoFoss (FOSS Analytical A/S, Hillerød, Denmark). After malolactic fermentation finished, decantation was carried out to remove thick lees. Afterwards, SO_2 was added as $K_2S_2O_5$ (Laffort Oenologie, France) at a final concentration of 35 mg L^{-1} free SO_2. Wines were stored for three months in 50 L stainless steel tanks at 13–15 °C. Finally, 48 green-glass bottles (750 mL volume) of each replicate (three per parcel) were fractionated and stored at 15 °C until analysis. Tin screw caps were used instead of natural cork as stoppers in order to prevent potential trichloroanisole contamination or variable oxygen incorporation.

2.2. Chemical characteristics of must and wines

The initial parameters in must were measured on the same day of harvest and before crushing the grapes in the winery. Approximated 100 berries were hand-crushed and then the juice was used for the chemical parameters determination. The concentration of sugars (°Brix) was measured in the juice with a Pen-Harvest digital pen refractometer (Atago Co., Ltd., Tokyo, Japan). Total acidity, expressed as g L^{-1} of tartaric was measured by titrating samples of juice (10 mL) with 0.1 N NaOH to a final pH value of 8.2. The pH value was measured in the juice using a portable pH meter.

Standard wine parameters including alcohol, total acidity, pH, volatile acidity and reducing sugar were analyzed according to FTIR method using WineScan (FOSS, Hillerød, Denmark). Levels of free SO_2 were measured pre-bottling and determined using TitraLab® automatic tritrator (Hach, Germany), that is an automatization of Ripper method. The absorbance at 280, 420 and 520 nm were determined one month after of bottling using a UV–VIS spectrophotometer Cary-50 (Varian Inc., Mulgrave, Australia). Wine color intensity and hue were calculated by adding the absorbance at 420 and 520 nm and by calculating the ratio of absorbance at 420 and 520 nm, respectively using quartz cuvettes with 1 mm pathlength. Total polyphenols in wine were estimated by the Total Polyphenol Index (TPI) using the absorbance at 280 nm using quartz cuvettes with 1 cm pathlength.

2.3. Climate data

The altitude (m.a.s.l.), growing degree day (GDD) and precipitation (rain) data for each wine region were obtained or calculated, respectively, to compare with the wine composition. Growing degree days were calculated using daily averages (in Celsius degrees) for the given periods and a base of 10 °C (Jones, Duff, Hall, & Myers, 2010), while precipitation was calculated as the sum of the daily rain (in mm) for the given periods. Data were obtained from the database of the Catena Institute of Wine and the Department of Agriculture and Climate

Table 1
Mendoza Malbec vineyard site information.

Department	n	Districts	Altitude (m above sea level)	Growing degree days*	Precipitation (mm)*
Luján de Cuyo	5	Agrelo, Anchoris and Ugarteche	959–1051	1577–1726	737–729
Maipú	2	Lunlunta	928	1778	494
Rivadavia	3	La Central and La Libertad	635–671	1833–1905	519–521
San Carlos	7	Altamira, El Cepillo and San Carlos	961–1100	1508–1611	518–719
Tunuyán	3	Chacayes and Los Arboles	1006–1135	1586–1601	417–517
Tupungato	7	Gualtallary and San José	1240–1510	1172–1633	466–737

* Growing degree days (GDD) and Precipitation (mm) calculated from 01-Oct-2015 to date of harvest.

Contingencies of Mendoza from October 2015 to date of harvest for each parcel. Climate data for some viticulture sites were not available; however, at least one station within all six Mendoza wine departments had accessible information that was generalized for all viticulture sites within that region.

2.4. Standards, solvents and sorbents

Standards of gallic acid (99%), 3-hydroxytyrosol (≥99.5%), (−)-gallocatechin (≥98%), caftaric acid (≥97%), (+)-catechin (≥99%), (−)-epicatechin (≥95%), caffeic acid (99%), polydatin (≥95%), syringic acid (≥95%), coumaric acid (99%), ferulic acid (≥99%), *trans*-resveratrol (≥99%), quercetin hydrate (95%), cinnamic acid (99%), quercetin 3-β-D-glucoside (≥90%), kaempferol-3-glucoside (≥99%) and astilbin (≥99%) were purchased from Sigma–Aldrich (St Louis, MO, USA). The standard of 2-(4-hydroxyphenyl) ethanol (tyrosol) (≥99.5%) was obtained from Fluka (Buchs, Switzerland). Stock solutions of standards were prepared in methanol at concentration levels of 1000 μg mL^{-1}. Further dilutions were prepared monthly in methanol and stored in dark-glass bottles at −20° C. Calibration standards used during optimization of high performance liquid chromatography with diode array detection (HPLC-DAD) conditions were dissolved in ultra-pure water (0.1% formic acid; FA)/Acetonitrile (MeCN) (95:5). HPLC-grade MeCN and FA were acquired from Mallinckrodt Baker Inc. (Pillispsburg, NJ, USA). Analytical grade sorbents (50 μm particle size) for dispersive solid phase extraction (d-SPE), including primary-secondary amine (PSA) and octadecylsilane (C_{18}) were both obtained from Waters (Milford, MA, USA). Reagent grade NaCl, anhydrous $MgSO_4$ and anhydrous $CaCl_2$ were purchased from Sigma–Aldrich. Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA).

2.5. HPLC-DAD analysis

Phenolic compounds were determined using a HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany). The HPLC instrument was a Dionex Ultimate 3000 consisting of vacuum degasser unit, autosampler, quaternary pump and chromatographic oven. The detector was a Dionex DAD-3000 (RS) model with an analytical flow cell operated with a data collection rate of 5 Hz, a band width of 4 nm and a response time of 1.000 s. The working wavelengths for quantification of the different families of phenolic compounds were 254 nm, 280 nm, 320 nm and 370 nm for non-anthocyanins (low molecular weight phenolic compounds, LMW-PPs) and 520 nm for anthocyanins. The Chromeleon 7.1 software was used to control all the acquisition parameters of the HPLC-DAD system and also to process the obtained data.

2.5.1. Anthocyanins

HPLC analysis was adapted from Fontana, Antoniolli, Fernández, and Bottini (2017) with minor modifications. Five hundred μL of wine were withdrawn from the bottle and evaporated to dryness and dissolved with 500 μL of initial mobile phase. Separation of different anthocyanins was carried out in a reversed-phase Kinetex C_{18} column (3.0 mm x 100 mm, 2.6 μm) Phenomenex (Torrance, CA, USA). The mobile phase consisted of ultrapure H_2O:FA:MeCN (87:10:3, v/v/v; eluent A) and ultrapure H_2O:FA:MeCN (40:10:50, v/v/v; eluent B) using the following gradient: 0 min, 10% B; 0–6 min, 25% B; 6–10 min, 31% B; 10–11 min, 40% B; 11–14 min, 50% B; 14–15 min, 100% B; 15–17 min, 10% B; 17–21 min, 10% B. The mobile phase flow was 1 mL min^{-1}, column temperature 25 °C and injection volume 5 μL. Quantifications were carried out by area measurements at 520 nm and the anthocyanin content was expressed as malvidin-3-glucoside equivalents using an external standard calibration curve (1–250 mg L^{-1}, R^2 = 0.998). The identity of anthocyanin compounds detected with HPLC-DAD was confirmed by comparison with the elution profile and identification of analytes achieved in our previous work using UPLC-MS (Antoniolli, Fontana, Piccoli, & Bottini, 2015).

2.5.2. Non-anthocyanins

Sample preparation conditions was adapted from Fontana et al. (2017) with minor modifications. In brief, 5 mL of wine was placed into a 15 mL PTFE centrifuge tube and acidified with FA (1%) by adding 57 μL of 88% w/v solution. Then, 2.5 mL MeCN were added and the tube was vigorously hand-shaken for 30 s to ensure adequate homogenization of sample material with the extraction solvent. For phase separation, 1.5 g of NaCl and 4 g of $MgSO_4$ were added; the tubes were shaken for 1 min and centrifuged for 10 min at 3000 rpm (900 rcf). Thereafter, 1 mL aliquot of the upper MeCN phase was transferred to a 2 mL dispersive solid-phase extraction (d-SPE) clean-up tube containing 150 mg $CaCl_2$, 50 mg PSA and 50 mg C_{18}. The mixture was then vortexed 30 s and centrifuged 2 min at 12,000 rpm (8400 rcf). Finally, an aliquot of 500 μL extract was evaporated to dryness under gentle N_2 stream, the residue reconstituted with 500 μL of initial mobile phase and analyzed by HPLC-DAD. The injection volume was 5 μL. HPLC separations/quantifications were carried out in reversed-phase Kinetex C_{18} column (3.0 mm × 100 mm, 2.6 mm) Phenomenex (Torrance, CA, USA). Ultrapure H_2O with 0.1% FA (A) and MeCN (B) were used as mobile phases. Analytes were separated using the following gradient: 0–2.7 min, 5% B; 2.7–11 min, 30% B; 11–14 min, 95% B; 14–15.5 min, 95% B; 15.5–17 min, 5% B: 17–20, 5% B. The mobile phase flow was 0.8 mL min^{-1}. The column temperature was 35 °C. Non-anthocyanins present in samples were quantified by using an external calibration with pure authentic standards to achieve unambiguous identification of analytes. Linear ranges between 0.5 and 40 mg L^{-1} with coefficient of determination (r^2) higher than 0.998 were obtained for all the studied non-anthocyanin compounds.

2.6. Descriptive sensory analysis

The sensory characteristics of wines were analyzed 6 months after bottling. All replicates of each parcel were smelled and tasted by experienced winemakers of Catena Zapata Winery to determine if any fermentation possessed major faults or off-flavors. The tasting showed that all wines did not have faults, so one replicate was chosen at random for the panel descriptive sensory analysis (DA).

All wines were characterized by a descriptive sensory analysis (Lawless & Heymann, 2010) by a trained panel of 8 volunteers (two

Table 2
Composition of sensory reference standards used to define aroma and taste attributes.

Attribute/Description	Composition
Aroma	
Plum	10 g fresh plums + 1 tsp plum jam (Product of Argentina, Distributed by Carrefour Argentina)
Chocolate	2 g shaved dark chocolate. Aguila, 60% cacao (Product of Argentina)
Earthy	4 g potting soil in 5 mL of water
Red fruit	1 g fresh strawberries + 5 g fresh cherries in small pieces (Product of Argentina, Distributed by Carrefour Argentina)
Roses	1 mL rose water Laborit (Product of Argentina)
Raisins	3 g raisins (Product of Argentina, Distributed by Carrefour Argentina)
Blueberries	6 sliced blueberries (Product of Argentina, Distributed by Carrefour Argentina)
Figs	5 g dried figs (Product of Argentina, Distributed by Carrefour Argentina)
Black pepper	0,1 g ground black pepper Pepe Nero in 10 mL hot water. (Product of Italy)
Herbaceous	1 mL asparagus cooking water + 2 g fresh chopped green peppers (Product of Argentina, Distributed by Carrefour Argentina)
Tobacco	2.3 g tobacco Marlboro Cigarette (Product of Argentina, Distributed by Massalin Particulares SRL, Bs. As)
Taste/Mouthfeel	
Hot (M)	15% V/V vodka Absolut (Product of Sweden)
Astringent (M)	468 mg alum in 500 mL water (aluminium ammonium sulfate) Anedra Research AG S.A (Product of Argentina)
Bitter (T)	0,1 g caffeine in 500 mL water Merck (Darmstadt, Germany)
Sweet (T)	2 g sucrose in 1000 mL water Chango Pure Cane Sugar granulated white (Product of Salta, Argentina).
Acid (T)	0.5 g tartaric acid in 500 mL water/(L-(+)-tataric acid) Derivados vinificos (Mendoza, Argentina)

men and six women) between 25 and 66 years old. Panelists were selected based on availability, motivation and previous experience on descriptive sensory panels.

The training consisted of 18 h for introduction in wine sensory analysis, attribute generation, discussion, consensus in reference standards and practice in scale use. In the period of training sessions, the panelist used the same sample of the project to create, refine and gain consensus on the attributes. The panelists evaluated 27 parcels of Malbec wines in duplicate over 11 sessions, equating to 4–5 wines per session presented in randomized block design. Prior to each evaluation session, the reference standards were available if the panelist need to refresh the memory.

The panel rated eleven aromas, three tastes and two mouthfeel descriptor. Table 2 shows details of the attributes and reference standards used in DA.

Each session was conducted in individual booths. A 30 mL sample of each wine was presented at room temperature in black tasting glasses (ISO 3591-1977) covered with plastic lids, which were coded with a three-digit random number. For each of the descriptors, panelists were directed to rate the intensity of each wine on a 15 cm unstructured line scale anchored with the terms “low” and “high” at either end of the scale. Between each sample, panelists measured with a rule each descriptor with the idea that every panelist take a relaxing period and refresh the palate with water and unsalted crackers.

2.7. Data analysis

Data were analyzed using software platform R 3.2.2 (R Foundation for Statistical Computing, 2016). The chemical data were analyzed using one-way analyses of variance (ANOVAs) with main effects of region. Canonical variate analysis (CVA) was performed for combined phenolic compounds (anthocyanins and non-anthocyanins) with 95% confidence ellipses, and was used to illustrate the relationships among different regions and departments of Mendoza. For the sensory data, MANOVA analysis was perform using three-way MANOVA (panelists, locations, rep and all two-way interactions) on all attributes. Three-way ANOVA with two-way interactions to analyze the descriptors attributes of without the missing values was used. The missing values were imputed with “missMDA” package (Josse & Husson, 2016). Principal component analysis (PCA) was applied on the wine sensory data, including the panelists. Confidence ellipses indicating 95% confidence intervals were based on the multivariate distribution of the Hotelling’s test for $p < 0.05$ and constructed using SensoMineR panellipse function on R (Pagés & Husson, 2005). A tanglegram was generated to illustrate similarities and divergences evaluating the associations and putative co-divergence between the two dendrograms, using the Euclidean distance and ward’s method for hierarchical cluster analysis on normalized data (Galili, 2015). The dendograms (sensory profile and chemical data) are drawn opposite each other, using auxiliary lines to connect samples and establish a network of interactions.

3. Results and discussion

3.1. Anthocyanins and non-anthocyanins

Table 3 shows the concentration of individual anthocyanin’s. As can

Table 3
Anthocyanin’s quantified [Mean (mg L^{-1}) ± SD] in Malbec wines from different locations of Mendoza.

Compound	Tupungato	Maipú	Tunuyán	San Carlos	Luján de Cuyo	Rivadavia
Delphinidin 3-O-glucoside	51.7 ± 16.9 a	23.0 ± 7.7 b	17.9 ± 9.9 b	19.9 ± 11.2 b	9.8 ± 7.4 b	11.7 ± 1.7 b
Cyanidin 3-O-glucoside	5.5 ± 2.1 a	4.4 ± 1.3 ab	1.4 ± 0.7 c	2.5 ± 1.7 bc	1.7 ± 0.3 c	1.4 ± 0.2 c
Petunudin 3-O-glucoside	65.9 ± 16.5 a	41.9 ± 7.2 b	38.5 ± 12.9 b	34.2 ± 15.3 b	23.9 ± 11.8 b	4.1 ± 3.1 c
Peonidin 3-O-glucoside	19.9 ± 10.0 a	18.0 ± 6.9 ab	11.5 ± 6.7 ab	10.6 ± 6.7 b	9.5 ± 6.2 b	13.4 ± 5.0 ab
Malvidin 3-O-glucoside	358.4 ± 59.1 a	360.4 ± 5.8 a	351.4 ± 38.7 a	325.6 ± 47.6 ab	277.6 ± 64.2 b	173.9 ± 30.4 c
Delphinidin 3-O-acetylglucoside	11.0 ± 3.4 a	9.0 ± 3.3 ab	7.3 ± 1.7 bc	5.5 ± 2.1 bc	6.6 ± 3.4 bc	3.7 ± 1.5 c
Petunidin 3-O-acetylglucoside	13.3 ± 2.6 a	8.1 ± 1.2 b	9.2 ± 2.8 b	8.3 ± 2.9 b	6.3 ± 3.7 b	1.3 ± 0.6 c
Peonidin 3-O-acetylglucoside	8.2 ± 1.8 a	2.9 ± 0.6 c	5.4 ± 1.7 b	3.0 ± 1.4 c	2.2 ± 1.8 c	0.2 ± 0.2 d
Malvidin 3-O-acetylglucoside	26.8 ± 7.8 ab	39.6 ± 3.3 a	36.4 ± 9.3 a	32.0 ± 12.1 ab	23.0 ± 13.1 b	10.2 ± 5.4 c
Petunidin 3-O-p-coumaroylglucoside	10.2 ± 3.5 a	4.3 ± 1.0 b	8.7 ± 2.7 a	4.5 ± 1.6 b	2.9 ± 2.4 bc	0.4 ± 0.3 c
Peonidin 3-O-p-coumaroylglucoside	9.3 ± 2.5 a	8.3 ± 2.0 ab	8.8 ± 3.1 a	5.4 ± 2.2 bc	3.4 ± 2.1 cd	1.5 ± 0.7 d
Malvidin 3-O-p-coumaroylglucoside	22.9 ± 8.6 a	16.2 ± 2.6 abc	18.5 ± 6.5 ab	9.8 ± 5.3 c	10.6 ± 7.1 bc	8.0 ± 5.9 c

Different letters within the same row indicate significant differences ($p < 0.05$) according to a Tukey HSD test.

be observed, Tupungato showed the maximum levels of anthocyanins, while Rivadavia showed the minimum levels amongst all departments. These results are comparable with those found for Malbec wines from Mendoza and California (Buscema & Boulton, 2015; Fanzone et al., 2010). The predominant anthocyanin was malvidin-3-O-glucoside, with concentrations ranged between 55% and 82% and an average of 68%, followed by petunidin-3-O-glucoside with an average of 7.3% for the majority of locations. These profiles of anthocyanins were similar to those of different grape cultivars around the world, as Grenache, Syrah, Carignan Noir, Cencibel, Mourvedre, Counoise and Alicante Bouchet (Ky, Lorrain, Kolbas, Crozier, & Teissedre, 2014). As has been reported previously (Alonso, Berli, Fontana, Piccoli, & Bottini, 2016; Berli, Fanzone, Piccoli, & Bottini, 2011; Gil et al., 2013; Yamane, Seok, Goto-Yamamoto, Koshita, & Kobayashi, 2006), the relative concentrations of anthocyanins are affected by environmental factors such as temperature, exposure to light and water availability; so it may be the case of Mendoza where climatic factors show great variability amongst the regions studied (Table 1). Several studies revealed that high temperatures decreased total anthocyanin content in Malbec (~28–41% reduction) and Cabernet Sauvignon (~50%) berries (de Rosas et al., 2017; Mori, Goto-Yamamoto, Kitayama, & Hashizume, 2007). The results of the present study (see Table 1) show that warm areas such as Rivadavia (1833–1903 GDD, 635–671 m.a.s.l) had a decrease of approximately ~53% in total anthocyanins as compared to cooler zones with higher altitudes like Tupungato (1171–1633 GDD, 1240–1510 m.a.s.l.). Additionally, the altitude may also increase the wine phenolic composition as a consequence of higher levels of UV-B radiation (Alonso et al., 2016; Berli et al., 2011). Besides of no changes in the profiles of anthocyanins based on type of derivative (non acylated, acylated and coumarylated) and by the type of anthocyanidin were observed between departments, some differential changes in compounds levels were observed for Rivadavia wines. In fact, the relationship between delphinidin 3-O-glucoside and petunidin 3-O-glucoside in Rivadavia is different to the other departments. The only department that had more concentration of delphinidin 3-O-glucoside and petunidin 3-O-glucoside was Rivadavia, showing a significant change in anthocyanin profiles for this department.

Among the non-anthocyanins (Table 4), the compounds with higher concentrations were caffeic acid, tyrosol, (+)-catechin, (−)-epicatechin and *p*-coumaric acid, with average concentrations of 23.4 mg L^{-1}, 32.8 mg L^{-1}, 25 mg L^{-1}, 22.1 mg L^{-1} and 19.8 mg L^{-1}, respectively. Wines from Rivadavia department (the Eastern warmer region) had higher amounts of caffeic acid, while Tupungato and Tunuyán (cooler zones on the Andes Piedmont) had higher amounts of tyrosol and *trans*-revesratrol. The Quercetin compound was higher in departments at a high altitude such as Tupungato, while considerable lower concentrations in Rivadavia were observed. This may be due to the difference in altitude, where at higher altitudes the sun exposure (as by consequence the UV-B irradiance) is higher and may increase the concentration of quercetin (Price, Breen, Valladao, & Watson, 1995).

The *trans*-resveratrol showed significant differences between departments, where higher altitude departments and colder areas have higher concentrations. The increase in the concentration of anthocyanins and resveratrol could be due to the higher UV-B exposure of the plants located at higher altitudes (Berli et al., 2008). Due to the fact that these wines were elaborated using the same winemaking, these differences in content can be clearly attributed to the environment/location differences.

3.2. Combined statistical analysis of phenolic compounds

Twenty-seven out of the thirty variables evaluated were different among the Malbec wines from the 6 departments of Mendoza using a p-value = 0.05. Regarding non-anthocyanins, three compounds had a p-value greater than 0.05, which are (+)-catechin, caftaric acid and quercetin-3-glucoside with a p-value of 0.36, 0.07 and 0.09, respectively.

A one-way ANOVA (MANOVA) test indicated that at least one of the 6 regions was significantly different from the others when compared on the phenolic variables ($p < 0.0001$). Fig. 1 shows a canonical variate analysis (CVA) using all the phenolics variables with significantly difference. The departments Rivadavia, Maipú, Tupungato and Tunuyán were different. The other departments formed one group, where the pair Luján de Cuyo-San Carlos were closely associated. The variables with the highest loading on the CV1 axis were tyrosol (0.69), petunidin-3-O-glucoside (0.62), petunidin-3-O-acetylglucoside (0.68), peonidin-3-O-acetylglucoside (0.66), petunidin-3-O-p-coumaroylglucoside (0.64) and malvidin-3-O-glucoside (0.62) on left side associated with the departments of Luján de Cuyo, San Carlos, Tunuyán and Tupungato; and gallic acid (−0.68), syringic acid (−0.87), caffeic acid (−0.85), p-coumaric acid (−0.85) and ferulic acid (−0.68) on the right side associated with the department of Rivadavia; while on the CV2 axis delphinidin-3-O-glucoside (−0.53), peonidin-3-O-acetylglucoside (−0.47), astilbin (−0.51), malvidin-3-O-acetylglucoside (0.34) and (−)-epicatechin (0.39) had the highest loads (Fig. S2).

Chemometrics has been used effectively in wine differentiation and classification by geographic origin (Buscema & Boulton, 2015; Fanzone et al., 2010; Kallithraka, et al., 2001; Rodríguez-Delgado, González-

Table 4
Non-anthocyanins quantified [Mean (mg L^{-1}) ± SD] in Malbec wines from different locations of Mendoza.

Compound	Tupungato	Maipú	Tunuyán	San Carlos	Luján de Cuyo	Rivadavia
Gallic acid	20.0 ± 7.8 bc	28.2 ± 4.2 ab	15.5 ± 1.5 c	20.6 ± 2.6 bc	25.3 ± 6.3 b	37.8 ± 11.2 a
OH-tyrosol	1.7 ± 0.6 c	1.6 ± 0.4 c	3.1 ± 1.2 ab	2.8 ± 0.6 b	3.6 ± 1.4 ab	4.0 ± 0.7 a
Tyrosol	37.8 ± 4.4 a	29.1 ± 4.1 a	40.2 ± 2.0 a	35.1 ± 7.2 a	30.9 ± 11.9 a	14.8 ± 9.5 b
Procyanidin B1	22.2 ± 9.1 a	9.1 ± 9.3 b	17.8 ± 8.5 ab	15.5 ± 10.1 ab	11.6 ± 10.1 ab	11.6 ± 12.8 ab
(+)-Catechin	28.6 ± 13.6 a	26.2 ± 7.8 a	25.3 ± 5.9 a	26.5 ± 4.8 a	24.6 ± 8.9 a	20.2 ± 8.6 a
Syringic acid	9.2 ± 0.1 c	13.4 ± 1.8 b	12.2 ± 1.3 b	12.6 ± 1.7 b	14.4 ± 3.7 b	27.8 ± 2.2 a
(−)-Epicatechin	17.2 ± 6.6 b	24.9 ± 7.3 ab	15.3 ± 1.8 b	23.2 ± 4.9 ab	29.7 ± 12.2 a	23.0 ± 3.5 ab
Astilbin	8.8 ± 1.3 a	4.3 ± 2.4 b	7.7 ± 1.8 a	4.7 ± 1.5 b	4.2 ± 1.7 b	3.6 ± 1.3 b
Caftaric acid	11.3 ± 5.3 a	6.9 ± 2.9 a	9.4 ± 5.0 a	12.4 ± 8.9 a	10.4 ± 9.9 a	4.1 ± 1.6 a
Caffeic acid	3.1 ± 1.8 c	10.0 ± 4.0 b	4.1 ± 2.3 c	9.3 ± 4.4 b	11.8 ± 4.7 b	29.9 ± 4.7 a
p-coumaric acid	5.9 ± 2.4 d	17.7 ± 4.5 bc	15.0 ± 6.9 c	22.4 ± 9.5 bc	26.2 ± 8.6 b	40.6 ± 8.1 a
Ferulic acid	0.8 ± 0.6 b	1.0 ± 0.1 b	1.0 ± 0.1 b	0.9 ± 0.4 b	1.1 ± 0.4 b	2.5 ± 0.4 a
Polydatin	4.9 ± 2.2 a	3.9 ± 1.8 ab	1.8 ± 2.5 bc	0.5 ± 0.3 c	1.4 ± 2.3 bc	1.1 ± 1.0 bc
Trans-resveratrol	7.7 ± 0.2 a	4.4 ± 1.4 bc	7.3 ± 2.0 ab	5.7 ± 1.6 bc	4.9 ± 1.8 bc	4.1 ± 1.15 c
Quercetin-3-glucoside	0.6 ± 0.2 a	0.8 ± 0.1 a	0.8 ± 0.2 a	0.8 ± 0.3 a	0.6 ± 0.2 a	0.6 ± 0.1 a
Kaempferol-3-Glucoside	0.9 ± 0.1 a	0.6 ± 0.1 c	0.9 ± 0.1 ab	0.6 ± 0.2 c	0.7 ± 0.2 bc	0.6 ± 0.1 c
Quercetin	11.7 ± 2.2 a	9.9 ± 4.3 ab	12.5 ± 1.5 a	7.2 ± 3.3 b	8.6 ± 5.7 ab	6.8 ± 1.3 b
(−)-Gallocatechin	14.9 ± 3.7 ab	14.6 ± 4.0 ab	11.5 ± 1.8 b	12.7 ± 2.4 b	11.9 ± 3.9 b	18.3 ± 3.3 a

Different letters within the same row indicate significant differences ($p < 0.05$) according to a Tukey HSD test.

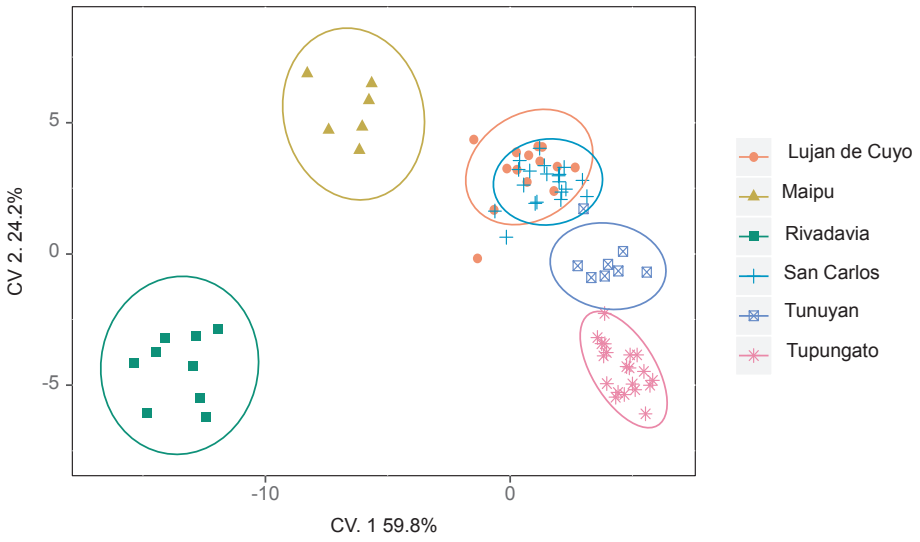

Fig. 1. Canonical variate analysis (CVA) plot of combined phenolics measured in individual fermentation replicates of Malbec wines from 6 departments in Mendoza, Argentina. Ellipses that overlap are not significantly different from one another at the 95% level.

Hernández, Conde-González, & Pérez-Trujillo, 2002; Saurina, 2010). In this study, the application of CVA to phenolic profile of Malbec shows a good discrimination among wines from different regions. That is, Rivadavia which is located in the East with low altitude and warmer temperatures was separated from Tupungato and Tunuyán having higher altitudes and cooler temperatures (see Table 1 for climate information). This finding might suggest that Tupungato and Tunuyán wines would exhibit a more intense color, as anthocyanins are responsible for this property. Additionally, wines with higher concentrations of phenolic compounds may be associated with wines with greater antioxidant capacity and greater storage potential both associated with higher quality and consumer acceptability (Jaffré et al., 2009).

A previous report for Malbec from four Mendoza regions showed similar results to this study, achieving good discrimination between departments, although the objective was to discriminate wines from Mendoza and California (Buscema & Boulton, 2015). Another study with wines from four Canary Islands (Spain) had a good differentiation among wines according to their production areas, applying linear discriminant analysis (LDA) to the wines phenolic compositions (Rodríguez-Delgado et al., 2002). Wines from three provinces of Argentina were correctly classified by variety and origin using phenolic profile and multi-element composition on wines (Di Paola-Naranjo et al., 2011).

3.3. Sensory descriptive analysis

A three-way ANOVA of the sensory results showed that the red fruit, raisins, black pepper, herbaceous, tobacco, hot and sweet had a significant effect ($p \leq 0.05$) and were used for principal component analysis (PCA) with confidence ellipses based on multivariate distribution of hotelling's test for $p \leq 0.05$ indicating 95% confidence intervals (Fig. 2). Table 5 show the results of descriptive sensory analysis for aroma, taste and mouthfeel attributes.

The first two dimensions accounted for 94% of the cumulative variance. The first dimension accounted for 55% of the variance and primarily separated Rivadavia department, which was positively correlated with the herbaceous, tobacco, black pepper and sweet. The second dimension accounted for 39% of the variance and characterized the difference in departments by the red fruit, hot and raisins notes, which were positive associated with Maipú (Fig. S3). The first two dimensions show that San Carlos, Luján de Cuyo and Tunuyán are not different from each other, while Tupungato overlap with Luján de Cuyo. Maipú and Rivadavia are clearly different from the other departments.

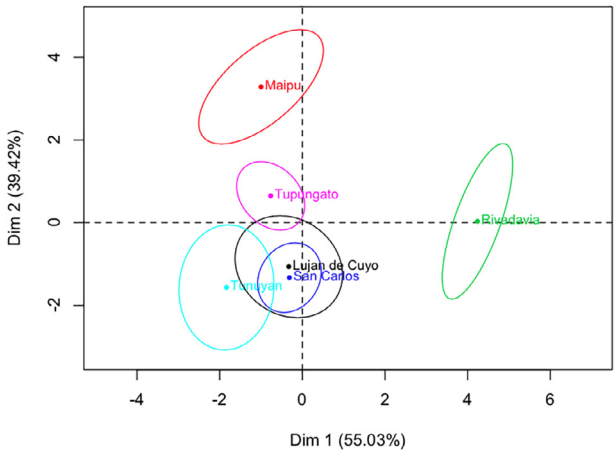

Fig. 2. Principal components analysis with descriptive sensory data of Malbec wines evaluated by a trained panel (n = 8): confidence ellipses based on multivariate distribution of Hotelling's test for $p < 0.05$ indicating 95% confidence intervals.

In a previous study published by King et al. (2014), it shows similar results were obtained with respect to the department of Maipú, where the wines had high values of red fruit. Other studies showed that Luján de Cuyo had high values of plum and floral aroma, while wines from the Uco Valley (San Carlos, Tunuyán and Tupungato) had high values of red fruit and astringency (Aruani, Quini, Ortiz, Videla, & Murgo, 2002). In our study did not find significant differences in astringency between departments. Goldner and Zamora (2007) showed that wines from Luján de Cuyo and Maipú were associated with pungency, sweet pepper, bitter and astringency, while wines from the Uco Valley were associated with cooked fruit, raisin, floral and sweetness.

The results obtained through the analysis of phenols and sensory profile show that the departments of San Carlos and Luján de Cuyo are closely associated with each other. The departments of Rivadavia and Maipú, in both analysis, showed that they are different from the rest (Figs. 1 and 2).

In terms of distance between the evaluated localities, San Carlos, Tunuyán and Tupungato constitute the Uco Valley. The Uco Valley is characterized by having vineyards at higher altitudes and cooler areas.

Table 5
Descriptive sensory analysis results rated by trained panelists for Malbec wines from different locations of Mendoza, Argentina for aroma attributes and taste and mouthfeel attributes.

Departments	Aroma attributes										
	Plum	Chocolate	Earthy	Red Fruit[a]	Roses	Raisins[a]	Blueberries	Figs	Black pepper[a]	Herbaceous[a]	Tobacco[a]
Luján de Cuyo	4.09 ± 2.2	2.14 ± 1.8	0.8 ± 1.47	2.59 ± 2.34	1.98 ± 1.98	1.85 ± 1.82	1.64 ± 1.65	2.62 ± 2.18	1.99 ± 2.12	1.67 ± 1.95	1.09 ± 1.49
Maipú	4.59 ± 2.47	1.62 ± 1.8	1 ± 2.26	3.43 ± 2.51	1.16 ± 1.66	3.28 ± 2.21	1.19 ± 1.58	2.8 ± 2.6	1.3 ± 1.66	1.39 ± 1.83	0.92 ± 1.37
Rivadavia	4.47 ± 2.42	1.98 ± 1.69	1.2 ± 1.56	2.94 ± 2.64	1.37 ± 1.62	2.8 ± 2.54	1.48 ± 1.74	3.31 ± 2.52	2.87 ± 2.19	2.48 ± 2.35	1.94 ± 1.77
San Carlos	4.1 ± 2.27	1.83 ± 1.53	0.76 ± 1.25	2.48 ± 2.29	1.16 ± 1.41	1.77 ± 1.67	1.07 ± 1.38	2.35 ± 1.95	2.46 ± 2.45	1.57 ± 1.63	0.97 ± 1.32
Tunuyán	3.77 ± 2.17	2.43 ± 1.66	1 ± 1.41	2.2 ± 2.34	1.26 ± 1.92	1.7 ± 1.84	1.02 ± 1.34	2.96 ± 2.17	2.27 ± 2.07	1.09 ± 1.44	0.83 ± 1.16
Tupungato	3.87 ± 2.22	1.79 ± 1.72	0.97 ± 1.53	3.03 ± 2.48	1.31 ± 1.67	2.16 ± 1.97	1.24 ± 1.48	2.56 ± 1.97	1.94 ± 1.97	1.54 ± 1.93	0.88 ± 1.22

Departments	Taste and mouthfeel attributes				
	Hot[a]	Sweet[a]	Acid	Astringency	Bitter
Luján de Cuyo	3.42 ± 2.55	1.96 ± 1.94	6.62 ± 3.32	5.31 ± 3.28	2.97 ± 2.07
Maipú	4.66 ± 3.68	1.82 ± 1.78	7.04 ± 3.23	5.14 ± 3.38	2.87 ± 2.42
Rivadavia	3.39 ± 2.84	2.76 ± 2.19	7.22 ± 3.13	6.37 ± 3.41	3.18 ± 2.28
San Carlos	3.83 ± 2.56	1.91 ± 1.89	6.95 ± 3.23	5.49 ± 2.96	2.88 ± 2.11
Tunuyán	3.66 ± 2.49	1.62 ± 1.77	7.01 ± 2.96	5.35 ± 2.63	2.85 ± 2.16
Tupungato	4.13 ± 3.2	1.76 ± 1.85	6.72 ± 3.14	5.74 ± 3.07	3.14 ± 2.3

[a] Indicate significant differences ($p < 0.05$).

The departments of Luján de Cuyo and Maipú form the region called "First Zone", being located near the Mendoza River, approximately 80 km from Uco Valley. Rivadavia is located in the eastern part of Mendoza, at an altitude of 650 m.a.s.l. and is classified as a warm zone (Table 1Fig. S1). It can be clearly seen that Rivadavia, in the case of chemical and sensorial profiles, was always separated from the rest of the departments since the environmental conditions (altitude and climate) are very different and have different terroir characteristics to places located near the Andes mountains. In the first zone and Uco Valley, a greater heterogeneity of climatic conditions (Tonietto & Zanus, 2007) and edaphological characteristics (Mehl, 2011) can be found. Actual legislation in Argentina define each wine geographical areas by historic or political limits. However, the results presented here suggest that the actual geographical indications in Mendoza, may not be the proper way to classify wines since a great influence of environment characteristics in each location was observed.

3.4. Tanglegram

To study the relationships among departments, a tanglegram (that is, the degree of intricacy amongst departments) was constructed using sensory and phenolic profiles (Fig. S4). The tanglegram is presented as two rooted dataset trees that are linked according to profile trends within each sample. The entanglement value (0.34) indicates that sensory and phenolic trees are partially stackable, and in general the chemical profile showed dissimilar from the sensory one. In the tree of the sensory profile we can clearly see the formation of three groups, where the first includes San Carlos, Luján de Cuyo, Tunuyán and Tupungato. San Carlos and Luján de Cuyo have similar sensory profiles. Maipú and Rivadavia are separated from the rest of the departments. In the phenolic profile tree, the formation of three groups can be also observed. San Carlos, Luján de Cuyo and Maipú have similar anthocyanin and non-anthocyanin compounds profiles. Tupungato and Tunuyán are associated with each other. Rivadavia is different from the other groups. Comparing both trees generated by the sensory and chemical profiles, it can be observed that there are coincidences in the grouping in some departments, while others are different. The groups that coincide are San Carlos and Luján de Cuyo, where the two profiles match in having similarities and the departments of Rivadavia and Tunuyán are the least similar in both profiles evaluated.

4. Conclusions

This study shows for the first time an exhaustive study of Malbec wines from different locations of Mendoza by evaluating the phenolic and sensory profiles with their possible associations. The data obtained was used to discriminate the places where grapes where cultivated using chemometrics and sensometrics methods. Results indicate that geographical origin exert influence on phenolic composition and sensory attributes of Malbec wines, which are influenced also by environmental factors. The results of multivariate analysis showed that Maipú and Rivadavia were clearly separated from the rest, and they have the same behavior using sensory and phenolic profiles. However, by using the chemical data a clearer separation of each location is better than those obtained by means of sensory data. Using unsupervised statistical methods such as cluster analysis and comparing them through the tanglegram, the groups obtained agree that San Carlos and Luján de Cuyo are clearly associated in their sensory profiles and phenolics compounds, and the same results were showed by the CVA and PCA analysis. The overall data, have enological and viticulture impact for winemaking industry, expanding the current knowledge of Malbec wines and its geographical origin. The present study also affords new insights related to vineyard site selection helping to understand the effects of climate and geographical origin of grapes on the quality parameters of wines such as phenolic compounds and sensory profiles.

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Conflict of interest statement

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.05.083.

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