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Fingerprints for Main Varieties of Argentinean Wines: Terroir Differentiation by Inorganic, Organic, and Stable Isotopic Analyses Coupled to Chemometrics

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ABSTRACT: Our main goal was to investigate if robust chemical fingerprints could be developed for three Argentinean red wines based on organic, inorganic, and isotopic patterns, in relation to the regional soil composition. Soils and wines from three regions (Mendoza, San Juan, and Córdoba) and three varieties (Cabernet Sauvignon, Malbec, and Syrah) were collected. The phenolic profile was determined by HPLC-MS/MS and multielemental composition by ICP-MS; $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ were determined by TIMS and IRMS, respectively. Chemometrics allowed robust differentiation between regions, wine varieties, and the same variety from different regions. Among phenolic compounds, resveratrol concentration was the most useful marker for wine differentiation, whereas Mg, K/Rb, Ca/Sr, and $^{87}Sr/^{86}Sr$ were the main inorganic and isotopic parameters selected. Generalized Procrustes analysis (GPA) using two studied matrices (wine and soil) shows consensus between them and clear differences between studied areas. Finally, we applied a canonical correlation analysis, demonstrating significant correlation ($r = 0.99$; $p < 0.001$) between soil and wine composition. To our knowledge this is the first report combining independent variables, constructing a fingerprint including elemental composition, isotopic, and polyphenol patterns to differentiate wines, matching part of this fingerprint with the soil provenance.

KEYWORDS: wine fingerprint, elements, isotopic ratio, phenolics, geographical origin, varietals

INTRODUCTION

The authenticity and origin of food products are topics of great interest in the food industry, not only for consumers but also for producers and distributors. Additionally, the use of geographical designations allows producers to obtain market recognition and often a premium price.[1] Wine is a product widely consumed around the world and has been extensively investigated because of frauds, including adulteration, false declaration of age, and geographical origin. The huge diversity of production areas poses a challenge in establishing the provenance of wine as the properties of wine are influenced by factors such as their history, grape variety, soil and climate, yeast, enological practices, transport, and storage.[2−12] To take marketing advantage of the recent large improvements in wine quality in several "New World" wine areas, many local producers have changed to declare the specific region of origin rather than just naming the grape variety.[13] The denomination origin controlled (DOC) system is applied in many countries to control and ultimately guarantee the origin and quality and to prevent fraud. Chemical characterization is one of the requirements to obtain DOC certification. Nowadays, there is a wide range of combined techniques to identify wine authenticity[14] by the content of organic constituents, by the elemental composition of metals, and by analysis of stable isotopes.[15]

The inorganic chemical pattern of a wine is a reflection of the local geochemistry of the soil, climate, and processing. The elemental composition is mainly influenced by the bioavailability of inorganic compounds of the soil and the demands of the plants.[16] The initial concentration of elements can be modified during the winemaking process by the addition of bentonite and similar compounds, used to clarify the wine,[5] or by coprecipitation of a fraction of inorganic elements with organic complexing agents present in the must.[2] Not all elements are metabolized or modified during the winemaking process.[14,17] Thus, selected elements can be considered as good markers of the geographical

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origin of wines. Galgano et al.[18] identified Li and Rb as elements facilitating a successful classification from three southern Italian wine-producing regions. Likewise, Gonzalez et al.[19] were able to discriminate wines from two different Valencian areas in Spain by using Li and Mg contents. However, factors such as environmental pollution, agricultural practices, climatic change, and vinification process may change the multielemental composition of the wine. Almeida and Vasconcelos[2] found that contamination during vinification as well as treatment with organic complexing agents influenced a wine's elemental composition. Nevertheless, significant correlations were obtained between wines and their provenance soils. A statistically significant dependence between the elemental composition of vineyard soil and wine has been demonstrated in both Czech[7] and Argentinean wines,[17,20] pointing to Mg as a good chemical marker for the provenance of wine.

Evaluation of natural abundance isotope ratios provides information on plant type or animal diet (carbon ratios) and geographical origin (strontium, deuterium and oxygen isotopic ratios).[21] However, the $^{13}C/^{12}C$ ratios of plants are affected not only by the botanical origin (C3 and C4 plants) but also by environmental and physiological factors that influence water use efficiency in the leaves. Stomatal conductance and intercellular and ambient CO_2 concentrations are influenced by humidity, temperature, amount of precipitation, water stress, plant age, and maturation.[22] In general, $\delta^{13}C$ values in leaves showed an increasing (less negative) trend with decreasing precipitation.[23] Strontium has four stable isotopes, and only one isotope, ^{87}Sr, is formed through radiogenic decay of ^{87}Rb (half-life $\sim 4.88 \times 10^{10}$ years).[24,25] Differences in the abundance of ^{87}Sr vary with geological age, and the Rb/Sr ratio and consequently strontium isotope ratios in bedrock and soil vary according to the local geology. Weathering processes actually modify this ratio, and bioavailable Sr isotope ratios are different from bulk soil/rock ratios. Isotopic abundances of the other Sr isotopes such as ^{84}Sr, ^{86}Sr, and ^{88}Sr can also vary due to mass-dependent isotopic fractionation through various physicochemical reactions in nature. However, in the conventional isotopic analysis, a $^{88}Sr/^{86}Sr$ ratio of 0.1194 is used for normalization, and this corrects any kind of experimental or natural mass-dependent fractionation, making $^{87}Sr/^{86}Sr$ an ideal tracer of the source.

Wine is an excellent source for various classes of polyphenols, including phenolic acids, flavonols, anthocyanins, flavan-3-ols, and stilbenes. These compounds give quality attributes to the wine, contributing to the color and sensory properties such as flavor and astringency. Also, they manifest a wide range of beneficial health effects including anti-inflammatory, antiviral, anticarcinogenic, and antiatherogenic activities.[26] The polyphenolic profile of a given grape cultivar and, consequently, the wines produced from it is subject to tight genetic (varietal) control, but environmental issues, including type of soil, sun irradiation, and climate, may be of equal importance in this regard.[27] One example of this is *trans*-resveratrol, which is synthesized in response to infections or other stress conditions of the grape berry. Thus, its presence in wine could be significantly affected by such events.[28] The potential of polyphenolic profile analysis for the differentiation of wines by grape variety and geographical origin in the context of food authentication has been recognized.[29−31] For instance, Rastija et al.[32] established flavanols and *trans*-resveratrol patterns as the basis for the classification of samples according to their geographical origin and type of wine. Makris et al.[33] and Kallithraka et al.[27] determined major and minor polyphenolic constituents in young red wines from the main viticultural areas of Greece, yielding satisfactory categorization of such wines on the basis of varietal and geographical origin.

Verification of wine authenticity may be accomplished by the combined use of two or more groups of parameters, such as isotopes, major and minor elements, and polyphenols. However, the use of combined methods produces a lot of data, from which the essential information must be extracted by multivariate statistical techniques (chemometrics).[14] Among other chemometric tools, principal components analysis (PCA), cluster analysis (CA), discriminant analysis (DA), canonical correlation analysis (CCA), and related methods are currently used for discrimination, classification, modeling, and correlation.[15]

It is worth mentioning that many classifications of wines from several origins rely mainly on the analysis of one group of parameters: either elements and isotopes or organic constituents. Additionally, many studies do not consider the association between the local soil and the final composition of wine. To our knowledge there are no papers combining elemental composition, isotopic, and polyphenol patterns or association between stable isotopes and elements from growing soils to characterize wines. Furthermore, these variables are not related between them; for instance, phenolic profiles are not controlled by underlying geology, and $^{87}Sr/^{86}Sr$ values are not controlled by grape variety.

Particularly, for grape varieties cultivated in Argentina and their corresponding wines, the polyphenolic and isotopic composition has not been examined in detail. There are two previous papers on the evaluation of 11 elements in soil, juice, and wine samples from Argentina.[17,20] These papers show that some elements allow differentiation between different geographical regions[20] and between wine varieties from the same region.[17]

Our main goal was to obtain a reliable fingerprint from typical Argentinean red wines on the basis of organic, inorganic, and isotopic patterns, considering the influence of provenance soil. Thus, we measured 33 elements, $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ isotopic ratios, and 10 phenolic compounds, of three selected wine varieties and soils from three different geographical regions and applied chemometrics for data analysis.

MATERIALS AND METHODS

Reagents and Materials. Ultrapure water (<5 $\mu g\ L^{-1}$ TOC) was obtained from a purification system, Arium 61316-RO plus Arium 611 UV (Sartorius, Goettingen, Germany). Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (Edo. de México, Mexico) and Fluka (Steinheim, Germany), respectively. Commercial standards of (+)-catechin, (−)-epicatechin, ferulic acid, and caffeic acid were obtained from Extrasynthèse (Genay, France). Kaempferol, *p*-coumaric acid, and quercetin were purchased from Fluka (Dorset, U.K.). Myricetin, *trans*-resveratrol, and rutin were obtained from Sigma-Aldrich (Buenos Aires, Argentina), and gallic acid was purchased from Riedel-de-Haën (Seelze, Germany). Inductively coupled plasma multielement standard solution Merck VI CertiPUR was obtained from Merck Química Argentina (Buenos Aires, Argentina). The composition and concentration of the Merck VI standard was as described in the accompanying certificate of analysis. Nitric acid (63.7%) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distiller, Córdoba, Argentina). The purity of the nitric acid was verified by ICP-MS. Filters (0.45 μm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). Ion exchange chromatography Sr Spec resin was obtained from Eichrom Technologies (Darien, IL). All other reagents were of analytical grade.

Sampling. Wine and soil samples were collected from the three major wine production regions of Argentina: Mendoza, San Juan, and Córdoba. The sampling area in the province of Mendoza is located

between 32° 55′−33° 09′ south latitude and 68° 15′−68° 29′ west longitude, the San Juan sampling area is located between 31° 30′−32° 03′ south latitude and 68° 14′−68° 34′ west longitude, and the Córdoba area is located between 30° 75′−31° 10′ south latitude and 63° 64′−64° 06′ west longitude. The altitude varies from 460 m above sea level in Córdoba to 580 m above sea level in San Juan to 640 m above sea level in Mendoza. The main varieties of grapes cultivated in these areas are Cabernet Sauvignon, Malbec, and Syrah.

The selected wine production regions of Argentina were Mendoza, San Juan, and Córdoba with different geological settings. Northern Mendoza production areas are underlaid by a Tertiary sedimentary sequence, composed of conglomerates and sandstones covered by Quaternary piedmont units. The geological setting of San Juan production areas are represented by a clastic sedimentay Tertiary sequence, overlaid by Quaternary alluvial and eolian units. This region is located near the outcrops of the Cambrian-Ordovician thick carbonatic succession of Pre-Andes range area. Finally, Córdoba production areas belong to the Pampean ranges, with dominant Paleozoic acid magmatic rocks.

Wine samples were obtained from different cellars situated in the three wine production regions. THe "Cavas de 1930" cellar produces wines from four vineyards situated in San Martín and Junín areas (province of Mendoza). "Augusto Pulenta" and "Casa Montes" cellars produce wines from three vineyards located in Valle de Tulum (province of San Juan). The "Group Pro-Vid" cellar produces wines from two vineyards situated in Colonia Caroya (province of Córdoba). The studied wines were produced using grapes from the same parcels that we visited during the soil sampling campaign.

All wines were obtained directly from producers having both GMP (good manufacturing practices) and traceability systems. Thus, each wine can be traced to a specific vineyard where the grapes were grown. Forty-eight samples from two vintages (2007 and 2008) were selected and analyzed: 21 samples from Mendoza (Cabernet Sauvignon, Malbec, and Syrah), 21 samples from San Juan (Cabernet Sauvignon, Malbec, and Syrah), and 9 samples from Córdoba (Cabernet Sauvignon and Malbec). Samples were collected in 750 mL glass bottles after bottling and stabilization (at least 6 months), transported to the laboratory, and stored in the dark at 4−8 °C until analysis. All samples were analyzed within 1 month. The alcoholic content ranged from 12 to 13% v v^{-1} ethanol.

Soil samples were collected using stainless steel shovels and were stored in individual black plastic bags (darkness). Soils were sampled in depths from 10 to 20 cm to avoid surface-soil pollution arising from the surrounding environment and to reduce the effects of fertilizers and variable organic matter content[2] and 50 cm from the side of the plot to reduce the effects of fertilizers and variable organic matter content.[34] One hundred soil samples were analyzed, 37 from the province of Mendoza, 39 from the province of San Juan, and 24 from the province of Córdoba.

Elemental Analyses. Wine samples were mineralized using a microwave oven (Anton Paar 3000); 5 mL was introduced in quartz vessels, followed by the addition of 6 mL of concentrated nitric acid. Vessels were kept open until no fumes were observed (2−3 h). Afterward, vessels were cap-closed and heated using the following power sequence: starting a 15 min ramp until reaching 350 W, holding for 45 min (maximal T = 169 °C; max pressure = 75 bar), and a final 15 min step disabling power to reach pressure equilibration. Mineralized samples were quantitatively transferred to 25 mL volumetric flasks, completing the volume with ultrapure water, followed by filtration using 0.45 μm filters. This process was done in duplicate for all samples. Three samples were spiked to verify recovery percentages of different elements. Therefore, spiked samples were prepared by adding variable amounts of individual standard solutions (1000 mg L^{-1} in 1% nitric acid) doubling the starting concentration for each element. The rest of the procedure was the same used for nonspiked samples. All recoveries were between 86 and 114%.

The bioavailable soil fraction was prepared for elemental analysis as follows: Samples were dried at 40 °C during 2 days. Afterward, soils were homogenized and sieved through a 2 mm acrylic sieve, followed by further drying at 40 °C overnight. Twenty grams of dried sieved soil was weighed into an Erlenmeyer flask, adding 50 mL of NH_4NO_3 (1 M). The resulting suspension was shaken for 2 h at room temperature. Subsequently, the suspension was allowed to settle for 1 h, filtered through 0.45 μm filters, and acidified with 0.5 mL of concentrated nitric acid (sub-boiling grade). This process was done in duplicate for all samples. Three spiked samples were also prepared. Variable amounts of individual standard solutions (1000 mg L^{-1} in 1% nitric acid) were added to 40 g of dried sieved soil sample to double the starting concentration for each element. The rest of the procedure was the same as used for nonspiked samples. All recoveries were between 80 and 120%.

Thirty-three elements were quantified in wine and soil samples. The analysis was carried out by quadrupole inductively plasma mass spectrometry (Q-ICPMS) for all elements except sodium. A Thermo-Elemental X7 series (Thermo Fisher Scientific, Bremen, Germany), equipped with an ASX-100 autosampler model (CETAC Technologies, Omaha, NE), was used. The sample introduction system consisted of a microflow concentric nebulizer, a Peltier cooled spray chamber, and a 1.5 mm i.d. fixed injector torch. The RF forward power was 1350 W for all of the experiments, and the interface was fitted with Ni sampling and skimmer cones designed for low polyatomics formation. Two operation modes were used: with and without collision cell technology (CCT). CCT mode measurements were performed for Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Cd, Ba, Pb, and U. The collision cell was flushed with 7% H_2 in a He high-purity mixture. The elements Li, B, Al, Cs, La, Ce, Nd, Sm, Eu, Yb, Lu, and Tl were measured without operation of the collision cell with gas and, thus, full sensitivity was obtained. The oxide ratio and double-charged species were maintained below 1% in both modes of operation. All of the Q-ICPMS measurements were performed using Sc, In, and Re as internal standards. Sodium measurements were carried out by flame atomic absorption spectrometry (FAAS) using a Perkin-Elmer 3030 in an air−acetylene flame. All of the wine and soil samples were diluted 10-fold using a HNO_3 1%−HCl 0.5% mixture before Q-ICPMS measurements. Standards and blanks were prepared using the same mixture (HNO_3 1%−HCl 0.5%). Instrumental and procedural blanks were determined together with samples, and the mean of five runs was obtained for each sample. Full quantitative analysis was performed against calibration standards for each element. All samples were analyzed in duplicate.

Isotopic Analysis. The ratio $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ were measured in both wine and soil samples.

$^{87}Sr/^{86}Sr$. Strontium isotopic ratios for analyzed samples were measured using a thermal ionization mass spectrometer (TIMS) Finnigan MAT-262 (actually Thermo Fischer, Bremen, Germany). It is equipped with a sample magazine for 13 samples and 7 Faraday cups and a secondary electron multiplier as collectors. The measurements were carried out using a double-filament rhenium ion source. NBS SRM 987 was employed as standard to determine the instrumental bias at each set of analysis, whereas an Eimer & Amend Standard (Massachusetts Institute of Technology) was regularly analyzed to check for proper operation of the mass spectrometer. Measured ratios were corrected for mass fractionation using $^{88}Sr/^{86}Sr$ = 8.375209. ^{85}Rb was monitored at each block of data to quantify any interference from ^{87}Rb. To prevent contamination, all laboratoryware was soaked in 50% (v/v) HNO_3 for at least 24 h, rinsed several times with deionized water, and dried in a Class 100 laminar flow hood. Sample manipulation was carried out in a clean room with Class 100 filtered air. During the course of measurements for this study, SRM 987 gave a value of 0.71008 (1σ = 0.00026 for n = 50) to evaluate the reproducibility and accuracy of the measurements.

Wine samples were processed by the dry ashing technique. Briefly, 250 mL aliquots of each sample were placed in porcelain crucibles at a low-temperature hot plate and heated overnight. Crucibles with residues were then introduced in a high-temperature muffle furnace and heated at

550 °C during 18−20 h until ash formed. After cooling, residues were treated with concentrated nitric acid on a hot plate. After that, samples were transferred to the muffle furnace during 18 h at 550 °C. White ashes obtained were dissolved in 1 M nitric acid and loaded into the ion exchange chromatography column and measured as described above. To avoid contamination, only new crucibles were used, and they were controlled for stress fractures or fissures.

A bioavailable soil fraction was obtained as described for elemental analysis. Fifty milliliters of this solution was evaporated to dryness and redissolved in 1 M nitric acid. Afterward, Sr was separated by cation-exchange chromatography and measured as described above.

$\delta^{13}C$. The isotope ratios were determined against internal reference materials calibrated against international standards supplied by the International Atomic Energy Agency and were referred to Vienna-Pee Dee Belemnite (V-PDB), using the international δ‰ notation.

We used 5−10 mg wine sample for isotopic measurements. Briefly, each sample was placed in a borosilicate glass tube containing 1 g of CuO. The glass tube was attached to a high-vacuum rack, evacuated using a mechanical pump, and sealed in a vacuum (ca. 5 mbar) with a torch. Subsequently, sealed tubes were placed in a furnace and combusted at 550 °C for 8 h. Afterward, tubes were allowed to cool inside the oven and attached to a vacuum purification line, equipped with a cryogenic trap to remove water and non-CO_2 gases. The purified CO_2 was collected in a glass collection tube, using a liquid nitrogen trap, and analyzed in a Multiport Dual Inlet IRMS (Finnigan MAT Delta S), using a reference CO_2 gas. The uncertainty of isotopic determinations was ±0.1‰.

Soil samples were weighed into a tin capsule. The capsule was sealed and dropped into the reaction tube of a Carlo Erba elemental analyzer. The combustion took place at 1020 °C, and the products were separated on a packed gas chromatographic (GC) column. The GC effluent flew into the stable isotope ratio mass spectrometer Finnigan MAT Delta V Plus via a ConFlo IV interface. Helium was used as carrier gas. The uncertainty of the isotopic determinations was ±0.2‰.

Phenolic Analysis. Phenolic compounds were analyzed in wine samples by a HPLC-ESI-MS/MS method, using a HPLC system (Varian Prostar Dinamax 24), equipped with a binary gradient pump, solvent degasser (Metachem Technologies, USA), and autosampler (Varian, Prostar 410). The chromatographic separation was achieved on a Luna (Phenomenex, Torrance, CA) reversed-phase C18 column (5 μm, 250 mm × 4.60 mm i.d.). The mobile phase consisted of 0.5% (v v^{-1}) formic acid (solvent A) and methanol (solvent B), starting with 20% and changing to 50% B during 3 min, kept for 5 min, followed by a second ramp to 80% B in 5 min, maintained for 17 min, a third ramp to 20% B in 1 min, remaining at this last condition for 10 min before the next run. The flow rate was 0.4 mL min^{-1}, injecting 10 μL on column. This HPLC system was coupled to a Varian 1200 triple-quadrupole tandem mass spectrometer, equipped with an electrospray ionization (ESI) interface. The working conditions for the ionization source were as follows: negative ionization mode; capillary voltage, −40 V; needle voltage, −3800 V; shield voltage, −175 V; drying gas temperature, 350 °C. Nitrogen and argon were used as nebulizing and collision gases, respectively. Varian MS Workstation version 6.6 software was used for data acquisition and processing.

For quantitative analysis of phenolics, the MS parameters were operated in multiple reaction monitoring (MRM) scan mode. The following product ions were specified: m/z 125 for gallic acid, m/z 245 for (+)-catechin and (−)-epicatechin, m/z 135 for caffeic acid, m/z 134 for ferulic acid, m/z 119 for *p*-coumaric acid, m/z 185 for *trans*-resveratrol, m/z 178 for myricetin, m/z 179 for quercetin, and m/z 285 for kaempferol. The retention time of each reference compound was also determined to provide additional identification of eluted compounds. Quantification of phenolics was performed by linear regression from calibration curves, using peak areas of product ions. Thus, a standard solution contained a mix of *p*-coumaric acid, (+)-catechin, (−)-epicatechin, myricetin, quercetin, and resveratrol (10 mg L^{-1} each), whereas gallic acid, caffeic acid, and ferulic acid had a concentration of 5 mg L^{-1} and kaempferol (1 mg L^{-1}) was prepared in methanol. Afterward, calibration curves were performed by diluting standard mix with HPLC mobile phase (80 A:20 B) at eight different concentrations. All solutions were filtered through 0.45 μm filters before injection and analyzed in triplicate. Several validation tests were performed to evaluate accuracy, precision, linearity, analytical range, limit of detection (LOD), and limit of quantification (LOQ).[35] The lineal analytical range was between 0.015 and 7.00 mg mL^{-1}, showing correlation coefficients (r^2) > 0.98. Coefficients of variation (CV) were below 10%. LODs ranged from 0.003 to 0.050 mg L^{-1}, whereas LOQs varied from 0.009 to 0.185 mg L^{-1}. Accuracy and matrix effect were verified by spiked samples: thus, three samples were spiked to double the starting concentration of polyphenols, prepared and analyzed in the same way, obtaining recovery percentages between 88 and 110%.

Wine samples were analyzed by dilution with methanol (1:5, v v^{-1}), filtered (0.45 μm), and injected in HPLC-ESI-MS/MS. All samples were analyzed in duplicate.

Statistical Analysis. Multivariate statistical methods were applied to data sets. The statistical package Statistica 7.1 from StatSoft Inc. (2005) was used. Concentrations of elements and phenolics, in addition to isotopic ratios, were used as chemical descriptors for wine and soil samples.

Analysis of variance (ANOVA) was performed with each single variable and, in case of significance ($p < 0.05$), a DGC [36] comparison test was performed to reveal paired differences between means.

DA in stepwise mode was performed to evaluate whether wine and soil samples could be mathematically distinguished according to their geographical origin and/or variety. Selection of the most significant variables was performed by backward stepwise analysis according to *F* value. The robustness of the classification model was evaluated by a cross-validation test, using the "leave-one-out" procedure.

Generalized Procrustes analysis (GPA) was applied to assess the relationship between wine and soil data. Specifically, GPA constructs the consensus configuration of a group of data sets by applying transforms in an attempt to superimpose them. In this work we used the Gower algorithm that minimizes the within-samples variance by applying translation, scaling, and rotation to generate a *p*-dimensional average configuration **Y**c. Following this, a *q*-dimensional group average space ($q \leq p$) is constructed from **Y**c by PCA.[37] Therefore, GPA theory and algorithms can be applied to match wine elemental and isotopic data to the corresponding soil data.

CCA was used for assessing the relationship between data sets. Specifically, this method allowed us to evaluate the relationship between soils and wines studied during this work. In addition to CCA, multi-elemental correlation plots were obtained after normalization of elemental concentrations in individual soils to the mean of the entire soil data set and the concentration ranges to a variance of 1. The same procedure was also applied to individual wines in the wine data set.

RESULTS AND DISCUSSION

Analysis of Wine Composition. The mean contents of inorganic and organic constituents for each varietal from the three studied regions are presented in Table 1. In addition to element concentrations, isotopic ratios, and phenolics compounds, we included values for K/Rb and Ca/Sr ratios. The K/Rb ratio can greatly differ among various rocks and soils.[38] On the other hand, the Ca/Sr ratio has been used mainly as a chemical tracer in geochemistry, hydrogeochemistry, and bioavailability studies.[39,40] As far as we know, there are no reports of the use of these ratios as tracers of the geographical origin of food.

Elemental analysis shows that K is the most abundant element in studied wines, followed by Na, Mg, Ca, Mn, and B. These results are consistent with those reported by Fabani et al.[17] for

Table 1. Means and Standard Deviations of Measured Elements, Isotope Ratios, and Polyphenols Content Corresponding to Each Wine Variety and Region[a]

	Cabernet Sauvignon			Malbec			Syrah	
variable	Córdoba (n = 6)	Mendoza (n = 9)	San Juan (n = 3)	Córdoba (n = 3)	Mendoza (n = 9)	San Juan (n = 3)	Mendoza (n = 3)	San Juan (n = 15)
Li	41 ± 6 a	164 ± 6 c	207 ± 1 d	30.42 ± 0.02 a	136 ± 8 b	230 ± 1 e	152 ± 3 c	257 ± 38 e
B	8188 ± 127 b	9033 ± 240 b	24659 ± 379 d	6877 ± 12 a	8834 ± 593 b	24080 ± 214 d	9056 ± 180 b	22552 ± 1838 c
Na	(9.9 ± 0.8) × 10^4 a	(8.4 ± 1.3) × 10^4 a	(9.5 ± 0.1) × 10^4 a	(6.2 ± 0.1) × 10^4 a	(7.9 ± 0.4) × 10^4 a	(19.4 ± 0.1) × 10^3 a	(7.7 ± 0.3) × 10^4 a	(0.9 ± 0.9) × 10^5 a
Mg	(8.0 ± 0.3) × 10^4 a	(9.8 ± 0.4) × 10^4 b	(108.7 ± 0.5) × 10^3 c	(80.1 ± 0.9) × 10^3 a	(9.9 ± 0.8) × 10^4 b	(10.6 ± 0.1) × 10^4 b	(9.9 ± 0.2) × 10^4 b	(11.5 ± 0.7) × 10^4 d
Al	240 ± 41 a	459 ± 193 b	398 ± 4 b	276 ± 3 a	432 ± 114 b	589 ± 7 c	724 ± 162 c	506 ± 239 b
K	(14.6 ± 0.2) × 10^5 a	(1.2 ± 0.3) × 10^6 a	(137.1 ± 0.6) × 10^4 a	(13.7 ± 0.1) × 10^5 a	(1.2 ± 0.3) × 10^6 a	(13.2 ± 0.2) × 10^5 a	(13.6 ± 0.3) × 10^5 a	(1.2 ± 0.1) × 10^6 a
Ca	(6.6 ± 0.6) × 10^4 b	(8.1 ± 1.8) × 10^4 b	(69.9 ± 0.9) × 10^3 b	(51.3 ± 0.6) × 10^3 a	(8.5 ± 2.1) × 10^4 b	(9.7 ± 0.2) × 10^4 b	(8.7 ± 0.3) × 10^4 b	(7.1 ± 1.0) × 10^4 b
V[b]	<LOD	23 ± 10 b	<LOD	<LOD	15 ± 16 b	<LOD	27 ± 7 b	5 ± 11 a
Mn	2499 ± 1545 c	991 ± 317 b	1048 ± 4 b	2746 ± 21 c	1133 ± 355 b	1074 ± 12 b	470 ± 25 a	967 ± 83 b
Co[b]	52 ± 57 b	0.8 ± 1.2 a	<LOD	<LOD	0.8 ± 1.2 a	2.7 ± 0.4 a	<LOD	2 ± 3 a
Ni	10 ± 2 a	18 ± 23 a	7 ± 6 a	3 ± 3 a	9 ± 5 a	9 ± 5 a	13 ± 5 a	7 ± 5 a
Cu	54 ± 30 a	246 ± 63 b	61 ± 4 a	68 ± 3 a	339 ± 169 b	39 ± 5 a	232 ± 21 b	104 ± 63 a
Zn	159 ± 143 a	1004 ± 1218 a	1125 ± 16 a	326 ± 7 a	868 ± 274 a	1010 ± 22 a	540 ± 155 a	1135 ± 550 a
Ga[b]	<LOD	0.06 ± 0.13 a	<LOD	<LOD	0.03 ± 0.07 a	<LOD	<LOD	0.02 ± 0.09 a
Se[b]	<LOD	1 ± 2 a	<LOD	2 ± 4 a	1 ± 2 a	5 ± 1 a	1 ± 2 a	1 ± 2 a
Rb	547 ± 156 b	500 ± 34 b	940 ± 13 c	364 ± 4 a	501 ± 81 b	395 ± 1 a	388 ± 4 a	544 ± 152 b
Sr	859 ± 19 b	1386 ± 37 d	486 ± 2 a	875 ± 16 b	1408 ± 81 d	1674 ± 16 e	1484 ± 24 d	1229 ± 381 c
Mo	3.5 ± 0.5 a	7 ± 1 b	2.4 ± 0.3 a	0.3 ± 0.5 a	6 ± 3 b	<LOD	8 ± 2 b	6 ± 5 b
Cd[b]	<LOD	58 ± 168 a	<LOD	<LOD	0.02 ± 0.07 a	0.3 ± 0.1 a	<LOD	0.1 ± 0.2 a
Cs	3 ± 2 a	0.7 ± 0.3 a	4.3 ± 0.2 a	1.80 ± 0.02 a	2 ± 6 a	(33.0 ± 0.1) × 10^{-2} a	0.53 ± 0.12 a	4 ± 5 a
Ba	161 ± 12 c	60 ± 24 b	67 ± 2 b	156 ± 1 c	49 ± 5 a	55 ± 3 b	59 ± 4 b	74 ± 14 b
La	6 ± 1 a	1 ± 1 a	4.3 ± 0.1 a	5.7 ± 0.2 a	0.4 ± 0.3 a	0.1 ± 0.1 b	1.0 ± 0.5 a	2 ± 3 a
Ce	0.06 ± 0.11 a	0.7 ± 0.5 b	1.9 ± 0.1 d	0.2 ± 0.1 a	0.4 ± 0.3 a	0.42 ± 0.04 a	1.5 ± 0.7 c	0.2 ± 0.1 a
Nd	0.02 ± 0.04 a	0.34 ± 0.19 b	0.02 ± 0.03 a	0.06 ± 0.06 a	0.18 ± 0.16 a	0.08 ± 0.04 a	0.84 ± 0.37 c	0.05 ± 0.06 a
Sm	0.01 ± 0.01 a	0.06 ± 0.07 a	0.01 ± 0.03 a	0.04 ± 0.01 a	0.04 ± 0.04 a	0.02 ± 0.00 a	0.15 ± 0.07 b	0.01 ± 0.02 a
Eu[b]	0.02 ± 0.02 a	0.01 ± 0.02 a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.02 a	<LOD	0.06 ± 0.02 b	0.01 ± 0.01 a
Yb	0.02 ± 0.02 a	0.04 ± 0.02 a	0.04 ± 0.02 a	0.04 ± 0.03 a	0.04 ± 0.03 a	0.03 ± 0.02 a	0.12 ± 0.05 b	0.02 ± 0.02 a
Lu	(0.4 ± 1.0) × 10^{-2} a	0.04 ± 0.05 a	(1.0 ± 0.4) × 10^{-2} a	(0.01 ± 0.5) × 10^{-2} a	0.04 ± 0.05 a	0.11 ± 0.00 a	0.01 ± 0.01 b	0.05 ± 0.05 a
Tl[b]	0.10 ± 0.12 b	0.01 ± 0.02 a	0.02 ± 0.04 a	0.05 ± 0.04 a	0.01 ± 0.02 a	<LOD	0.05 ± 0.03 a	0.04 ± 0.05 a
Pb[b]	4 ± 3 a	30 ± 9 c	91 ± 1 d	<LOD	33 ± 14 c	17.2 ± 0.1 b	39 ± 2 c	9 ± 6 a
U[b]	1.97 ± 2.16 c	1.25 ± 0.29 b	<LOD	<LOD	0.88 ± 0.43 b	(0.06 ± 3.50) × 10^{-3} a	3.19 ± 0.85 d	0.13 ± 0.24 a
K/Rb	2914 ± 161 b	2621 ± 915 b	2847 ± 23 b	3770 ± 15 c	2740 ± 610 b	1395 ± 73 a	2537 ± 863 b	2318 ± 591 b
Ca/Sr	72 ± 22 a	56 ± 4 a	51 ± 1 a	59 ± 1 a	65 ± 13 a	54 ± 2 a	76 ± 42 b	68 ± 37 a
$\delta^{13}C$	−28.70 ± 0.00 a	−27.71 ± 0.06 d	−26.30 ± 0.00 e	−28.40 ± 0.00 b	−27.80 ± 0.05 d	−28.20 ± 0.00 c	−27.56 ± 0.05 d	−28.08 ± 0.48 c
$^{87}Sr/^{86}Sr$	0.7093 ± 0.0001 c	0.7073 ± 0.0002 a	0.7082 ± 0.0000 b	0.7092 ± 0.0000 c	0.7074 ± 0.0010 a	0.7084 ± 0.0000 b	0.7071 ± 0.0002 a	0.7081 ± 0.0006 b
p-coumaric acid	3.6 ± 0.6 a	4.8 ± 0.9 a	2.1 ± 0.2 a	4.7 ± 0.3 a	8.2 ± 4.0 b	7.2 ± 0.1 b	11.8 ± 2.3 c	2.0 ± 1.2 a
gallic acid	14.5 ± 1.9 b	22.9 ± 2.9 b	19.9 ± 0.8 b	5.2 ± 0.4 a	32.3 ± 15.5 b	40.9 ± 0.9 d	44.6 ± 7 d	27.5 ± 12.4 b
caffeic acid	6.3 ± 1.2 a	6.4 ± 1.3 a	8.0 ± 0.4 b	5.2 ± 0.5 a	8.9 ± 3.0 b	7.40 ± 0.0 b	13.7 ± 2.1 c	4.1 ± 1.3 a
ferulic acid[c]	2.5 ± 2.7 a	4.0 ± 2.1 a	<LOD	<LOD	4.4 ± 2.8 a	4.2 ± 1.4 a	3.7 ± 3.1 a	2.3 ± 3.2 a
trans-resveratrol[c]	152 ± 6.0 c	7.8 ± 3.8 b	<LOD	30.4 ± 2.2 d	10.6 ± 4.7 b	6.1 ± 0.2 b	8.4 ± 1.3 b	2.1 ± 1.8 a
kaempferol	0.17 ± 0.03 a	0.64 ± 0.35 b	0.64 ± 0.03 b	0.41 ± 0.02 a	0.82 ± 0.48 b	0.58 ± 0.03 b	0.40 ± 0.08 a	0.61 ± 0.30 b
(+)-catechin	22.2 ± 1.9 b	30.0 ± 9.9 b	19.4 ± 0.3 b	9.6 ± 1.1 a	44.2 ± 19.4 c	41.4 ± 0.1 c	49.8 ± 7.1 c	28.8 ± 12.2 b
(−)-epicatechin	11.4 ± 2.7 a	24.1 ± 9.5 b	19.3 ± 1.0 b	6.1 ± 0.4 a	34.1 ± 18.5 b	30.1 ± 3.2 b	35.7 ± 7.8 b	23.4 ± 7.1 b
quercetin	1.7 ± 1.9 a	7.9 ± 3.0 b	5.1 ± 0.2 a	4.7 ± 0.4 a	10.9 ± 6.1 a	13.8 ± 0.5 b	9.5 ± 1.6 b	8.6 ± 3.5 b
myricetin	3.8 ± 1.8 a	6.9 ± 4.0 a	2.4 ± 0.1 a	3.9 ± 0.3 a	9.9 ± 8.7 a	13.7 ± 0.1 b	7.2 ± 2.1 a	3.1 ± 1.5 a

[a] Element values are reported in $\mu g\ L^{-1}$. Polyphenol values are reported in mg L^{-1}. $^{13}C/^{12}C$ ratios are expressed in delta units (‰, per thousand). Different letters in a row indicate significant differences ($p < 0.05$). [b] LOD ($\mu g\ L^{-1}$): V (0.15), Co (0.003), Ga (0.007), Se (0.04), Cd (0.01), Eu (0.001), Tl (0.006), Pb (0.003), and U (0.001). [c] LOD (mg L^{-1}): ferulic acid (0.035) and resveratrol (0.027). LOQ (mg L^{-1}): ferulic acid (0.115) and resveratrol (0.09).

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wines from the province of San Juan. The elements show different patterns in accordance with wine provenance. For instance, Li presents the major concentration in wines from San Juan but the lowest in samples from Córdoba. The same pattern is observed for B and Mg. On the other hand, Mn shows the major concentration in the Córdoba region, whereas Cu has the highest level in Mendoza (Table 1). The median annual temperature in San Juan province is 17 °C, and rainfall is scarce (90 mm per year).[17] Consequently, the higher concentration and bioavailability of Li and B might be attributed to the hydrothermal input in the main tributaries of the San Juan River at the high mountain range of San Juan (Los Andes range) and the use of these waters for irrigation. Besides this, the upward movement along with other soluble salts, associated with arid climatic zones (such as San Juan), contributes to the anomaly. Magnesium is supplied by the conspicuous and nearby Cambrian-Ordovician rocks of the central western pre-Andes range of Argentina. This thick carbonate succession is mainly composed by marls, limestones, dolomitic limestones, and dolostones with $MgCO_3$ contents reaching 44%.[41,42]

Isotopic analysis of wines shows that the $^{87}Sr/^{86}Sr$ ratio presents the highest values in Córdoba, the lowest being in Mendoza, with San Juan showing intermediate values and the biggest dispersion. Furthermore, $^{87}Sr/^{86}Sr$ ratios in wines are coincident with soils (Table 2, discussed later).

The analysis of $\delta^{13}C$ in wine samples evidences the lowest values for Córdoba, the highest for Mendoza, and intermediate values for San Juan, with the biggest dispersion for San Juan (Table 1). This geographical variation could be related to climatic conditions as Córdoba has the higher mean annual precipitation, but we cannot disregard other factors previously mentioned that may also play a role. The concentration of carbon-13 in soils is a result of inputs of organic material from C3 and C4 plants and depends on the relative abundance of each plant type, rather than the absolute abundance of C3 or C4 plants.[43] This can explain the $\delta^{13}C$ offset between wine (plant) and soil.

With respect to phenolic constituents, the most abundant compound in studied wines is (+)-catechin followed by gallic acid. Our current results show that the content of (+)-catechin in studied red wines ranged between 20 and 41 mg L^{-1}, whereas gallic acid ranged between 21 and 34 mg L^{-1}. These results agree with several reports on phenolic compounds in wines from different regions and varieties.[32,44,45] In our case, wines from Mendoza show the highest values for most of the studied compounds, with the exception of resveratrol, which presents the highest concentration in wines from Córdoba (Table 1).

Next, we consider the ability of inorganic, isotopic, and organic patterns to predict the geographic region, then grape varietals, and then the combination of region and varietal.

Geographical Origin Based Classification. To assess the potential of organic, inorganic, and isotopic patterns for classification of the wines according to their geographical origin, we used chemometrics. Among different statistical methods used to evaluate differences between groups, multivariate analysis[14,15] affords the best results by considering the interaction between multiple variables. Therefore, we applied DA to identify those variables that could help to distinguish naturally occurring groups.

The application of backward stepwise DA allowed 100% discrimination between wines from the three studied regions, selecting 19 significant variables of 45. A graphical representation of differences between samples from three areas is presented in Figure 1A. It is noteworthy that the discrimination was possible including variables of the three groups analyzed, organic (*trans*-resveratrol, kaempferol, and (+)-catechin), inorganic components (B, Na, Mg, Ca, Mn, Co, Ni, Cu, Rb, Sr, Ba, La, Pb, and Ca/Sr), and isotopic ratios ($\delta^{13}C$ and $^{87}Sr/^{86}Sr$).

Between selected variables, Mg concentration increases from Córdoba to San Juan, with Mendoza showing intermediate values. The same pattern is observed for Li, B, Zn, and Lu (Table 1). Although K presents the highest value in wine from Córdoba, with similar values in San Juan and Mendoza, whereas Rb shows the highest values in wines from San Juan, with similar values in Córdoba and Mendoza, the K/Rb ratio is highest in

Table 2. Means and Standard Deviations for Elements and Isotopic Ratios Measured in Soils from the Three Studied Regions[a]

	province		
variable	Córdoba (n = 24)	Mendoza (n = 37)	San Juan (n = 39)
Li	165 ± 70 a	139 ± 65 a	422 ± 346 b
B	295 ± 197 a	864 ± 2228 b	2141 ± 3658 c
Na	59 ± 76 a	363 ± 781 b	802 ± 1202 c
Mg	491057 ± 112150 b	210788 ± 138696 a	511404 ± 308749 c
Al	74 ± 92 b	43 ± 61 a	39 ± 40 a
K	1230769 ± 1713153 b	416728 ± 279390 a	428512 ± 320591 a
Ca	3076922 ± 4282884 b	1041820 ± 698474 a	1071281 ± 801478 a
V	3 ± 7 a	11 ± 7 c	7 ± 6 b
Mn	1417 ± 4579 b	168 ± 184 a	107 ± 87 a
Fe	52 ± 52 a	57 ± 67 a	49 ± 26 a
Co	3 ± 5 b	1 ± 1 a	2 ± 3 b
Ni	6 ± 13 b	5 ± 5 a	5 ± 5 a
Cu	43 ± 66 a	232 ± 130 c	67 ± 58 b
Zn	33 ± 28 c	18 ± 12 b	12 ± 8 a
Ga	0.12 ± 0.16 a	0.09 ± 0.12 a	0.11 ± 0.13 a
As	8 ± 20 a	38 ± 19 b	49 ± 36 c
Se	5 ± 3 a	7 ± 10 a	11 ± 13 b
Rb	873 ± 533 c	474 ± 187 b	288 ± 96 a
Sr	17415 ± 8787 a	22968 ± 10502 b	23429 ± 9298 c
Mo	24 ± 102 a	25 ± 18 a	20 ± 21 a
Cd	1 ± 1 a	12 ± 16 b	0.5 ± 0.6 a
Cs	29 ± 13 a	53 ± 173 b	24 ± 12 a
Ba	19627 ± 6999 c	6806 ± 2988 a	10499 ± 5671 b
La	55 ± 164 b	12 ± 38 a	11 ± 29 a
Ce	1.6 ± 2.1 b	0.7 ± 0.6 a	1.5 ± 1.6 b
Nd	1.2 ± 1.6 c	0.4 ± 0.5 a	0.9 ± 1.1 b
Sm	0.3 ± 0.3 b	0.3 ± 0.1 b	0.2 ± 0.2 a
Eu	1.9 ± 0.8 b	0.9 ± 0.4 a	1. ± 0.6 a
Yb	0.1 ± 0.1 c	0.04 ± 0.03 a	0.07 ± 0.06 b
Lu	0.02 ± 0.01 b	0.11 ± 0.01 c	0.01 ± 0.01 a
Tl	4 ± 3 c	3.4 ± 0.6 b	1.6 ± 0.5 a
Pb	0.4 ± 0.7 b	1.5 ± 0.5 c	0.2 ± 0.4 a
U	1 ± 3 a	13 ± 4 b	16 ± 13 c
K/Rb	1207 ± 622 b	910 ± 499 a	1582 ± 1362 c
Ca/Sr	186 ± 266 b	46 ± 23 a	45 ± 23 a
$\delta^{13}C$	−12.9 ± 9.4	−15.2 ± 2.7	−15.5 ± 2.8
$^{87}Sr/^{86}Sr$	0.7104 ± 0.0031 c	0.7072 ± 0.0004 a	0.7081 ± 0.0011 b

[a] Element values are reported in $\mu g\,kg^{-1}$. $^{13}C/^{12}C$ ratios are expressed in delta units (‰, per thousand). Different letters in a row indicate significant differences ($p < 0.05$).

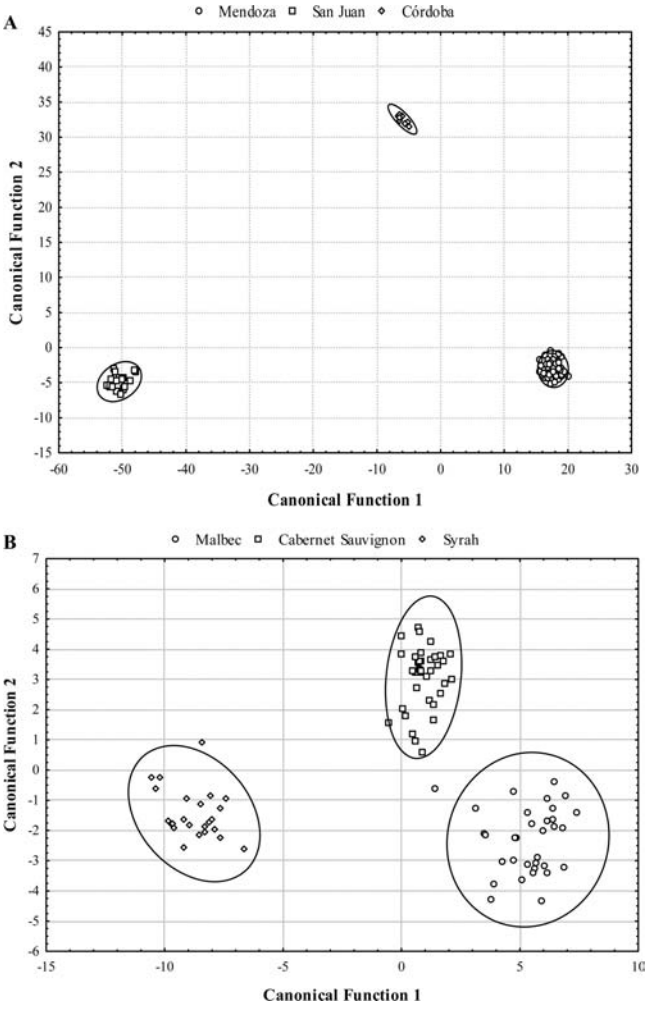

Figure 1. Discriminant analysis of wine samples by region (A) and grape variety (B).

wines from Córdoba and lowest from San Juan, with intermediate values for Mendoza. Therefore, the K/Rb ratio presents a pattern inverse to that observed for Mg. Our current results show that the Mg content in Córdoba and San Juan wines (Table 1) are similar to those of previous studies,[20] but with significant differences among the three studied areas.

Our current results show a characteristic pattern for both $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ ratios. $^{87}Sr/^{86}Sr$ presents the highest values for Córdoba, lowest levels for Mendoza, with intermediate values for San Juan.

In the same way, phenolic composition show different patterns for diverse wine production areas. As can be seen in Table 1, *trans*-resveratrol shows the highest values correspond to Córdoba, whereas the lowest value is observed in San Juan. Phenolics content has been also proposed to differentiate between wines from different regions. Makris et al.[33] reported that flavanols and flavonols exerted a profound influence on geographical origin based differentiation of Greek wines. Additionally, Rastija et al.[32] reported that flavonols and *trans*-resveratrol levels were the basis for the classification of Croatian wines according to their geographical origin. These results are in agreement with our present outcomes, because both (+)-catechin and kaempferol belong to flavanol and flavonol subclasses, respectively.

Wine Variety Based Classification. The main wine varieties produced in Argentina are Cabernet Sauvignon, Malbec, and Syrah. Thus, we were also interested in analyzing differences between varietals by considering organic, inorganic, and isotopic patterns. In most cases, Syrah presents the major concentration of elements, whereas Malbec shows the lowest amounts. Therefore, Syrah shows the highest values for Li, B, Mg, Al, Nd, Eu, Tl, and the Ca/Sr ratio, but the lowest values for Mn and Pb. Cabernet Sauvignon shows the highest values for Ni, Rb, Ba, La, Ce, Pb, and U and the lowest values for Sr and the Ca/Sr ratio. Likewise, Malbec presents the major values for Cu and Sr, and the minor values for Ni, Rb, Ba, La, Ce, Nd, Tl, and U. Also, the average concentrations of K, Na, and Zn do not show significant difference among studied wines. This result is consistent with those reported by Fabani et al.,[17] except for Syrah, which presented the highest sodium concentration during such study.

Backward stepwise DA allows us to correctly classify 100% of the wines analyzed from three varieties (Figure 1B), pointing out 21 significant variables to obtain such discrimination: ferulic acid, kaempferol, (+)-catechin, Li, Mg, Al, K, Ca, Co, Cu, Zn, Rb, Cd, La, Ce, Lu, Pb, U, Ca/Sr, $\delta^{13}C$, and $^{87}Sr/^{86}Sr$. It is worth mentioning that parameters belonging to three studied groups (organic, inorganic, and isotopic ratios) were included by DA. Among variables pointed out by DA, Mg shows a distinctive pattern, with the major amount for Syrah and minor values for Cabernet Sauvignon, with intermediate values for Malbec. A similar pattern is observed for Al, Cs, and the Ca/Sr ratio. Fabani et al.[17] reported that Mn, Mg, Na, Zn, K, and Ca were useful to distinguish between different varietals by DA. Our current results agree with those reported by Fabani et al.[17] for Mg, Zn, K, and Ca, whereas Mn and Na were not selected by the DA in our current study. Moreover, several papers designate K as a good descriptor of different classes of wines,[4] which reinforces our current results.

Catechin also shows the major amount for Malbec, the minor amount for Cabernet Sauvignon, and intermediate values for Syrah. A pattern similar to that of (+)-catechin is observed for other phenolics such as *p*-coumaric acid, caffeic acid, (−)-epicatechin, and quercetin, which are not selected by DA. Likewise, DA selects ferulic acid and kaempferol for wine classification, which can be explained by considering that these last compounds have different patterns from the corresponding to (+)-catechin. The phenolic pattern has been the basis for the classification of wines according to their grape variety in several studies.[27,31,33,46] The production of polyphenols is genetically influenced, which is one reason to use them to characterize wines according to their variety.[14] Nevertheless, phenolic profiles exhibit notable variations, even in wines made from the same cultivar, evidencing an important impact of cultural practices, local environmental conditions, and vinification techniques.[27,46] This is the main reason leading us to use phenolic profile in combination with elemental profile and isotopic ratios to improve the differentiation by constructing a wider data set (fingerprint).

Wine Variety−Province Based Classification. So far, from our previous discussion, wines can be differentiated by provenance area and by variety. Now, we are interested in testing if wines can be also differentiated by production region and grape varieties, together. Therefore, we applied backward stepwise DA to the data set that allows distinguishing between eight groups with 100% accuracy (Figure 2A). DA afforded 24 descriptors: caffeic acid, *trans*-resveratrol, kaempferol, Li, B, Na, Mg, Al, K, Ca, Mn, Co, Ni, Cu, Zn, Rb, Sr, Ba, Lu, Pb, U, Ca/Sr, $\delta^{13}C$, and $^{87}Sr/^{86}Sr$. We constructed box and whisker plots to facilitate the graphical visualization of patterns corresponding to some parameters pointed

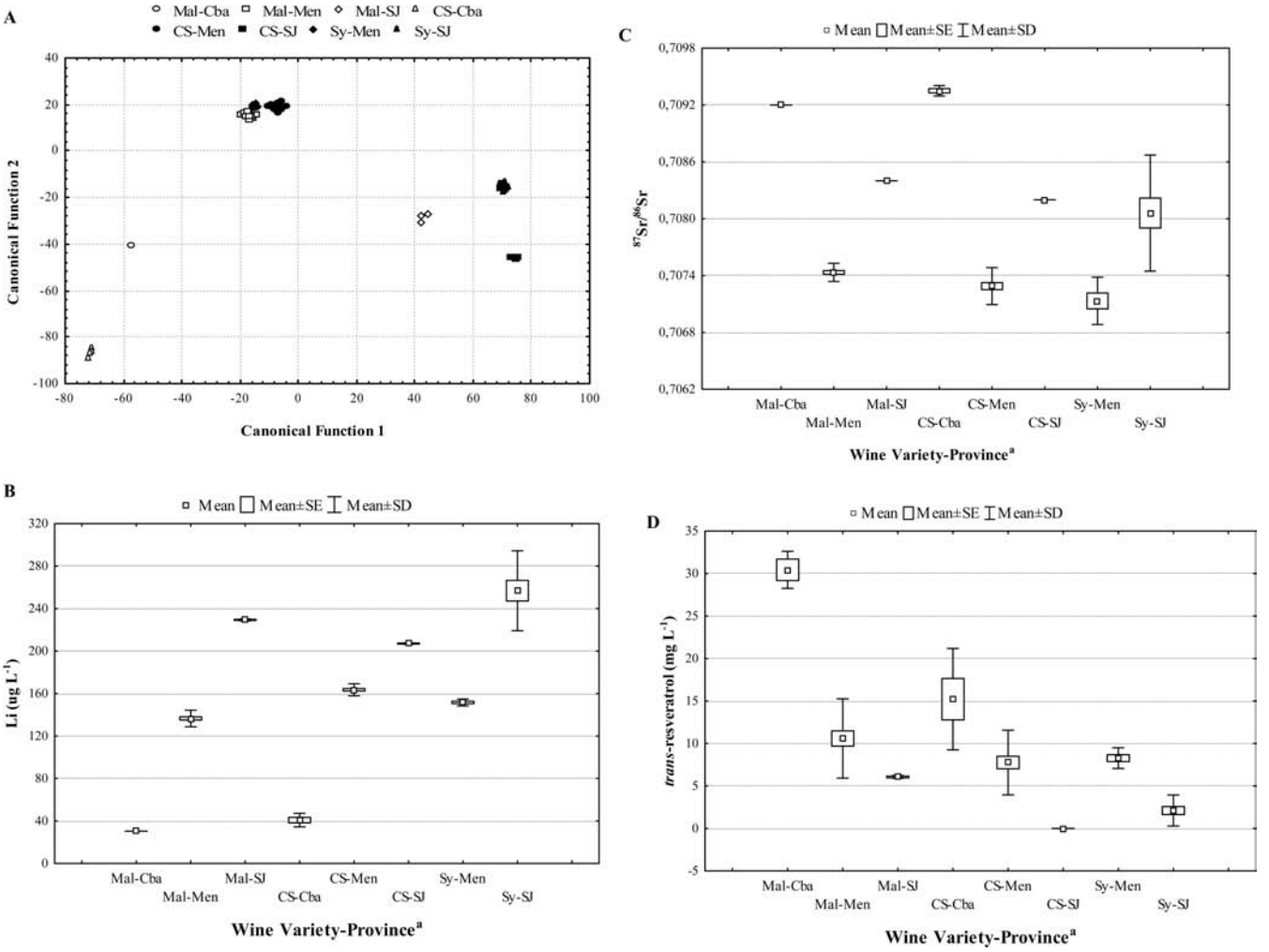

Figure 2. (A) Discriminant analysis of wine by wine variety and geographical origin. (B−D) Box and whisker plots showing means and standard deviations of some parameters pointed out by DA according to wine variety and geographical origin. [a]Wine varieties: Mal (Malbec), CS (Cabernet Sauvignon); Sy (Syrah). Provinces: Cba (Córdoba); Men (Mendoza); SJ (San Juan).

out by DA (Figure 2B−D). Li and $^{87}Sr/^{86}Sr$ distinguish between regions from the same varietal, but cannot distinguish between varietals belonging to the same region (Figure 2B−C). Mg follows the same pattern as Li (data not shown). Our current results coincide with those reported by Kment et al.[7] and Fabani et al.,[17] who proposed Mg as a chemical marker of wine provenance applicable in this case. *trans*-Resveratrol was also selected by DA; its use allows differentiation between three production areas. Additionally, *trans*-resveratrol allows distinguishing Malbec from Córdoba and Cabernet Sauvignon from Córdoba and San Juan from the rest of studied wines (Figure 2D). These results reinforce the idea that the combined use of elemental, isotopic, and phenolic analyses allows constructing a wider data set (fingerprint), which enables more confidence and accuracy in the evaluation of wine varieties in combination to production areas.

Analysis of Soil Composition. Descriptive statistics (mean and standard deviation) for measured soils are reported in Table 2. It can be seen that the composition of inorganic components shows differences among three sampling areas. Concentrations of K, Al, Ca, Mn, La, and Eu and the Ca/Sr ratio were highest in Córdoba and similar in soils from Mendoza and San Juan. On the other hand, levels of B, Na, As, Se, and U are lower in soils from Córdoba, whereas soils from San Juan present the highest contents, with intermediate values in Mendoza. On the other hand, Fe, Ni, Ga, Mo, and Sm do not show significant differences between the three studied regions.

The K/Rb ratio was lower in soils than in wines (Table 2). K is essential for plants, whereas Rb, a trace element with properties similar to those of K, has no biological function. Although Rb can substitute at K sites, there is a significant difference in uptake of these metals in plants, and Rb cannot substitute for K in metabolic processes. The uptake of Rb is controlled by soil acidity, which limited K availability and increases Rb uptake.[38,47] This results in higher K/Rb ratios in biological materials than in soils.

As in the K/Rb case, Sr and Ca can compete with each other, but Sr does not replace Ca in biochemical functions. Sr is not a micronutrient, and it is absorbed following the plant's metabolic requirements for Ca.

The $^{87}Sr/^{86}Sr$ ratio shows a different pattern, with the highest values in Córdoba, followed by San Juan, and Mendoza in coincidence with previously discussed results for wines.

Backward stepwise DA of soil data set allows distinguishing among the three studied regions with 99% certainty, pointing out

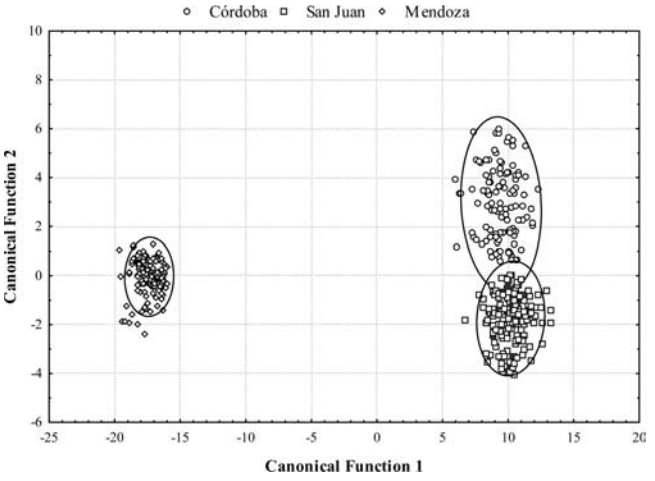

Figure 3. Distribution of soil samples in the plane defined by the two first canonical functions of DA.

18 variables: B, Na, Mg, Al, Ca, Mn, Fe, As, Mo, Ba, Ce, Eu, Yb, Lu, Tl, Pb, Ca/Sr, and $^{87}Sr/^{86}Sr$. Figure 3 shows a graphical representation of differences among the three studied regions. So far, soils where vines grew are clearly different at the three areas considered in this study. Our next challenge is to demonstrate if soil composition shows good correlation with wine produced at each area.

Correlation between Soil and Wine Composition. Recent papers demonstrate that some parameters found in wines can be associated with vineyard soil, whereas others cannot.[17] Therefore, we were interested in evaluating soils corresponding to vineyard areas used during this study as well as the association between chemical profiles of soils and wines. Elemental composition and isotopic analysis were performed on the bioavailable fraction of soils, because the composition of this fraction has been considered more directly correlated with the multielement composition of the vine leaves and grapes.[2] Some elements exhibit a good correspondence between its content in both soil and wine for the three provinces under study. For instance, Ba shows the highest values in Córdoba, lowest values in Mendoza, and intermediate values in San Juan soils. The same trend was observed for wine samples (Figure 4A). This behavior was also observed for K and La (data not shown). Likewise, B, V (Figure 4B−C), and Cd show the same pattern for both wine and soil samples from the same vineyard area. Ca shows a good correspondence between soils and wines from Mendoza and San Juan, but we did not observe a good association between soils and wines from Córdoba. This could be explained because Ca concentrations can be affected by enological processes, as the precipitation from wine during aging, with formation of calcium tartrate crystals.[12] Figure 5 presents normalized values for multiple elements measured for both wines and soils from the three studied areas, showing the correspondence between both data sets after normalization to avoid differences arising from the different magnitudes measured.

Looking for additional evidence on the correspondence between two studied matrices, we decided to apply GPA. GPA produces a configuration of the different geographical regions that reflects the consensus among the wines and soils. The result is a consensus alignment that uses all elements and isotopes from the two data sets. In Figure 6, the consensus configuration projected onto the plane defined by its first and second principal

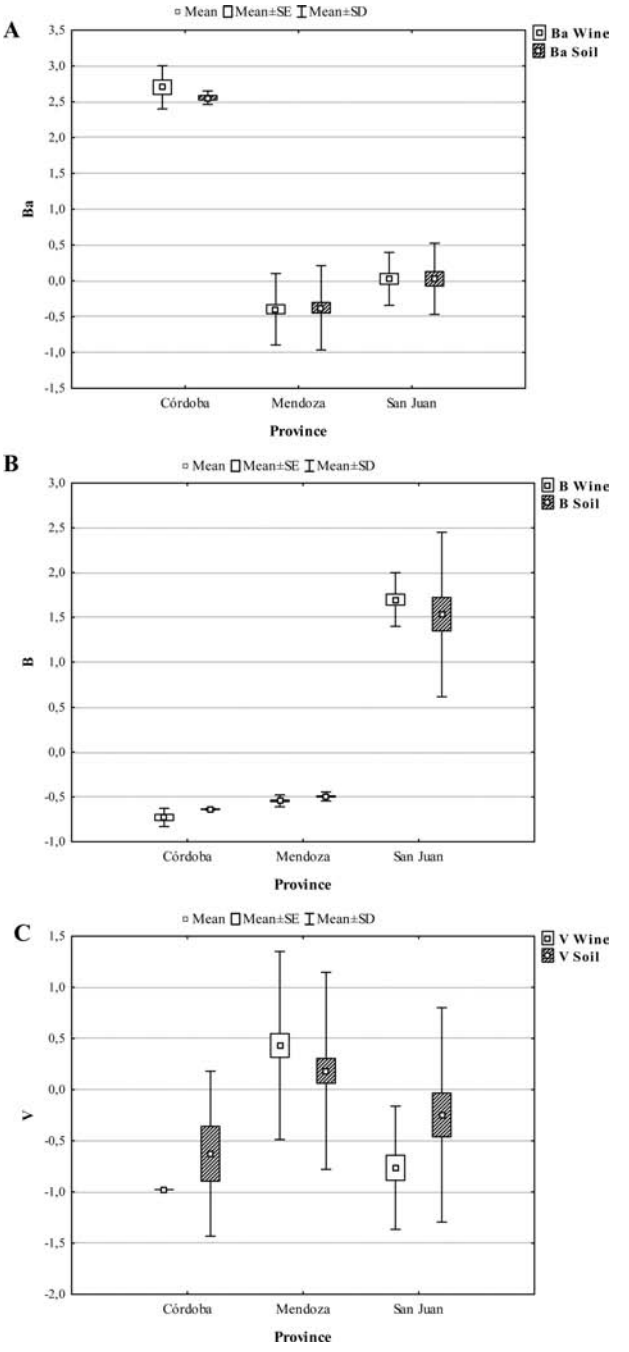

Figure 4. Correspondence between levels of several elements in wine and soil samples from different geographical areas (normalized values).

axes is shown, explaining 100% of variability between samples. We observe that the three geographical origins are well separated on the basis of the elements and isotopes of soil and wine samples. This result shows that data obtained from wine have a significant consensus (98.8%) with those corresponding to soil, as the two data sets project the regions in the same way onto the plane defined by its first and second principal axes. This last result gives further indication of the connection between soil and wine data sets.

Finally, we applied a CCA to assess the correspondence between soil and wine composition. For this purpose, two sets of variables were defined, selecting those parameters that DA

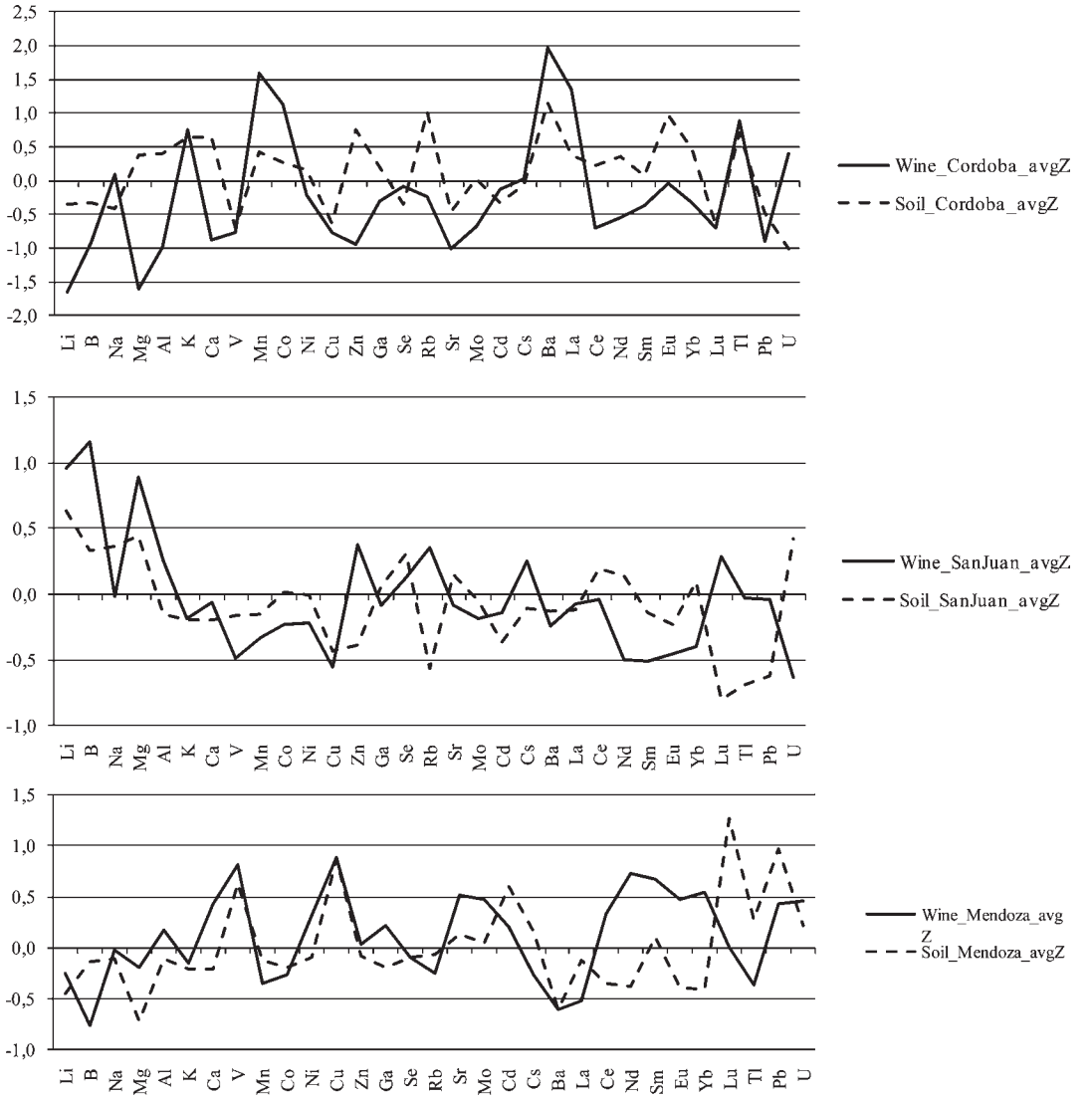

Figure 5. Normalized values for multiple element measured in both soil and wine at three sampling areas.

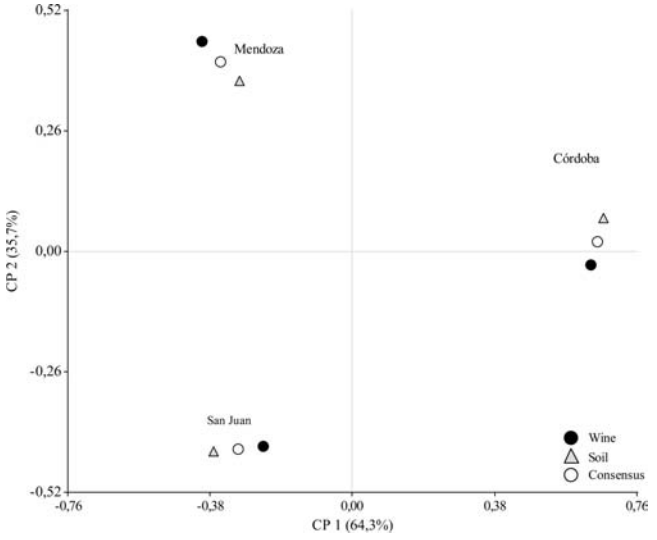

Figure 6. Configuration of the different geographical regions that reflects the consensus between the two matrices studied (wines and soils).

pointed out as the most significant to discriminate between wine varieties and regions. The CCA shows a significant correlation ($r = 0.99$; $p < 0.001$) between soil and wine data sets. This last result indicates that 99% of variability observed between wines could be attributed to the vineyard soil with consideration of its environment ("*terroir*"). Contents of Co, Ba, $^{87}Sr/^{86}Sr$, and Tl in wine as well as Ba content in soil show substantial loadings on the first canonical factor; that is, they correlate highly with this factor, meaning that these variables are those that mainly contribute to the correlation between soil and wine (data not shown).

We conclude that both elemental and isotopic compositions including geochemical ratios such as K/Rb and Ca/Sr allow a good differentiation among wine-producing regions. Mg concentrations and $^{87}Sr/^{86}Sr$ values were the best discriminators of wine provenance in the studied regions. Moreover, the inclusion of the phenolics profile allows a better differentiation between wine varieties from the same region, resveratrol being one of the most significant organic components for this purpose. It is worth remarking that, in this case study, DA gives satisfactory results for the wine differentiation, proving an important data reduction, selecting the most important variables for discrimination. Therefore, the use of combined

analytical sources (organic, inorganic, and isotopic components) presents a powerful strategy to obtain a reliable fingerprint for the evaluation of wine provenance in association with the characteristics of its terroir. Furthermore, GPA and CCA allow matching the wine profile with the soil composition.

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