Analytical Methods

Intra-regional classification of grape seeds produced in Mendoza province (Argentina) by multi-elemental analysis and chemometrics tools

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ABSTRACT

The feasibility of the application of chemometric techniques associated with multi-element analysis for the classification of grape seeds according to their provenance vineyard soil was investigated. Grape seed samples from different localities of Mendoza province (Argentina) were evaluated. Inductively coupled plasma mass spectrometry (ICP-MS) was used for the determination of twenty-nine elements (Ag, As, Ce, Co, Cs, Cu, Eu, Fe, Ga, Gd, La, Lu, Mn, Mo, Nb, Nd, Ni, Pr, Rb, Sm, Te, Ti, Tl, Tm, U, V, Y, Zn and Zr). Once the analytical data were collected, supervised pattern recognition techniques such as linear discriminant analysis (LDA), partial least square discriminant analysis (PLS-DA), k-nearest neighbors (k-NN), support vector machine (SVM) and Random Forest (RF) were applied to construct classification/discrimination rules. The results indicated that nonlinear methods, RF and SVM, perform best with up to 98% and 93% accuracy rate, respectively, and therefore are excellent tools for classification of grapes.

1. Introduction

The identification of the geographical origin of wine grapes (*Vitis vinifera* L.) is of remarkably important in today's globalized trade, because it is directly related with wine provenance and wine-making practices belonging to a specific region. Likewise, the knowledge of grape origin is highly valuable to winemakers in order to assure the quality of the wines being produced. In South America, Argentina is one of the main producers and exports high quality wines to different countries around the world. Among several regions of Argentina, Mendoza province is the most important grape and wine producing region, representing more than 75% of national production (Castex, Tejeda, & Beniston, 2015; International Organization of Vine and Wine (OIV), 2016). The natural conditions of Mendoza province, such as altitude, arid and rocky soils, and differentiated thermal amplitude between day and night, are ideal for the successful growing of several varieties of high quality grapes (white, red, and rose cultivars) (Fundación ProMendoza, 2016). However, besides high quality, ensuring the geographical origin of grapes is considered an important aspect by consumers, and hence, of great strategic value to the economies of the regions producing grapes and wines. Within this framework,

the traceability of grapes and wines produced in Argentina is being intensively controlled in recent years to certify their origin.
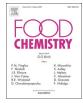
Recently, regional classification using chemometrics has been widely explored for wines from different parts of the world. Among the several factors that are strongly correlated and affect wine composition, mention can be made of the variety or varieties of grapes, the location where grapes are grown, the seasonal weather conditions, agricultural practices in vineyards, and the techniques used by winemakers, among others. However, the most influential variable on wine flavor is the grape variety due to differential chemical composition (Reynolds, 2010; van Leeuwen, 2010). Grapes show a high complex matrix composed of water, sugars, organic acids, phenolic compounds, vitamins, and minerals (Jackson, 2014). Moreover, it is well known that grapes are used for wine production and play an important role in the quality of this alcoholic beverage. In general, the elemental composition of wines reflects the geochemistry of the producing area, chemical composition of raw materials, as well as manufacture factors such as agricultural practices, or winemaking processes (Versari, Laurie, Ricci, Laghi, & Parpinello, 2014). Inorganic elements have several advantages as chemical markers because they are not metabolized and remain unchanged or transformed during enological processes (Pohl, 2007;

Saurina, 2010). Considering these aspects, the study of mineral profiles along with chemometrics tools could be used for classification of grapes. In fact, it could be a powerful strategy to discriminate wines and other grape-products coming from different geographical origins. Furthermore, trace elements in grapes are mainly located in their seeds (Fabani, Toro, Vázquez, Díaz, & Wunderlin, 2009; Rogiers, Greer, Hatfield, Orchard, & Keller, 2006), which is also very important because they are major end-products resulting from wine industry, accounting for 15% of the solid waste (Lachman et al., 2013; Mironeasa, Leahu, & Codină, Stroe, & Mironeasa, 2010).

Fingerprinting techniques combine chemical analysis with multivariate statistical analysis or advanced data mining methods to solve complex problems that require multidisciplinary approaches (Galgano, Favati, Caruso, Scarpa, & Palma, 2008; González-Centeno et al., 2010; Mutihac & Mutihac, 2008). In general, to generate reliable data on mineral contents of different kind of samples, elemental mass spectrometry is used. Currently, ICP-MS is the most used technique due to its high sensitivity and robustness for reliable multi-elemental determinations (Tanner & Günther, 2009). However, although several works have reported the application of ICP-MS along with chemometrics to discriminate wines, the classification of grapes according to their geographical origin has not been extensively studied (Cugnetto et al., 2014; Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2013; Versari et al., 2014). Moreover, to the knowledge of the authors, there are no studies where grape seeds have been used for fingerprinting grapes. Most probably, due to the fact that normally the entire grape berry is analyzed, without establishing clearly which part is more suitable to analyze in order to develop the chemometrics classification.

Accordingly, the purpose of the current work was to assess the potential use of several multivariate statistics tools combined with multi-element data obtained by ICP-MS analysis of grape seeds as an original source for the differentiation of grapes according to their geographical origin. The potential of this strategy was tested to discriminate grapes cultivated in nearby locations within Mendoza province. Thus, this work is different from others reporting the classification of grapes sourced from regions separated by large distances, where significant differences in soil composition might be expected and had a remarkable impact on chemical composition of grapes and their easy classification. Finally, this is the first work reporting the classification of grapes grown in Mendoza province according to their geographical origin.

2. Materials and methods

2.1. Instrumentation

Analytical measurements were performed using an inductively coupled plasma mass spectrometer, PerkinElmer-SCIEX, ELAN DRC-e model (Thornhill, Canada) fitted with an HF-resistant and high performance perfluoracetate (PFA) nebulizer, coupled to a baffled quartz-made cyclonic spray chamber, cooled with the PC^3 system from ESI (USA). Nickel sampler and skimmer cones were used.

The instrumental conditions were as follows: auto lens mode on, peak hopping measure mode, dwell time of 50 ms, 10 sweeps per readings, 1 reading per replicate and each analysis consisted of 3 replicates. After optimization of the instrument, the following conditions were chosen for all determinations: 1000 W RF power, sample introduction flow of $0.8 \, \mathrm{mL \, min^{-1}}$, nebulizer gas flow rate of $0.85 \, \mathrm{L \, min^{-1}}$. The instrument was calibrated against external certified standard solutions. To correct non-spectral interferences, Rh was used as internal standard for all determinations. The isotopes detected, in order of mass number, were as follows: ^{47}Ti, ^{51}V, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{69}Ga, ^{75}As, ^{85}Rb, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{98}Mo, ^{107}Ag, ^{130}Te, ^{133}Cs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{142}Nd, ^{152}Sm, ^{153}Eu, ^{158}Gd, ^{169}Tm, ^{175}Lu, ^{205}Tl, and ^{238}U.

2.2. Reagents and standards

All the reagents used were of analytical grade. Ultrapure water (18 MΩ·cm) obtained from a Milli-Q water purification system (Millipore, Paris, France) was used in the preparation of all solutions. Ultrapure concentrated nitric acid (65% v/v) purchased from Merck (Darmstadt, Germany) and hydrogen peroxide (30% v/v) from Biopack (Argentina) were used throughout. All the glassware was washed in $0.5 \, \mathrm{mol \, L^{-1}}$ HNO_3 solution for 24 h and later rinsed with ultrapure water before use. Argon (99.996%) from Air Liquide (Córdoba, Argentina) was used for ICP-MS determinations. Certified multi-element standard solutions 2, 3 and 5, and rhodium (^{103}Rh) mono-elemental standard solution from Perkin Elmer Pure Plus Atomic Spectroscopy Standards, (Norwalk, USA) were used.

2.3. Sample collection and analytical procedure

Vitis vinifera L. grapes were collected from vineyards located in five winemaking regions of Mendoza (Argentina): Rivadavia (33°11′S, 68°28′W), San Martín (33°04′S, 68°19′W), Guaymallén (32°54′S, 68°47′W), Junín (33°15′S, 68°43′W) and Maipú (32°58′S, 68°46′W), during the 2011 growing season. Sampling locations are shown in Fig. 1. The following grapevine varieties were collected: Cabernet Sauvignon, Malbec, Bonarda, Aspirant Bouchet (as red cultivars), Chardonnay, Sauvignon Blanc, and Pedro Jiménez (as white cultivars).

In order to obtain representative samples, individual bunches were randomly collected from each specific vineyards plot, obtaining a total of 408 samples. The grape bunches were manually harvested at their optimum maturity stage with an appropriate pair of scissors, cutting them carefully at the point of insertion of peduncle with vine shoot. Subsequently, the samples were refrigerated and carried immediately to the laboratory for analysis.

The grapes collected for multi-elemental determination were first washed with tap water and then rinsed with Milli-Q water. Grape seeds were separated from the rest of the whole bunches using plastic tweezers, and then washed with Milli-Q water to remove pulp residues. Then, seeds were lyophilized and finally pulverized with a mill.

Lyophilized seed samples were mineralized following the next procedure: 0.5 g were weighted and added with 6 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2. This mixture was left at room temperature for 12 h. Afterwards the following heat treatment was applied using a heating plate: 60 min at 50 °C, 90 min at 100 °C, and finally 90 min at 150 °C. The digested samples were left to cool until room temperature was reached and then transferred into a volumetric flasks and taken to a final volume of 25 mL with Milli-Q water. Subsequently, aliquots of 2 mL of the previous solution were taken and placed into a 50 mL flask and the volume was completed with Milli-Q water. These solutions were finally analyzed by ICP-MS.

2.4. Chemometrics techniques

All seeds samples were quantitatively analyzed by ICP-MS and characterized by twenty-nine descriptors (element concentrations). The data matrix for the chemometric treatment contained 408 rows and 29 columns. The matrix rows represented the number of samples, and the columns corresponded to trace element concentrations. The pre-processing of the dataset in the matrix was autoscaled due to wide differences in data dimensionality. For further chemometrical processing, grape seed samples of the different producing regions were identified as follows: SM for San Martín, RV for Rivadavia, JN for Junín, GY for Guaymallén, and MP for Maipú.

Multivariate statistical methods allowed verification of the contribution of each variable to the model and their ability to discriminate the different categories. Basic chemometrical characterization of the investigated grape seed samples was made by principal component analysis (PCA), which is an unsupervised technique. It was used as an