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Anthocyanic composition of Brazilian red wines and use of HPLC-UV–Vis associated to chemometrics to distinguish wines from different regions ☆

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ABSTRACT

This study determined and correlated the anthocyanin profile of Brazilian tropical (São Francisco Valley in Pernambuco), and temperate wines (Rio Grande do Sul), and temperate Chilean wines (Valle del Colchagua and Central Valley), with their geographical origins, using high performance liquid chromatography (HPLC) combined with a chemometric method, by applying Principal Component Analysis (PCA). The concentrations and the percentage of the nine anthocyanins (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside-acetate, malvidin-3-glucoside-acetate, peonidin-3-glucoside coumarate and malvidin-3-glucoside coumarate) were obtained and the values varied greatly according to the cultivar, vintage and country. The results demonstrated that wines from Rio Grande do Sul showed the highest levels of anthocyanin glucosides, which served as the discrimination factor for the chemometric analysis of the samples. Wine samples from the São Francisco Valley preferentially seem to follow the biosynthetic route Naringenin → Kaempferol → Cyanidin-3-Glucoside → Peonidin-3-Glucoside, unlike the samples from the other two regions (Chile and Rio Grande do Sul) that seem to follow the route Naringenin → Kaempferol → Delphinidin-3-Glucoside → Petunidin-3-Glucoside → Malvidin-3-Glucoside. The samples of the Syrah variety from the São Francisco Valley had higher concentrations of individual and total anthocyanins than the Chilean, suggesting the potential for successful adaptation of the cultivation of this grape to the terroir of the Brazilian northeast.

1. Introduction

Red wine is composed of polyphenolics, such as anthocyanin and flavonoid compounds, which have special importance due to their antioxidant properties, contributing also to the grape pigmentation and red color [1,2]. When subjected to acid hydrolysis, anthocyanins release their aglycones, called anthocyanidins. The most common anthocyanins found in foods are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, while in fine wines from *Vitis vinifera* L., the most important is malvidin [3].

The variation of anthocyanin composition and concentration in grapes is influenced by many viticultural factors, such as climate, seasonal influences, soil, vine nutrition, training and trellis systems, water management, and the winemaking process [4–7]. During the maturation stage of the grapes, the anthocyanin content increases progressively until it reaches a maximum value, known as phenolic maturation. At this point, there is a balance of color and structure of tannins, in the skins and seeds, indicating the optimum period for their harvest. However, in places with high temperatures, phenolic maturation is not completely reached in the hot seasons, principally for tannins in the seeds, that are hard and vegetal, which results in elaborated or unbalanced soft wines [8,9].

The term “terroir” has been used worldwide to describe the influence of the climate, soil, and human intervention on the chemical and metabolic compositions of the wines [10–15]. The studies revered to have established the composition of anthocyanins as information about typicality from various geographical origins [16]. Winegrowing areas with different climate and soil conditions can advance or delay the grape harvest, and allow the vines to get more or less water, which directly affects the composition of grape anthocyanins and consequently the wine produced from these grapes.

The “terroirs” of the winegrowing regions worldwide influence grapes and quality dry wines in different ways under different conditions: in temperate climates, for example, which have favorable thermal amplitude between night and day temperatures and low rainfall at maturity provide optimal conditions for phenolic maturation [1,17,18]. Different terroirs are found around the world. For example,

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in Chile, grape maturation occurs under low rainfall conditions, permitting a good maturation of the main red varieties, like Carmenere and Cabernet Sauvignon. In the South of Brazil, in the state of Rio Grande do Sul in the *Serra Gaucha* highlands, grapes are harvested between December and February, the period that has the highest rainfall in the South, decreasing the enological potential of the red wines, but providing good conditions for sparkling wines. In the São Francisco Valley, in the Northeast of Brazil, it is possible to have two harvests per vine a year, because the region has a high annual average temperature, intense solar radiation and water availability for irrigation. Wines can also change composition according to the month in which they were harvested [19–21].

To determine the anthocyanin profile, high performance liquid chromatography is the analytical technique of choice. Its use has been frequently reported in the literature, aiming at an analysis of the separation and determination of anthocyanin compounds isolated in grapes and wines from South Africa [22], Greece [23], China [24], Chile [25], Australia and New Zealand [26], France and Germany [27]. Several papers have reported on the use of the technique of high performance liquid chromatography (HPLC) combined with statistical methods for identifying geographical origin and harvest [28–34].

García-Beneytez et al. reported on the effectiveness of HPLC coupled to a mass analyzer for the detection 20 anthocyanins in grapes and experimental wines [35]. Otteneder et al. employed the technique for the quantification of anthocyanins in Portugieser wines and identification of adulteration by the ratio between anthocyanin with acetyl and coumaryl groups [36]. Walker et al. performed comparative studies between enzymatic and chromatographic methods for determining wine composition, demonstrating the potential of the HPLC technique for this kind of assay [37].

For the processing of a large amount of data, the use of chemometric methods, which facilitate the treatment of multivariate statistical data for extracting latent information and classification of samples, has proved a powerful tool for metabolomic studies [16,38–42]. Principal Component Analysis (PCA) is a method mainly used to describe samples present in an n-dimensional space order for pattern recognition and is able to extract the relevant information from a given data set of a multivariate nature to aid in understanding the model. PCA has been reported in the literature to describe various problems involving food and agricultural matrices, including grapes and wines [16,38,43–45]. This is an unsupervised exploratory technique which reduces the dimensions of an initial multivariate dataset to a smaller number of uncorrelated variables with maximized variance, i.e., that permits the analysis of a dataset using the most important variables.

The aim of this study was to determine and to correlate the anthocyanin profile of Brazilian tropical (São Francisco Valley in Pernambuco), and temperate wines (Rio Grande do Sul), and temperate Chilean wines (Valle del Colchagua and Central Valley), with their geographical origins, using high performance liquid chromatography (HPLC) combined with a chemometric method, by applying Principal Component Analysis (PCA).

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (HPLC-grade) was obtained from Baker (J.T. Baker, Phillipsburg, New Jersey, USA), HPLC grade formic acid (Sigma-Aldrich, USA) and malvidin-3-glucoside were supplied by *Sigma-Aldrich* (St. Louis, MO, USA) and HPLC water was purified with a Milli-Q system (Millipore, USA).

2.2. Standard solutions

Standard stock solution of malvidin-3-glucoside, the major anthocyanin in wines [33], was prepared by dilution of an appropriate volume of water to a final concentration of 150 mg L^{-1}. This solution was stored under refrigeration at −20 °C until analysis. Working solutions with concentrations of 2.0; 5.0; 10.0; 50.0 and 100.0 mg L^{-1} of malvidin-3-glucoside were used to construct the calibration curve [29].

2.3. Sample solutions

Thirty eight samples of dry red wines from Pernambuco (Brazil), Rio Grande do Sul (Brazil) and Vale del Colchagua/Vale Central (Chile) of different vintages, between 2009 and 2010 were purchased from commercial markets in Recife (Pernambuco, Brazil) as shown in Supplementary data (Table S1).

The red wines from Pernambuco, in the Northeast of Brazil, were: one sample of Cabernet Sauvignon (2008); one of Cabernet Sauvignon (2009); three of Cabernet Sauvignon (2010); one of Syrah (2008); two of Syrah (2009); two of Syrah (2010); five of Tannat (2009). The red wines from Rio Grande do Sul, in the south of Brazil, were: three samples of Cabernet Sauvignon (2009); three of Cabernet Sauvignon (2010); three of Merlot (2009); two of Merlot (2010); one of Tannat (2009); two of Tannat (2010).

The red wines from Chile were: three samples of Merlot (2009); three of Merlot (2010); two of Syrah (2009); one of Cabernet Sauvignon (2010).

All wines were stored in a dark room, at 5 °C, until analysis.

2.4. Sample preparation

The first aliquot of each wine was discarded and three other aliquots of 1.8 mL from each sample wine were collected. Samples were filtered through 0.45 μm membrane filters from Millipore (Brazil), transferred to vials, and 50 μL of each solution was injected into HPLC.

2.5. Equipment

The chromatography system consisted of a *Prominence* LC-20A series high performance liquid chromatograph from *Shimadzu* (Kyoto, Japan), equipped with a binary pump (LC-6AD), on-line degasser (DGU-14A), autosampler (SIL-20A), furnace (CTO-20A) thermostatized at 40 °C, and UV–VIS detector (SPD-10AVvp). Chromatographic separation for anthocyanins was performed in an YMC Pack CLC-ODS (M) column (250 mm × 4.6 m, 5 μm). The column temperature was set at 40 °C. Data acquisitions were performed using LC Solution v.1.21 software.

2.6. HPLC analysis

The methods presented in the literature were tested [28,31,35,46] and the methodology proposed by the *Organisation International de la Vigne et du Vin* (*OIV, 1990*), described in the *Resolution OENO 22/2003* [47] was used. The chromatographic separation of the anthocyanins was achieved using a mobile phase containing a solvent A of water: formic acid:acetonitrile (87:10:3, v/v/v) and a solvent B of water: formic acid:acetonitrile (40:10:50, v/v/v). Gradient elution of 94:6 A:B v/v to 70:30 A:B v/v (0–15 min); 70:30 A:B (v/v) to 50:50 A:B (v/v) (15–30 min); 50:50 A:B (v/v) to 40:60 A:B (v/v) (30–35 min) then to 94:6 A:B (v/v) (35–41 min) was used. The flow rate was 0.8 mL min^{-1} and the detection was performed at 518 nm.

2.7. Statistical analysis

Statistical analyses were applied to separate the wine samples in natural groupings and classify them by geographical location, using *Unscrambler 9.7*, software. The data matrix was composed of 38 samples × 9 variables. All data were auto-scaled before processing.