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Determination of metabolite profiles in tropical wines by ^{1}H NMR spectroscopy and chemometrics

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Traditionally, wines are produced in temperate climate zones, with one harvest per year. Tropical wines are a new concept of vitiviniculture that is being developed, principally in Brazil. The new Brazilian frontier is located in the northeast region (São Francisco River Valley) in Pernambuco State, close to the equator, between 8 and 9 °S. Compared with other Brazilian and worldwide vineyards, the grapes of this region possess peculiar characteristics. The aim of this work is a preliminary study of commercial São Francisco River Valley wines, analyzing their metabolite profiles by ^{1}H NMR and chemometric methods.

Keywords: ^{1}H NMR; wine; chemometric methods

Introduction

Wines have been produced in different countries for many centuries. Traditionally, the vitivinicultural regions are located in temperate zones in the north and south hemispheres, where it is possible to harvest grapes to make wines once a year. For instance, Europe, United States, Chile, Argentina, South Africa, New Zealand, Australia and southern Brazil (Rio Grande do Sul state).

Nowadays, other regions are being known as new producers of quality wines, extracted from grapes of *Vitis vinifera* L, like northeast Brazil, which is commonly referred as São Francisco River Valley. Commercial wine production in this region started approximately 20 years ago using few cultivars. This area presents a climate with intra-annual variability, with an annual average temperature of 26.4 °C (21.0 °C minimum and 31.7 °C maximum temperatures), and it is located at 350 m above sea level, in a flat landscape.[1–3] The rainy season occurs from December to March, with about 567 mm of normal rainfall.[2] The heliothermic availability (about 3000-h luminosity year^{-1}) allows continuous vegetative development, and grapevine cropping is possible throughout the year. But wineries prefer to produce wines from April to December (outside the rainy season) to make the phytosanitary control in the field easier, and to enhance grape quality.

In 2003, new cultivars were introduced in the region by Embrapa (the Brazilian Agricultural Research Corporation), in an experimental area located in three commercial wineries, to evaluate the potential of new cultivars to enhance wine quality and find a typicity for tropical wines. At this moment, the total area cultivated in the São Francisco River Valley is about 700 ha, and the cultivars used for red wines are Syrah (S), Cabernet Sauvignon (CS), Barbera (B), Castelão (C), Petit Verdot (PV), Tannat (T), Alicante Bouschet (AB), Ruby Cabernet (RB), Periquita (P), Touriga Nacional (TN) and, more recently, Tempranillo (TL). For white wines, the cultivars used are Chenin Blanc (CB), Moscato Canelli (MC), Viognier (VG) and also more recently, Sauvignon Blanc (SB). In 2007, the production of fine wine in this area was about 7 million liters.

The aim of this work was to determine metabolite profiles of tropical wines produced in northeast Brazil, by using ^{1}H NMR spectroscopy in combination with chemometric methods to analyze and discriminate wine samples from 2007 vintages produced by different wineries.

Experimental

Nine wine samples were obtained commercially. The red wine cultivars were PV, C, B and P and white wine cultivars VG and CB, all produced in the São Francisco River Valley in 2007.

In order to prepare the samples, 20 ml of each wine was evaporated under vacuum to remove the ethanol. The extracts were lyophilized for 6 h to reduce the residual water signal in ^{1}H NMR spectra. Finally, the samples were dissolved in D_2O, and the NMR analyses were carried out. The pH of these solutions was always equal to 4.0.

All NMR measurements were performed in a 5-mm tube, D_2O (99.9%, Aldrich) on a Varian Unity plus 300 spectrometer, operating at 299.0 MHz for ^{1}H at 20 °C. Sodium 3-(trimethylsilyl) propionate-d_4 (TSP 98%, Aldrich) was used as an internal chemical shift reference for ^{1}H NMR spectra. Each spectrum consisted of 32 scans of 32 K data points with a spectral width of 4500 Hz, an acquisition

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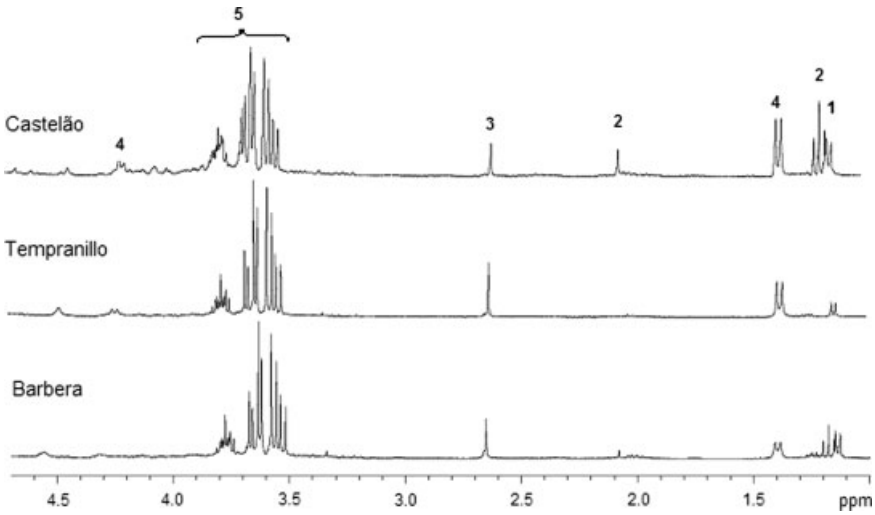

Figure 1. Part of ^{1}H NMR spectra of Castelão, Tempranillo and Barbera in D_2O, where 1 corresponds to butylene glycol, 2 to ethyl acetate, 3 to succinic acid, 4 to lactic acid and 5 to glycerol.

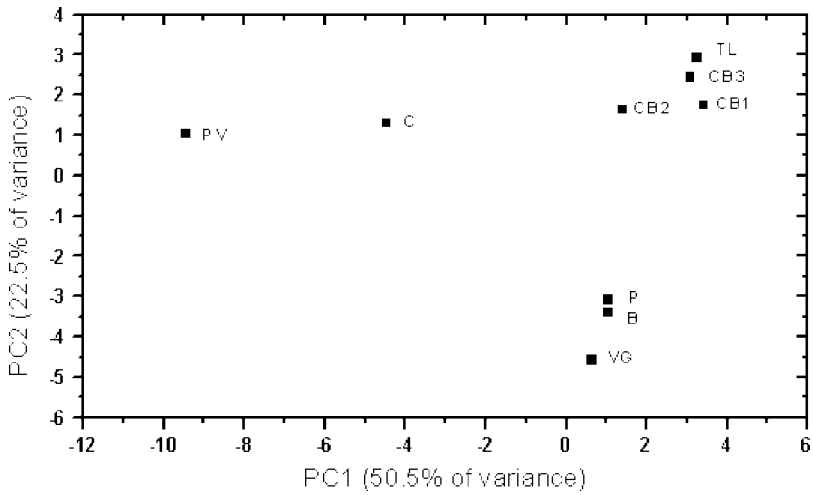

Figure 2. PC1 *versus* PC2 scores for 100 spectral bins of 1D ^{1}H NMR spectra plot of commercial wines (PV, C, B, P, TL, CB and VG) produced in São Francisco River Valley, Brazil.

time of 3.64 s, and a recycle delay of 3 s per scan. The pulse angle was 90°.

Before the statistical analyses, the 1D ^{1}H NMR spectra were segmented into about 100 spectra domains of 0.04 ppm (buckets) between 0.545 and 5.99 ppm (100 variables). The resonances between 4.8 and 5.0 ppm, associated mainly with a residual water signal, as well as other regions without NMR signal, were removed. Also, the data were converted to Excel software format, and further processed by STATISTICA® 6.0 software for PCA analyses. During preprocessing the data were autoscaled, i.e. each element on a column was subtracted by the average and scaled to unit variance on the column data.

Results and Discussion

Figure 1 shows three examples of representative ^{1}H NMR spectra. The spectra were well resolved and the principal components were identified.[4] The doublet at 1.41 ppm and the quartet at 4.24 ppm were attributed to lactic acid. The signals between 3.50 and 3.80 ppm were assigned to glycerol. Succinic acid was recognized as a singlet at 2.62 ppm. The doublet at 1.15 ppm was identified as butylene glycol. The signals at 1.19 ppm (triplet), 4.20 ppm (quartet – very small) and 2.08 ppm (singlet) were attributed to ethyl acetate. In comparison with other reported findings,[5–9] the diversity of compounds in our samples was similar.

According to previous research, grapes and wines in a temperate zone are easily discriminated by using ^{1}H NMR data and chemometric methods, where the metabolite variations are due to amino acids (proline, arginine, valine and isoleucine), organic acids (tartaric, malic and lactic), sugars (glucose, fructose and sucrose) and phenolic compounds.[10–12]

Figure 2 shows the score plot of the first two principal components that contain, approximately, three quarters of the original information. The graphic describes the data in three groups of analyzed wine samples. The model explains 50.5% of total variance in the first principal component, PC1, whereas the second principal component, PC2, explains 22.5% of the variance. The first group is formed by samples PV and C, both red wines. They are located on the negative side of PC1 and positive side

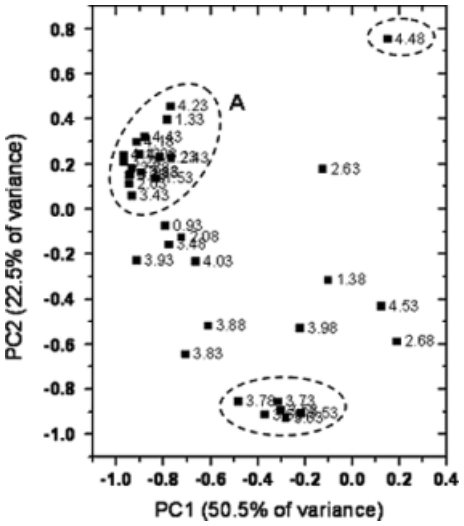

Figure 3. PC1 *versus* PC2 loading plot for commercial wines (PV, C, B, P, TL, CB and VG) produced in the São Francisco River Valley, Brazil. The labels correspond to the center chemical shift of the bucket.

of PC2. The second group is located on the positive side of PC1, represented by the other wines. This group can still be separated in two different ones along PC2. The first group, formed by samples of three wines (P, B and VG), is located on the negative side of PC2. The second group is made up of four samples, i.e. three samples of CB (CB1, CB2 and CB3) obtained from different wineries and one of TL.

The analyses of the 2D loading plot (Fig. 3) show that the separation along PC1 is dominated by the ^{1}H NMR chemical shift domain at 3.2–5.5 ppm associated with sugars (region A in Fig. 3). In this way, PC1 could distinguish dry and sweet wines, due to differential sugar content. The separation along PC2 is associated with chemical shifts at 4.48 ppm (tartaric acid) and 3.52–3.77 ppm, associated with glycerol. The signal at 4.48 ppm is responsible for the discrimination on the positive side of PC2, corresponding to wines CB and TL. Finally, the negative side is based on the spectra domains of glycerol.

On the other hand, amino acids played no important role in the clustering, although they were detected in small quantities in all samples. Small amounts of polyphenols were also observed; however, at the limit of NMR sensitivity at 300 MHz, their signals were too weak to be used as parameters for the chemometric analysis.

Tropical wines elaborated in northeast Brazil have been studied by routine analytical methods for the past 3 years. Many factors are being compared to understand the effects of *terroir* on grape and wine quality. A previous work has shown that rootstocks can play an important role in chemical composition.[13] Moreover, the intra-annual climate variability can modify grape and wine composition, being necessary to use different winemaking techniques for each condition.[14]

In association with routine analytical analyses, NMR spectroscopy has given us more information concerning the metabolic profile of wines in a new producer region with particular conditions, as described above. This knowledge is important to advance in order to obtain the Designation of Origin Certificate of São Francisco River Valley Wine. In addition, further work is necessary and is being carried out, also comparing wines from other regions in Brazil, like Rio Grande do Sul, Santa Catarina and Minas Gerais (located in temperate zones). We hope to find metabolic markers that could be used to systematically discriminate various wines, provide a tool for quality control and also to explain major differences between temperate and tropical brands.

Conclusion

This work describes the first preliminary ^{1}H NMR and chemometric study of samples from wine originating from the São Francisco River Valley. The use of ^{1}H NMR spectroscopy in combination with multivariate statistical techniques allowed us to distinguish wines according to their composition. The metabolite profile discriminated the sweet and dry wines, according to sugar content, while glycerol and tartaric acid were responsible for separation and clustering along PC2. The amino acids and polyphenols were not discriminating factors in this analysis due to the current level of sensitivity of the NMR measurements. Further work is being carried out to widen the scope and quality of wine analysis and characterization.

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