^{1}H NMR SPECTROSCOPY DATA TO DISCRIMINATE PETIT VERDOT WINES FROM THREE DIFFERENT SOIL TYPES IN THE SÃO FRANCISCO VALLEY, BRAZIL

Giuliano Elias Pereira[1,*] Fernando Hallwass[2,*]; Raphael Soares[3]; Marcos Martins Masutti[4]; Juliane Barreto de Oliveira[5]

[1]Enology Researcher, Brazilian Agricultural Research Corporation – Embrapa Grape & Wine/Tropical Semiarid, P.O. Box 23, Zip Code 56.302-970, Petrolina-PE, Brazil; [2]Department of Chemistry, Federal University of Pernambuco, Av. Luiz Freire s/n, 50.740-540, Recife-PE, Brazil; [3]IF Ipojuca, PE 60, km 14, Califórnia, Zip Code 55590-000, Ipojuca-PE, Brazil; [4]IF Sertão Pernambucano, Zip Code 56.300-000, Petrolina-PE, Brazil.

[*]E-mails: giuliano.pereira@embrapa.br and hallwass@ufpe.br

Abstract

Tropical wines have been produced in the São Francisco river Valley thirty years ago, in the Northeast of Brazil. The main grape cultivar used for red tropical wines is 'Syrah', but wines have presented fast evolution, if they were made in the first or second semester, due to the high values of pH in grapes and wines and high climate temperatures. In the region, vine can produce twice a year, because annual average temperature is 26.5°C, with high solar radiation and water from irrigation. Petit Verdot cultivar was used commercially in one winery until 2011, when this one stopped to produce fine wines (*Vitis vinifera* L.) to produce table wines (*Vitis labrusca*). This cultivar presents a high powerful to help tropical wines increasing their stability, due to the high acidity, low pH e high phenolic concentration in the grapes and red wines. NMR spectroscopy is a powerful tool allowing in a single analysis to find many analytical compounds in grapes and wines. PCA multivariate statistical analysis applied on NMR data allows to discriminate samples and to identify markers compounds from the variables evaluated. The aim of this work was to evaluate Petit Verdot wines harvested in three different soils, the first one sandy, the second one gravelly cambisoil and the third one sandy-clayey argisoils, by using 1H NMR spectroscopy data. Vines were planted in 2002 in the winery on six hectars, conducted on traditional lyre, grafted onto 101-14 Mgt and irrigated by drip. Grapes were harvested in January 2014 and wines were elaborated by traditional red winemaking, then analyzed one month after bottling by 1H NMR spectroscopy. It was possible to determine alcohols, organic and amino acids, and phenolics in the Petit Verdot wines. Results showed a significantly difference in terms of metabolic compounds, of the wines evaluated from the three different soils. PCA was able to find markers from each soil type. Results are discussed according to the enological potential of each plot.

Keywords: Vitis vinifera L.; grapes; tropical wines; ^{1}H NMR spectroscopy; metabolic fingerprint; multivariate statistical analyses.

1 INTRODUCTION

In the Northeast of Brazil, fine wines started to be elaborated in the middle of the 80's by using grapes to red and white wines. The main characteristics of the region are high temperatures throughout the year, with annual average of 26.6°C, with high solar radiation and water available for irrigation from the São Francisco river. According to these characteristics, one vine can produce twice a year, and wineries can choose when they want to cut and harvest grapes, which decision depends mainly of the market (Lima et al. 2014).

Until the end of the 90's, these wines were well known from the region, and then other wineries arriving decided to produce sparkling wines, because Brazilian market asking these products. This change was also because red wines from Chile and Argentine present low prices and Brazilian consumers prefer imported wines than national wines. Tropical red wines have presenting fast evolution due to the high values of the pH in the soils, and also because high temperatures in the second semester cause decreasing of acidity and increasing wine pH, and phenolic degradation. Enologists use adding of acids to reduce pH and maintain acidity in these cases.

The main variety used for red and some rosés wines in the region is cv. Syrah, followed by Tempranillo, Touriga Nacional, Ruby Cabernet and Petit Verdot. But the last one, since 2014, have stopped to be used for commercial wines by the winery, that started to produce table wines, using *Vitis labrusca* varieties (Pereira et al. 2011). Petit Verdot wines have presented interesting characteristics in the region, like high acidity, low pH and could help to maintain wine stability.

Soils can influence strongly grape and wine characteristics as showed in many works (Van Leeuwen et al. 2004). To evaluate grape and wine compounds, ^{1}H NMR is used successful with multivariate statistical analyses, as showed in our papers (Pereira et al. 2005, 2006 and 2007).

In this context, the aim of this work was to determine metabolic profiles of wines using ^{1}H NMR and chemometrics, to discriminate samples from three different soils, in the São Francisco Valley, Northeast of Brazil.

2 MATERIALS AND METHODS

Winery is located in Lagoa Grande, Pernambuco State, at 09° South Hemisphere, belonged to a Frenchman until 2008. Petit Verdot vines, which clone was brought from Bordeaux France, were planted in traditional lyre, grafted onto 101-14 Mgt in 2002 and irrigated by drip, in six hectars. Studies carried out in the areas showed three different soils that were well characterized. The first one is as Abruptic eutrophic plinthosol, classified as sandy-clayey soil, a yellow argisoil (68% sand and 15% clay). The second one is an Eutrophic soil, classified as an Haplic Cambisoil, and it is a sandy gravelly soil (67% sand and 15% clay). And the last one was described as an Abruptic eutrophic plinthosol, a red-yellow argisoil, and it is a sandy soil (83% sand and 7% clay).

A total of ninety vines were randomized marked in each soil type, three times thirty vines, and grapes were harvested to winemaking in January 2014. Three wines were elaborated from each soil type, in glass thanks of 20 liters each one. Traditional red winemaking was adopted, with only ten days of maceration, and alcoholic and malolactic fermentations were developed in good conditions. At the end, wines were corrected with sulphur dioxide, then bottled and left thirty days in the cellar before analyses.

Analyses were made in the nine wines, three from each soil type, to determine density, pH, total and volatile acidities, alcoholic degree, total and frre sulphur dioxide, total anthocyanins, total phenolic index and colours at 420, 520 and 620 nm (OIV 1990). And also ^{1}H NMR spectroscopy was used to evaluate metabolic compounds (Pereira et al. 2007, Gomes Neto et al. 2009). 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 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 signals, 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 (Pereira et al. 2005 and 2007).

3 RESULTS AND DISCUSSION

Grapes were analyzed and presented significant differences according to the soils (data not shown). 1D ^{1}H NMR spectroscopy allowed us to obtain nine spectra that can be view in the Figure 1. The main compounds found in the 1D ^{1}H NMR spectra of the wines were glycerol, between 3.5 and 3.8 ppm (number 2), succinic and acetic acids recognized as a singlet at 2.6 ppm (number 3) and 2.1 ppm (number 4), respectively, lactic acid identified as a doublet at 1.4 ppm and the quartet at 4.2 ppm (number 5), and finally 2,3-butanediol, identified as the signal at 1.1 ppm (number 6).

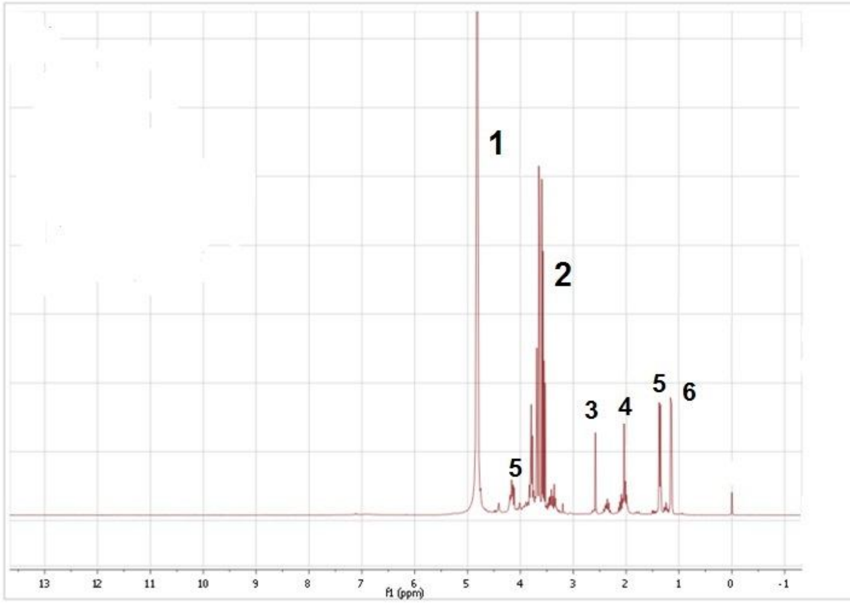

Figure 1 shows an example of representative ^{1}H NMR spectrum of Petit Verdot wine. The signal at number one in spectrum corresponds to residual water. The signals between 3.5 and 3.8 ppm (number 2) were assigned to glycerol. Succinic acid and acetic acid was recognized as a singlet at 2.6 ppm (number 3) and 2.1 ppm (number 4), respectively. The doublet at 1.4 ppm and the quartet at 4.2 ppm were attributed to lactic acid (number 5). Finally, the signal at 1.1 ppm (number 6) was attributed to the presence of 2,3-butanediol.

Wines presented differences as showed in the Table 1. It is possible to see that soils influenced significantly classical analyses made in nine Petit Verdot wines, three replicates for each soil type.

Table 1: Classical analyses of Petit Verdot wines from three soil types (sandy-clayey, sandy gravelly and sandy soils), in the Northeast of Brazil, which grapes were harvested in January 2014. TPI: total polyphenol index. Each result is the mean obtained from three wines for each soil type.

Parameter	Sandy-clayey soil	Sandy-gravelly soil	Sandy soil
1. Density	0.995a	0.993a	0.994a
2. pH	3.6a	3.5a	3.7a
3. Alcoholic degree (v/v)	17.8a	16.7b	16.4b
4. Total acidity (g L^{-1} tartaric acid)	8.2a	8.6a	8.4a
5. Volatile acidity (g L^{-1} acetic acid)	0.3a	0.3a	0.4a
6. Total SO2 (g L^{-1})	31.2c	33.2b	38.1a
7. Free SO2 (g L^{-1})	25.2b	25.6b	30.9a
8. Total anthocyanins (mg L^{-1})	1,030.8a	975.1b	965.4b
9. TPI	120.9a	117.4b	112.8c
10. Color 420 nm	12.1a	11.3b	10.9c
11. Color 520 nm	17.8a	18.1a	16.5b
12. Color 620 nm	5.1a	5.1a	4.8b

Means followed by the same letter did not differ at 5% probalility by Tukey Test.

Values determined did not differ for density, pH, total and volatile acidities. In the case oh pH, it is interesting to see that even the harvest made in January, considered between October and January the most warmest months of the year, the values were very low, as compared to results obtained in our previous works (Pereira et al 2011 and Gomes Neto et al 2009). The low values can suggest that Petit Verdot must and wines could be blended with other red wines, helping a natural control for decreasing pH of commercial wines from the São Francisco river Valley. In relation to the other parameters, all presented significant differences according to the soil type. The alcohol degree was high to the wines from sandy-gravelly soil, that was confirmed by the highest values of sugars found in the grapes (data not shown), as compared to the wines from sandy-gravelly and sandy soils. Van Leeuwen et al (2004) showed that the main effect influencing on grape quality was climate, followed by soil and then cultivar. It seems that high percentage of clay found in the sandy clayey and sandy-gravelly soils resulted in the most concentrated wines. This fact can be confirmed on phenolic concentration of the wines. Wines from sandy-clayey soils presented the highest values for total anthocyanins, total polyphenol index and colors, not differed of wines from sandy-gravelly for color index at 520 and 620 nm. These results can be confirmed by phenolics determined on grapes by high performance liquid chromatography (data not shown).

Figure 2 shows results of the principal component analysis-PCA applied on ^{1}H NMR data, obtained from nine wines from three different soils, which harvest was in January 2014. Principal component-PC 1 explained 67.7% of total variability, which PC2 explained 18.9% of the variability. It can be observed that Petit Verdot wines from sandy-clayey and sandy gravelly soils were separated of wines from sandy soils, in PC1. The main compounds explaining the variability were glycerol and 2,3-butanediol, in the positive side of PC1, confirming the highest values of alcohol content in wines from sandy-clayey soils than wines from sandy-gravelly and sandy soils. In the negative side of PC1, characterizing Petit Verdot wines from sandy soils, it was identified acetic acid, that can be confirmed by the highest values of Petit Verdot wines (0.4 g L^{1} of acetic acid) as compared to Petit wines from the other two soil types, despite no statistical differences were found between the wines (Table 1). These results were different as compared to our previous work made with Castelão, Tempranillo, Barbera and Petit Verdot experimental wines in 2007 (Gomes Neto el al. 2009).

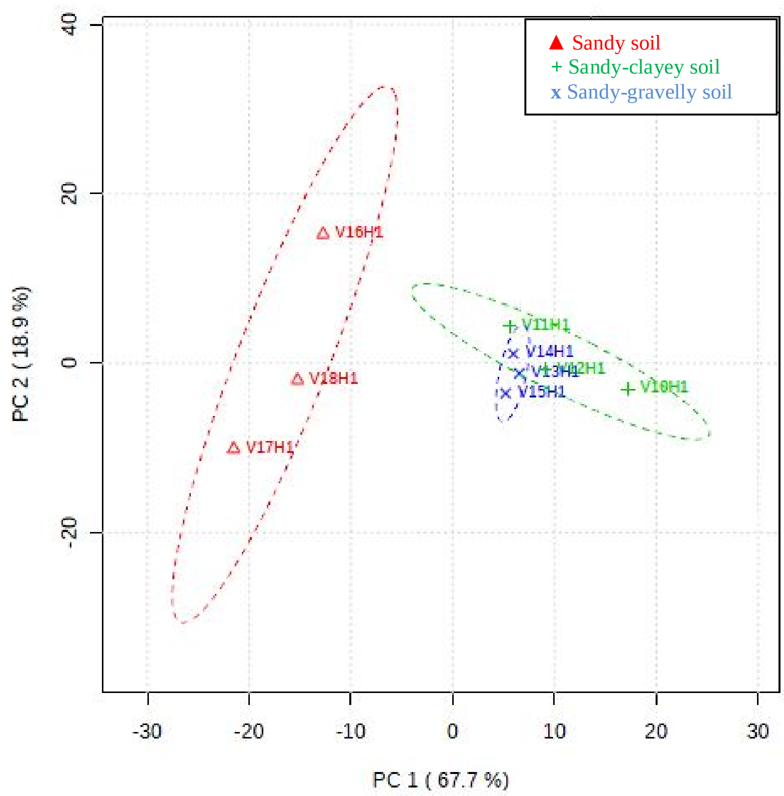

Figure 2 shows the principal component analysis-PCA applied on ^{1}H NMR data, obtained from nine wines from three different soils. Principal component-PC 1 explained 67.7% of total variability, which PC2 explained 18.9% of the variability.

4 CONCLUSION

Petit Verdot wines presented different enological potential according to the soil type. Wines from grapes cultivated in a sandy-clayey soil presented the highest values of alcohol degree than wines from sandy-gravelly and sandy soils. pH of the wines were low for this period of the year, being possible to be used to a natural correction of pH and acidity in blends with other red wines. Phenolics of the wines from sandy-clayey and sandy-gravelly presented high values. Chemometrics applied on 1D ^{1}H NMR data obtained from nine Petit Verdot wines, elaborated with grapes from three different soil types, allowed us to discriminate tropical wines. The main compounds identified explaining clusterings were glycerol, 2,3-butanediol and acetic acid.

Acknowledgments (Times New Roman, bold, italic, 10pt, left aligned)

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