Microchemical Journal 110 (2013) 256–262

Contents lists available at SciVerse ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

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

Raphael Henrique Soares de Andrade [a,b], Lígia Samara do Nascimento [a,b], Giuliano Elias Pereira [c], Fernando Hallwass [a], Ana Paula Silveira Paim [a,*]

[a] Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, Av. Jornalista Aníbal Fernandes, s/n, Cidade Universitária, CEP 50.740-560 Recife, PE, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia de Pernambuco, Campus Ipojuca, PE 60, km 14, s/n, Califórnia — Ipojuca, CEP 55.559-000 PE, Brazil
[c] Embrapa Uva e Vinho/Semiárido, BR 428, km 152, CP 23, CEP 56.302-970 Petrolina, PE, Brazil

ARTICLE INFO

Article history:
Received 16 January 2013
Received in revised form 20 March 2013
Accepted 3 April 2013
Available online 26 April 2013

Keywords:
Anthocyanin
Malvidin
Wines
HPLC
PCA
Geographical origin

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.

© 2013 Elsevier B.V. All rights reserved.

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,

☆ Paper presented at 5th Ibero-American Congress of Analytical Chemistry 2012.

* Corresponding author. Tel.: +55 81 21267469; fax: +55 81 2126 8442.
E-mail addresses: anaspaim@pq.cnpq.br, anaspaim@ufpe.br (A.P.S. Paim).

0026-265X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2013.04.003

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.

3. Results and discussion

3.1. Chromatographic analysis

The main goal of the method was the separation of the nine anthocyanins in a single run, using a UV–VIS detector and measuring each analyte at its maximum absorption wavelength. The methodology, proposed by *Organisation International de la Vigne et du Vin* (*OIV*), described in *Resolution OENO 22/2003* [46,47], resulted in an adequate, efficient and selective means for the separation of the analytes under study (Fig. 1).

The anthocyanins determined in the wine sample and numbered in Fig. 1 constitute three groups of substances: anthocyanidin-3-glucoside non-acylated (Del-3-Gl, Cya-3-Gl, Pet-3-Gl, Peo-3-Gl and Mal-3-Gl); anthocyanidin-acetyl-3-glucoside (Peo-3-AcGl and Mal-3-AcGl) and anthocyanidin coumaril-3-glucoside (Peo-3-CouGl and Mal-3-CouGl). The most abundant anthocyanin in all samples was Mal-3-Gl (peak 5). The chromatograms of an individual identification of 100 mg L^{-1} malvidin-3-glucoside and a Cabernet Sauvignon wine sample are shown in Fig. 2.

As shown in Figs. 1 and 2(b), the wine samples were composed of nine anthocyanins and the malvidin (Mal-3-Gl) was the phenolic compound that presented the signal with the highest intensity.

As the concentration of malvidin determined in the wines was about 30 mg L^{-1}, the analytical curve from 2.0 to 100 mg L^{-1} malvidin was chosen, presenting a coefficient (R^2) of 0.9978. The regression equation was $Y = 7 \cdot 10^{-6}X + 3.919$, where Y is the area and X is the *malvidin-3-glucoside* concentration. All compounds were quantified in relation to the malvidin-3-glucoside, used as standard.

3.2. Statistical analysis

The results presented in this study were preliminary and made it possible to evaluate trends of group formation with the samples analyzed. PCA was applied in order to ascertain trends or group formations of wine samples from different regions and to show and to explain the wine variability according to different metabolites identified (Figs. 3 and 4). The samples presented different concentrations and nine anthocyanins were identified.

The wine selected to represent the three regions studied (Rio Grande do Sul, Chile and Pernambuco) was Cabernet Sauvignon, as this grape is commonly used for winemaking in many commercial wines. PCA with full cross-validation of the data was performed with the information from data which had been previously auto-scaled. Fig. 3 shows the graph of the scores of the first and second principal components. As we can see, the first principal component (PC1) accounts for 64% of the total variability in the original data. The second principal component (PC2) accounts for 24% of the variability, i.e., together, PC1 and PC2 account for 88% of the total variance.

It is apparent that samples showed grouping trends in three distinct groups. The first grouping trend, located on the central portion of PC1 and PC2, is constituted from the Brazilian samples from Rio Grande do Sul; the second grouping trend, located in the negative portion of PC1 and PC2, is formed from the Brazilian samples from the São Francisco Valley; and the third grouping trend, in the positive portion of PC1 and negative of PC2, contains the Chilean wines.

The loadings, illustrated in Fig. 4, are the coefficients that define the weight of each original variable in the PCs, and can be used to understand which anthocyanins are responsible for the differences among the wine samples from the three geographic regions.

A comparison of scores and loadings for PC1 demonstrates that the content of Pet-3-Gl, Peo-3-Gl and Mal-3-Gl have higher importance (on the right side), and Peo-3-AcGl, Peo-3-CouGl and Mal-3-CouGl have lesser importance; while the PC2 is represented by the content of Peo-3-CouGl and Peo-3-AcGl (the positive side) and Mal-3-Gl (the negative side). We observed that the levels of anthocyanin in glycosidic form pigments (Mal-3-Gl, Del-3-Gl, Cya-3-Gl, Peo-3-Gl and Pet-3-Gl) were higher in samples from Chile than in samples from the São Francisco Valley.

Apparently, the climatic conditions of Chile and RS promote the synthesis and accumulation of anthocyanins, which is consistent with the literature that affirms that these processes are favored under appropriate conditions of temperature, light and intra-day thermal amplitude [48].

The concentrations and the percentage of the nine anthocyanins were obtained. The content percentage was calculated from the ratio of the concentration of each anthocyanin in relation to the total content of the same in its respective classes. Thus, for the glycosylated anthocyanin, the content of each sample was determined relative to the total content of this class. The same procedure was followed for acetylated and coumarylated anthocyanin as shown in Table 1. The values vary highly according to the cultivar, vintage and country.

The interval of individual and total anthocyanin concentrations in Cabernet Sauvignon wines indicates that, in general, the wine samples from the SFV were of a lower quantity than those from RS and Chile. Studies have demonstrated that the influence of warm weather and sunlight on the grapes causes rapid maturation of pigments and therefore reduces concentration in the berry [49].

However, in the samples of Syrah from the Northeastern of Brazil, the anthocyanin concentrations were higher than those from other regions, suggesting that the adaptation of this cultivar happened satisfactorily with respect to the aspect being evaluated. Syrah is a very plastic cultivar, which can adapt to many different terroirs [50].

With regard to the Merlot cultivar, the individual or total anthocyanin concentration ranges were more pronounced in samples from RS than those from Chile suggesting that the terroir of Southern Brazil

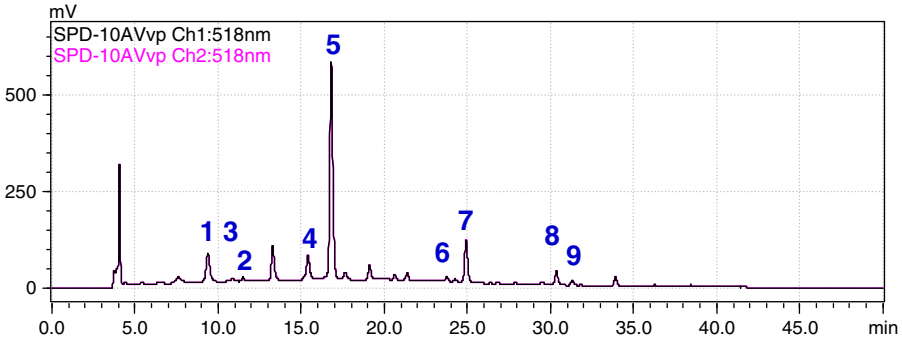

Fig. 1. HPLC chromatogram of the Syrah wine from Northeast of Brazil, showing the follow compounds identified: (1) delphinidin-3-glucoside (Del-3-Gl); (2) cyanidin-3-glucoside (Cya-3-Gl); (3) petunidin-3-glucoide (Pet-3-Gl); (4) peonidin-3- glucoside (Peo-3-Gl); (5) malvidin-3-glucoside (Mal-3-Gl); (6) peonidin-3-glucoside-acetate (Peo-3-AcGl); (7) malvidin-3- glucoside-acetate (Mal-3-AcGl); (8) peonidin-3-glucoside coumarate (Peo-3-CouGl) and (9) malvidin-3-glucoside coumarate (Mal-3-CouGl).

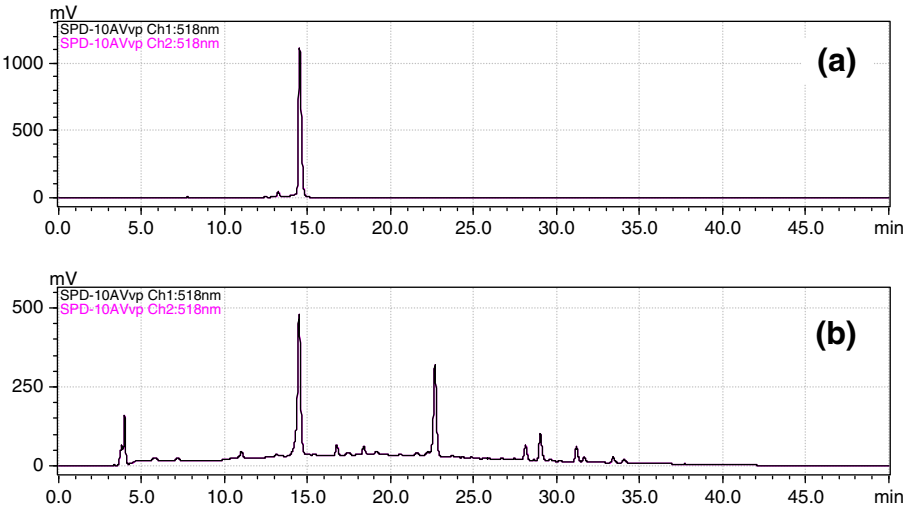

Fig. 2. HPLC Chromatograms obtained from (a) a standard solution 100 mg L^{-1} *malvidin-3-glucoside* and (b) a wine sample of *Cabernet Sauvignon* from Chile.

is more favorable for the synthesis and/or accumulation of these pigments.

The percentual data interpretation was based on the biosynthetic pathway of anthocyanins, in accordance with some reports in the literature on the relationship between aspects of the terroir to the chemo-sensory characteristics of the grapes and wines made from them.

In the synthesis of these pigments, the precursor naringenin is converted to dihydrokaempferol, which, in turn, can follow three different biosynthetic pathways, resulting in three anthocyanic forms such as free and subsequently glycosylated cyanidin, pelargonidin and delphinidin. The route of cyanidin-3-glucoside finishes with its bioconversion to peonidin-3-glucoside; the route of pelargonidin goes no further. The route of delphinidin-3-glucoside can generate malvidin-3-glucoside or petunidin-3-glucoside. The petunidin-3-glucoside may be converted into malvidin-3-glucoside as well. This complex scheme is dependent on the regulatory activities of the genetic expression and enzymatic catalysts involved [48,51].

The amount of Cya-3-Gl (Gl%) and Peo-3-Gl (Gl%) in cultivars from the São Francisco Valley, compared to the respective cultivars from other regions, was slightly higher, while the percentages of Pet-3-Gl (Gl%) and Mal-3-Gl (Gl%) in cultivars from other regions were higher than the SFV samples, except for the Syrah varieties from SFV.

This observation can be explained because the anthocyanin glucosides of the SFV samples preferably follow the route: naringenin → kaempferol → cyanidin-3-glucoside → peonidin-3-glucoside. This would be related to soil and climatic conditions of the semi-arid region of the northeastern part of Brazil, which is characterized by high temperatures, and high solar radiation, among other factors. These results were in agreement with those reported in the literature [51].

The values obtained for the Mal-3-Gl (% Gl) of the Cabernet Sauvignon red wine varieties produced in Rio Grande do Sul were slightly lower and higher than those produced in Chile and SFV, respectively. Besides, the RS wines presented significantly higher *Del-3-Gl (% Gl)* and *Pet-3-Gl (% Gl)* values than the wines from Chile, while the opposite occurred with the Merlot samples. Thus, the varieties of Cabernet Sauvignon from the Chilean terroir indicated the enzymatic activities of conversion *Del → Mal* and/or *Pet → Mal*; while the terroir of southern Brazil favored the Merlot varieties in this bioconversion.

The varieties Syrah presented higher values of *Mal-3-Gl (% Gl) and lower values of Del-3-Gl (% Gl)* and *Cya-3-Gl* (%Gl) in wine samples from the SFV than the other varieties. This occurs due to the transformation of *Del-3-Gl → Mal-3-Gl* and *Cya-3-Gl → Cya-3-AcGl*, due to the terroir of Brazil northeast.

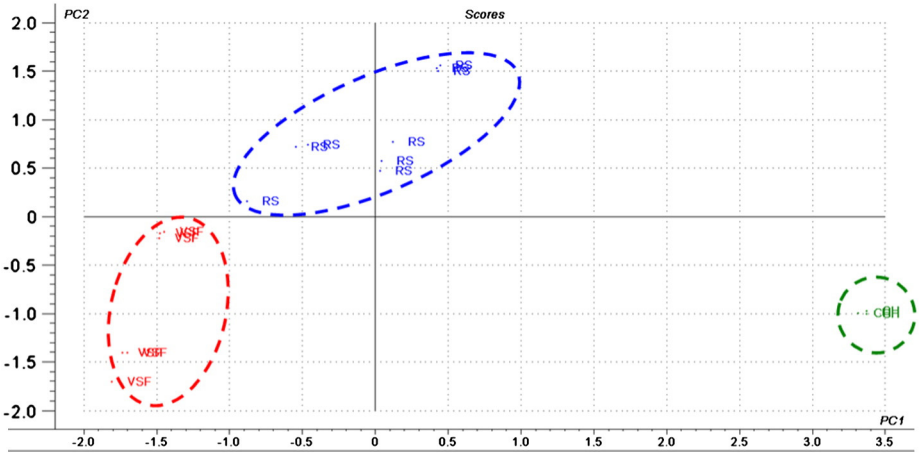

Fig. 3. Scores plot of PC1 × PC2 obtained from anthocyanin data of the *Cabernet Sauvignon* wines from Rio Grande do Sul (RS), São Francisco Valley — Pernambuco (SFV) and Chile (CH), explaining 88% of total variability.

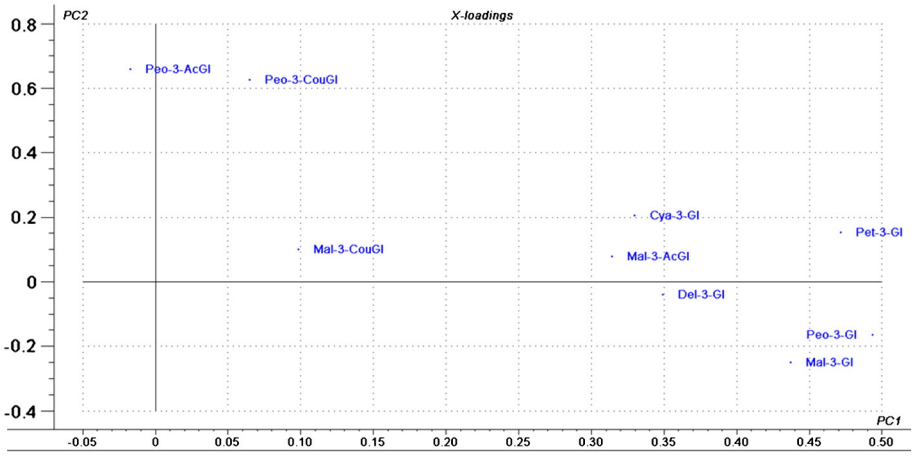

Fig. 4. Loadings of *PC1* × *PC2* obtained from anthocyanin data of the *Cabernet Sauvignon* wines from Rio Grande do Sul (RS), São Francisco Valley — Pernambuco (SFV) and Chile (CH).

Furthermore, the values of acetylated and coumarylated anthocyanins had higher percentual values in wine samples from the SFV than those from the other regions evaluated, particularly as regards the cultivar Cabernet Sauvignon. This could be explained based on soil and climatic conditions of this region (warm climate with high exposure of the grape berries to sunlight) that collaborate with the enzymatic activity of anthocyanin acyltransferase, that converts the glucoside forms into acetylate and, consequently, improves the stability of the pigments and color of the fruit [48].

The glucosidic anthocyanins are more stable than their free forms. Naturally, these pigments are glycosylated in the grape pulp and acylation occurs, producing the acetyl-glucosidic form, thus elevating the stability of anthocyanins and preserving the color of fruits [48].

Studies have demonstrated that factors such as pH, temperature, sunlight exposure, structure, and enzymes affect the stability and biosynthesis of anthocyanins. These occur because these factors can modify both the expression of the structural and regulatory genes and the activity of the enzyme involved in the biosynthetic pathway [48].

4. Conclusions

The samples of 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. This can be explained by climatic conditions favorable to the synthesis/accumulation of these

Table 1
Concentration interval (mg L^{-1}) and percentage of individual anthocyanin concentrations (m/m) determined in wines from three regions.

Origin of wine	São Francisco Valley			Rio Grande do Sul			Valle Del Colchagua and Central Valley		
Cultivar	Cab Sauv	Sy	Ta	Cab Sauv	Me	Ta	Cab Sauv	Sy	Me
Intervals of anthocyanin concentration									
Del-3-Gl	6.26–22.4	6.81–16.5	6.27–15.2	11.8–34.1	11.3–21.9	6.32–16.4	19.3	7.47–22.0	9.75–9.85
Cya-3-Gl	4.04–8.12	6.58–9.24	4.69–11.5	6.46–13.9	6.29–9.86	7.14–7.99	10.2	4.69–10.8	6.70–7.04
Pet-3-Gl	6.38–10.9	13.3–25.8	7.88–16.2	14.0–38.3	12.2–28.9	14.8–18.2	24.2	7.65–19.5	13.1–14.6
Peo-3-Gl	5.70–20.3	13.1–31.3	5.68–19.1	9.38–24.2	9.89–20.4	6.49–14.6	24.9	5.64–19.6	6.49–10.3
Mal-3-Gl	23.1–60.3	21.5–205	19.5–46.5	33.3–125	23.6–141	8.34–86.5	124	22.7–79.7	36.6–40.5
Peo-3-AcGl	4.67–9.08	6.13–11.8	4.33–10.5	6.32–11.3	5.06–8.20	5.29–6.72	5.37	4.23–8.92	7.96–8.78
Mal-3-AcGl	7.57–29.7	9.25–46.3	6.68–11.5	11.4–35.4	9.47–29.0	12.6–16.4	22.7	10.4–19.9	12.4–12.5
Peo-3-CouGl	4.82–8.01	8.38–12.7	4.85–10. 3	4.95–9.77	4.98–7.48	6.15–6.71	6.46	4.73–7.90	6.48–7.33
Mal-3-CouGl	6.83–11.0	7.04–28.6	4.42–20.4	6.82–17.2	6.33–23.3	8.02–10.0	9.76	7.01–9.75	9.40–9.77
Total Gluc	45.5–122	61.3–287	44.0–109	75.0–236	63.3–222	43.1–144	202	48.2–152	72.6–82.3
Total Acet	12.2–38.8	15.4–58.1	11.0–22.0	17.8–46.7	14.5–37.2	17.9–23.1	28.1	14.6–28.8	20.4–21.3
Total Coum	11.7–19.0	15.4–41.3	9.27–30.7	11.8–26.9	11.3–30.8	14.2–16.7	16.2	11.7–17.7	15.9–17.1
Total Antoc	69.4–179	92.1–386	64.3–162	94.6–310	89.1–310	75.2–184	246	74.5–199	109–121
Total Gluc = Del-3-Gl + Cya-3-Gl + Pet-3-Gl + Peo-3-Gl + Mal-3-Gl; Total Acet = Peo-3-AcGl + Mal-3-AcGl; Total Coum = Peo-3-CouGl + Mal-3-CouGl; Total Antoc = Total Gluc + Total Acet + Total Coum									
Percentage of individual anthocyanin into the group									
Del-3-Gl (% Gl)	17.11	6.69	14.03	14.76	11.64	12.14	9.55	14.72	12.65
Cya-3-Gl (% Gl)	7.26	4.54	10.58	6.55	5.66	8.09	5.05	7.74	8.87
Pet-3-Gl (% Gl)	10.32	11.23	15.74	16.82	14.41	17.64	11.98	13.56	17.88
Peo-3-Gl (% Gl)	15.52	12.75	16.20	10.80	10.62	11.27	12.33	12.61	10.84
Mal-3-Gl (% Gl)	49.79	65.03	43.14	50.90	57.69	50.69	61.39	51.15	49.77
Peo-3-AcGl (% AcGl)	26.96	24.39	44.94	27.32	25.65	29.29	19.11	30.30	40.14
Mal-3-AcGl (% AcGl)	73.08	75.58	55.09	72.56	74.41	70.73	80.78	69.82	59.71
Peo-3-CouGl (% CouGl)	41.79	37.18	24.27	38.04	29.60	41.62	39.88	42.96	41.85
Mal-3-CouGl (% CouGl)	58.08	62.86	62.10	62.07	70.38	58.32	60.25	57.01	58.09
Gluc Totals (% AntTot)	67.43	72.85	67.61	76.87	71.49	72.18	82.11	73.20	67.35
Acet Totals (% AntTot)	20.53	15.37	14.58	15.94	12.95	15.82	11.42	15.87	18.13
Coum Totals (% AntTot)	12.36	11.86	17.66	9.57	10.55	11.92	6.59	10.75	14.35
Del-3-Gl (% Gl) = Del-3-Gl / Total Gluc; Peo-3-AcGl (% AcGl) = Peo-3-AcGl / Total Acet; Peo-3-CouGl (% CouGl) = Peo-3-CouGl / Total Coum; Gluc Totals (% AntTot) = Total Gluc / Total Antoc									

Cab Sauv: Cabernet Sauvignon; Ta: Tannat; Me: Merlot; Sy: Syrah.

pigments in these vintages, being consistent with the literature, which reports that these processes are favored, under appropriate conditions of temperature, light and night-day thermal amplitude.

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 soil and climatic conditions of the semi-arid region of northeast Brazil, which are characterized by high temperatures and high solar exposure, among other factors, account for these differences. However, more specific studies are needed to detect exactly in what way.

The samples of the Syrah variety from the São Francisco Valley had higher concentrations of individual and total anthocyanins than the Chilean Syrah, suggesting that the cultivation of this grape may be successfully adapted to the terroir of the Brazilian northeast.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.microc.2013.04.003.

Acknowledgments

The authors thank the CNPq (Proc. 477584/2010-1) and FACEPE (APQ-0701-1.06/10) for financial support.

References

[1] R.G.V. Bramley, J. Ouzman, P.K. Boss, Variation in vine vigour, grape yield and vineyard soils and topography as indicators of variation in the chemical composition of grapes, wine and wine sensory attributes, Aust. J. Grape Wine Res. 17 (2011) 217–229.
[2] R. De Andreäs-De Prado, M. Yuste-Rojas, X. Sort, C. Andreäs-Lacueva, M. Torres, R.M. Lamuela-Raventoäs, Effect of soil type on wines produced from *Vitis vinifera* L. Cv. Grenache in commercial vineyards, J. Agric. Food Chem. 55 (2007) 779–786.
[3] F. Galvano, L. Fauci, G. Lazzarino, V. Fogliano, A. Rietieni, S. Ciappellano, N.C. Battistini, B. Tavazzi, G. Galvano, Cyanidins: metabolism and biological properties, J. Nutr. Biochem. 15 (2004) 2–11.
[4] S. Kounduras, V. Marinos, A. Gkoulioti, Y. Kotseridis, C. Van Leeuwen, Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components, J. Agric. Food Chem. 54 (2006) 5077–5086.
[5] M.-I. López, M.-T. Sànchez, P. Ramírez, J. Morales, Effect of a deficit irrigation regime on the quality of wines made from White grapes (*Vitis vinifera* L.) grown in semiarid areas, J. Food Qual. 32 (2009) 481–503.
[6] G. Chavarria, H. Bergamaschi, L.C. da Silva, H.P. dos Santos, F. Mandelli, C.C. Guerra, C.A. Flores, J. Tonietto, Water relations, yield and phenolic compounds of grapevines cv. Cabernet Sauvignon in three soil types, Bragantia 70 (3) (2011) 481–487.
[7] G. Mazza, Anthocyanins in grapes and grape products, Crit. Rev. Food Sci. Nutr. 35 (1995) 341–371.
[8] E. Peynaud, Connaissance et travail du vin, Ed. Dunod, Paris, 1997.
[9] L. Usseglio-Tomasset, Chimie Oenologique, Technique & Documentation, 2 ed., 1995.
[10] Resolution OIV/VITI 333/2010, Definition of vitivinicultural "Terroir", The General Director of the OIV, General assembly Tbilisi (Georgia), June 2010. 1.
[11] V.A. Carey, D. Saayman, E. Archer, G. Barbeau, M. Wallace, Viticulture terroirs in Stellenbosch, South Africa: I. The identification of natural terroir units, J. Int. Sci. Vigne Vin 42 (2008) 169–183.
[12] V.A. Carey, E. Archer, G. Barbeau, D. Saayman, Viticulture terroirs in Stellenbosch, South Africa. II. Spatialisation of viticulture and oenological potential for Cabernet–Sauvignon and Sauvignon Blanc by means of a preliminary model, J. Int. Sci. Vigne Vin 43 (1) (2008) 1–12.
[13] C. Van Leeuwen, G. Seguin, The concept of terroir in viticulture, J. Wine Res. 17 (1) (2006) 1–10.
[14] C. Van Leeuwen, F. Friant, X. Chone, O. Tregoat, S. Kounduras, D. Dubourdieu, The influences of climate, soil and cultivar on terroir, Am. J. Enol. Vitic. 55 (3) (2004) 207–217.
[15] E. Vaudour, A.B. Shaw, A worldwide perspective on viticultural zoning, S. Afr. J. Enol. Vitic. 26 (2) (2005) 106–115.
[16] G.E. Pereira, J.-P. Gaudillere, C. Van Leeuwen, G. Hilbert, M. Maucourt, C. Deborde, A. Moing, D. Rolin, ^{1}H NMR metabolic profile of wines from three cultivars, three soil types and two contrasting vintages, J. Int. Sci. Vigne Vin 41 (2) (2007) 103–109.
[17] A. Deloire, E. Vaudur, V. Carey, V. Bonnardot, C. Van Leeuwen, Grapevine responses to terroir: a global approach, J. Int. Sci. Vigne Vin 39 (4) (2005) 149–162.
[18] E. Goulet, R. Morlat, The use of surveys among wine growers in vineyards of the middle-Loire Valley (France), in relation to terroir studies, Land Use Policy 28 (2001) 770–782.
[19] J. Tonietto, G.E. Pereira, A concept for the viticulture of tropical wines. In: Proceedings of the IX International Terroir Congress. 25–29/06/2012, France, 2012, p. 34–37.
[20] G.E. Pereira, A.J.B. Araújo, J. Santos, R. Vanderlinde, L.L.A. Lima, Chemical and aromatic characteristics of Brazilian tropical wines, Acta Hortic. 910 (2011) 135–140.
[21] L.L.A. Lima, G.E. Pereira, N.B. Guerra, Physicochemical characterization of tropical wines produced in the Northeast of Brazil, Acta Hortic. 910 (2011) 131–134.
[22] A. Villier, G. Vanhoenacker, P. Majek, P. Sandra, Determination of anthocyanins in wine by direct injection liquid chromatography–diode array detection–mass spectrometry and classification of wines using discriminant analysis, J. Chromatogr. A 1054 (2004) 195–204.
[23] S. Kallithraka, A.A.-A. Mohdaly, D.P. Makris, P. Kefalas, Determination of major anthocyanin pigments in Hellenicnative grape varieties (*Vitis vinifera* sp.): association with antiradical activity, J. Food Compos. Anal. 18 (2005) 375–386.
[24] F. Fang, J.-M. Li, Q.-H. Pan, W.D. Huang, Determination of red wine flavonoids by HPLC and effect of aging, Food Chem. 101 (1) (2007) 428–433.
[25] N.H. Beltran, M.A. Duarte-Mermoud, S.A. Salah, M.A. Bustos, A.I. Peña-Neira, E.A. Loyola, J.W. Jalocha, Feature selection algorithms using Chilean wine chromatograms as examples, J. Food Eng. 67 (4) (2005) 483–490.
[26] A.Z. Berna, S. Trowell, D. Clifford, W. Cynkarc, D. Cozzolino, Geographical origin of Sauvignon Blanc wines predicted by mass spectrometry and metal oxide based electronic nose, Anal. Chim. Acta 648 (2) (2009) 146–152.
[27] B. Berente, M. Reichenbacher, K. Danzer, Improvement of the HPLC analysis of anthocyanins in red wines by use of recently developed columns, Fresenius J. Anal. Chem. 371 (1) (2001) 68–72.
[28] J. Bakker, N.W. Preston, C.F. Timberlake, The determination of anthocyanins in aging red wines: comparison of HPLC and spectral methods, Am. J. Enol. Vitic. 37 (2) (1986) 121–126.
[29] G. González-Neves, L. Barreiro, G. Gil, J. Franco, M. Ferrer, M. Moutounet, A. Carbonneau, Anthocyanic composition of Tannat grapes from the south region of Uruguay, Anal. Chim. Acta 513 (1) (2004) 197–202.
[30] O.D. Prestes, M.A. Presta, D.I.S. Kolberg, R. Zanella, S.B. Rossato, N.G. Penna, L.H.R. Hecktheuer, Desenvolvimento e validação de um método analítico para a determinação de histamina em vinhos utilizando Cromatografia Líquida de Alta Eficiência com detecção por Fluorescência, Quim. Nova 30 (1) (2007) 18–21.
[31] H. Kelebek, A. Canbas, S. Selli, HPLC-DAD–MS analysis of anthocyanins in rose wine made from cv. Öküzgözü grapes, and effect of maceration time on anthocyanin content, Chromatographia 66 (3–4) (2007) 207–212.
[32] Z.-M. Jin, J.-J. He, H.-Q. Bi, X.-Y. Cui, C.-Q. Duan, Phenolic compound profiles in berry skins from nine red wine grape cultivars in northwest China, Molecules 14 (12) (2009) 4922–4935.
[33] J. Burns, W. Mullen, N. Landrault, Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes, J. Agric. Food Chem. 50 (14) (2002) 4096–4102.
[34] G. González-Neves, J. Franco, L. Barreiro, G. Gil, M. Moutounet, A. Carbonneau, Varietal differentiation of Tannat, Cabernet–Sauvignon and Merlot grapes and wines according to their anthocyanic composition, Eur. Food Res. Technol. 225 (1) (2007) 111–117.
[35] E. García-Beneytez, F. Cabello, E. Revilla, Analysis of grape and wine anthocyanins by HPLC–MS, J. Agric. Food Chem. 51 (19) (2003) 5622–5629.
[36] H. Otteneder, R. Marx, M. Zimmer, Analysis of the anthocyanin composition of Cabernet Sauvignon and Portugieser wines provides an objective assessment of the grape varieties, Aust. J. Grape Wine Res. 10 (1) (2004) 3–7.
[37] T. Walker, J. Morris, R. Threlfall, G. Main, Analysis of wine components in Cynthiana and Syrah wines, J. Agric. Food Chem. 51 (6) (2003) 1543–1547.
[38] G.E. Pereira, J.-P. Gaudillere, C. Van Leeuwen, G. Hilbert, O. Lavialle, M. Maucourt, C. Deborde, A. Moing, D. Rolin, ^{1}H NMR and chemometrics to characterize mature grape berries in four wine-growing areas in Bordeaux-France, J. Agric. Food Chem. 53 (2005) 6382–6389.
[39] E.K. Kemsley, Discriminant Analysis and Class Modelling of Spectroscopic Data, John Wiley & Sons, Chichester, U.K., 1998
[40] M. Anastasiadi, A. Zira, P. Magiatis, S.A. Haroutounian, A.L. Skaltsounis, E. Mikros, ^{1}H NMR based metabonomics for the classification of Greek wines according to variety, region, and vintage, comparison with HPLC data, J. Agric. Food Chem. 57 (23) (2009) 11067–11074.
[41] I.J. Kosir, J. Kidric, Use of a modern magnetic resonance spectroscopy in wine analysis: determination of minor compounds, Anal. Chim. Acta 458 (2007) 77–84.
[42] H.O. Son, G.S. Hwang, K.I.M. Kim, H.J. Ahn, W.M. Park, F. Van den Berg, Y.S. Hong, C.H.J. Lee, Metabolomic studies on geographical grapes and their wines using ^{1}H NMR analysis coupled with multivariate statistics, J. Agric. Food Chem. 57 (2009) 1481–1490.
[43] G.E. Pereira, J.-P. Gaudillere, G. Hilbert, J.-P. Soyer, C. Van Leeuwen, O. Lavialle, A. Moing, C. Deborde, M. Maucourt, D. Rolin, ^{1}H NMR metabolic fingerprints of grape berries produced in different plots in Bordeaux-France, Acta Hortic. 689 (2005) 257–263.
[44] G.E. Pereira, J.-P. Gaudillere, C. Van Leeuwen, G. Hilbert, M. Maucourt, C. Deborde, A. Moing, D. Rolin, ^{1}H NMR metabolite fingerprintings of grape berry: comparison of vintage and soil effects in Bordeaux grapevine growing areas, Anal. Chim. Acta 563 (2006) 346–352.
[45] G.E. Pereira, J.-P. Gaudillere, C. Van Leeuwen, G. Hilbert, M. Maucourt, C. Deborde, A. Moing, D. Rolin, Microclimate influence on mineral and metabolic profiles of grape berries, J. Agric. Food Chem. 54 (2006), (6765-7-6775).
[46] Organisation Internationale de la Vigne et du Vin (OIV), Recueil des méthodes internationales d'analyse des vins et des moûts, Paris, 1990.
[47] Resolution OENO 22/2003, OIV, 2003. 1–12.

[48] F. He, L. Mu, G.-L. Yan, N.-N. Liang, Q.-H. Pan, J. Wang, M.J. Reeves, C.-Q. Duan, Biosynthesis of anthocyanins and their regulation in colored grapes, Molecules 15 (12) (2010) 9057–9091.
[49] P. Ribéreau-Gayon, Y. Glories, A. Maujean, D. Dubordieu, Traité d'oenologie: Chimie du vin, Stabilisation et traitements, 5 ed., Ed. Dunod, Paris, 2004.
[50] U.A. Camargo, G.E. Pereira, C.C. Guerra, Wine grape cultivars adaptation and selection for tropical wines, Acta Hortic. 919 (2011) 121–129.
[51] C. Vergara, D. Von Baer, I. Hermosín, A. Ruiz, M.A. Hitschfeld, N. Castillo, C. Mardones, Anthocyanins that confer characteristic color to red copihue flowers (*Lapageria rosea*), J. Chil. Chem. Soc. 54 (2) (2009) 194–197.