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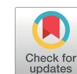
Climate effects on physicochemical composition of Syrah grapes at low and high altitude sites from tropical grown regions of Brazil



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ABSTRACT

The major areas of the world where viticulture is practiced enjoy temperate or cool temperature climates. When wine grapes are grown in tropical regions, edaphoclimatic factors result in distinct grape quality attributes, and production techniques also require significant adjustment. The objective of this study was to characterize the chemical compositions, in particular of phenolic compounds, of Syrah grapes grown in two location in northeast Brazil - these are also at widely different altitudes. A range of methods of phenolic extraction were used, along with classical chemical analyses including for organic acids, sugars, monomeric anthocyanins, flavonols, stilbene, condensed tannins and some of the monomeric and small oligomeric procyanidins. The regions and their diverse environments had a larger influence than harvest year. The grapes at higher altitude (Bahia, 1.100 m asl (metres above sea level) were characterized by higher levels of malic acid, anthocyanins and condensed tannins in the skins. The low-altitude grapes (Pernambuco, 350 m asl (metres above sea level) had higher levels of glucose, fructose, 3-*O*-acetylglucoside anthocyanins and condensed tannins in the seeds. Fruit composition was highly influenced by the region. In the low-altitude region, the grapes were characterized by higher tartaric and citric acid in the must, also of flavonols in skins and of tannins in the seeds. Meanwhile, the fruit from the high altitude, contained higher levels of malic and succinic acid in the must, and of anthocyanins and condensed tannins in the skins.

1. Introduction

A very large number factors affect grapevine production and these can be grouped into cultural factors and permanent factors. The permanent ones may be further grouped into natural factors (climate, soil and biological environment) and induced factors (variety, rootstock, planting density, location, canopy management, within-row and between-row spacings). Of the natural permanent factors, climate exerts one of the strongest influences, determining the viticultural potential of an area in relation to the requirements of particular grape cultivars (Hidalgo, 1993). In a review of climate change and the potential effects on grape quality and wine production, Orduña (2010) confirms that climate and microclimate are key determinants of winegrape quality, exerting considerable influence on berry composition, including sugars, malic acid and anthocyanins. Van Leeuwen et al. (2004) comment that annual variations in the weather are well known in viticulture as the 'vintage effect'. Variations in grapevine behavior and in grape ripening from one year to the next in a given location reflect the effects of weather alone, because the soil and plant type can be considered constant. The distribution of viticulture round the world is determined by solar radiation, air temperature, air humidity and soil moisture – these determine the photosynthetic activity and evapotranspiration of the grapevine and also, critically, flowering and fruit set and the incidence and severity of pest and disease problems, usually these that render some sites unsuitable for grape growing.

During fruit maturation, complex physiological and biochemical changes occur that are responsible for the chemical constitution of the fruit. Grape berry constituents include: sugars, organic and phenolic acids, minerals, nitrogenous substances, phenolic compounds (such as anthocyanins, stilbenes, flavonols, tannins), pectic substances, fiber,

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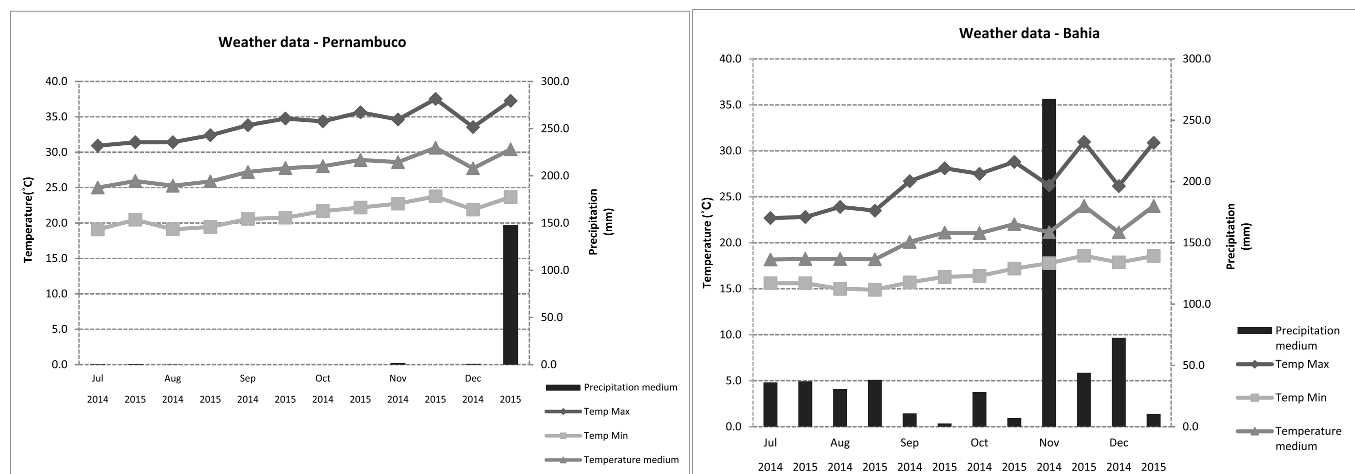

Fig. 1. Monthly temperatures and precipitation at Pernambuco (350 m asl) and Bahia (1100 m asl) during the 2014-15 study period. Figure 1A_Source of the meteorological data Embrapa (Semi-arid). Figure 1B_Source of the meteorological data INMET. Temp Max (Maximum temperature); Temp Min (Minimum temperature); Jul (July); Aug (August); Sep (September); Oct (October); Nov (November); Dec (December).

vitamins and aromatic compounds (Jackson, 2008). The concentrations in the must of sugars and organic acids are related to the physiological aspects of grape maturation, natural climatic factors, soil and agronomic production practices. Changes recorded in these constituents are used to follow the fruit maturation process (Rizzon & Sganzerla, 2007; Winkler, Cook, Kliewer, & Lider, 1974).

The sugars of the must have a fundamental role in the composition of the wines since they are transformed into alcohol and carbon dioxide during the alcoholic fermentation, being the alcohol a most abundant volatile compound, responsible for sensory sensations, interaction with other compounds and aging (Jordão, Vilela, & Cosme, 2015). Organic acids and their salts act as buffers, thus ensuring that the wine maintains a relatively low pH; this helps to protect it against bacterial attack and subsequent spoilage, acids influence the organoleptic properties of wines and help in the conserve wine color and influence esterification with a consequent impact on bouquet (Jackson, 2008; Ribéreau-gayon, Dubourdieu, Donèche, & Lonvaud, 2006).

The phenolic compounds in grapes and wines are a heterogeneous group of substances that comprise several classes of components. These are particularly important in enology and are present in the skin and seed of the grape (Andres et al., 2007). The amounts of the phenolic compounds in grapes depend on cultivar and is highly influenced by terroir (Rastija, Srenik, & Sáric, 2009; Tarko, Duda-Chodak, Sroka, Satora, & Jurasz, 2010). The impact of terroir on grape phenolic compounds has been extensively investigated (Ledda, Sanna, Manca, Franco, & Porcu, 2010; Mateus, Marques, Gonçalves, Machado, & De Freitas, 2001). Research has shown that irradiance, water deficit and day/night temperature differences can all regulate the expression of genes related to flavonoid metabolism, thus significantly affecting the content of these (Kennedy, Matthews, & Waterhouse, 2002; Tonietto, Ruiz, & Gómez-Miguel, 2012).

The response of grapevines to climate and effects on biosynthesis, translocation, degradation and accumulation of substances in the berry are transferred to the wine, being mainly responsible for defining the color (intensity and matrix), aroma (intensity and profile) and flavor (body, acidity, structure, complexity and persistence) of the wine (Lima, Pereira, & Guerra, 2011).

The region of the São Francisco Valley (Sub-middle) is located in northeast Brazil (Southern Hemisphere) between the 8 and 9° parallels at an altitude of 350 m asl. This region has a semi-arid tropical climate, with edaphoclimatic characteristics that make it possible to stagger winegrape production throughout the year. Moreover, vines can produce two harvest each year, mainly due to the prevailing high temperatures (average annual temperature is 26 °C) and also to high insolation rates and the availability of abundant, high-quality irrigation water (Pereira, de Santos, Guerra, & Alves, 2008). Morro do Chapéu, Bahia, is also located in the Brazilian northeast, but is located at the 11° parallel in the Chapada Diamantina at an altitude of 1.100 m asl. Here the climate is classified as 'tropical at altitude'. Recently, the municipality been carrying out studies with grape adaptation and wine production, as a possible new option for regional development. Here, grape production can occur only once a year but requires two pruning interventions - one for training and the other for production. The average annual temperature in this region is 19 °C. Hence, although the two regions are similarly located in northeast Brazil, their climates and soil conditions are quite different. The aim of this study is to compare the berry compositions of a single grape cultivar, *Vitis vinifera* L. cv. Syrah, grown at widely different altitudes in a tropical climate. This would seem to be a pioneer study made possible by the location and aided by the wide analytical approach we used. The knowledge about the composition of the grapes in the two regions allow the productive sector to direct the type of wine to be produced, as well as to adapt the elaboration methods ensure a better extractability of the compounds.

2. Materials and methods

2.1. Vineyard locations and harvests

The first study area is in the municipality of Lagoa Grande, state of Pernambuco. It lies between latitudes 8 and 9° parallels at 350 m asl. The soils are classified as red-yellow Argisols (Embrapa Solos, 2006). Usually these are of medium natural fertility. The climate is classified as 'semi-arid tropical' and is characteristic of an intra-annual winegrowing climate where the wine is generally classed according to the period of the year during which the grapes are produced (Tonietto et al., 2012). The weather data during the study period in Fig. 1A. The commercial vineyards are planted with Syrah grapes grafted on 1103P rootstock, trained to an ascending vertical system with spacings of 3.0 m (between-row) and 1.0 m (in-row), rows are oriented north-south and irrigation is by drippers. The vines are about 10 year-old and produce twice a year (first and second semesters).

The second vineyard is located in the municipality of Morro do Chapéu, Chapada Diamantina region, Bahia, at the 11° latitude parallel, at 1.100 m asl. Soils are classified as red-yellow Latosols deep and with pH around 4.8 (Embrapa Solos, 2006). The climate is classified as 'tropical at altitude', with rain distributed throughout the year. Weather data presented in Fig. 1B. The vineyard is part of a trial of Syrah grapes grafted on 1103P rootstocks, planted in January 2011 (so, about 4 year

old), the training is also to an ascending vertical system and with drip irrigation. In this region production is only one annual harvest due to heavy rainfall in the period around November/December.

In Bahia grapes were harvested in September (2014) and in October (2015). In Pernambuco, the harvests were in December 2014 and in September 2015. Cluster samples of good maturity and health were collected in the field from randomly-marked vines (total of 25 plants). In the laboratory, the berries for measurement were removed from different parts of the cluster to ensure representative sampling.

2.2. Extraction methods

Three methods of extraction for phenolic compounds were used.

The first was as proposed by Carbonneau and Champagnol (1993) in which the skins and seeds were macerated for 24 h at 20 °C with ethanol and buffer (pH 3.2). Extracts were subjected to spectrophotometry to measure: total phenols (Singleton, Sullivan, & Kramer, 1971), non-flavonoids (Ribéreau-Gayon & Stonestreet, 1965), flavonoids (Ribéreau-Gayon & Stonestreet, 1965), total anthocyanins (Ribéreau-Gayon, 1970), color intensity and tonality (OIV, 2014), tanning power (De Freitas & Mateus, 2001) and individual monomeric anthocyanins (Roggero, Coen, & Ragonnet, 1986). The classical analyses were also carried out on must obtained from the pulp: pH, total soluble solids and total acidity determined by the methods of OIV (2014).

The second extraction method, separated and weighed the skins, pulp and seeds, and the phenolic compounds were extracted from each sample using the method described by Bourzeix, Weyland, and Heredia (1986) employing three solvents of different polarity - methanol, water and acetone - and with different contact times for successive macerations. The first extraction was overnight at −24 °C in methanol. Further macerations are carried out under nitrogen: 4 h at room temperature in methanol:water (80:20, v:v); 4 h at room temperature in methanol:-water (50:50, v:v); 15 h at 24 °C in distilled water; 1 h at room temperature in acetone:water (75:25, v:v). At each extraction the liquid obtained was collected and stored at 4 °C under nitrogen. The extracts were used to analyze the fraction of flavanols as a function of their molecular weight (Sun, Leandro, Ricardo-da-Silva, & Spranger, 1998) and the low molecular weight flavanols (Ricardo-da-Silva, Rosec, Bourzeix, & Heredia, 1990).

The third method of extraction followed the Pereira et al. (2006) separate extracts of skins and pulp were prepared using 96% ethanol, these were then centrifuged and filtered. These extracts were analyzed using liquid chromatography to quantify sugars and organic acids in the pulp and stilbene (trans-resveratrol) and flavonols in the skins.

2.3. Determination of glucose and fructose by HPLC

For sugar determination (glucose and fructose) the method of Corrêa et al. (2012), was used. The pulp extract was prepared using the method of Pereira et al. (2006). To reduce interference, 2 mL pulp aliquots were evaporated using a concentrator and re-suspended in 40 mL ultrapure water. After filtration using a 0.45 μm membrane, the sample was injected into a high efficiency liquid chromatography, model e2695 (Waters, USA) coupled to a refractive index detector, model 2414 (Waters, USA). We used a Rezex RCM-monosaccharide CA^{+2} column, 300 mm × 7.8 mm (Phenomenex, USA) and a Carbo H pre-column, 4.0 mm × 3.0 mm (Phenomenex, USA). Ultrapure water was used as the mobile phase, with an isocratic flow of 0.75 mL min^{-1}, the oven temperature of 65 °C, the run time was 15 min and 10 μL of the samples were injected in triplicate. The standards used to prepare the calibration curve were glucose and fructose.

2.4. Quantification of organic acids by high performance liquid chromatography

The quantification of tartaric, malic, citric and succinic acids was by high performance liquid chromatography (HPLC) using a chromatograph coupled with a diode detector (DAD), model Aliance e2695 (Milford, USA). The pulp samples prepared using the extraction method from grapes by Pereira et al. (2006) were filtered through a 0.45 μm membrane and injected in triplicate. For the determination, the DAD wavelength was maintained at 210 nm, with a run time of 15 min, flow rate 0.6 mL min^{-1}, temperature at 26 °C and volume injection of 10 μL, in triplicate. The column used was a Gemini-NX C18, dimensions 150 mm × 4.60 mm, with internal particles 3 μm and Gemini-NX C18 pre-columns 4.0 mm × 3.0 mm (Phenomenex, USA). The mobile phase comprised a 0.025 M KH_2-PO_4 solution acidified with H_3PO_4 to pH 2.6 using the method described by Natividade, Corrêa, Souza, Pereira, and Lima (2013).

2.5. Separation and quantification of individual monomeric anthocyanins by HPLC

To separate the individual monomeric anthocyanins, a Perkin-Elmer HPLC (USA) was used. This consisted of a pump (Series 200) and detector (LC95 Uv/Visible). The separation occurred in a column C18 (250 mm × 4 mm), with reverse phase of 5 μm of compaction, protected by a pre-column consisting of the same material, both from LichroCart, Merck-Germany. The solvents were: A (40% formic acid and 60% bi-distilled water), B (acetonitrile PA) and C (bi-distilled water). Methanol:water (50:50, v:v) was used to wash the column after the analyses. Samples were prepared according to Carbonneau and Champagnol (1993).

The initial conditions used were: 25% A, 6% B and 69% C for 15 min, followed by a 25% linear gradient of A, 25.5% B and 49.5% C for 70 min. Finishing with 20 min of 25% A 25.5% B and 49.5% C. The flow was 0.7 mL min^{-1}, using a detector with wavelength at 520 nm. Both the samples and the solvents were filtered under the same conditions. The volume injected was 20 μL and the analyses were carried out in triplicate. Identification followed the method described by Roggero et al. (1986) and quantification was based on the standard malvidin 3-*O*-glucoside curve.

The following individual anthocyanin molecules were quantified: delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, malvidin 3-*O*-glucoside, delphinidin 3-*O*-acetylglucoside, cyanidin 3-*O*-acetylglucoside, peonidin 3-*O*-acetylglucoside; petunidin 3-*O*-acetylglucoside, malvidin 3-*O*-acetylglucoside, peonidin 3-*O*-coumarylglucoside, petunidin 3-*O*-coumarylglucoside, delphinidin 3-*O*-coumarylglucoside and malvidin 3-*O*-coumarylglucoside.

2.6. Separation and quantification of individual flavonols and stilbene by HPLC

Individual flavonols and stilbene were determined by HPLC on a Waters (Aliance e2695) chromatograph equipped with solvent pump and automatic injector, coupled with DAD, following the method of Natividade et al. (2013) with skin extracts prepared following the method of Pereira et al. (2006).

Data collection and analysis were carried out using Empower ™ 2 software (Mil-ford, USA). In DAD, the detection of the compounds was done at 320 nm for trans-resveratrol and 360 nm for analysis of flavonols: kaempferol 3-*O*-glucoside, myricetin 3-*O*-glucoside, quercetin-3-*O*-glucoside, rutin (quercetin-3-*O*-rutiniside) and isorhamnetin 3-*O*-glucoside.

The column was a Gemini-NX C18, 150 mm × 4.60 mm, with internal particles of 3 μm and the pre-column was a Gemini-NX C18, 4.0 mm × 3.0 mm (Phenomenex, USA). The oven temperature was maintained at 40 °C, the injection volume was 10 μL (the extracts being previously filtered through a 0.45 μm membrane) and the flow rate was 0.5 mL min^{-1}. The mobile phase consisted of a solution of 0.85% phosphoric acid (Solvent A) and acetonitrile (solvent B). The elution

Table 1
Characterization of Syrah grapes cultivated at the two sites, Pernambuco and Bahia, in 2014 and 2015.

Region	Bahia (1100 m asl)		Pernambuco (350 m asl)		ANOVA
Harvest year	2014	2015	2014	2015	(*p*-values)
Classic analyses					
pH	3.66[c] ± 0.01	3.76[b] ± 0.01	4.29[a] ± 0.02	3.44[d] ± 0.02	***
Total acidity ($g L^{-1}$)	5.80[b] ± 0.09	5.14[c] ± 0.24	5.58[b] ± 0.10	6.42[a] ± 0.09	***
SST (°Brix)	21.17[b] ± 0.15	21.53[a] ± 0.06	20.87[c] ± 0.06	18.20[d] ± 0.10	***
Sugars					
Glucose ($mg kg^{-1}$)	196.01[b] ± 8.45	209.05[b] ± 15.38	271.67[a] ± 25.8	192.96[b] ± 20.77	*
Fructose ($mg kg^{-1}$)	139.66[c] ± 2.71	177.95[ab] ± 17.68	192.44[a] ± 15.49	158.67[bc] ± 17.09	*
Organic acids					
Tartaric ($g kg^{-1}$)	4.93[b] ± 0.17	4.26[c] ± 0.14	5.54[a] ± 0.30	4.70[b] ± 0.13	**
Malic ($g kg^{-1}$)	2.87[a] ± 0.24	2.75[a] ± 0.12	2.41[b] ± 0.06	2.43[b] ± 0.24	**
Citric ($mg kg^{-1}$)	2.30[c] ± 0.20	2.55[c] ± 0.41	76.67[a] ± 5.77	35.03[b] ± 4.58	***
Succinic ($mg kg^{-1}$)	42.03[c] ± 6.83	158.10[a] ± 2.01	115.67[c] ± 6.66	154.61[b] ± 3.20	**

*Means followed by the same letter in a row did not differ by Tukey test ($p \leq 0.05$). Standard deviation of triplicate analysis. SST = total soluble solids. For sugars and acids, the values are relative to fresh weight; n.s. (not significant).
* Significant differences ($p \leq 0.05$).
** Significant differences ($p \leq 0.01$).
*** Significant differences ($p \leq 0.001$).

gradient was: 100% A; 0–10 min: 93% A and 7% B; 20 min: 90% A and 10% B, 30 min: 88% A and 12% B; 40 min: 77% A and 33% B; 45 min: 65% A and 35% B and 55 min: 100% B. The analysis was carried out in triplicate for all samples.

2.7. Separation of proanthocyanidins in Sep-Pak C18 cartridges and quantification of the fractions by the vanillin assay

Separation of the flavanols was carried out using a Sep-Pak C18 cartridge (Waters, USA) according to their degree of polymerization in three fractions: monomeric, oligomeric and polymeric fractions, following the method of Sun, Ricardo da Silva, and Spranger (1998). Extractions of skins and seeds followed the method of Bourzeix et al. (1986).

The flavanol content of each fraction was determined using the vanillin assay according to the method of Sun, Ricardo da Silva, and Spranger (1998). The quantification was carried out using standard curves prepared from flavanol monomers, oligomers and polymers of grape-seed isolates as described by Sun, Ricardo da Silva, and Spranger (1998); Sun, Ricardo-da-Silva, and Spranger (2001). Extractions on the C18 cartridge and the readings after reaction with vanillin were in triplicate.

2.8. Fractionation of low molecular weight flavanols by polyamide column chromatography and further quantification by HPLC

Extracts (5 mL) of seeds and skins were prepared according to Bourzeix et al. (1986) and fractionated on a polyamide column (Macherey-Nagel, Germany) as described by Ricardo-da-Silva et al. (1990). Phenolic acids were first eluted with 80 mL of phosphate buffer, pH 7.0. The monomeric flavanols were eluted with 50 mL of ethyl acetate:water (30:70, v:v) and oligomeric procyanidins with 50 mL of acetone:water (75:25, v:v). The fractions were dried, dissolved in 1.2 mL of methanol:water (50:50, v:v), filtered through a 0.45 µm membrane and injected onto the HPLC column. Following the above procedure, a new polyamide column was used for each sample, the analyses were carried out in triplicate.

The HPLC equipment comprised a UV–Vis detector (Waters 2487) and a Merck L-7100 pump, USA. The separation was carried out on a Lichrosphere C18 reverse phase column (Merck – Germany) 250 mm × 4.6 mm × 5 µm, at ambient temperature.

For monomeric flavan-3-ols, a gradient consisting of solvent A (water:acetic acid, 97.5:2.5, v:v) and solvent B (acetonitrile:solvent A, 80:20, v:v) was applied at a flow rate of 0.9 mL min^{-1} as: 7–25% B linear from 0 to 31 min, followed by washing (methanol:water, 50:50, v:v) 32–50 min and the rebalancing of the column from 51 to 65 min under the initial gradient conditions. For oligomeric procyanidins, a solvent gradient A (distilled water) and solvent B (water:acetic acid, 90:10, v:v) was applied at a flow rate of 1.0 mL min^{-1} as: 10–70% linear B 0–45 min, 70–90% linear B 45–70 min, 90% B isocratic 70–82 min, 90–100% linear B 82–85 min, 100% B isocratic 85–90 min, followed by washing (methanol:water, 50:50, v:v) 91–100 min. And rebalancing the column from 101 to 120 min under the initial conditions of the gradient. Detection was carried out at 280 nm absorbance and injections were in triplicate.

The following flavanol molecules were quantified: gallocatechin; (+)-catechin; (−)-epicatechin; (−)-epigallocatechin; procyanidin dimers B1, B2, B3, B4, B1–3-*O*-gallate, B2–3-*O*-gallate, B2–3′-*O*-gallate, trimers C1 and trimer 2 (T2). Identification of compounds was according to Rigaud, Pérez-Ilzarbe, Ricardo-da-Silva, and Cheynier (1991) and Ricardo-da-Silva, Darmon, Fernandez, and Mitjavila (1991) and the quantification of monomeric flavan-3-ol and oligomeric procyanidins (some dimers and trimers) was based on standard curves of (+) catechins for the monomers and of B2 for the other compounds.

2.9. Data analysis–Statistics

All analyses were carried out in triplicate. To verify sample differences based on the two regions, an analysis of variance test (ANOVA) was carried out. The differences in each treatment level were tested by a multiple mean comparison test (HSD Tukey) at a significance level of 5%. A Principal Component Analysis (PCA) was applied to the anthocyanins, flavonols, condensed tannins and small flavanols data to evaluate its effects on grapes cultivate in the two altitudes. Both analyses were carried out in the STATISTIX 9.0 program analytical software (Florida, USA).

3. Results and discussion

3.1. Chemical composition of grape musts

The results of must characterization by the classical methods, sugars and organic acids are presented in Table 1. Based on the classical parameters (pH, total soluble solids and total acidity), we note that maturation in Bahia (higher altitude) was more uniform in the two years of study, than that in Pernambuco.

Concentrations of acidity in the must, analyzed between regions ranged from 5.14 to 6.42 $g\,L^{-1}$ of tartaric acid. In the literature, the total acidity of grape must in traditional regions usually varies between 5.0 and 16.0 $g\,L^{-1}$ of tartaric acid. These values are known to be influenced by the cultivar, climate, cultural practices and fruit maturity (Reynolds, 2010). Some studies with the Syrah variety in the tropical region, state Pernambuco (350 m asl), report concentrations of total acidity ranging from 4.2 to 7.4 $g\,L^{-1}$ of tartaric acid (Leão, Nunes, & Lima, 2015; Lima, Leite, Sampaio, Vianello, & Lima, 2015).

The levels of tartaric acid varied from a minimum of 4.26 $g\,kg^{-1}$ (year 2015, Bahia) to a maximum of 5.54 $g\,kg^{-1}$ of fresh fruit (year 2014, Pernambuco). The difference in climate, as affected by altitude, does not seem to affect the concentration of tartaric acid significantly. A number of investigations have analyzed the influences on grape berry acid composition, of temperature and irradiance. In general, the concentration of tartaric acid in mature grape berries is not much affected by temperature (Schultz & Lider, 1964).

The concentrations of malic acid were 2.43 $g\,kg^{-1}$ in Pernambuco, in both seasons. In Bahia, higher concentrations were observed: 2.87 $g\,kg^{-1}$ in 2014 and 2.75 $g\,kg^{-1}$ in 2015. According to Toda (1991) and Conde et al. (2007), high temperatures result in markedly greater degradation of malic acid in grapes. This may have been the cause of the low malic acid concentrations in Pernambuco.

Citric acid was higher at Pernambuco (lower altitude), with values varying between harvests, from a minimum of 35.33 $mg\,kg^{-1}$ in 2014, to a maximum of 61.00 $mg\,kg^{-1}$ fresh weight in 2015. In Bahia, the values were significantly lower, ranging from 4.40 in 2014 to 4.52 mg kg, in 2015. Low concentrations of citric acid at higher altitude may be related to its use in the biosynthesis of malic acid. According to the literature, the citric acid content in grapes is related to three factors: it can be converted to malic acid in the Krebs cycle; it can be reduced due to the formation of acid salts; and it can be diluted by increased berry volume (Moreno & Peinado, 2012; Riberéau-Gayon et al., 2006).

High concentrations of succinic acid were observed, with slightly higher values in Bahia (158.10 $mg\,kg^{-1}$) than in Pernambuco (154.67 $mg\,kg^{-1}$) both 2015. Few studies on the synthesis of succinic acid have been reported in grapes, but their presence in *V. vinifera* cultivars has been reported (Kliewer, 1966). According to Lamikanra, Inyang, and Leong (1995), succinic acid declines rapidly during maturation. This implies it is rapidly converted to other compounds during the late stages of berry development. The possible involvement of succinic acid as a precursor or intermediate product in the formation of other compounds has received little attention. Maybe this is because it is present only at low concentrations in grapes in the main viticultural regions of the world (Kliewer, 1977).

The main sugars present in *V. vinifera* L. cultivars are glucose and fructose. Together these account for about 99% of total carbohydrates in the must and between 12 and 27% of the fresh weight of the mature grapes (Winkler et al., 1974). In Pernambuco, in 2014, glucose and fructose were present at high concentration (glucose 271.7 $mg\,kg^{-1}$ fresh weight and fructose 192.4 $mg\,kg^{-1}$ fresh weight). In the high altitude region, Bahia, glucose levels varied from 196.01 to 209.05 $mg\,kg^{-1}$ fresh fruit, and fructose (2014) about 177.95 $mg\,kg^{-1}$ fruit fresh. Like other cellular constituents, sugar accumulation varies with cultivar, maturity and environmental conditions (Jackson, 2008). Temperature influences berry composition strongly - within the critical limits, the higher the temperature, the higher the sugar concentration (Winkler et al., 1974).

3.2. Color and phenolic index

The color and phenolic index of the fruit (skins and seeds) over the two seasons are shown in Table 2. There were significant differences in the parameters measured, in 2014, in Bahia, concentrations of these compounds was highest: total phenols (1.44 $g\,kg^{-1}$), non-flavonoids (0.20 $g\,kg^{-1}$), flavonoids (1.24 $g\,kg^{-1}$), total anthocyanins (0.89 $g\,kg^{-1}$) and color intensity (26.7 u.a), followed by the 2015 harvest also in the region of highest altitude. This suggests the synthesis of compounds related to colorimetric parameters was greater at higher altitude, and possibly is related to the climatic characteristics of this region, characterized with greater thermal amplitude day / night and maximum temperatures below 30 °C during the productive cycle, that promote a greater synthesis and preservation of these compounds during the maturation of the grape.

3.3. Monomeric anthocyanins in skins

Nine of the fourteen monomeric anthocyanins identified (see Table 2) were at higher concentration at the higher altitude (Bahia) especially for the 2014 harvest, followed by 2015 one. Four anthocyanins, 3-*O*-glucosides, ranged from 2.28 $g\,kg^{-1}$ fresh weight for delphinidin and 15.91 $g\,kg^{-1}$ fresh weight for malvidin. Cyanidin 3-*O*-acetylglucoside (0.48 $g\,kg^{-1}$), delphinidin 3-*O*-acetylglucoside (0.30 $g\,kg^{-1}$) and anthocyanins 3-*O*-coumarylglucoside, peonidin (0.74 $g\,kg^{-1}$), delphinidin (0.87 $g\,kg^{-1}$) and malvidin (2.62 $g\,kg^{-1}$). These are all from the 2014 harvest, followed by the 2015 one.

At the lower altitude (Pernambuco) in 2014, four anthocyanins were at higher concentration: 3-*O*-acetylglucoside: peonidin (0.61 $g\,kg^{-1}$), petunidin (0.34 $g\,kg^{-1}$) and malvidin (4.09 $g\,kg^{-1}$), in addition to petunidin 3-*O*-coumarylglucoside (0.66 $g\,kg^{-1}$). It is suggested that climate, rather than soil, is the main factor resulting in the higher concentrations of anthocyanins in Bahia and lower in Pernambuco. There is considerable evidence that anthocyanin production is positively correlated with levels of light and temperature (Kliewer, 1977; Spayd, Tarara, Mee, & Ferguson, 2002). Mateus, Silva, Santos-Buelga, Rivas-Gonzalo, and De Freitas (2002) studied Touriga Nacional and Touriga Francesa grapes, from two vines at different altitudes, over three consecutive years in the Douro Valley. They found that higher altitudes increased the levels of anthocyanins the grapes and wines. Meanwhile, Liang et al. (2012) studied four grape cultivars in five viticultural regions in China and reported higher concentrations of anthocyanins in regions of high altitude. According to Winkler et al. (1974) the color of grapes is temperature dependent, with very-cold or very-hot temperatures being associated with poor color development. The optimal temperature range for anthocyanin accumulation is 17–26 °C. Low temperatures, particularly at night, increase coloration in red grapes (Kliewer & Torres, 1972) and temperatures above 35 °C general inhibit anthocyanin synthesis (Mateus et al., 2002; Liang et al., 2012; Azuma, Yakushiji, Koshita, & Kobayashi, 2012). Therefore, the high concentrations of total monomeric anthocyanins found in grapes of the region of 1100 m asl may be related to the maximum temperatures during the productive cycle that remained below 30 °C, favoring the accumulation of anthocyanins and avoiding their degradation.

3.4. Flavonols in skins

We quantified the flavonol glycosides: kaempferol, isorhamnetin, myricetin, quercetin and rutin (Table 2). The highest concentrations were of quercetin-3-*O*-glucoside, ranging from 0.22 $g\,kg^{-1}$ fresh weight (Bahia, 2015) to 0.43 $g\,kg^{-1}$ fresh weight (Pernambuco, 2014). The low altitude region showed higher concentrations of the flavonols myricetin (0.06 $g\,kg^{-1}$) and rutin (0.04 $g\,kg^{-1}$). Agronomic and environmental factors strongly affect flavonol quantities and thus the flavonol profile.

The synthesis of flavonols in plants is strongly influenced by sunlight. In general, it would be expected that the grapes more exposed to sunshine would have increased biosynthesis of all flavonoids (Azuma et al., 2012; Flamini, Mattivi, Rosso, Arapitsas, & Bavaresco, 2013; Koyama, Ikeda, Poudel, & Goto-Yamamoto, 2012). According to some authors, temperature has less influence on flavonol accumulation than light exposure. However, ultraviolet (UV) radiation, particularly UV-B, stimulates flavonol biosynthesis, so any practice that favors fruit exposure to sunlight has positive influences on flavonol concentration

Table 2
Phenolic compounds, anthocyanins and flavonols in skin extracts of Syrah grapes, cultivated in Pernambuco and Bahia. Two tropical regions in northeast Brazil.

Region	Bahia (1100 asl)		Pernambuco (350 m asl)		ANOVA
Harvest year	2014	2015	2014	2015	(*p*-values)
Color and global phenolic compounds					
Total phenols ($g\,kg^{-1}$)	$1.44^{a} \pm 0.00$	$1.23^{b} \pm 0.01$	$0.45^{c} \pm 0.00$	$0.41^{c} \pm 0.00$	***
Non- flavonoids ($g\,kg^{-1}$)	$0.20^{a} \pm 0.00$	$0.14^{c} \pm 0.00$	$0.13^{c} \pm 0.00$	$0.08^{d} \pm 0.00$	**
Flavonoids ($g\,kg^{-1}$)	$1.24^{a} \pm 0.00$	$1.10^{b} \pm 0.01$	$0.32^{c} \pm 0.00$	$0.34^{c} \pm 0.00$	***
Total anthocyanins ($mg\,L^{-1}$)	$0.89^{a} \pm 0.00$	$0.57^{b} \pm 0.00$	$0.35^{c} \pm 0.00$	$0.25^{d} \pm 0.00$	***
Color Intensity (u.a)	$26.75^{a} \pm 0.11$	$16.06^{b} \pm 0.07$	$10.12^{c} \pm 0.06$	$7.67^{d} \pm 0.04$	***
Tonality (u.a)	$2.50^{b} \pm 0.00$	$2.73^{a} \pm 0.002$	$2.10^{c} \pm 0.05$	$2.05^{c} \pm 0.01$	***
Monomeric anthocyanins ($mg\,kg^{-1}$)					
Cyanidin 3-*O*-glucoside	$0.65^{ab} \pm 0.04$	$0.44^{ab} \pm 0.07$	$0.66^{ab} \pm 0.06$	$0.45^{ab} \pm 0.02$	n.s.
Delphinidin 3-*O*-glucoside	$2.28^{a} \pm 0.06$	$1.56^{b} \pm 0.06$	$0.00^{d} \pm 0.00$	$0.20^{c} \pm 0.01$	***
Peonidin 3-*O*-glucoside	$4.14^{a} \pm 0.07$	$3.12^{b} \pm 0.15$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	***
Petunidin 3-*O*-glucoside	$2.55^{a} \pm 0.08$	$1.89^{b} \pm 0.15$	$0.71^{c} \pm 0.04$	$0.44^{c} \pm 0.03$	**
Malvidin 3-*O*-glucoside	$15.91^{a} \pm 0.35$	$11.36^{b} \pm 0.32$	$6.35^{c} \pm 0.33$	$4.72^{d} \pm 0.27$	**
Peonidin 3-*O*-acetylglucoside	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.61^{a} \pm 0.04$	$0.25^{b} \pm 0.03$	***
Petunidin 3-*O*-acetylglucoside	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.34^{a} \pm 0.04$	$0.30^{b} \pm 0.01$	**
Cyanidin 3-*O*-acetylglucoside	$0.48^{a} \pm 0.03$	$0.39^{b} \pm 0.05$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	**
Delphinidin 3-*O*-acetylglucoside	$0.30^{a} \pm 0.04$	$0.33^{a} \pm 0.04$	$0.05^{b} \pm 0.01$	$0.03^{b} \pm 0.00$	*
Malvidin 3-*O*-acetylglucoside	$2.81^{b} \pm 0.07$	$2.36^{d} \pm 0.06$	$4.09^{a} \pm 0.17$	$2.57^{c} \pm 0.08$	***
Peonidin 3-*O*-coumarylglucoside	$0.74^{a} \pm 0.03$	$0.65^{b} \pm 0.04$	$0.52^{c} \pm 0.06$	$0.12^{d} \pm 0.02$	**
Petunidin 3-*O*-coumarylglucoside	$0.26^{b} \pm 0.01$	$0.20^{c} \pm 0.01$	$0.66^{a} \pm 0.04$	$0.31^{b} \pm 0.01$	***
Delphinidin 3-*O*-coumarylglucoside	$0.87^{a} \pm 0.05$	$0.71^{b} \pm 0.04$	$0.00^{c} \pm 0.00$	$0.04^{c} \pm 0.01$	*
Malvidin 3-*O*-coumarylglucoside	$2.62^{a} \pm 0.07$	$1.88^{b} \pm 0.14$	$1.85^{b} \pm 0.16$	$0.87^{c} \pm 0.03$	*
Total monomeric anthocyanins	$32.90^{a} \pm 0.76$	$24.89^{b} \pm 0.86$	$15.78^{c} \pm 0.79$	$10.34^{d} \pm 0.25$	**
Flavonols ($g\,kg^{-1}$)					
Kaempferol 3-*O*-glucoside	$0.06^{a} \pm 0.00$	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.00$	$0.05^{b} \pm 0.00$	*
Isorhamnetin 3-*O*-glucoside	$0.19^{a} \pm 0.01$	$0.13^{b} \pm 0.00$	$0.08^{c} \pm 0.00$	$0.05^{d} \pm 0.02$	***
Myricetin 3-*O*-glucoside	$0.01^{d} \pm 0.00$	$0.02^{c} \pm 0.00$	$0.05^{b} \pm 0.00$	$0.06^{a} \pm 0.00$	***
Quercetin-3-*O*-glucoside	$0.29^{b} \pm 0.01$	$0.22^{c} \pm 0.02$	$0.43^{a} \pm 0.02$	$0.31^{b} \pm 0.00$	**
Rutin (Quercetin-3-*O*-rutiniside)	$0.02^{b} \pm 0.00$	$0.02^{b} \pm 0.00$	$0.04^{a} \pm 0.00$	$0.03^{b} \pm 0.00$	*
Total flavonols	$0.57^{b} \pm 0.00$	$0.45^{c} \pm 0.00$	$0.66^{a} \pm 0.00$	$0.50^{c} \pm 0.00$	*
Stilbene ($mg\,kg^{-1}$)					
Trans-resveratrol	$4.11^{c} \pm 0.30$	$4.72^{c} \pm 0.44$	$5.71^{b} \pm 0.68$	$8.17^{a} \pm 1.01$	**

*Means followed by the same letter in a row did not differ by Tukey test ($p \leq 0.05$). Standard deviation of triplicate analysis. Region: BA = Bahia and PE = Pernambuco; u.a: absorbance unit. Concentrations expressed in $g\,kg^{-1}$ fresh weight of grape, except stilbene ($mg\,kg^{-1}$); n.s. (not significant).

* Significant differences ($p < 0.05$).

** Significant differences ($p < 0.01$).

*** Significant differences ($p < 0.001$).

(Downey, Harvey, & Robinson, 2003; Flamini et al., 2013; Pfeifer, Koepke, & Reuder, 2006).

3.5. Stilbene in skins

Many studies have shown that the levels of UV radiation reaching the Earth's surface increase with altitude and decrease with increasing latitude (towards the poles). Thus, higher resveratrol content was expected in samples collected in Bahia (1100 m) but the effect was the opposite. Grapes from Pernambuco (350 m) had resveratrol higher concentrations. At the higher altitude, the resveratrol concentrations ranged from $4.11\,mg\,kg^{-1}$ (2014) to $4.72\,mg\,kg^{-1}$ (2015) while at the lower altitude values were from $5.71\,mg\,kg^{-1}$ (2014) to $8.17\,mg\,kg^{-1}$ (2015). A possible explanation is that temperatures had stronger influences on resveratrol than UV radiation. Maximum diurnal temperatures above 30 °C in Pernambuco (low altitude) during growth and maturation stressed the vines and consequently raised the levels of resveratrol. This fits with results of Langcake and Pryce (1976) and Threlfall, Morris, and Mauromoustakos (1999) who report resveratrol synthesis in grapes is raised in response to microbial infections or stresses. Resveratrol levels are also raised following chemical treatments, such as herbicide or fungicide applications, or upon exposure to UV light.

3.6. Tannins in skins and seeds

Levels of tannins and flavanols in the skins (Table 3), reveal high levels for most of these compounds (except for B3 dimer in Pernambuco) at the higher altitude. This finding is contrary to that of Mateus et al. (2001) at altitudes of 100 to 300 m asl. They conclude that low altitudes favor the synthesis of flavan-3-ol monomers of grape procyanidin dimers, trimer C1, also of extracellular proanthocyanidins. The dissimilarity may be due to the greater difference in altitude between our regions. Recently, Xing, He, Xiao, Duan, and Pan (2014) concluded that biosynthesis of flavan-3-ol in grapes occurs primarily at the onset of ripening and there appears to be a limited effect of altitude on their synthesis.

Seeds at the lower altitude (Pernambuco) contained higher levels of monomeric, oligomeric and polymeric 3-flavanol, determined by the vanillin reaction (Table 4). Higher values for condensed tannins (oligomeric and polymer phase) were found in 2015. But here, the degree of maturity may have influenced the composition of condensed tannins in the seeds. This is consistent with other studies (Jordão, Ricardo-da-Silva, & Laureano, 2001; Ó -Marques, Reguinga, Laureano, & Ricardo-da-Silva, 2005). The concentrations of the proanthocyanidins varied between the two regions and years. The monomer, epicatechin, was similarly abundant in the skins and seeds in both regions. This suggests, there was no significant effect of altitude under our conditions.

The levels of monomers (gallocatechin and epigallocatechin) and of dimers (B1, B2, B3 and B4) in the seeds were higher in Bahia (2014). In

Table 3
Concentration of condensed tannins in skins of Syrah grapes, in Pernambuco and Bahia. Two tropical regions in northeast Brazil.

Region	Bahia (1100 asl)		Pernambuco (350 m asl)		ANOVA
Harvest	2014	2015	2014	2015	(*p*-values)
Condensed tannins (mg g^{-1})					
Monomeric	0.08^{b} ± 0.01	0.09^{a} ± 0.02	0.05^{c} ± 0.00	0.02^{c} ± 0.00	**
Oligomeric	0.83^{a} ± 0.03	0.67^{b} ± 0.03	0.24^{c} ± 0.01	0.12^{d} ± 0.03	***
Polymeric	1.91^{a} ± 0.05	1.42^{b} ± 0.04	1.36^{b} ± 0.02	0.72^{d} ± 0.04	***
Total condensed tannins	2.81^{a} ± 0.09	2.18^{b} ± 0.08	1.65^{c} ± 0.03	0.86^{d} ± 0.01	***
Monomeric and small oligomeric (mg kg^{-1})					
(+) Gallocatechin	1.06^{a} ± 0.09	0.00^{b} ± 0.001	0.00^{b} ± 0.00	0.00^{b} ± 0.00	*
(+) Catechin	2.81^{c} ± 0.24	8.17^{a} ± 0.37	5.30^{b} ± 1.10	2.64^{c} ± 0.62	**
(−) Epicatechin	82.64^{a} ± 3.31	34.17^{d} ± 1.61	73.45^{b} ± 2.60	49.39^{c} ± 1.98	**
(−) Epigallocatechin	12.64^{a} ± 0.62	1.68^{c} ± 0.16	3.82^{b} ± 0.98	4.29^{b} ± 0.79	***
B1	4.29^{b} ± 1.94	7.45^{a} ± 1.41	3.74^{bc} ± 0.21	2.35^{c} ± 0.05	**
B2	18.97^{a} ± 4.33	11.92^{b} ± 0.25	13.45^{ab} ± 2.71	8.23^{b} ± 1.15	*
B3	4.84^{bc} ± 2.48	5.11^{b} ± 0.17	9.58^{a} ± 0.43	2.35^{c} ± 0.33	*
B4	2.01^{c} ± 0.18	3.74^{a} ± 0.17	3.09^{b} ± 0.23	1.20^{d} ± 0.08	**
B1 3-O-gallate	19.41^{a} ± 1.45	10.18^{b} ± 2.55	0.02^{d} ± 0.01	4.49^{c} ± 0.83	***
B2 3-O-gallate	3.91^{c} ± 0.97	13.80^{a} ± 0.18	5.02^{bc} ± 0.27	8.12^{b} ± 1.71	**
B2 3'-O-gallate	23.13^{a} ± 0.90	5.08^{b} ± 0.19	3.79^{b} ± 0.65	0.44^{c} ± 0.76	***
Epicatechin 3-O-gallate	0.77^{ab} ± 0.59	1.23^{a} ± 0.04	0.45^{b} ± 0.15	0.29^{b} ± 0.08	***
C1	9.12^{a} ± 1.53	2.37^{bc} ± 0.42	0.00^{c} ± 0.00	4.93^{b} ± 2.11	*
Trimer 2	0.01^{c} ± 0.00	11.59^{a} ± 1.07	1.07^{b} ± 0.06	0.04^{c} ± 0.01	***
Total small flavanols	185.60^{a} ± 8.14	116.49^{c} ± 3.73	122.78^{b} ± 3.15	88.76^{d} ± 6.01	*

*Means followed by the same letter in a row did not differ by Tukey test ($p < 0.05$). Standard deviation of triplicate analysis. Concentrations expressed in mg g^{-1} fresh weight of grape for condensed tannins and mg kg^{-1} fresh weight of grape for monomeric and small oligomeric; n.s. (not significant).
* Significant differences ($p < 0.05$).
** Significant differences ($p < 0.01$).
*** Significant differences ($p < 0.001$).

Pernambuco (2015), the seeds had high concentrations of catechin and B2 dimers esterified with gallic acid. In grapes, the concentration of proanthocyanidins depends on cultivar and vintage, is also influenced by viticultural and environmental factors such as shading and canopy temperature (Chira, Pacella, Jourdes, & Teissedre, 2011; Cohen & Kennedy, 2010). Also, levels of flavan-3-ol and proanthocyanidins in the skins are more affected by environmental conditions, including sunlight, than in the seeds (Downey, Harvey, & Robinson, 2004; Koyama et al., 2012).

3.7. Principal component analysis (PCA)

Fig. 2A and B shows discrimination among berry samples from the two tropical winegrowing regions, in different elevations. Results are

Table 4
Concentration of condensed tannins in Syrah grapes seeds in Pernambuco and Bahia. Two tropical regions in northeast Brazil.

Region	Bahia (1100 asl)		Pernambuco (350 m asl)		ANOVA
Harvest	2014	2015	2014	2015	(*p*-values)
Condensed tannins (mg g^{-1})					
Monomeric	0.65^{c} ± 0.03	0.53^{d} ± 0.03	2.30^{a} ± 0.03	2.06^{b} ± 0.07	***
Oligomeric	6.45^{c} ± 0.09	5.14^{d} ± 0.04	7.48^{b} ± 0.04	14.07^{a} ± 0.12	***
Polymeric	17.35^{c} ± 0.21	11.16^{d} ± 0.30	25.12^{b} ± 0.38	43.10^{a} ± 0.20	***
Total condensed tannins	24.45^{c} ± 0.23	16.83^{d} ± 0.31	34.90^{b} ± 0.32	59.23^{a} ± 0.31	**
Monomeric and small oligomeric (mg kg^{-1})					
(+) Gallocatechin	97.16^{a} ± 2.33	16.90^{b} ± 2.45	19.19^{b} ± 2.53	7.88^{c} ± 0.88	***
(+) Catechin	46.32^{c} ± 3.03	187.57^{b} ± 1.46	55.71^{c} ± 2.01	207.21^{a} ± 2.12	***
(−) Epicatechin	158.87^{b} ± 0.22	732.43^{a} ± 7.83	129.94^{c} ± 1.43	775.30^{a} ± 5.10	*
B1	229.80^{a} ± 9.17	80.07^{c} ± 3.11	71.50^{c} ± 7.07	92.88^{b} ± 2.68	***
B2	256.86^{a} ± 3.17	175.85^{c} ± 5.36	86.31^{d} ± 2.35	209.56^{b} ± 3.92	**
B3	74.32^{a} ± 10.09	51.87^{b} ± 0.35	32.87^{c} ± 0.89	55.20^{b} ± 2.26	**
B4	97.32^{a} ± 0.97	42.07^{b} ± 4.18	42.03^{b} ± 3.40	47.08^{b} ± 1.85	***
B1 3-O-gallate	6.60^{c} ± 1.21	22.56^{a} ± 1.52	15.87^{b} ± 1.47	23.35^{a} ± 4.41	*
B2 3-O-gallate	6.24^{c} ± 0.58	16.57^{b} ± 0.84	17.59^{b} ± 1.78	19.94^{a} ± 1.01	***
B2 3'-O-gallate	101.96^{c} ± 3.43	108.77^{b} ± 3.66	56.90^{d} ± 2.67	129.58^{a} ± 2.71	**
Epicatechin 3-O-gallate	1.69^{b} ± 0.07	0.95^{c} ± 0.21	3.58^{a} ± 0.26	1.14^{c} ± 0.25	**
C1	22.48^{b} ± 2.03	2.56^{c} ± 0.43	142.85^{a} ± 2.32	1.31^{c} ± 0.03	***
Trimer 2	40.09^{ab} ± 6.00	36.56^{b} ± 1.55	48.61^{a} ± 4.54	42.49^{ab} ± 2.36	**
Total small flavanols	1139.71^{b} ± 2.10	1474.73^{b} ± 1.87	722.95^{c} ± 1.45	1612.92^{a} ± 2.09	*

*Means followed by the same letter in a row did not differ by Tukey test ($p < 0.05$). Standard deviation averages of triplicate. Concentrations expressed in mg g^{-1} fresh weight of grape for condensed tannins except for monomeric and small oligomeric, with values in mg kg^{-1}; n.s. (not significant).
* Significant differences ($p < 0.05$).
** Significant differences ($p < 0.01$).
*** Significant differences ($p < 0.001$).

Fig. 2. Principal component analysis (PCA) with monoglycosylated anthocyanins and flavonols in Syrah grapes produced at two altitudes of the Brazilian Northeast. 1100 m (region with 1100 m of altitude); 350 m (region with 350 m of altitude); 14 and 15 (years of the experiment); R1, R2 and R3 (repetition); Delf3Gluc (delphinidin 3-*O*-glucoside); Cian3Gluc (cyanidin 3-*O*-glucoside); Petun3Gluc (petunidin 3-*O*-glucoside); Malv3Gluc (malvidin 3-*O*-glucoside); Peon3GlAc (peonidin 3-*O*-acetylglucoside); Petun3GlAc (petunidin 3-*O*-acetylglucoside); Cian3GlAc (cyanidin 3-*O*-acetylglucoside); Delf3GlAc (delphinidin 3-*O*-acetylglucoside); Malv3GlAc (Malvidin 3-*O*-acetylglucoside); Peon3GlCum (peonidin 3-*O*-coumarylglucoside); Petn3GlCum (petunidin 3-*O*-coumarylglucoside); Delf3GlCum (delphinidin 3-*O*-coumarylglucoside); Malv3GlCum (malvidin 3-*O*-coumarylglucoside); AntTOT (total anthocyanins); Quer (quercetin); Isorhm (isorhamnetin); Rut (rutin); Myr (myricetin).

Fig. 3. PCA analysis of condensed tannins and small flavanols with Syrah grapes cultivated at two altitudes of the Brazilian Northeast. 1100 m (region with 1100 m of altitude); 350 m (region with 350 m of altitude); 14 and 15 (years of the experiment); R1, R2 and R3 (repetition of the analysis); Gall (gallocatechin); Cat (catechin); Epicat (epicatechin); Epigal (epigallocatechin); B1, B2 and B3 (Procyanidins dimer); B1 3 Gal (B1 3-*O*-gallate), B2 3 Gal (B2 3-*O*-gallate) and B2 3LGal (B2 3′-*O*-gallate) (Procyanidin dimers gallate); Epicat_Gal (epicatechin 3-*O*-gallate); C1 and T2 (Procyanidins trimers); Tan MON (monomeric tannins); Tan OLI (oligomeric tannins); Tan POL (polymeric tannins); Tan TOT (total tannins).

based on monomeric anthocyanins and flavonols analyzes by HPLC, showed in the Table 2. The first two principal components explained 95.31% of total variability (PC1 vs. PC2), where PC1 was responsible by 71.27% and PC2 explained 24.04%. The main factor explaining grape variability was the altitude effect, followed by harvest season. PC1 separated samples from lower altitudes (350 m asl Pernambuco), located in the positive side of x axis, from those located in higher altitudes (1100 m asl Bahia), in the negative side of PC1. Samples from Pernambuco were characterized by the compounds trans-resveratrol, myricetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside and delphinidin 3-*O*-acetylglucoside. Samples from Bahia were characterized mainly by the compounds delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, peonidin 3-*O*-coumarylglucoside, and delphinidin 3-*O*-coumarylglucoside. PC2 separated samples inside each winegrowing region, according to the harvest date. In the positive side of y axis are located samples from 2015 in both regions, characterized by the compounds trans-resveratrol, myricetin, quercetin, rutin, total flavonols, petunidin 3-*O*-acetylglucoside, peonidin 3-*O*-acetylglucoside, malvidin 3-*O*-acetylglucoside and petunidin 3-*O*-coumaryl-glucoside, while in the negative side are samples from 2014 vintage. In general, there are four different groups represented by samples from two different regions according to elevation, and two different vintages for each region.

All data of condensed tannins and small flavanols in seeds and skins of grapes were analyzed also by PCA. Fig. 3A shows the grapes samples and 3B the variables, composed by principal components (PC1 × PC2), that explained 70.51% of the total variance. PC1 explained 44.89% and separated samples lower altitudes (350 m asl-Pernambuco), located in

the positive side of x axis, from those located in higher altitudes (1100 m asl-Bahia), only from 2014 vintage, in the negative side of PC1. Samples from Pernambuco were characterized by following compounds of the seeds: oligomeric and polymeric tannins, catechin, B1 3-O-gallate, B2 3-O-gallate, in the positive side, while from 2014 vintage in Bahia, by the compounds monomeric tannins, T2 and C1 trimers, epicatechin-3-*O*-gallate, besides the compound B3 from the skins (Fig. 3).

PC2 explained 25.62% of total variability, and separated samples from Pernambuco 2014 vintage, located in the negative side of y axis, from the other samples, in the positive side of PC2. Samples from Pernambuco 2014 vintage were characterized by the compounds in seeds epicatechin, dimer B3 and trimer 2.

In general, PCA was very useful to discriminate samples from two different winegrowing regions and two vintages. After comparing results of PCAs from monomeric anthocyanins and flavonols to those obtained from condensed tannins and small flavanols, first set of compounds were most important and sensitive, allowing to separate berry samples. Results showed that anthocyanins and flavonols are strongly influenced of the climate and vintage as compared to tannins and small flavonols, in this study.

4. Conclusions

The results show that differences in altitude and associated climatic factors combine to determine the chemical composition of Syrah winegrapes in northeast Brazil. In Pernambuco (350 m asl), the must tends to be high in sugars, tartaric and citric acid, the skins high in flavonols, trans-resveratrol and 3-*O*-acetylglucoside anthocyanins and the seeds in condensed tannins. Compared with grapes from Pernambuco (350 m asl), those from Bahia (1100 m asl) showed higher levels of malic and succinic acids in the must, and of anthocyanins and condensed tannins in the skins.

The profile and concentration of anthocyanins and flavonols in the skins, and of condensed tannins in skins and seeds were strongly influenced by the different environments of the two regions. According to the literature environmental factors are key for determining regional influences on the phenolic profiles of winegrapes. Further studies on the behavior and quality attributes of Syrah grapes in these two tropical regions are being carried out in relation to winemaking quality.

In this study, PCA applied on HPLC results of skins and seeds showed that anthocyanins and flavonols were most distinguishable according to the winegrowing region (elevation) and vintage than condensed tannins and small flavanols.

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