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Effects of successive harvesting in the same year on quality and bioactive compounds of grapes and juices in semi-arid tropical viticulture

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ABSTRACT

The technological and polyphenolic parameters of grapes for juice processing obtained from successive harvests were studied in semi-arid tropical viticulture. Red grapes "BRS Violeta" and "Isabel Precoce" were harvested during the same year and analyzed based on a multivariate approach that included climatic variations. The grapes were marked by variations in flavonoid compounds, particularly anthocyanins. Temperature, global radiation, air velocity and evapotranspiration rates were associated with higher accumulation of bioactive polyphenols in grape berries and juices. The temperatures in February were associated with higher antioxidant activity in grape peels, while the highest thermal amplitude in August and November favored the accumulation of anthocyanins up to 564 mg kg^{-1}. Flavanols and procyanidin compounds were more abundant in seeds when the maximum thermal amplitude was 13.8 °C. The climatic data together with the polyphenolic results point out useful information about appropriate periods for harvesting grapes with high phenolic content in tropical areas.

1. Introduction

Brazil is one of the largest fruit producers in the world and grapes are the fourth most produced fruit in the country (OCDE-FAO, 2015). Although grape cultivation in Brazil is recent when compared to traditional grape-growing countries, there is a diversified potential for viticulture due to the environmental conditions in different regions of the territory, where areas of grape production are characterized by different types of climate, such as temperate, subtropical and tropical (Camargo, Tonietto, & Hoffmann, 2011). The South and Southeastern regions are characterized by temperate and subtropical climates and have one grape harvest per year, as is the case of most grape producing regions in the world. The semi-arid tropical viticulture practiced in the São Francisco Valley (SFV) located in the Northeastern region of Brazil allows grape harvest throughout every month of the year (Camargo et al., 2011).

The SFV is located between parallels 8 and 9 °S and 40 to 42 °W and is characterized by unique climatic conditions with low annual precipitation rates (about 540 mm per year) concentrated between November and April, relatively dry air (67% average relative humidity), high insolation with higher values in the period from August to November (8.7 h per day), and average annual temperature of 26 °C with the minimum from April to September (18.2–22.2 °C) and maximum from October to March (29.6–34 °C) (Teixeira, 2010). These conditions are responsible for the differentiation in the physiological behavior of the grapevine, because the absence of low temperatures and the constant sun exposure cause the plant to vegetate during the whole year, which makes it possible the production and pruning that, together with irrigation from the San Francisco river, allows grape harvest throughout the year (Teixeira, Scherer-Warren, Hernandez, Andrade, & Leivas, 2013; Padilha et al. 2017). In addition to the production of table grapes and fine wines (*Vitis vinifera* L.), hybrid grapes and *Vitis labrusca* L. varieties are cultivated in the region for the production of high quality grape juices (Coelho et al., 2018, Dutra et al., 2018).

The potentiality of a region for the adaptation and production of grapevines is strongly dependent on the climate that interacts with other components of the environment such as grape variety, soil and agronomic techniques (Silva et al., 2019). Until recently, many efforts have been focused on combining environmental and viticulture practices to improve the quality and bioactive potential of grape products.

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Those are important to achieve high quality derivatives and sustainability, as well as to identify *terroirs* and establish better agricultural practices. Previous studies that dealt with grape management and its contribution to the quality or to the phytochemical characteristics of grape juices and wines used mostly grapes from only one annual harvest due to the climatic characteristics of the regions (Xu, Zhang, Zhu, Huang, & Lu, 2011; Acuña-avila, Vásquez-murrieta, Hernández, del López-Cortéz, 2016; Sartor, Malinovski, Caliari, Silva, & Bordignon-Luiz, 2017).

One way of measuring the influence of exogenous factors such as the climatic conditions of the region and the growing systems on the quality and typicity of grapes and grape derivatives is the utilization of chemometrics tools and multivariate analysis (Granato, Koot, Schnitzler, van Ruth, 2015a). These techniques allow the differentiation of regions of origin as a function of chemical markers such as phenolic compounds or antioxidant activity (Granato, Margraf, Brotzakis, Capuano, van Ruth, 2015b; Margraf, Santos, de Andrade, van Ruth, & Granato, 2016). The evaluation of grape characteristics in relation to the climatic conditions of a region is of great importance for the improvement of the quality of the beverage obtained from this fruit. Even in tropical regions, where there is little annual climatic variability, factors influencing grape cultivation, particularly the successive harvesting, may be determinant of the quality of grapes and grape juices. In this study, the effects of successive harvesting periods during the same year on the quality of grapes and grape juices from a semi-arid tropical viticulture were evaluated. The red grape varieties BRS Violeta (hybrid grape) and Isabel Precoce (*V. labrusca*) were harvest at each of four months of harvesting to study the effects on physico-chemical parameters, organic acids, sugars and polyphenolic composition, and antioxidant activity. These grapes are abundant in the SFV region and were chosen for their importance for grape juice production. The quality of their grape juices produced in an industrial process was also evaluated. Additionally, the chemical data was studied using chemometric techniques to assess the influence of climatic factors. This provided the possibility of differentiating the bioactive potential of the grapes and the chemical changes in response to environmental conditions, which finds application in grape and wine industries.

2. Material and methods

2.1. Chemicals and reagents

The following reagents were purchased from Merck (Darmstadt, Germany): ethyl alcohol, phosphoric and sulfuric acids, monopotassium phosphate, potassium persulfate, hydrogen peroxide and Folin-Ciocalteu phenol reagent. The polyphenols standards ((−)-epicatechin, kaempferol 3-*O*-glucoside, malvidin 3-*O*-glucoside, delphinidin 3-*O*-glucoside, peonidin 3-*O*-glucoside) were obtained from Extrasyntese (Genay, France).

The stilbenes *trans*-resveratrol and *cis*-resveratrol were obtained from the Cayman Chemical Company (Michigan, USA). Hesperidin, naringenin, (+)-catechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, procyanidin B1, procyanidin B2, rutin, quercetin 3-glucoside, malvidin 3,5-diglucoside, cyanidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, caffeic acid, chlorogenic acid, *trans*-caftaric acid, ρ-coumaric acid, syringic acid, gallic acid, glucose, fructose, rhamnose, maltose, 6-hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid (Trolox), 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and methanol were purchased from Sigma-Aldrich (St. Louis, USA). The organic acids, tartaric, malic, lactic, citric, succinic and acetic, were obtained from Vetec (Rio de Janeiro, Brazil). Water used for the mobile phase was purified in a Milli-Q Water Purification system (Millipore, Bedford, MA, USA).

2.2. Grapes harvesting and sampling

Two grape varieties were used in this study: the hybrid grape BRS Violeta (BRS Rubea × IAC 1398-21) and Isabel Precoce (*V. labrusca*), designated in the study as BV and IP samples, respectively. The grapes were cultivated in two commercial vineyards from the Sasaki Farm – Cooperativa Agrícola Nova Aliança (COANA), located in Juazeiro, Bahia, Brazil (09°24′42″S and 40°29′55″W, 370 m above sea level). The mean plant age was 6 years and the plants were grafted on the rootstock IAC 766, planted in the field with 3.0 and 2.0 m of spacing between lines and plants, respectively, irrigated by microaspersion and conducted in a trellis system. Both varieties were cultivated using the same agronomic practices.

The commercial vineyard was divided to conduct two sets of experiments with both of the grape varieties in order to have two successive harvests at different periods in the same year. In the first vineyard, two prunings were performed on October 2015 and April 2016, and the harvest took place on February and August of 2016. In the second vineyard, the vines were pruned in January and July of 2016, and the harvest was performed in the months of May and November of 2016. The harvesting dates were determined from the parameters of industrial maturity (pH, soluble solids content, titratable acidity).

The meteorological data was collected from the automatic weather station of the Brazilian Agricultural Research Corporation (EMBRAPA – Semi-Arid Station), located in Juazeiro, Bahia, Brazil. The meteorological parameters collected were: maximum and minimum air temperature (°C), global solar radiation (MJ m^{-2}), relative humidity (%), wind speed (m s^{-1}), rainfall (mm) and evapotranspiration (mm) (Table 1).

2.3. Physical and technological maturity parameters

Diameter and average weight of the grape samples were determined using 150 berries for each grape variety. A digital caliper was used to determine the diameter. The average weight of berries was obtained by dividing the total mass by the number of heavy berries. The must resulting from the crushing of the samples was analyzed for soluble solids content, pH and titratable acidity (g L^{-1} tartaric acid) according to the Organization Internationale de la Vigne et du Vin (OIV, 2011). A digital refractometer HI 96,801 (Hanna®, Woonsocket, USA) was used to measure the content of total soluble solids and pH measurements were performed with a pH analyzer potentiometer (Tecnal®, São Paulo, Brazil).

Table 1
Climatic parameters during the harvest cycle of grapes BRS Violeta and Isabel Precoce in four distinct months of the same year.

Harvest period	Temperature (°C)			Thermal amplitude (°C)	Relative humidity (%)			Global radiation (MJ)	Wind speed (m/s)	Rainfall (mm)	Evapotranspiration (mm)
	Maximum	Medium	Minimum		Maximum	Medium	Minimum				
February	35.4	28.3	22.4	13.1	82.7	64.6	34.0	26.4	2.61	2.3	5.8
May	34.0	27.3	21.5	12.5	89.2	71.6	40.4	21.0	2.32	0.1	5.2
August	32.3	25.2	19.0	13.3	90.7	72.4	37.6	18.9	2.78	0.0	4.7
November	34.5	27.1	20.7	13.8	83.4	64.2	31.9	22.6	2.81	0.3	6.1

2.4. Preparation of grape's seed and peel extracts

Grape seed and grape peel extracts were prepared in triplicate using 50 berries of each grape variety collected at the four harvesting periods. The seeds and peels were manually removed from the berries and the extracts were prepared separately by manual maceration with 100 mL of a mixture of methanol:water:acetic acid (50:48.5:1.5, v/v/v). The extracts were filtered through a number 1 Whatman filter paper and centrifuged (Edutec®, Curitiba, Brazil) at 3000 *g* for 5 min (Barcia, Pertuzatti, Gómez-Alonso, Godoy, & Hermosín-Gutiérrez, 2014). The collected supernatant was used for the spectrophotometric and chromatographic determinations.

2.5. Grape juices

The grape juices were produced at the industrial plant of the Cooperativa Agrícola Nova Aliança (COANA), located at the Senador Nilo Coelho Irrigation Project, 02 Lot 551, Petrolina, PE, Brazil, according to the process described by Lima et al. (2014).

Because the grape juice is typically produced using blends of the varieties, 3000 kg of grapes in the proportion of 80% Isabel Precoce and 20% BRS Violeta were processed at each of the four months of harvest to produce 2000 L of red grape juice. The juice was obtained by hot extraction with the addition of Pectofruit PR pectinase (Spindal-Pascal Biotech, France) at 3.0 mL 100 kg^{-1} in an industrial juice processing line manufactured by JAPA® (Garibalde, Brazil). For each harvest, three bottles of grape juice (1L) were collected, with a total of 12 bottles of grape juice used in the study.

2.6. HPLC analysis for organic acids, sugars and polyphenolic compounds

An 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) with diode array detector (DAD) and refractive index detector (RID) was used to determine organic acids, sugars and phenolic compounds in the samples. Data was processed using the OpenLAB CDS ChemStation Edition software (Agilent Technologies).

The analysis of organic acids and sugars in grape juices was performed using the method described by Coelho et al. (2018). The column used was a Hi-Plex H (300 × 7.7 mm) ion exchange with internal particles of 8.0 μm protected by a PL Hi-Plex H (5 × 3 mm) guard column (Agilent Technologies). The temperature of the column compartment was maintained at 70 °C and the RID flow cell at 50 °C. The flow was set at 0.5 mL min^{-1} with a total run time of 20 min. The mobile phase was composed of H_2SO_4 0.004 mol L^{-1} in ultrapure water.

The quantitative analysis of phenolic compounds in grape extracts and grape juices followed the method of Padilha *et al.* (2017) adapted by Dutra et al. (2018). The column and pre-column used were a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm) and a Zorbax C18 (12.6 × 4.6 mm, 5 μm), respectively (Agilent Technologies). The column temperature was set at 35 °C. The mobile phase consisted of a solution of phosphoric acid 0.1 mol L^{-1}, pH = 2.0 (A) and methanol acidified with 0.5% H_3PO_4 (B). The flow rate was 0.8 mL min^{-1} and 20 μL was the injection volume of the sample. Before sample injection, grape peel extracts were diluted with the mobile phase A, and filtered through a 0.45 μm membrane (Millex Millipore, Barueri, SP, Brazil). The elution gradient used was 0–5 min: 5% B; 5–14 min: 23% B; 14–30 min: 50% B; 30–33 min: 80% B. Detection was performed at 220, 280, 320, 360 and 520 nm, and quantification was performed by comparison with external standards.

2.7. Antioxidant activity assays

The antioxidant activity was evaluated using the following assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method (Kim, Guo, & Packer, 2002); ABTS (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid)) radical scavenging method (Re et al., 1999), hydrogen peroxide scavenging method (Ruch, Cheng, & Klaunig, 1989) and Folin-Ciocalteu reducing capacity method (FC) (Singleton & Rossi, 1965). The analyzes were carried out on a UV–Visible UV 2000A spectrophotometer (Instrutherm, São Paulo, Brazil).

The samples were diluted with the extraction solution (seed and peel extracts) or with water (grape juice) for the analysis. Solutions of the radicals ABTS (1 mmol L^{-1}) and DPPH (100 μmol L^{-1}) were prepared in ethanol and absorbance measurements were taken before and after the addition of the samples at 734 and 517 nm, respectively. Absorbances were measured at time zero (no sample), and after 30 min for DPPH and 6 min for ABTS (with added sample). The results were expressed as Trolox equivalents (μmol TE) using Trolox as calibration standard.

The hydrogen peroxide scavenging activity was assessed using a solution of hydrogen peroxide (0.4 mol L^{-1}) in phosphate buffer (pH 7.4). The samples were mixed with the hydrogen peroxide solution and absorbances were determined spectrophotometrically at 230 nm after 10 min. Phosphate buffer was taken as blank sample and the results were expressed as Trolox equivalents (μmol TE).

The Folin-Ciocalteu reducing capacity was measured by mixing 100 μL of diluted samples with 7.9 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. 1.5 mL of sodium carbonate solution (20% m/v) was added after 3 min, and the absorbance of the mixture was determined at 765 nm after 2 h in the dark. Results were expressed as mg of gallic acid equivalents (mg GAE).

2.8. Statistical analysis

Statistical analysis was performed using the SPSS statistics software version 17.0 (IBM, New York, USA). One-way analysis of variance and Tukey's test were used to assess statistical differences ($p < 0.05$) among samples. The multivariate statistics was carried out using principal component analysis (PCA) with pretreatment of the data for normalization and scaling.

3. Results

3.1. Physical and technological parameters of the grapes

Table 2 shows the physico-chemical characteristics of the grapes obtained in the harvests of February, May, August and November of the same year. The results for berry size and weight showed that BV grapes had smaller berries (13.9–14.8 mm and 2.0–2.3 g, respectively) when compared to IP grapes (14.2–16.8 mm and 2.3–2.9 g, respectively) and that both of them had few variations in these parameters in different months of the year. The pH varied significantly from 3.24 to 3.41 and from 3.44 to 3.70 in IP and BV varieties, respectively, and some variations were observed among the harvest months.

The content of total soluble solids ranged from 18.1 to 23.4 °Brix for BV grapes and from 18.6 to 23.8 °Brix for IP grapes, while the titratable acidity varied between 0.50 and 1.02 (% w/v as tartaric acid) for the varieties. The TSS/TA ratio varied from 30.2 to 37.5 for BV grapes and from 23.3 to 31.0 for IP grapes. These values are in accordance with Brazilian legislation for grape harvesting and were comparatively higher in the grapes harvest in May.

The grapes harvested in August had the highest values for titratable acidity (0.74 and 1.02 g 100 mL^{-1} for BV and IP, respectively). The lowest titratable acidity was found for the BV grapes harvested in February (0.50 g 100 mL^{-1}), whereas for IP grapes the lowest value was found in the samples harvested in May (0.67 g 100 mL^{-1}). In general, the samples obtained in the later months of the year (August and November) showed higher values of soluble solids and titratable acidity. This is consistent with the study of Ribeiro, de Lima, and Alves (2012) who reported that Isabel Precoce and BRS Magna grapes grown in the SFV region showed higher content of soluble solids when harvested in November.

Table 2
Quality parameters of grapes harvested in four months of the year.

Harvest period	Grapes	Parameters					
		Physical		Physico-chemical			
		Berry weight* (g)	Berry diameter* (mm)	pH	Total soluble solids (°Brix)	Titratable acidity (g/100 mL)	Ratio (TSS/TA)
February	BV	2.13	13.85	3.70 ± 0.01^{a}	18.1 ± 0.1^{g}	0.50 ± 0.00^{g}	36.1 ± 0.1^{b}
	IP	2.60	14.18	3.28 ± 0.01^{e}	18.6 ± 0.0^{f}	0.78 ± 0.01^{c}	24.0 ± 0.1^{g}
May	BV	2.31	14.31	3.59 ± 0.00^{b}	19.9 ± 0.2^{e}	0.53 ± 0.00^{f}	37.5 ± 0.0^{a}
	IP	2.56	15.98	3.41 ± 0.04^{c}	20.7 ± 0.1^{d}	0.67 ± 0.01^{e}	31.0 ± 0.2^{d}
August	BV	2.22	14.82	3.62 ± 0.01^{b}	22.3 ± 0.3^{c}	0.74 ± 0.01^{d}	30.2 ± 0.2^{e}
	IP	2.31	15.78	3.34 ± 0.01^{d}	23.8 ± 0.3^{a}	1.02 ± 0.01^{a}	23.3 ± 0.4^{g}
November	BV	2.00	13.92	3.44 ± 0.03^{c}	23.4 ± 0.1^{b}	0.68 ± 0.01^{e}	34.1 ± 0.1^{c}
	IP	2.92	16.76	3.24 ± 0.05^{e}	22.6 ± 0.1^{c}	0.80 ± 0.01^{b}	28.4 ± 0.4^{f}

Data expressed as the mean ± standard deviation ($n = 3$).
TSS, total soluble solids; TA, titratable acidity (expressed as tartaric acid equivalents); BV, BRS Violeta; IP, Isabel Precoce. Different letters in the same column indicate significant difference according to Tukey's test ($p < 0.05$).
* Represents the mean value of the analysis of 150 grape berries.

3.2. Changes on polyphenolic profile and in vitro antioxidant activity of grape peels and seeds

The polyphenolic profile and antioxidant activity in peel and seed extracts of BRS Violeta and Isabel Precoce grapes harvested in different periods of the same year are shown in Tables 3 and 4, respectively. Flavonols, anthocyanins, phenolic acids, flavanones, stilbenes, catechins and procyanidins were among the polyphenolic substances found in the grape extracts.

Anthocyanins were quantified in peel extracts and were the most abundant polyphenols in these samples with their total concentrations ranging from 704.2 to 5643.8 mg kg^{-1}, followed by flavanols (20.0–1383.2 mg kg^{-1}), phenolic acids (46.8–1076.9 mg kg^{-1}), flavanones (16.5–480.7 mg kg^{-1}), stilbenes (2.9–456.9 mg kg^{-1}) and flavonols (10.5–77.1 mg kg^{-1}). The seed extracts showed the highest concentrations of flavanols (2147.3–4757.6 mg kg^{-1}) and the lowest concentrations of phenolic acids (12.1–100.2 mg kg^{-1}), flavonols (1.3–36.4 mg kg^{-1}) and naringenin (3.5–7.2 mg kg^{-1}) (Table 4). The predominance of anthocyanins in the peels and of monomers and dimers of flavanols in the seed extracts was expected since these polyphenols are the main phenolic constituents of red grape peels and grape seeds (Lorrain, Ky, Pechamat, & Teissedre, 2013).

The antioxidant activity varied among grape samples and the months of harvest and was dependent of the analytical method. The values were high in seed extracts and ranged up to 213.89 and 653.32 μmol TE g^{-1} when using the ABTS and H_2O_2 methods, respectively. The Folin-Ciocalteu reducing capacity varied from 15.35 to 32.31 mg GAE g^{-1} in these samples. The antioxidant activity of grape peels ranged up to 161.81 in the ABTS method and up to 1175.1 μmol TE g^{-1} in the H_2O_2 method. The highest value for the Folin-Ciocalteu reducing capacity of peel extracts was 28.64 mg GAE g^{-1}. The high antioxidant activity found in samples when using the H_2O_2 method suggests that both BV and IP grapes have inhibitory potential against reactive oxygen species (ROS). It is known that the antioxidant capacity is related to protection mechanisms, including inhibition of ROS that are associated with several pathological conditions that lead to diseases such as cancer, diabetes and cardiovascular diseases (Pandey & Rizvi, 2009; Toaldo et al., 2016). The high antioxidant activity of grape seeds is mainly explained by the predominance of flavanols and procyanidins. These polyphenols exert strong antioxidant activity that is even higher than that of the anthocyanins, which are more abundant in peels (Muselik et al., 2007).

The BV grapes showed similar phenolic composition during the four months of harvest, which was not the case for the IP variety. It is important to emphasize that the BRS Violeta is a hybrid obtained through genetic improvement using several crosses, and may have acquired greater stability in the face of climatic variations. This variety was developed for cultivation in tropical areas, such as the São Francisco Valley (Camargo, Maia, & Nachtigal, 2005). The variety Isabel Precoce is a spontaneous somatic mutation of the traditional variety Isabel (Lima et al., 2014). Some variations were found for the phenolic composition of grapes throughout the successive harvests. Specific changes in the concentration levels of many types of phenolic compounds are presented below.

3.2.1. Phenolic acids

The sum of phenolic acids quantified in peels of IP samples ranged from 46.8 to 1076.9 mg kg^{-1} for the grapes harvested in May and February, respectively (Table 3). The harvesting period influenced ($p < 0.05$) their levels in peels of both varieties, with the highest concentrations observed in February and the lowest in May. *trans*-Caftaric acid was the main phenolic acid found in the peels of IP (39.2–965.9 mg kg^{-1}) and BV (84.3–385.7 mg kg^{-1}) grapes, followed by syringic acid for the BV variety (60.4–127.0 mg kg^{-1}) and chlorogenic acid for the IP variety (2.6–76.1 mg kg^{-1}). The ρ-coumaric acid was not detected in grape peels, regardless of harvest period. Studies with wines from the SFV region have described *trans*-caftaric as the predominant phenolic acid (Padilha et al., 2017; Dutra et al., 2018). Among the non-flavonoid polyphenols, the phenolic acids are the major compounds in grapes and grape beverages (Lorrain et al., 2013).

The concentrations of phenolic acids in seeds ranged from 12.1 to 100.2 mg kg^{-1} in BV grapes harvested in February and November, respectively (Table 4). The months of harvesting influenced ($p < 0.05$) their levels in grape seeds, particularly for the grapes harvested in November, which had the highest concentrations of these compounds, with the exception of ρ-coumaric acid. Syringic acid was the major phenolic acid in samples and was found in concentrations ranging from 4.4 to 37.8 mg kg^{-1}. The BV seeds showed higher values of phenolic acids when compared with the seeds of IP variety, regardless of harvest period. The levels of phenolic acids were increased in the later months of the year (harvests in August and November) and this was observed for both grape varieties.

3.2.2. Stilbenes

The stilbenes *cis*- and *trans*-resveratrol were found only in the peel extracts of BV and IP grapes (Table 3) not being detected in the seeds. Their concentrations reached up to 456.9 mg kg^{-1} in BV grapes harvested in May. Their concentrations varied greatly ($p < 0.05$) among the harvesting periods, with the highest levels observed in May and in November for BV and IP varieties, respectively. Consistently, the isomer

Table 3
Phenolic composition (mg kg^{-1} fresh weight) and antioxidant activity of grape peel extracts from BRS Violeta and Isabel Precoce harvested in different periods of the year.

Parameters	Harvest period							
	February		May		August		November	
	BV	IP	BV	IP	BV	IP	BV	IP
Phenolic acids								
trans-Caftaric acid	293.5 ± 0.9^{C}	965.9 ± 0.5^{A}	84.3 ± 0.3^{F}	39.2 ± 0.1^{G}	143.7 ± 1.6^{E}	182.4 ± 4.2^{D}	385.7 ± 2.4^{B}	176.4 ± 0.4^{D}
Syringic acid	127.0 ± 1.7^{A}	10.6 ± 0.1^{E}	73.9 ± 0.0^{C}	2.3 ± 0.0^{F}	117.0 ± 0.3^{B}	5.0 ± 0.1^{F}	60.4 ± 1.6^{D}	5.3 ± 0.4^{F}
Chlorogenic acid	91.8 ± 0.8^{A}	76.1 ± 0.1^{B}	13.4 ± 0.4^{F}	2.6 ± 0.3^{G}	35.3 ± 0.0^{D}	19.5 ± 0.4^{E}	41.5 ± 0.6^{C}	12.4 ± 0.1^{F}
Caffeic acid	39.3 ± 3.0^{A}	10.2 ± 0.0^{D}	19.9 ± 0.4^{C}	2.7 ± 0.0^{E}	24.6 ± 0.1^{B}	6.1 ± 0.1^{DE}	19.1 ± 0.1^{C}	5.8 ± 0.1^{E}
Gallic acid	24.4 ± 2.0^{A}	14.1 ± 0.1^{B}	ND	ND	4.2 ± 0.0^{D}	ND	8.1 ± 0.5^{C}	ND
ρ-Coumaric acid	ND	ND	ND	ND	ND	ND	ND	ND
Σ phenolic acids quantified	576.0 ± 8.4	1076.9 ± 0.8	191.5 ± 0.11	46.8 ± 0.4	324.8 ± 2.0	213.0 ± 4.8	514.8 ± 5.2	199.9 ± 0.1
Stilbenes								
cis-Resveratrol	353.5 ± 1.3^{C}	ND	439.5 ± 0.8^{A}	ND	381.9 ± 12.4^{B}	ND	331.7 ± 2.1^{D}	15.4 ± 0.1^{E}
trans-Resveratrol	8.3 ± 0.6^{D}	13.2 ± 0.2^{C}	17.4 ± 0.1^{A}	2.9 ± 0.0^{F}	6.5 ± 0.0^{E}	5.7 ± 0.0^{E}	16.1 ± 0.5^{B}	12.3 ± 0.1^{C}
Σ stilbenes quantified	361.8 ± 1.9	13.2 ± 0.2	456.9 ± 0.9	2.9 ± 0.0	388.4 ± 12.4	5.7 ± 0.0	347.8 ± 2.6	27.7 ± 0.2
Flavanols								
(+)-Catechin	10.4 ± 2.0^{B}	9.8 ± 0.1^{B}	10.1 ± 0.1^{B}	4.0 ± 0.6^{C}	12.7 ± 0.2^{B}	5.5 ± 0.6^{C}	48.9 ± 2.1^{A}	11.9 ± 0.0^{B}
(−)-Epicatechin	371.8 ± 7.0^{C}	14.5 ± 0.2^{E}	387.0 ± 0.1^{B}	6.9 ± 0.1^{E}	408.6 ± 0.5^{A}	11.5 ± 0.6^{E}	317.8 ± 0.2^{D}	5.4 ± 0.2^{E}
(−)-Epicatechin gallate	ND	34.4 ± 0.1^{A}	28.1 ± 0.1^{B}	2.1 ± 0.1^{C}	ND	ND	28.1 ± 2.1^{B}	3.1 ± 0.1^{C}
(−)-Epigallocatechin gallate	99.1 ± 4.1^{B}	9.4 ± 0.4^{E}	79.7 ± 0.1^{C}	2.2 ± 0.0^{F}	132.8 ± 0.2^{A}	3.7 ± 0.1^{EF}	68.8 ± 0.3^{D}	5.0 ± 0.1^{EF}
Procyanidin B1	16.5 ± 0.7^{A}	8.6 ± 0.1^{D}	7.1 ± 0.1^{E}	3.0 ± 0.0^{F}	6.2 ± 0.2^{E}	3.9 ± 0.1^{F}	14.2 ± 0.2^{B}	11.4 ± 0.0^{C}
Procyanidin B2	885.4 ± 2.3^{A}	5.5 ± 0.2^{F}	742.5 ± 1.2^{B}	1.8 ± 0.0^{F}	662.7 ± 0.8^{D}	3.9 ± 0.2^{F}	731.4 ± 1.4^{C}	24.2 ± 0.2^{E}
Σ flavanols quantified	1383.2 ± 16.1	82.2 ± 0.11	1254.5 ± 1.7	20.0 ± 0.8	1223.0 ± 1.9	28.5 ± 1.6	1209.2 ± 6.3	61.0 ± 0.6
Flavonols								
Quercetin 3-*O*-glucoside	15.1 ± 0.8^{D}	64.0 ± 0.1^{A}	33.9 ± 0.6^{B}	16.1 ± 0.1^{CD}	17.0 ± 0.1^{C}	0.8 ± 0.0^{E}	1.8 ± 0.1^{E}	1.4 ± 0.1^{E}
Rutin	6.3 ± 1.1^{B}	1.0 ± 0.0^{C}	10.8 ± 0.1^{A}	0.4 ± 0.0^{C}	11.5 ± 0.2^{A}	5.7 ± 0.0^{B}	9.9 ± 1.1^{A}	7.1 ± 0.1^{B}
Kaempferol 3-*O*-glucoside	ND	7.2 ± 0.2^{D}	ND	1.7 ± 0.0^{F}	48.6 ± 0.1^{A}	4.0 ± 0.1^{E}	46.4 ± 0.3^{B}	7.9 ± 0.2^{C}
Σ flavonols quantified	21.4 ± 1.9	72.2 ± 0.3	44.7 ± 0.7	18.2 ± 0.1	77.1 ± 0.4	10.5 ± 0.1	58.1 ± 1.5	16.4 ± 0.4
Flavanones								
Naringenin	161.5 ± 9.3^{D}	18.3 ± 0.0^{E}	368.5 ± 1.6^{B}	18.7 ± 0.0^{E}	419.1 ± 0.9^{A}	26.0 ± 0.1^{E}	241.5 ± 0.8^{C}	16.5 ± 0.1^{E}
Hesperidin	52.9 ± 1.9^{C}	ND	65.5 ± 1.8^{A}	ND	61.6 ± 0.4^{B}	27.2 ± 0.2^{D}	ND	ND
Σ flavanones quantified	214.4 ± 11.2	18.3 ± 0.0	434.0 ± 3.4	18.7 ± 0.0	480.7 ± 1.3	53.2 ± 0.3	241.5 ± 0.8	16.5 ± 0.1
Anthocyanins								
Malvidin 3-*O*-glucoside	ND	1483.0 ± 0.9^{A}	105.9 ± 0.2^{E}	468.6 ± 0.7^{D}	104.7 ± 0.2^{E}	1032.1 ± 0.9^{B}	ND	877.7 ± 0.3^{C}
Delphinidin 3-*O*-glucoside	144.8 ± 9.8^{C}	79.0 ± 0.4^{E}	219.2 ± 0.6^{B}	13.1 ± 0.1^{G}	382.6 ± 1.9^{A}	26.8 ± 0.4^{FG}	120.9 ± 0.9^{D}	33.0 ± 0.6^{F}
Peonidin 3-*O*-glucoside	ND	214.5 ± 1.3^{A}	20.4 ± 0.4^{E}	55.5 ± 0.0^{D}	17.5 ± 0.0^{F}	115.7 ± 0.6^{B}	ND	113.1 ± 0.7^{C}
Malvidin 3,5-diglucoside	2527.7 ± 7.6^{C}	515.3 ± 1.8^{E}	3035.3 ± 1.4^{B}	167.0 ± 0.8^{G}	4278.8 ± 51.3^{A}	275.2 ± 0.1^{F}	1900.5 ± 1.8^{D}	329.9 ± 1.3^{F}
Cyanidin 3,5-diglucoside	599.1 ± 2.4^{B}	11.8 ± 0.1^{E}	506.8 ± 1.1^{C}	ND	860.2 ± 0.1^{A}	ND	418.0 ± 1.6^{D}	9.8 ± 0.2^{E}
Pelargonidin 3,5-diglucoside	ND	ND	ND	ND	ND	ND	ND	ND
Σ anthocyanins quantified	3271.6 ± 19.8	2303.6 ± 4.5	3887.6 ± 3.7	704.2 ± 1.6	5643.8 ± 53.5	1449.8 ± 2.0	2439.4 ± 4.3	1363.5 ± 3.1
Antioxidant activity								
DPPH (μmol TE g^{-1} FW)	113.39 ± 3.61^{A}	46.80 ± 2.81^{D}	90.14 ± 0.82^{B}	35.47 ± 0.56^{E}	89.39 ± 1.2^{2B}	16.44 ± 0.42^{G}	75.62 ± 3.42^{C}	23.34 ± 1.34^{F}
ABTS (μmol TE g^{-1} FW)	161.81 ± 5.44^{A}	47.60 ± 2.69^{D}	97.85 ± 1.88^{C}	6.87 ± 0.41^{F}	98.70 ± 3.30^{C}	19.33 ± 0.42^{EF}	114.30 ± 12.65^{B}	26.98 ± 2.97^{E}
H_2O_2 (μmol TE g^{-1} FW)	593.02 ± 0.41^{C}	280.29 ± 1.92^{E}	532.65 ± 0.17^{D}	193.81 ± 1.24^{F}	790.73 ± 19.24^{B}	149.83 ± 1.48^{G}	1175.1 ± 9.97^{A}	614.58 ± 9.97^{C}
Folin Ciocalteu (mg GAE g^{-1})	28.64 ± 0.66^{A}	14.3 ± 1.43^{D}	21.86 ± 1.73^{B}	2.82 ± 0.26^{E}	23.59 ± 0.58^{B}	4.47 ± 0.16^{E}	18.12 ± 1.14^{C}	3.39 ± 0.35^{E}

Data represent the mean values for each sample ± standard deviation ($n = 3$); Different letters in the same row indicate significant difference according to Tukey's test ($p < 0.05$); BV, BRS Violeta; IP, Isabel Precoce; TE, Trolox equivalents; GAE, gallic acid equivalents; FW, fresh weight; ND, not detected.

Table 4
. Phenolic composition (mg kg^{-1} fresh weight) and antioxidant activity of grape seed extracts from BRS Violeta and Isabel Precoce harvested in different periods of the year.

Parameters	Harvest period							
	February		May		August		November	
	BV	IP	BV	IP	BV	IP	BV	IP
Phenolic acids								
trans-Caftaric acid	ND	10.0 ± 0.1^{C}	ND	ND	5.9 ± 0.0^{D}	ND	35.6 ± 0.0^{A}	18.6 ± 0.4^{B}
Syringic acid	4.4 ± 1.4^{F}	14.0 ± 0.1^{D}	11.2 ± 0.4^{E}	15.5 ± 0.0^{D}	14.7 ± 0.1^{D}	22.9 ± 0.1^{C}	28.5 ± 0.2^{B}	37.8 ± 0.1^{A}
Chlorogenic acid	3.2 ± 0.1^{E}	5.6 ± 0.0^{B}	ND	ND	3.2 ± 0.0^{E}	3.9 ± 0.1^{D}	6.7 ± 0.1^{A}	5.4 ± 0.1^{C}
Caffeic acid	ND	ND	ND	ND	2.0 ± 0.1^{C}	ND	$3.2 \pm 0.1A$	2.4 ± 0.1^{B}
Gallic acid	4.50 ± 1.1^{G}	12.5 ± 0.0^{D}	20.7 ± 0.1^{C}	13.9 ± 0.0^{D}	10.0 ± 0.0^{E}	7.5 ± 0.0^{F}	26.2 ± 0.2^{B}	28.1 ± 0.3^{A}
ρ-Coumaric acid	ND	ND	ND	ND	8.2 ± 0.1	ND	ND	ND
Σ phenolic acids quantified	12.1 ± 2.6	42.1 ± 0.2	31.9 ± 0.5	29.4 ± 0.0	44.0 ± 0.3	34.3 ± 0.2	100.2 ± 0.6	92.3 ± 1.0
Flavanols								
(+)-Catechin	2316.3 ± 6.6^{C}	969.3 ± 13.1^{G}	2879.3 ± 7.7^{B}	1011.3 ± 0.1^{F}	1598.1 ± 1.4^{D}	1008.7 ± 5.0^{F}	2931.0 ± 1.1^{A}	1499.6 ± 0.4^{E}
(−)-Epicatechin	884.9 ± 16.5^{E}	1590.9 ± 50.3^{B}	1025.9 ± 6.2^{D}	786.1 ± 0.5^{F}	713.5 ± 3.2^{F}	1109.8 ± 3.3^{C}	1184.6 ± 0.4^{C}	1868.2 ± 0.4^{A}
(−)-Epicatechin gallate	54.2 ± 2.4^{B}	154.9 ± 0.3^{A}	ND	ND	33.0 ± 0.1^{C}	57.6 ± 0.3^{B}	5.7 ± 0.1^{D}	7.0 ± 0.1^{D}
(−)-Epigallocatechin gallate	16.6 ± 0.6^{D}	49.9 ± 0.6^{A}	38.2 ± 0.8^{B}	26.9 ± 0.3^{C}	28.5 ± 0.2^{C}	40.4 ± 0.5^{B}	17.4 ± 0.2^{D}	50.5 ± 0.9^{A}
Procyanidin B1	163.2 ± 3.4^{E}	188.4 ± 0.6^{D}	229.8 ± 3.1^{B}	103.9 ± 1.2^{G}	113.0 ± 1.4^{F}	119.0 ± 0.8^{F}	260.0 ± 0.2^{A}	195.6 ± 0.1^{C}
Procyanidin B2	193.4 ± 3.8^{F}	407.2 ± 2.1^{B}	349.5 ± 5.9^{C}	219.1 ± 1.3^{E}	195.7 ± 1.4^{F}	288.0 ± 1.9^{D}	358.9 ± 1.1^{C}	473.2 ± 1.1^{A}
Σ flavanols quantified	3628.6 ± 33.3	3360.6 ± 67.0	4522.7 ± 23.7	2147.3 ± 3.4	2681.8 ± 7.7	2623.5 ± 11.8	4757.6 ± 3.1	4094.1 ± 3.0
Flavonols								
Quercetin 3-*O*-glucoside	4.9 ± 1.1^{C}	3.2 ± 0.1^{D}	6.0 ± 0.0^{C}	1.7 ± 0.0^{D}	ND	2.2 ± 0.0^{D}	27.2 ± 0.2^{A}	14.0 ± 0.3^{B}
Rutin	3.1 ± 0.8^{E}	11.3 ± 0.0^{A}	5.1 ± 0.1^{D}	4.9 ± 0.1^{D}	ND	6.1 ± 0.0^{CD}	6.9 ± 0.1^{C}	9.4 ± 0.3^{B}
Kaempferol 3-*O*-glucoside	1.40 ± 0.3^{AB}	1.3 ± 0.0^{AB}	2.1 ± 0.6^{AB}	1.2 ± 0.0^{B}	1.3 ± 0.1^{B}	1.6 ± 0.1^{AB}	2.3 ± 0.0^{A}	1.4 ± 0.1^{AB}
Σ flavonols quantified	9.4 ± 2.2	15.8 ± 0.1	13.2 ± 0.7	7.8 ± 0.1	1.3 ± 0.1	9.9 ± 0.1	36.4 ± 0.3	24.8 ± 0.7
Flavanones								
Naringenin	3.5 ± 0.4^{C}	ND	ND	ND	ND	ND	7.2 ± 0.1^{A}	5.7 ± 0.1^{B}
Hesperidin	ND	ND	ND	ND	ND	ND	ND	ND
Σ flavanones quantified	3.5 ± 0.4	–	–	–	–	–	7.2 ± 0.1	5.7 ± 0.1
Antioxidant activity								
DPPH (μmol TE g-1 FW)	172.98 ± 19.74^{BC}	204.69 ± 15.71^{AB}	212.41 ± 7.00^{A}	170.25 ± 19.02^{C}	101.61 ± 8.40^{D}	149.51 ± 4.32^{C}	145.08 ± 2.85^{C}	161.30 ± 4.34^{C}
ABTS (μmol TE g-1 FW)	198.47 ± 19.67^{AB}	196.83 ± 19.37^{AB}	149.45 ± 0.46^{CD}	213.89 ± 9.96^{A}	99.27 ± 4.79^{E}	120.26 ± 2.33^{DE}	156.09 ± 3.55^{C}	172.88 ± 9.15^{BC}
H_2O_2 (μmol TE g-1 FW)	525.61 ± 0.83^{D}	576.26 ± 0.79^{C}	609.61 ± 0.16^{B}	469.83 ± 2.43^{E}	454.12 ± 1.29^{F}	577.99 ± 1.40^{C}	653.32 ± 8.40^{A}	652.95 ± 5.36^{A}
Folin Ciocalteu (mg GAE g^{-1})	23.77 ± 0.52^{C}	24.94 ± 0.42^{BC}	32.31 ± 0.10^{A}	20.87 ± 0.73^{D}	15.35 ± 0.35^{E}	20.27 ± 1.30^{D}	27.27 ± 0.98^{B}	30.32 ± 1.92^{A}

Data represent the mean values for each sample ± standard deviation ($n = 3$); Different letters in the same row indicate significant difference according to Tukey's test ($p < 0.05$); BV, BRS Violeta; IP, Isabel Precoce; TE, Trolox equivalents; GAE, gallic acid equivalents; FW, fresh weight; ND, not detected.

cis-resveratrol was only detected in the last month of harvest in IP grapes. These findings corroborate that not only grape variety but also exogenous factors such as the variable climatic conditions of the harvesting periods influence the composition of stilbenes in grapes. Several studies have suggested that increases in concentrations of these polyphenols are associated with stress conditions in the grapevine (Wang et al., 2010; Acuña-avila et al., 2016; Sartor et al., 2017).

The molecules *cis*- and *trans*-resveratrol are interchangeable isomers. *cis*-Resveratrol was present at high concentrations (331.7 to 439.5 mg kg^{-1}) in all BV samples. Previous studies on juices produced in the SFV region reported the prevalence of this compound in BRS Violeta grapes (Dutra et al., 2018). It is known that resveratrol is more abundant and stable in the *trans* configuration, as this is the main end product generated in the biosynthesis of resveratrol. However, *trans-cis* isomerization occurs when *trans*-resveratrol is exposed to solar or ultraviolet radiation (Moreno, Castro, Falqué, 2008). This may suggest that the high concentrations of *cis*-resveratrol found in the samples may be associated with the high radiation (18.9–26.4 MJ) observed during the harvesting periods (Table 1).

3.2.3. Flavanols, procyanidins, flavonols and flavanones

The concentrations of monomeric flavanols and their dimers (proanthocyanidins) differed greatly among grapes varieties and harvesting periods. Levels of flavanols ranged from 20.0 to 1383.2 mg kg^{-1} in peels (Table 3) and from 2147.3 to 4757.6 mg kg^{-1} in seeds (Table 4). The grapes harvested in November showed the highest levels of flavanols. Catechin, epicatechin and procyanidins were the predominant polyphenols found in seeds and the most abundant flavanols in peels of both varieties. Their concentrations were variably affected ($p < 0.05$) throughout the harvesting periods. Other studies have also reported procyanidins, catechin and epicatechin as the main flavanols present in grapes (Garcia-Jares et al., 2015; Liu, Yan, Li, Wang, & Shi, 2018).

Rutin and 3-*O*-monoglucosides of quercetin and kaempferol were the flavonols found in the samples. Their total concentrations ranged from 10.5 to 77.1 mg kg^{-1} in peels of IP and BV grapes, respectively, and from 1.3 to 36.4 mg kg^{-1} in seeds of BV grapes. The presence of flavonols and their concentrations varied significantly between grapes and among the harvesting periods. The BV grapes harvested in November had the highest concentrations of flavonols with a total sum of 94.5 mg kg^{-1} considering peels and seeds. A high content of kaempferol was found in the peel (up to 46.4 mg kg^{-1}), while the seeds had up to 27.2 mg kg^{-1} of quercetin. For IP grapes, it was observed that February was the most favorable month for flavonols production, when the levels of quercetin reached up to 64 mg kg^{-1} in peels and the levels of rutin were up to 11.3 mg kg^{-1} in seeds. The concentration levels of flavonols found in the samples may be related to stress caused by high solar radiation observed in the study region. According to Flamini, Mattivi, De Rosso, Arapitsas, and Bavaresco (2013), flavonols are mainly present in the grape peel, acting effectively as photoprotectors by absorbing ultraviolet radiation, particularly UV-A and UV-B. In addition, the amount of flavonols is also influenced by the size and thickness of the berry, which may explain the higher concentrations of these polyphenols found for the BV grapes (Table 2).

The flavanone naringenin was identified in peel extracts of both grape varieties, while it was almost absent in the seeds. For both BV and IP grapes, naringenin concentrations were higher in relation to hesperidin, which was not detected in the seeds. For the IP variety, hesperidin was found only in grapes harvested in August (27.2 mg kg^{-1}). This is consistent with the study of Dutra et al. (2018) who reported concentrations of hesperidin and narigenin in grape juices and wines of up to 4.93 and 5.64 mg L^{-1}, respectively.

Total concentrations of flavanones in grape peels varied between 16.5 mg kg^{-1} in November and 480.7 mg kg^{-1} in August. Although some studies report the antioxidant activity of flavanones, this group of phenolic compounds is not commonly studied in grapes, as they are more abundant in citrus fruit (Domínguez, 2016).

3.2.4. Anthocyanins

Five anthocyanins (3-*O*-glucosides and 3,5-diglucosides) were quantified in peel extracts of the grapes (Table 3). Malvidin in its 3,5-diglucoside and 3-*O*-monoglucoside forms were the major anthocyanins in BV and IP grapes, and their levels accounted for up to 78.1% and 71.2% of total anthocyanins found in these grapes, respectively. The diglucoside of pelargonidin was not detected in samples, regardless of harvest period. Statistical differences were observed among the harvesting periods in relation to anthocyanins. BV grapes collected in August had the highest levels of anthocyanins in peels, with a total concentration of 5643.8 mg kg^{-1}. For IP grapes, the highest levels were observed in February (2303.6 mg kg^{-1}). The lowest total concentrations of anthocyanins were observed in November (2439.4 mg kg^{-1} for BV) and May (704.2 mg kg^{-1} for IP). Taken together, these variations on anthocyanin concentrations indicate great influence of exogenous factors during the harvesting periods. Apart from the influence of grape variety, factors such as high luminosity and mild temperatures are important for the synthesis and accumulation of anthocyanins in the berry. In fact, temperatures around 35 °C are associated with difficulties in anthocyanins synthesis by the plant as well as with the activation of oxidative enzymes that lead to degradation of these compounds (He et al., 2010).

The concentration levels of all anthocyanins were significantly lower in the last month of harvest (November). In that month, the monoglucosides of malvidin and peonidin were not detected in the BV grapes. In addition, concentrations of these compounds decreased consistently in peels of IP grapes throughout the harvesting periods. Hence, the results pointed out specific behaviors of these grapes in relation to anthocyanins concentrations and profiles. It is important to remark that in the SFV region the grapes BRS Violeta and Isabel Precoce are used to produce grape juice by mixing these varieties, and that the first is used to provide a better coloring characteristic. The results showed that the harvest in August led to obtention of grapes more rich in anthocyanins. This month presented the lowest temperatures and the lowest evapotranspiration, which corroborates the importance of mild temperatures for anthocyanins synthesis and accumulation.

3.3. Association between successive harvesting and polyphenolic composition

Data of the polyphenolic and antioxidant characteristics of grape seeds and peels was used to explore the effects of harvesting periods on grape quality and bioactive composition through multivariate analysis. The PCA score and loading plots for grape peels (Fig. 1) and grape seeds samples (Fig. 1) are presented. The score plots illustrate the groups among grape varieties and harvesting periods, while the loading plots indicate the compositional variables for grouping of samples.

The data set of the grape peels was represented in the first two principal components (PC1 × PC2) that explained 73.6% of the total data variability. The PC1 accounted for the maximum variability (52.46%) and depicted a clear separation according to the harvest month and polyphenols composition. Grapes harvested in February and August (PC1 > 0) were separated in the PC 1 axis from those harvested in May and November (PC1 < 0) and associated with most of the individual phenolics. This reflects the high weight of these variables, harvest period and phenolic composition in explaining the original data. The major contribution variables in the first principal component (loading > 0.70) were as follows: syringic acid (Sra: 0.985), epigallocatechin gallate (EgG: 0.985), cyanidin (CyD: 0.977), caffeic acid (Cfc: 0.974), malvidin 3,5-diglucoside (MaD: 0.973), *cis*-resveratrol (Cres: 0.965), epicatechin (Epc: 0.965), procyanidin B2 (PB2: 0.964), delphinidin 3-O-glucoside (Del: 0.910), naringenin (Nar: 0.890), hesperidin (Hes: 0.855), maximum temperature (Tmax: 0.747), mean temperature (Tmea:0.747), minimum temperature (Tmin: 0.747) and

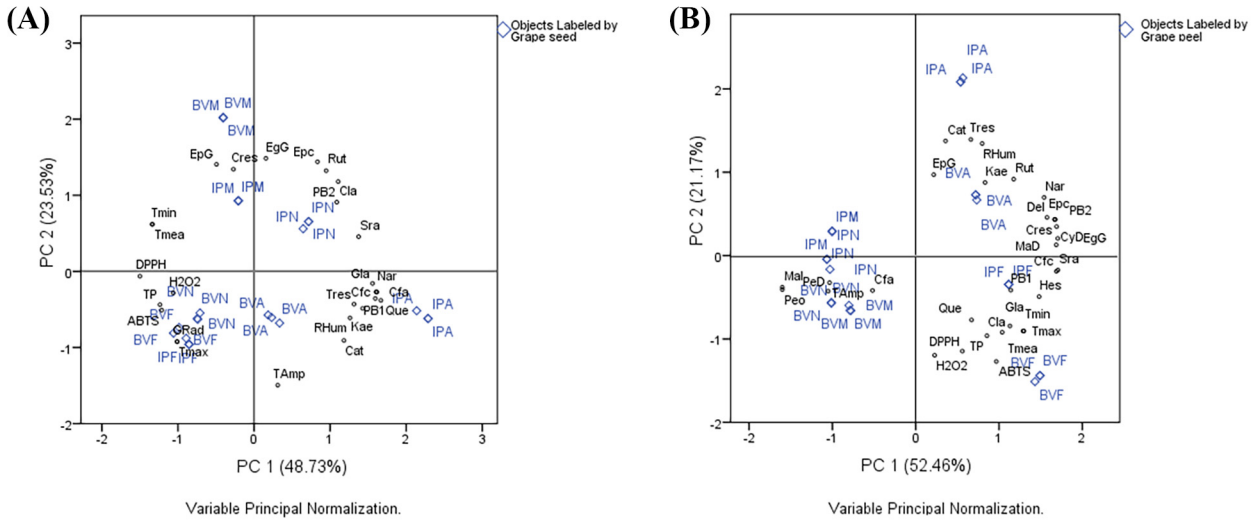

Fig. 1. Principal components analysis (PCA) using the results of phenolic compounds and antioxidant activity, and climatic parameters for grape seeds (A) and peels (B) from grapes harvested in the months of February, May, August and November 2016. Cfa-Caftaric acid; Sra-Syringic acid; Cla-Chlorogenic acid; Cfc-Caffeic acid; Gla-Gallic acid; Cres-*cis*-resveratrol; Tres-*trans*-resveratrol; Cat-Catechin; Epc-Epicatechin; EpG-Epicatechin gallate; EgG-Epigallocatechin gallate; PB1-Procyanidin B1; PB2-Procyanidin B2; Que-Quercetin 3-glucoside; Rut-Rutin; Kae-Kaempferol 3-*O*-/glucoside; Nar-Naringenin; Hes-Hesperidin; Mal-Malvidin 3-*O*-glucoside; Del-Delfinidin 3-*O*-glucoside; Peo-Peonidin 3-*O*-glucoside; MaDMalvidin 3,5-diglucoside; CyD-Cyanidin 3,5-diglucoside; PeD – Pelargonidin 3,5-diglucoside; Tmean – Mean Temperature; Tmax – Maximum temperature, Tmin – Minimum Temperature; Tamp – Thermal amplitude ; Grad – Global radiation; RHum – Mean relative humidity.

global radiation (Grad: 0.747). The PC2 explained 21.17% of the total variability and the variables having the greatest contribution were *trans*-resveratrol (Tres: 0.804), catechin (Cat: 0.794), relative humidity (Rhum: 0.776) and antioxidant activity (ABTS: −0.733).

The PCA analysis of grape seeds data revealed that 72.3% of total variation was represented by the two main principal components. PC1 explained 48.73% of data variability, while 23.53% were explained by PC2. The variables and grape samples were found to be more evenly - distributed in the score plot of seed samples. The variables with the highest contribution (loading > 0.70) in the first component were quercetin 3-glucoside (Que: 0.949), caffeic acid (Cfc: 0.918), naringenin (Nar: 0.915), caftaric acid (Cfa: 0.915), procyanidin B1 (PB1: 0.907), gallic acid (Gla: 0.887), kaempferol 3-O-glucoside (Kae: 0.813), syringic acid (Sra: 0.783), *trans*-resveratrol (Tres; 0.748) and relative humidity (Rhum:0.718). These were strongly associated with the grapes BRS Violeta and Isabel Precoce harvested in August. The antioxidant activity measured by the DPPH (−0.854) and Folin Ciocalteu methods (−0.703) and the minimum and mean temperatures (Tmin and Tmea: −0.761) had a strong contribution in the PC1, which means that these variables were more associated with the IP and BV grapes harvested in May when the lowest thermal amplitude and mild radiation were recorded (Table 1). The multivariate approach revealed that to a variable extent all the endogenous and exogenous factors involved in grape composition and quality can be more or less influenced by successive harvesting, particularly due to the climate variations that occur during the months of the year.

3.4. Phenolic composition and antioxidant activity of blended grape juices

The phenolic profile and antioxidant activity of the blended grape juices made from the BRS Violeta and Isabel Precoce varieties are shown in Table 5. In general, it was observed that the harvest month had influence over the bioactive content of grape juices. The polyphenols epicatechin, procyanidin B2, cyanidin 3,5-diglucoside, caftaric acid and malvidin glucosides were the most abundant phenolics and their concentrations were affected ($p < 0.05$) by harvest period. Malic and tartaric acids were the predominant organic acids, while fructose and glucose were the main sugars found in juice samples.

The juices produced from the grapes harvested in August showed the highest concentrations of organic acids, which is consistent with the increased acidity of these juices. The same was observed for the concentrations of sugars, phenolic compounds and the antioxidant activity that were significantly higher for grapes and juices obtained in that month. These corroborate the results of the chemical characterization of grape samples and confirm that grape juice composition is dependent on grape variety and its cultivation conditions. Indeed, the quality of grape-based beverages is related to grape characteristics which are greatly influenced by endogenous and exogenous factors (Rizzon, Manfroi, & Meneguzzo, 1998).

In this study we observed that even in a region of low annual climatic variability as the SFV region, the successive harvesting during the same year led to grapes and juices with different contents of phenolic compounds and antioxidant activity. The main phenolic markers found in the grape samples and juices were *trans*-caftaric acid, procyanidin B2, malvidin 3,5-diglucoside and *cis*-resveratrol. These have also been reported in other grape juices produced in that region (Dutra et al., 2018, Lima et al., 2014). Noteworthy, the anthocyanins malvidin 3,5-diglucoside and malvidin 3-O-glucoside have been reported as phenolic markers of Brazilian and European grape juices, respectively (Granato, Koot et al., 2015a).

4. Conclusions

Following our previous study elucidating the phenolic quality of grape juices produced in a semi-arid tropical region (Lima et al., 2014), the results herein pointed out the effects of successive harvesting on grape composition as influenced by specific climate conditions, which have thus impact on juice quality. The variety and climatic-related factors exerted a significant influence on the quality and composition of grapes, as shown by the multivariate analysis. Isabel Precoce and BRS Violeta grapes showed similar polyphenolic profiles and comparable values for antioxidant activity, while those harvested in February and August had the highest accumulation of polyphenols. This was evident for anthocyanins and flavanols. The temperature, global radiation, air velocity and rate of evapotranspiration were the main climatic variables associated with higher accumulation of phenolic compounds in the samples, all these influencing the harvest period. The information provided in this study in terms of the relationship between the

Table 5
Physico-chemical parameters, organic acids, sugars, polyphenolic composition and antioxidant activity of grape juices made from BRS Violeta and Isabel Precoce grapes.

Parameters	Harvest period			
	February	May	August	November
Physico-chemical				
pH	3.4 ± 0.0^{b}	3.5 ± 0.0^{a}	3.4 ± 0.0^{b}	3.3 ± 0.0^{c}
Total soluble solids (°Brix)	17.7 ± 0.0^{d}	21.3 ± 0.2^{c}	23.9 ± 0.1^{a}	22.0 ± 0.4^{b}
Titratable acidity (g 100 mL^{-1})	0.7 ± 0.0^{c}	0.8 ± 0.0^{b}	1.1 ± 0.0^{a}	0.8 ± 0.1^{b}
Organic acids (g L^{-1})				
Tartaric acid	3.1 ± 0.1^{a}	2.4 ± 0.57^{a}	3.2 ± 0.59^{a}	2.2 ± 0.24^{a}
Malic acid	2.93 ± 0.04^{c}	3.17 ± 0.02^{b}	3.9 ± 0.00^{a}	2.8 ± 0.04^{d}
Citric acid	0.26 ± 0.01^{b}	0.29 ± 0.00^{b}	0.08 ± 0.07^{c}	0.49 ± 0.01^{a}
Succinic acid	0.50 ± 0.03 ^{a}	0.54 ± 0.00 ^{a}	0.60 ± 0.01 ^{a}	0.54 ± 0.00^{a}
Acetic acid	ND	ND	ND	ND
Lactic acid	ND	ND	ND	ND
Σ organic acid quantified	6.8 ± 0.2	6.4 ± 0.59	7.8 ± 0.67	6.0 ± 0.29
Sugars (g L^{-1})				
Maltose	0.10 ± 0.01^{b}	0.09 ± 0.02^{b}	0.14 ± 0.00^{a}	0.1 ± 0.00^{b}
Glucose	71.44 ± 4.76^{b}	90.43 ± 0.43^{a}	96.64 ± 0.43^{a}	95.01 ± 0.33^{a}
Fructose	80.11 ± 0.28^{d}	92.16 ± 0.00^{c}	98.22 ± 0.45^{a}	95.24 ± 0.23^{b}
Rhamnose	ND	ND	ND	ND
Σsugars quantified	151.7 ± 5.05	182.7 ± 0.45	195.0 ± 0.88	190.4 ± 0.56
Phenolic compounds (mg L^{-1})				
Flavanols				
(+)-Catechin	11.3 ± 0.1^{b}	10.3 ± 0.0^{b}	44.6 ± 0.1^{a}	11.3 ± 2.0^{b}
(−)-Epicatechin	14.6 ± 0.2^{d}	21.2 ± 0.0^{b}	49.8 ± 0.1^{a}	18.3 ± 0.7^{c}
(−)-Epicatechin gallate	1.5 ± 0.0^{b}	0.8 ± 0.0^{c}	2.3 ± 0.1^{a}	ND
(−)-Epigallocatechin gallate	4.0 ± 0.1^{c}	5.4 ± 0.0^{b}	10.4 ± 0.0^{a}	5.6 ± 0.1^{b}
Procyanidin B1	3.6 ± 0.1^{c}	3.0 ± 0.1^{c}	10.8 ± 0.1^{a}	5.3 ± 0.4^{b}
Procyanidin B2	24.1 ± 1.1^{c}	22.9 ± 0.1^{c}	48.0 ± 0.2^{a}	27.3 ± 0.6^{b}
Σ Flavanols quantified	59.1 ± 1.6	156.2 ± 0.2	165.9 ± 0.6	67.8 ± 3.8
Flavonols				
Quercetin 3-glucoside	9.4 ± 0.0^{a}	2.4 ± 0.0^{c}	9.0 ± 0.0^{a}	4.3 ± 0.3^{b}
Rutin	0.1 ± 0.0^{c}	0.8 ± 0.0^{b}	0.1 ± 0.0^{c}	1.0 ± 0.1^{a}
Kaempferol 3-O-glucoside	0.9 ± 0.0^{c}	ND	1.2 ± 0.0^{b}	2.0 ± 0.1^{a}
Σ Flavonols quantified	9.5 ± 0.0	3.2 ± 0.0	9.1 ± 0.0	5.3 ± 0.4
Flavanones				
Hesperidin	ND	ND	7.1 ± 0.2^{a}	1.6 ± 2.2^{b}
Naringenin	3.0 ± 0.0^{d}	5.2 ± 0.0^{b}	14.3 ± 0.0^{a}	4.7 ± 0.1^{c}
Σ Flavanones quantified	3.0 ± 0.0	5.2 ± 0.0	21.4 ± 0.2	6.3 ± 2.3
Anthocyanins				
Cyanidin 3,5-diglucoside	12.8 ± 0.0^{c}	17.7 ± 0.1^{b}	41.5 ± 0.1^{a}	16.9 ± 0.8^{b}
Malvidin 3,5-diglucoside	77.7 ± 1.7^{c}	103.0 ± 0.3^{b}	256.2 ± 1.3^{a}	99.1 ± 3.7^{b}
Pelargonidin 3,5-diglucoside	ND	ND	ND	ND
Peonidin 3-*O*-glucoside	9.4 ± 0.1^{b}	3.9 ± 0.0^{d}	12.6 ± 0.0^{a}	6.8 ± 0.2^{c}
Malvidin 3-*O*-glucoside	61.1 ± 0.7^{b}	26.9 ± 0.1^{d}	99.6 ± 0.1^{a}	47.4 ± 1.6c
Delphinidin 3-*O*-glucoside	6.9 ± 0.0^{b}	6.2 ± 0.1^{c}	19.2 ± 0.0^{a}	6.3 ± 0.3^{bc}
Σ Anthocyanins quantified	167.9 ± 2.5	157.7 ± 0.6	429.1 ± 1.5	176.5 ± 6.6
Phenolic acids				
Gallic acid	8.2 ± 0.1^{a}	5.4 ± 0.0^{c}	8.1 ± 0.0^{a}	7.0 ± 0.1^{b}
Syringic acid	5.5 ± 0.1^{c}	6.1 ± 0.1^{b}	12.7 ± 0.0^{a}	6.4 ± 0.1^{b}
ρ-Coumaric acid	4.7 ± 0.1b	3.1 ± 0.1^{b}	8.9 ± 0.2^{a}	4.3 ± 0.9^{b}
Caffeic acid	4.9 ± 0.2^{c}	4.5 ± 0.0^{c}	9.7 ± 0.1^{a}	5.6 ± 0.1^{b}
Caftaric acid	192.8 ± 6.0^{b}	205 ± 0.5^{b}	330.9 ± 0.6^{a}	344.1 ± 35.3^{a}
Chlorogenic acid	17.6 ± 0.6^{b}	15.9 ± 0.0^{b}	27.0 ± 0.0^{a}	26.3 ± 2.6^{a}
Σ Phenolic acids quantified	233.7 ± 7.1	240.0 ± 0.7	397.3 ± 0.9	393.7 ± 39.1
Stilbenes				
trans-Resveratrol	1.8 ± 0.0^{a}	0.5 ± 0.^{0d}	1.7 ± 0.0^{b}	0.9 ± 0.0^{c}
cis-Resveratrol	5.7 ± 0.4^{c}	9.1 ± 1.8^{bc}	16.8 ± 0.^{2a}	11.3 ± 0.2^{b}
Σ Stilbenes quantified	7.5 ± 0.4	9.6 ± 1.8	18.5 ± 0.2	12.2 ± 0.2
Antioxidant activity				
DPPH (mmol TE L^{-1})	7.9 ± 0.3^{bc}	9.8 ± 0.2^{b}	26.2 ± 2.0^{a}	6.6 ± 0.2^{c}
ABTS (mmol TE L^{-1})	8.0 ± 0.6^{e}	14.5 ± 0.4^{b}	27.3 ± 1.3^{a}	11.6 ± 0.2^{c}
H_2O_2 (mmol TE L^{-1})	32.9 ± 0.3^{c}	52.7 ± 0.2^{b}	142.2 ± 1.3^{a}	51.5 ± 0.9^{b}
Folin Ciocalteu (mg GAE L^{-1})	2249.1 ± 46.8^{b}	2146.9 ± 14.2^{b}	4935.5 ± 112.6^{a}	1905.3 ± 147.0^{c}

Data represent the mean values for each sample ± standard deviation ($n = 3$); Different letters in the same row indicate significant difference according to Tukey's test ($p < 0.05$); Titratable acidity expressed as tartaric acid equivalents; TE, Trolox equivalents; GAE, gallic acid equivalents; ND, not detected.

polyphenolic composition of varietal grapes and climatic variations may assist growers in producing grapes and grape juices with high bioactive quality in regard to levels of some polyphenols in semi-arid and tropical areas.

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Declaration of Competing Interest

None.

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