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Original Research Article

Characterization of the phenolic ripening development of 'BRS Vitoria' seedless table grapes using HPLC–DAD–ESI-MS/MS

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ABSTRACT

Determining the harvest date of table grapes is very important to achieve high-quality bunches with adequate soluble solids content, low titratable acidity, and high concentrations of polyphenols. Table grape consumption has increased worldwide due to its phenolic compound content and its beneficial effects on human health. Thus, this study aimed to characterize the phenolic ripening of 'BRS Vitoria' seedless table grapes at different ripening stages using HPLC–DAD–ESI-MS/MS. For this purpose, a trial was carried out during 2016 in a commercial vineyard of 'BRS Vitoria' seedless grape located in Marialva, state of Parana (Southern Brazil). Berry samples were assessed weekly, starting at *véraison* until full ripeness. At each ripening stage, the berries were analyzed to determine their physicochemical characteristics and polyphenolic profile. It was observed that 'BRS Vitoria' grapes can be harvested approximately 28 days after *véraison*, when the berries reach soluble solids content higher than 15°Brix and low titratable acidity. The grapes presented a typical anthocyanin profile of hybrid grapes, composed of 3-glucoside and 3,5-diglucoside derivatives. In addition, pelargonidin traces were also observed, and this aglycone is rarely detected in grapes. The total anthocyanin concentration, as malvidin-3,5-diglucoside equivalents, is close to 596.9 mg kg^{-1}. Concerning flavonols, myricetin and quercetin are detected in greater proportions, and this cultivar can also be considered an important source of proanthocyanidins.

1. Introduction

Grapes are an important source of antioxidants, such as phenolic compounds that change their concentration during ripening. Phenolic compounds are divided into two groups: flavonoid (anthocyanins, flavan-3-ols, condensed tannins and flavonols) and non-flavonoid compounds (phenolic acids and stilbenes). Each polyphenol family group is directly responsible for the important characteristics of specific grape varieties and their products (Andjelkovic et al., 2013).

Technological and phenolic ripening are used to determine the harvest date of table grapes when berries reach the characteristics of each cultivar. Technological ripening is based mainly on grape total soluble solids content, titratable acidity and pH. On the other hand, phenolic ripening takes into account anthocyanins, tannins and total phenolic concentration (Ferrer-Gallego et al., 2012; Meléndez et al., 2013). The concentrations of anthocyanins and tannins, the most abundant polyphenols in colored grapes, are good indicators of phenolic ripening since they accumulate in the grape skin during the ripening process. Located in cell vacuoles, anthocyanins are easily released in the extraction medium when these vesicles are weakened by grape ripening

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(Boselli et al., 2004). In addition, flavonoids such as anthocyanins, flavonols and flavan-3-ols contribute to the organoleptic characteristics of grapes, such as color, astringency and bitterness (Wang et al., 2018).

In grapes, the accumulation of anthocyanins starts at *véraison*, the onset of ripening, and seems to be regulated, at least partially, by abscisic acid (ABA) (Owen et al., 2009). Anthocyanin accumulation in berry skin occurs in three stages: it starts with an initial slow anthocyanin accumulation, which is followed by a rapid increase ending in a stabilizing stage, and decreases at the end of the ripening process (Gholami, 2004; Mateus et al., 2002). In contrast, the flavonol concentration in grapes is higher at the flowering time, decreasing between flowering and berry set and then remaining constant throughout berry development (Downey et al., 2003a).

The phenolic compound content and profile recorded in different grapes may vary according to the species, cultivar, ripening and environmental conditions during crop growth (Downey et al., 2006; Stockley and Høj, 2005). However, there is limited information available about the phenolic composition of some new hybrid table grapes (Nixdorf and Hermosín-Gutiérrez, 2010; Santos et al., 2011). In this way, 'BRS Vitoria' seedless grapes (*Vitis* sp.) have not yet been characterized regarding its phenolic composition, especially at different ripening stages. 'BRS Vitoria' seedless grape can be grown under tropical and subtropical climates, presents excellent horticultural performance and tolerance to downy mildew (Colombo et al., 2018; Maia et al., 2014) and has become an important table grape cultivar for national and international markets (Colombo et al., 2020).

This study aimed to characterize the phenolic ripening development of 'BRS Vitoria' seedless grapes using HPLC–DAD–ESI-MS/MS. The pool of compounds considered in this work includes the most important phenolic compounds in terms of sensory characteristics (color, bitterness and astringency) and possible biological effects (e.g., antioxidant capacity) of table grapes and their products, such as anthocyanins, flavonols, hydroxycinnamic acid derivatives, stilbenes, flavan-3-ol monomers and dimers, and proanthocyanins.

2. Material and methods

2.1. Field trial and sample collection

The trial was conducted in a commercial vineyard located in Marialva, state of Parana (Southern Brazil) (23°29′S, 51°47′W, elevation of 570 m a.s.l.). The 'BRS Vitoria' grapevines (*Vitis* sp.) were grafted onto 'IAC 766 Campinas' rootstock in 2012, spaced at 2.5 × 5.0 m (800 vines per ha) and trained as an overhead trellis system covered with 18 % shading plastic net.

Vines were cane-pruned in the late winter of the 2016 season. The crop load was primarily adjusted to ca. 48 canes per vine, and 5 buds were retained per cane. Afterwards, hydrogen cyanamide was applied at 3% at the 2 apical buds to promote uniform budburst. When bunches were at pea size, the crop load of the vineyard was adjusted to 7 bunches m^{-2}, with an estimated yield of ~28 tons per ha.

The vineyard was divided into three plots, with 60 vines per plot. In each plot, 80 berries were collected weekly, at random, to perform the technological and phenolic ripening characterization of 'BRS Vitoria' seedless grapes, as described below.

The technological ripening development of 'BRS Vitoria' seedless grapes was assessed weekly, starting at *véraison* until full ripening. The following physicochemical analyses were evaluated: berry mass (g), soluble solids content (SS), pH, titratable acidity (TA) and maturation index (MI) (SS/TA). These evaluations were performed using three samples composed of 20 berries each. A digital refractometer with automatic temperature compensation (DR301-95 Model, Krüss Optronic, Germany) was used to determine SS content, and the results were expressed as °Brix. TA was calculated by titrating the grape juice with a standard 0.1 N NaOH solution in a semi-automatic titrator, adopting pH = 8.2 as the endpoint of the titration, and the results were expressed as % of tartaric acid (IAL, 2008).

The phenolic ripening of 'BRS Vitoria' was also assessed weekly. For anthocyanin and polyphenol analyses, 60 berries per plot were collected and divided into two subsamples of 30 berries. Berry skins of one subsample were manually and carefully peeled off, weighed, frozen at –80 °C, and freeze-dried. The other subsample was weighed and immediately crushed for two minutes with 50 mL of methanol and formic acid (98.5:1.5 v/v) solution, avoiding oxidation. This extract, from whole berries, was centrifuged at 5000 rpm for 5 min. The supernatant was separated, and a second extraction was prepared from the residue of the first extract using 30 mL of methanol, water and formic acid (50:48.5:1.5 v/v/v) solution, following the same procedure previously described. The centrifuged extracts obtained from both extractions were mixed, measured and frozen at –80 °C.

During this trial, all vineyard cultural practices, such as fertilization, weeds, pests and disease control, were carried out as recommended in the region according to Kishino et al. (2019), including berry thinning (Roberto et al., 2015).

2.2. Sample for HPLC analysis

2.2.1. Chemicals

All solvents were HPLC grade, and all chemicals were analytical grade (>99 %) and dissolved in ultrapure water, as previously described by Colombo et al. (2020). Commercial standards of malvidin 3-glucoside, malvidin 3,5-diglucoside, peonidin 3,5-diglucoside, caffeic acid, *p*-coumaric acid, *trans*-caftaric acid, *trans*-piceid, (−)-epigallocatechin, and (−)-gallocatechin were purchased from Phytolab (Vestenbergsgreuth, Germany). Cyanidin 3-glucoside, cyanidin 3,5-diglucoside, procyanidins B1 and B2, kaempferol, quercetin, isorhamnetin, myricetin, syringetin, and the 3-glucosides of kaempferol, quercetin, isorhamnetin, and syringetin were obtained from Extrasynthese (Genay, France). Gallic acid, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-gallate, and (−)-gallocatechin 3-gallate were collected from Sigma (Tres Cantos, Madrid, Spain). Other non-commercial flavonol standards (myricetin 3-glucoside, quercetin 3-glucuronide, and laricitrin 3-glucoside) were previously isolated from 'Petit Verdot' grape skins (Castillo-Muñoz et al., 2009a). Finally, a sample of procyanidin B4 was kindly provided by Prof. Fernando Zamora (Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Tarragona, Spain). The *trans*-isomers of resveratrol and its 3-glucoside (piceid) were transformed into their respective *cis*- isomers by UV-irradiation using 366 nm light for 5 min in a quartz cell of 25 % MeOH solutions of *trans*- isomers (Rebello et al., 2013).

All standards were used for identification. The quantification of each compound, expressed in mg kg^{-1} of berries, fresh weight – FW, was carried out as the equivalent of the most representative compounds for each family of the following phenolic compounds: malvidin 3,5-diglucoside was used for anthocyanidin 3,5-diglucosides; malvidin 3-glucoside for anthocyanidin 3-glucosides; caftaric acid for hydroxycinnamic acid derivatives; quercetin 3-glucoside for flavonol 3-glycosides and their free aglycones; (+)-catechin for polymeric flavan-3-ols (total proanthocyanidins); individual flavan-3-ol monomers and dimers by their corresponding standards, and their total sum as (+)-catechin equivalents. Although phenolic compounds were evaluated only in the berry skins, except for hydroxycinnamic acid derivatives, their final concentrations were converted to mg kg^{-1} of berries, FW.

2.2.1.1. Grape skin extract preparation. 'BRS Vitoria' freeze-dried berry skins were triturated in a grinder (IKA® A 10 Basic, Germany), weighed and used for further analysis of phenolic compounds. The samples (ca. 0.20 g) were immersed in 20 mL of methanol, water, and formic acid solution (50:48.5:1.5 v/v) and subjected to ultrasonic bar treatment for 3 min. Samples were centrifuged at 2500 rpm for 15 min at 5 °C. Afterwards, the supernatant was carefully poured into a flask. The solid

phase (residue) was again mixed with 20 mL of the methanol, water, and formic acid solution (50: 48.5: 1.5 v / v) and subjected to ultrasonic bath treatment for 3 min. The supernatant was mixed with that collected in the previous step. These extracts were mixed (ca. 40 mL) and filtered; the final extract volume was adjusted to 50 mL using the same solvent solution (methanol, water, and formic acid). Anthocyanin determination was performed according to Colombo et al. (2020); 1 mL of extract was collected and dried in a rotary evaporator (35 °C), and the dried extract was redissolved in 0.3 mL of HCl 0.1 N (1:10, v/v) and directly injected into the HPLC system - Agilent 1100 Series system (Agilent, Germany), equipped with DAD (G1315B) and an LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry system (ESI-MS^n).

PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa PCX, Agilent Technologies, USA) allowed the isolation of non-anthocyanin phenolic compounds from skin extracts, and these anthocyanin-free fractions were used to analyze flavonols. To prepare the samples, 3 mL of grape skin extracts were reduced to 1.5 mL in a rotary evaporator (35 °C) and diluted with 3 mL of HCl 0.1 N. The prepared samples were passed through the SPE cartridges, which were previously conditioned with 5 mL of methanol and 5 mL of water. Then, the cartridges were washed with 5 mL of HCl 0.1 N acid and 5 mL of water, and the anthocyanin-free fractions were eluted with 2 × 3 mL of 96 % ethanol. The eluate was dried in a rotary evaporator (35 °C) and redissolved in 1.5 mL of 80 % methanol in water and directly injected into the HPLC equipment, as previously reported.

Flavan-3-ols (monomers, B-type dimers, and proanthocyanidins) and stilbenes were isolated from skin extracts using SPE on C18 cartridges (Sep-Pak Plus C18, Waters Corp., Milford, USA) filled with 820 mg of adsorbent according to Rebello et al. (2013).

2.2.2. Whole berry extract preparation

Following the procedure described by Colombo et al. (2020) to analyze hydroxycinnamic acid derivatives, the extract obtained from whole berries (Section 2.1) was dried in a rotary evaporator (35 °C) to evaporate the methanol contents. To eliminate the sugars and other polar substances from the samples, an extraction procedure was carried out in solid phase (SPE) with Bond Elut C18 cartridges (500 mg, 3 mL; Agilent Technologies, Santa Clara, USA). For this purpose, the cartridges were conditioned by passing 5 mL of methanol followed by 5 mL of water. Then, the extracts (5 mL) were loaded into the cartridge, and the eluate was discarded. The cartridges were rinsed 3 times with 5 mL of water, and the adsorbed phenolic compounds were recovered with 5 mL of methanol 3 times and dried in a rotary evaporator (35 °C). Dried samples were redissolved in 5 mL of acetonitrile/water/formic acid (3:88.5:8.5, v/v) solution and directly injected into HPLC equipment.

2.3. HPLC–DAD–ESI-MSn phenolic compound identification

All chromatographic analyses were carried out at the Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad Castilla-La Mancha, Ciudad Real, Spain. Anthocyanins and non-anthocyanin phenolic compounds from grape skin and whole berries were separated using the methods described by Castillo-Muñoz et al. (2007, 2009a), Rebello et al. (2013) and Colombo et al. (2020).

The identification was mainly based on spectroscopic data (UV–Vis and MS/MS) obtained from authentic standards as previously reported (Castillo-Muñoz et al., 2009a; Lago-Vanzela et al., 2011a, b). For phenolic compound quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols) and 320 nm (hydroxycinnamic acid derivatives). Samples from each collection period were injected in triplicate.

2.4. Identification and quantification of flavan-3-ols and stilbenes using multiple reactions monitoring HPLC–ESI-MS/MS

Chromatographic analyses corresponding to flavan-3-ols and stilbenes were performed at the Instituto de la Vid y del Vino de Castilla-La Mancha (IVICAM), Tomelloso, Spain. The SPE-C18 extract (described in Section 2.2.2) was used to analyze flavan-3-ol monomers and B-type dimer procyanidins and stilbenes from grape skins. From SPE-C18 extract, 0.30 mL was taken and diluted in 1.50 mL of water and formic acid (98.5:1.5) in a sealed chromatographic vial and injected into HPLC equipment.

Concerning the analyses regarding the structural information about proanthocyanidins, the method of pyrogallol-induced acid-catalyzed depolymerization was used (Bordiga et al., 2009, 2013). For this purpose, 0.30 mL of pyrogallol reagent solution (100 g L^{-1} of pyrogallol and 20 g L^{-1} ascorbic acid in methanolic 0.3 N HCl) was added to 0.30 mL of SPE-C18 extract, and the mixture was then kept at 30 °C for 40 min. After, the reaction was interrupted with the addition of 1.20 mL of 67 μM sodium acetate, and the reaction mixture was injected into HPLC equipment (Colombo et al., 2020).

The analyses were performed using an Agilent 1200 Series HPLC System equipped with DAD (Agilent, Germany) and coupled with an AB Sciex 3200 Q TRAP (Applied Biosystems) Electrospray Ionization Mass Spectrometry System (ESI-MS/MS). The chromatographic system was managed by the Agilent ChemStation (version B.01.03) data-processing station. The mass spectra data were processed with Analyst MSD software (Applied Biosystems, version 1.5), and the analysis conditions were performed according to Rebello et al. (2013).

Initial concentration data of flavan-3-ol monomers, obtained before the depolymerization reaction, were used for the correction of released flavan-3-ol monomer (terminal subunits of polymeric proanthocyanidins) concentrations during the depolymerization reaction of proanthocyanidins. Identification and quantification of diverse flavan-3-ols and stilbenes were carried out using the same standards and methodology described by Lago-Vanzela et al. (2011a, b) and Rebello et al. (2013).

2.5. Statistical analyses

All recorded data were submitted to normality and homogeneity of variances tests before ANOVA. In the case of significance, the means were adjusted to the corresponding polynomial regression model. Significant characteristics were also submitted to a principal component analysis (PCA) using *R* package FactoMineR (Lê et al., 2008); and a heat map, considering Ward's hierarchical clustering analysis based on Euclidean distances, using *R* package Pheatmap (Kolde, 2013).

3. Results and discussion

3.1. Technological ripening characterization

For 'BRS Vitoria' seedless grapes, the ripening characterization was assessed weekly, from *véraison* until full ripening (28 days after *véraison* - DAV). During this period, the berries accumulated approximately 1 g of fresh mass, which corresponds to approximately 20 % of the total berry fresh mass of this grape cultivar (Fig. 1).

As the berry color changed, the total soluble solids content (SS) increased from 10.1°Brix at *véraison* to 15.4°Brix at full ripeness, characterizing the minimum technological harvest date for this table grape cultivar grown under subtropical conditions. For 'Plavac Mali' grapes harvested at different dates, comprising between *véraison* and full ripe (42 days), an increase close to 4°Brix was recorded (Mucalo et al., 2020); however, 'Plavac Mali' is a *V. vinifera* cultivar and, at *véraison,* the berries had close to 18°Brix, since these species usually have higher SS content than hybrid grapes. According to Brazilian legislation, table grapes must present a minimum of 14°Brix to be harvested (MAPA, 2002). 'BRS Vitoria' table grapes have the potential to reach higher total SS content (Maia et al., 2014); it was verified in previous reports that the harvest of this seedless grape cultivar can be performed when the berries presented total SS content ranging from ~16.0–18.0 °Brix (Roberto

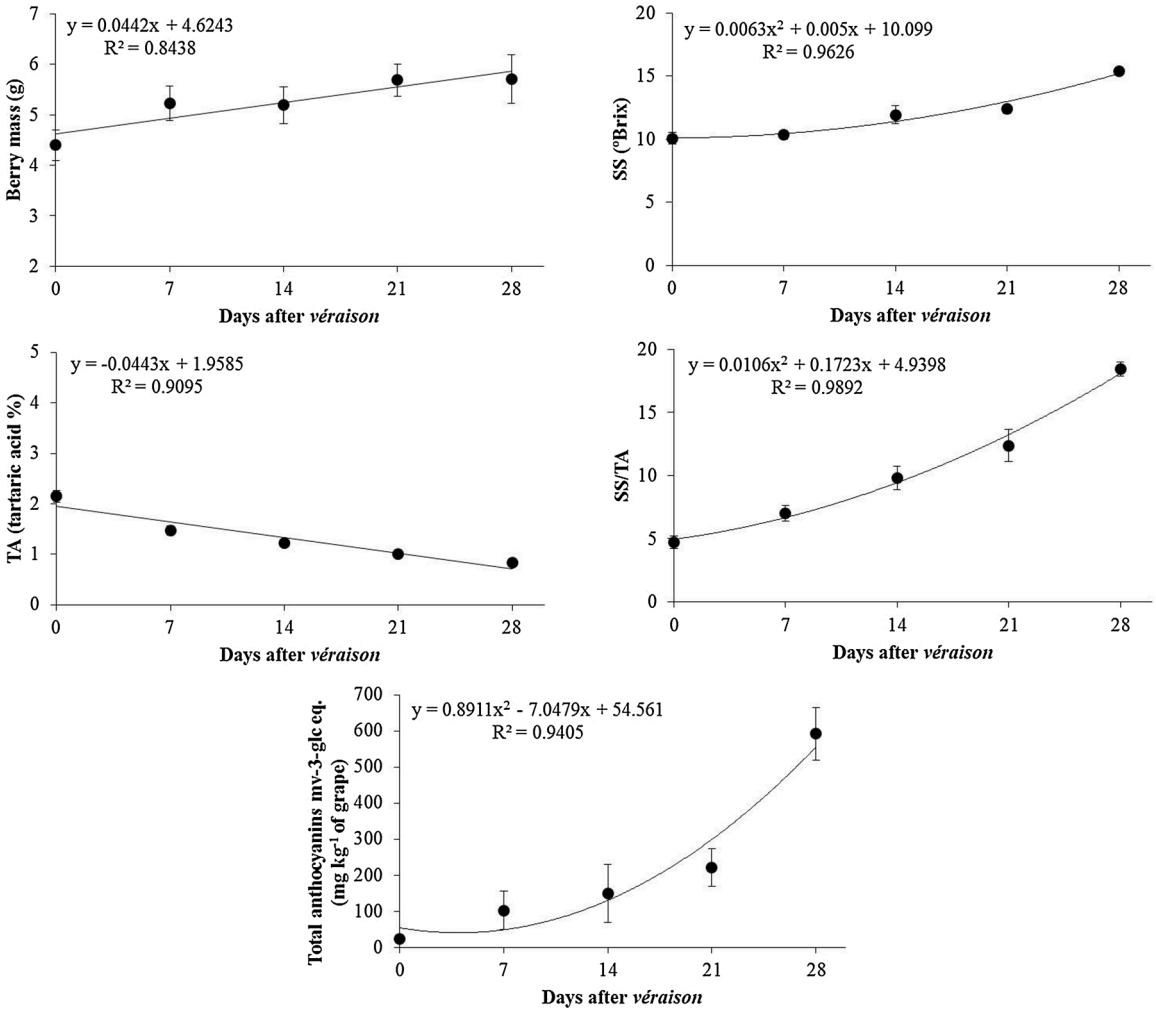

Fig. 1. Berry mass, soluble solids content (SS), titratable acidity (TA), maturation index (SS/TA) and total anthocyanins as malvidin-3-glucoside equivalents (mv-3-glc eq.) recorded in 'BRS Vitoria' seedless table grapes at different ripening stages.

et al., 2015; Youssef et al., 2015). This variation can be related to different seasons, as well as to the cultural practices and bunch management performed in a commercial 'BRS Vitoria' vineyard. The SS content values recorded in this trial are considered adequate for consumer acceptance and commercialization of this grape cultivar for domestic and international markets.

Concerning titratable acidity (TA), the 'BRS Vitoria' table grapes can be characterized as a low acidity cultivar, which contributes to an increase in its maturation index (SS/TA). In this study, bunches were harvested when the berries presented TA approximately 0.8 % (as tartaric acid equivalent), while Roberto et al. (2015) reported TA values of 0.6 % (as tartaric acid equivalent) for the same cultivar.

In addition to these changes, the berry pH increased during ripening, even though this increase was not significant. On the other hand, the total anthocyanin content, determined by UV–vis spectrophotometry (Peppi et al., 2006), increased significantly. Although this can be considered a simple method of evaluation, this methodology allows us to obtain a general result for the total anthocyanin content, expressed as malvidin-3-glucoside equivalent. Fig. 1 shows the total anthocyanin content changing from 25 mg kg^{-1} at *véraison* to 592 mg kg^{-1} at full ripe.

The bunch mass was recorded only at harvest (330 ± 50 g), and from this characteristic, together with bunch density, it was possible to estimate the yield of 'BRS Vitoria' table grape as 23.0 ± 3.3 tons ha^{-1}.

3.2. Phenolic ripening characterization

3.2.1. Anthocyanins

Grape anthocyanin profile determination at different ripening stages is important to comprehend the phenolic changes that occur in grape berries during their development. Anthocyanidins (aglycones), delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn) and malvidin (mv) were identified in 'BRS Vitoria' table grapes by their molecular weights (*m/z* 303, 287, 317, 301 and 331, respectively) obtained from ionization products in mass spectrometry (MS/MS), as shown in Table 1 and Fig. 2. Pelargonidin aglycone traces (pg, *m/z* 271) were also detected in the samples collected closest to the fully ripe stage.

In hybrid grapes, such as 'BRS Vitoria', anthocyanidins may be linked to substitution groups with one or two glucose molecules. Anthocyanin 3-glucoside or 3,5-diglucoside assignments were based on the fragmentation pattern obtained by mass spectrometry (MS/MS), according to Rebello et al. (2013). Thus, it was verified that the anthocyanin profile of 'BRS Vitoria' berries is very complex, mainly due to the presence of six aglycone types, which can be detected under different glycosylation levels. Five complete series of five aglycones (dp, cy, pt, pn and mv) detected in 'BRS Vitoria' berry skins were identified: non-acylated 3-glucosides, 3,5-diglucosides and their *p*-coumaroyl derivatives (in *cis*- and *trans*- conformations), as well as the 3-glucoside acetyl derivatives.

Regarding these compounds, a total of 25 anthocyanin compounds were identified by LC–MS in 13 table grape varieties; malvidin was the

Table 1
Chromatographic and spectroscopic (MS/MS spectra) characteristics of identified anthocyanins in 'BRS Vitoria' seedless table grapes at different ripening stages (days after *véraison* – DAV) by HPLC–DAD–ESI-MS/MS (positive ionization mode). Molar proportions (mean value, n = 3) and total concentration (as malvidin 3-glucoside or malvidin 3,5-diglucoside equivalents) in berry skin. Peak assignation in Fig. 2.

Peak	Assignation [a]	Molecular ion; product ions (*m/z*)	Rt (min)	*Véraison* (% molar)	7 DAV	14 DAV	21 DAV	28 DAV
3	dp-3-glc	465; 303	8.94	0.00 ± 0.00	10.80 ± 0.50	12.62 ± 1.41	10.97 ± 1.18	12.02 ± 0.48
5	cy-3-glc	449; 287	11.85	4.71 ± 0.66	3.51 ± 0.59	2.94 ± 0.45	2.29 ± 0.30	2.91 ± 0.28
7	pt-3-*cis*-glc	479; 317	14.58	15.51 ± 0.71	8.29 ± 0.39	8.85 ± 0.62	8.13 ± 0.51	8.32 ± 0.19
37	pt-3-*trans*-glc	479; 317	24.29	0.77 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
9	pn-3-glc	463; 301	17.00	7.54 ± 0.80	5.74 ± 0.56	4.38 ± 0.38	3.61 ± 0.21	3.99 ± 0.32
10	mv-3-*cis*-glc	493; 331	19.06	13.75 ± 0.73	14.89 ± 0.45	15.47 ± 0.18	14.44 ± 0.21	13.89 ± 0.05
13	mv-3-*trans*-glc	493; 331	21.11	1.72 ± 0.36	0.27 ± 0.02	0.20 ± 0.02	0.22 ± 0.03	0.31 ± 0.02
12	dp-3-acglc	507; 303	20.56	0.91 ± 0.21	0.83 ± 0.04	0.86 ± 0.11	0.74 ± 0.05	0.85 ± 0.01
16	cy-3-acglc	491; 287	23.29	0.63 ± 0.05	0.51 ± 0.03	0.42 ± 0.02	0.44 ± 0.04	0.39 ± 0.20
19	pt-3-acglc	521; 317	25.19	0.78 ± 0.03	0.76 ± 0.04	0.75 ± 0.09	0.67 ± 0.04	0.72 ± 0.03
27	mv-3-acglc	535; 331	29.43	0.00 ± 0.00	1.65 ± 0.05	1.47 ± 0.13	1.42 ± 0.06	1.40 ± 0.08
18	dp-3-*cis*-cmglc	611; 303	24.59	0.00 ± 0.00	0.66 ± 0.02	0.62 ± 0.06	0.68 ± 0.08	0.53 ± 0.01
25	dp-3-*trans*-cmglc	611; 303	27.80	10.22 ± 1.69	14.49 ± 0.72	15.73 ± 0.30	17.15 ± 0.26	17.46 ± 0.09
23	cy-3-*cis*-cmglc	595; 287	27.37	1.90 ± 0.03	0.33 ± 0.04	0.30 ± 0.01	0.36 ± 0.06	0.29 ± 0.06
29	cy-3-*trans*-cmglc	595; 287	30.48	12.42 ± 0.66	3.87 ± 0.43	3.29 ± 0.13	3.64 ± 0.10	3.76 ± 0.14
26	pt-3-*cis*-cmglc	625; 317	28.65	0.64 ± 0.03	0.58 ± 0.03	0.55 ± 0.09	0.59 ± 0.07	0.42 ± 0.04
32	pt-3-*trans*-cmglc	625; 317	32.10	6.94 ± 1.19	9.34 ± 0.26	9.68 ± 0.58	10.67 ± 0.40	10.24 ± 0.35
31	pn-3-*cis*-cmglc	609; 301	31.56	0.86 ± 0.17	0.41 ± 0.05	0.27 ± 0.08	0.30 ± 0.07	0.22 ± 0.06
34	pn-3-*trans*-cmglc	609; 301	35.01	6.42 ± 0.26	4.97 ± 0.37	3.94 ± 0.10	4.21 ± 0.19	4.26 ± 0.19
33	mv-3-*cis*-cmglc	639; 331	32.55	1.42 ± 0.15	1.18 ± 0.08	0.92 ± 0.21	1.02 ± 0.19	0.75 ± 0.09
35	mv-3-*trans*-cmglc	639; 331	36.24	12.87 ± 0.63	16.92 ± 0.86	16.73 ± 1.77	18.46 ± 1.38	17.26 ± 0.91
A	pn-3-acglc	505; 301	27.80	NQ	NQ	NQ	NQ	NQ
	Total (mg kg^{-1} of berries, mv-3-glc)			16.72 ± 5.45	63.53 ± 13.85	148.98 ± 34.95	163.27 ± 24.45	296.97 ± 44.22
1	dp-3,5-diglc	627; 465, 303	5.29	35.20 ± 3.97	1.74 ± 0.10	2.76 ± 0.68	2.24 ± 0.36	2.79 ± 0.33
2	cy-3,5-diglc	611; 449, 287	7.76	0.00 ± 0.00	0.95 ± 0.13	1.14 ± 0.24	0.83 ± 0.21	1.18 ± 0.13
4	pt-3,5-diglc-*cis*	641; 479, 317	10.55	3.38 ± 2.93	3.79 ± 0.14	5.32 ± 0.78	4.46 ± 0.71	5.11 ± 0.50
36	pt-3,5-diglc-*trans*	641; 479, 317	12.86	26.33 ± 2.70	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6	pn-3,5-diglc	625; 463, 301	13.13	0.00 ± 0.00	12.35 ± 0.82	11.26 ± 0.76	10.22 ± 0.50	11.12 ± 0.92
8	mv-3,5-diglc	655; 493, 331	14.96	0.00 ± 0.00	30.32 ± 3.09	34.34 ± 0.60	33.59 ± 0.94	33.28 ± 0.70
15	mv-3-acglc-5-glc	697; 535, 493, 331	22.32	3.51 ± 0.30	1.69 ± 1.05	2.01 ± 0.04	2.00 ± 0.02	2.26 ± 0.04
11	dp-3-*cis*-cmglc-5-glc	773; 611, 465, 303	19.66	0.00 ± 0.00	0.63 ± 0.09	0.66 ± 0.02	0.62 ± 0.06	0.56 ± 0.01
17	dp-3-*trans*-cmglc-5-glc	773; 611, 465, 303	24.01	3.62 ± 0.37	3.85 ± 0.20	4.55 ± 0.27	4.50 ± 0.34	4.89 ± 0.22
14	cy-3-*cis*-cmglc-5-glc	757; 595, 449, 287	21.99	0.00 ± 0.00	0.37 ± 0.09	0.23 ± 0.02	0.25 ± 0.04	0.28 ± 0.02
21	cy-3-*trans*-cmglc-5-glc	757; 595, 449, 287	26.21	3.80 ± 0.55	1.58 ± 0.18	1.55 ± 0.16	1.46 ± 0.07	1.50 ± 0.16
24	pt-3-cmglc-5-glc	787; 625, 479, 317	27.62	0.00 ± 0.00	3.98 ± 0.17	3.91 ± 0.22	3.91 ± 0.40	3.23 ± 0.63
20	pn-3-*cis*-cmglc-5-glc	771; 609, 463, 301	25.73	3.90 ± 0.80	1.10 ± 0.02	0.74 ± 0.07	0.79 ± 0.19	0.54 ± 0.04
28	pn-3-*trans*-cmglc-5-glc	771; 609, 463, 301	29.96	14.62 ± 2.22	6.55 ± 0.41	5.30 ± 0.22	5.93 ± 0.39	5.78 ± 0.11
22	mv-3-*cis*-cmglc-5-glc	801; 639, 493, 331	26.70	5.62 ± 1.17	3.21 ± 0.40	2.69 ± 0.52	2.83 ± 0.55	2.05 ± 0.28
30	mv-3-*trans*-cmglc-5-glc	801; 639, 493, 331	30.97	0.00 ± 0.00	27.90 ± 3.49	23.53 ± 2.54	26.37 ± 1.63	25.43 ± 1.75
	Total (mg kg^{-1} of berries, mv-3,5-diglc)			6.22 ± 2.20	41.69 ± 7.55	93.72 ± 17.02	114.56 ± 9.11	202.37 ± 13.40
	Ratio mv-3-glc/mv-3,5-diglc			2.71 ± 0.17	1.52 ± 0.09	1.58 ± 0.19	1.38 ± 0.12	1.46 ± 0.13

[a] Nomenclature abbreviation: dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; diglc, diglucoside; acglc, 6″-acetyl-glucoside; cmglc, 6″-*p*-coumaroyl-glucoside. NQ: detectable but not quantifiable; Rt: retention time (min). LOD and LOQ values for malvidin 3-glucoside: 0.012 mg L^{-1} and 0.041 mg L^{-1}, respectively. LOD and LOQ values for malvidin 3,5-diglucoside: 0.041 mg L^{-1} and 0.137 mg L^{-1}, respectively.

anthocyanidin with the highest number of derivatives, mainly glucosides and coumaroyl derivatives (Di Lorenzo et al., 2019). In addition, peonidin-glucoside and malvidin-glucoside were the anthocyanins detected in the highest number of samples. However, the phenolic profile of these varieties is less complex than the phenolic profile identified in the 'BRS Vitoria' table grape, since the varieties studied by these authors were represented by *V. vinifera*, while the 'BRS Vitoria' table grape is a hybrid between *V. vinifera* and *V. labrusca* varieties.

A new peak (Peak 31) was recorded in the samplings carried out from 17 DAV, which was tentatively identified as pelargonidin 3-coumaroyl-glucoside. The UV–vis spectrum of this compound showed the expected features based on its suggested structure: a visible maximum at 505 nm (attributable to B-ring mono-substitution pattern, pelargonidin), shoulder at approximately 431 nm (3-glucoside) and 314 nm (attributable to the coumaroyl moiety). On the other hand, the MS spectra showed the signal of the expected molecular ion (*m/z* 579) and a fragmentation signal at 271, implying the loss of 308 amu. This difference is due to the coumaroyl moiety loss (146 amu), while the other 162 amu correspond to the loss of a glucose moiety (Maldini et al., 2016). Its UV–vis, ESI-MS and ESI-MS/MS spectra are shown in Fig. 3. It was not possible to quantify them due to the presence of only small traces in the samples. The same compound was detected in the low molar ratio in *V. vinifera* grapes 'Garnacha Tintorera', corresponding to 0.03 % of its anthocyanin profile (Castillo-Muñoz et al., 2009b). In *Vitis amurensis* berries (skins), the anthocyanin pelargonidin 3,5-diglucoside was detected (Zhao et al., 2010), showing that this aglycone anthocyanidin occurs in a few grape species. In addition, the presence of pelargonidin-3-*O*-glucoside at trace levels in berry skins of *V. vinifera* grapes Cabernet Sauvignon and Pinot noir was demonstrated by He et al. (2010).

During the ripening process, a significant increase in total anthocyanins was observed in 'BRS Vitoria' berry skins, and these compounds were responsible for the visual changes in coloration observed on the berry skin. Both 3-glucoside and 3,5-diglucoside concentrations increased; however, the 3-glucoside:3,5-diglucoside ratio decreased from 2.7 to 1.5.

The total anthocyanin concentration expressed as malvidin-3,5-diglucoside equivalents observed at *véraison* was 28.4 mg kg^{-1}, while at full ripeness (28 DAV), it increased to 596.9 mg kg^{-1}, which implies an increase of 21 times in the total anthocyanin concentration (Table 1; Fig. 4). It is likely that this level would be even greater if the grapes were harvested later. However, due to the occurrence of high rainfall throughout the evaluated crop season, coinciding with the ripening

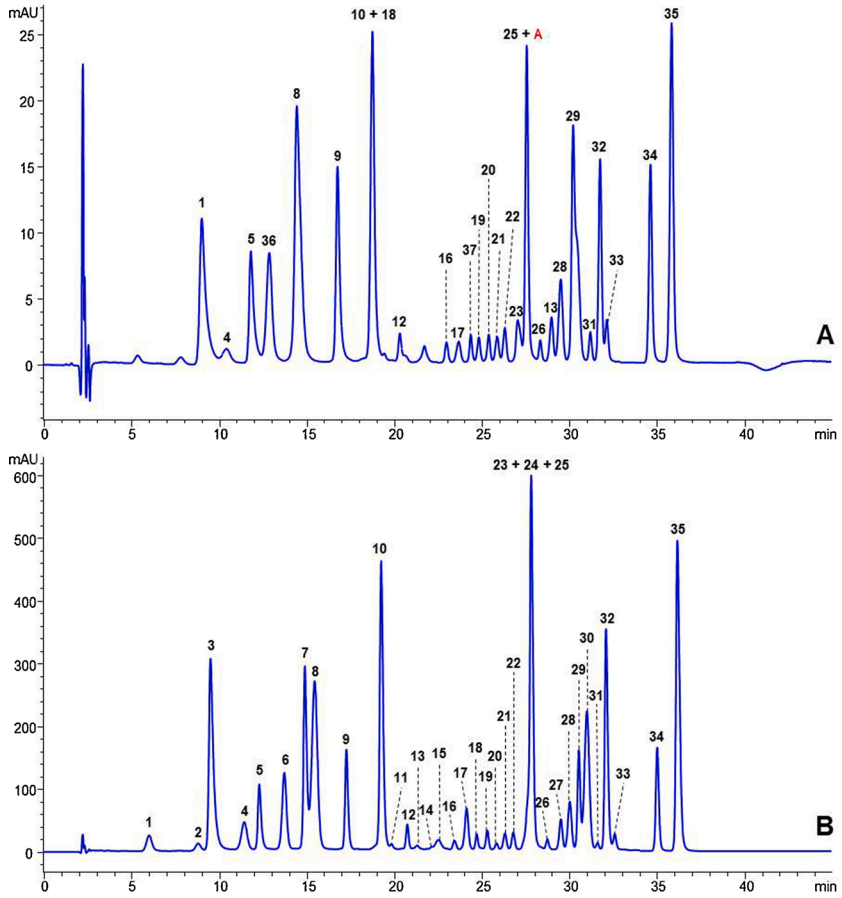

Fig. 2. HPLC–DAD chromatograms (detection at 520 nm) correspond to anthocyanins detected in 'BRS Vitoria' grape skin at different ripening stages. A: *véraison*; B: 28 days after *véraison* (full ripe or harvest). For peak assignation, see Table 1.

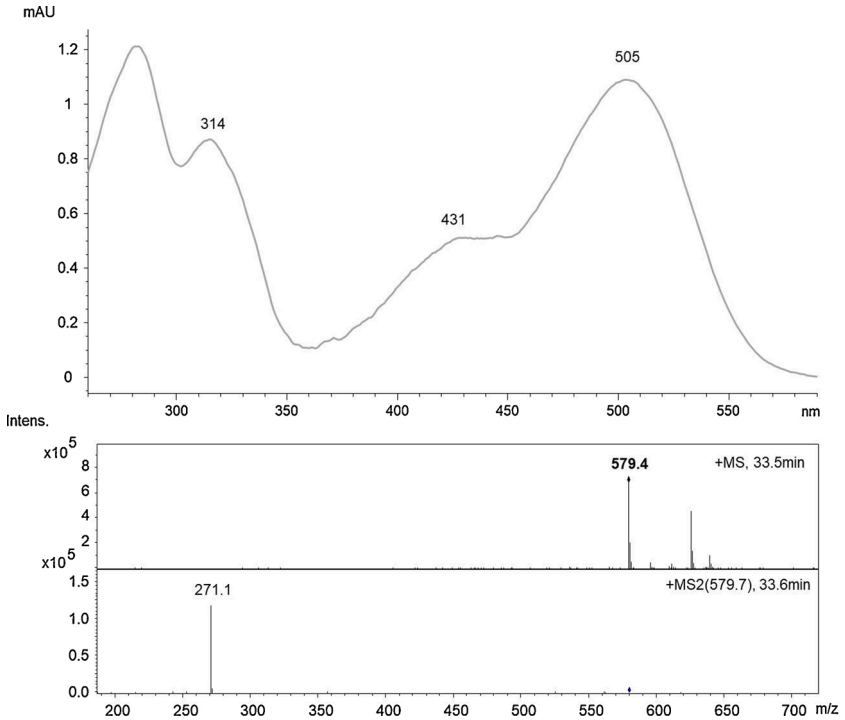

Fig. 3. DAD-UV–vis, ESI-MS and ESI-MS/MS spectra of pelargonidin 3-coumaroylglucoside detected in 'BRS Vitoria' grape skin.

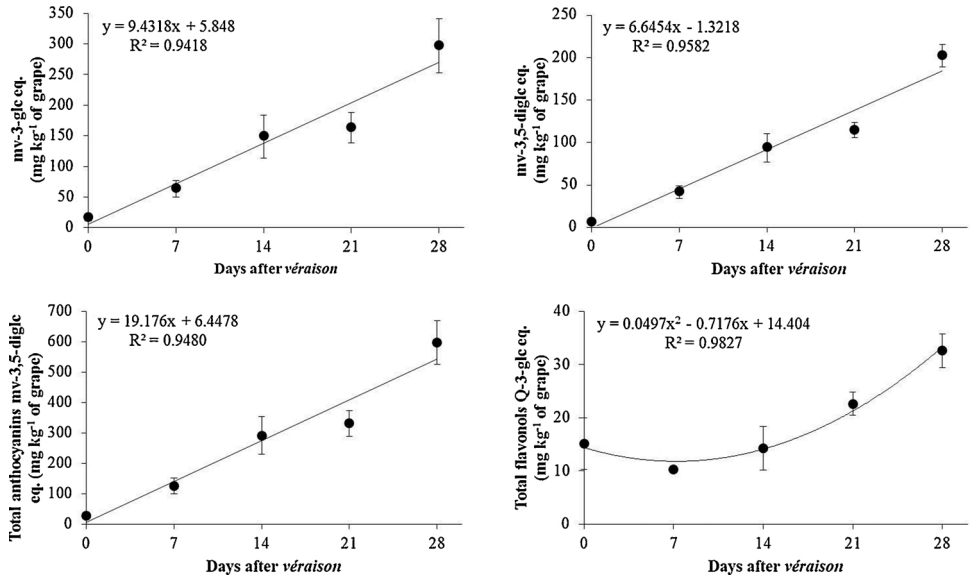

Fig. 4. Main phenolic compounds recorded in 'BRS Vitoria' seedless table grapes at different ripening stages. A: 3-glucoside series (expressed as mv-3-glc eq.); B: 3,5-diglucoside series (expressed as mv-3,5-diglc eq.); C: total anthocyanins (3-glc series + 3,5-diglc series, expressed as mv-3,5-diglc eq.); D: total flavonols (expressed as Q-3-glc eq.). *Note: mv: malvidin; Q: quercetin; glc: glucoside; diglc: diglucoside.*

period, it was necessary to anticipate the harvest in approximately 10 days to avoid bunches rotting and consequent losses in berry quality.

These anthocyanin concentrations are in agreement with those reported in non-vinifera grapes, such as 'Folha de Figo' (*Vitis labrusca*) and 'Niagara Rosada' (*V. labrusca*), 61–1,550 (in this case mg kg^{-1}, as cyanidin equivalents, which implies 146–3,779 mg kg^{-1} as mv-3,5-diglc equivalents) (Abe et al., 2007). However, 'BRS Vitoria' grapes presented lower total anthocyanin concentrations than 'BRS Violeta', another hybrid grape, which presented approximately 3950 mg kg^{-1}, as mv-3,5-diglc equivalents (Rebello et al., 2013). In addition, in non-vinifera grapes, the total anthocyanin concentrations [mg kg^{-1} of grapes (as mv-3,5-diglc equivalents)] ranged from 1116 to 2750 for 'Concord' (*V. labrusca*), 'Marechal Foch' (*Vitis rupestris* × *V. vinifera*) and 'Norton' grapes (*V. aestivalis*) (Muñoz-Espada et al., 2004).

3.2.2. Flavonols

The total flavonol concentration recorded in 'BRS Vitoria' table grape skins, expressed in mg kg^{-1} of berries, as quercetin 3-glucoside (Q-3-glc) equivalents, showed a variation during the ripening process (Table 2; Figs. 4 and 5). At *véraison*, the total flavonol concentration was higher than that observed at 7 DAV, increasing again until full ripening. At harvest (28 DAV), the grapes presented a total flavonol concentration of approximately 33 mg kg^{-1} (as Q-3-glc equivalents).

In 'BRS Vitoria' table grapes, the total flavonol concentration was lower than those obtained for 'BRS Violeta' grapes, approximately 153 mg kg^{-1}, as Q-3-glc equivalents (Rebello et al., 2013). However, in 'Bordô' (*V. labrusca*) grapes, the total flavonol concentration was approximately 153 μmol kg of grapes, which corresponds to 70.78 mg kg^{-1} of grapes, as Q-3-glc equivalents (Lago-Vanzela et al., 2011b), which corresponds to half of the concentration verified for 'BRS Vitoria' table grapes.

Concerning the flavonol profile described for 'BRS Vitoria' table grape skins, flavonol 3-*O*-glycosides were detected. These are the main derivatives of the six possible flavonol aglycones reported for grapes and verified for 'BRS Vitoria': myricetin (M), quercetin (Q), laricitrin (L), kaempferol (K), isorhamnetin (I) and syringetin (S), with M and Q found in greater proportions. These six structural flavonol aglycons are the same as those found in red wines made from *V. vinifera* cultivars

Table 2
Chromatographic and spectroscopic (MS/MS spectra) characteristics of identified flavonols in 'BRS Vitoria' seedless table grapes at different ripening stages (days after *véraison* – DAV) by HPLC–DAD–ESI-MS/MS (negative ionization mode). Molar proportions (mean value, n = 3) and total concentration (as quercetin 3-glucoside equivalents) in berry skin. Peak assignation in Fig. 5.

Peak	Assignation [a]	Molecular ion; product ions (*m/z*)	Rt (min)	*Véraison* (% molar)	7 DAV	14 DAV	21 DAV	28 DAV
38	M-glcU	493;317	16.59	0.59 ± 0.19	1.12 ± 0.17	1.21 ± 0.36	1.54 ± 0.54	1.20 ± 0.30
39	M-gal	479; 317	16.68	0.27 ± 0.23	1.39 ± 0.50	1.92 ± 0.38	1.43 ± 0.26	1.99 ± 0.13
40	M-glc	479; 317	18.71	4.49 ± 1.39	21.00 ± 2.85	38.92 ± 13.68	25.48 ± 8.23	39.62 ± 4.13
41	Q-gal	463; 301	26.34	1.70 ± 0.20	1.77 ± 0.23	1.01 ± 0.79	1.51 ± 0.38	1.34 ± 0.32
42	Q-glcU	477; 301	26.90	68.56 ± 0.91	38.36 ± 5.65	21.04 ± 14.05	34.01 ± 11.56	17.59 ± 1.46
43	Q-glc	463; 301	28.53	18.52 ± 0.85	27.10 ± 3.79	23.86 ± 7.37	26.97 ± 2.92	26.05 ± 3.30
44	Q-3-rhm-glc	609; 301	28.80	2.08 ± 0.25	2.27 ± 0.86	2.18 ± 1.20	1.02 ± 0.39	1.27 ± 0.39
45	L-glc	493; 331	31.69	0.59 ± 0.15	2.01 ± 0.21	3.50 ± 1.17	2.39 ± 0.70	3.66 ± 0.40
46	K-gal	447; 285	32.54	0.30 ± 0.21	0.79 ± 0.10	1.39 ± 0.49	0.84 ± 0.23	1.88 ± 0.22
47	K-glcU	461; 285	34.59	0.66 ± 0.14	0.67 ± 0.09	0.30 ± 0.26	0.44 ± 0.14	0.31 ± 0.07
48	K-glc	447; 285	35.77	1.16 ± 0.13	1.15 ± 0.13	1.43 ± 0.52	1.75 ± 0.50	1.74 ± 0.58
49	I-glc	477; 315	39.10	0.93 ± 003	1.66 ± 0.19	1.81 ± 0.43	1.87 ± 0.40	2.17 ± 0.26
50	S-glc	507; 345	40.69	0.12 ± 0.08	0.72 ± 0.04	1.42 ± 0.88	0.74 ± 0.17	1.18 ± 0.15
	Total (mg kg^{-1} of berries, Q-3-glc)			15.16 ± 4.83	10.25 ± 0.62	14.26 ± 8.27	20.54 ± 4.22	32.60 ± 7.33

[a] Nomenclature abbreviation: M, myricetin; Q, quercetin; K, kaempferol; L, laricitrin; I, isorhamnetin; S, syringetin; glcU, glucuronide; glc, glucoside; gal, galactoside; rhm, rhamnose; Rt: retention time (minutes). LOD and LOQ values for quercetin 3-glucoside: 0.027 mg L^{-1} and 0.089 mg L^{-1}, respectively.

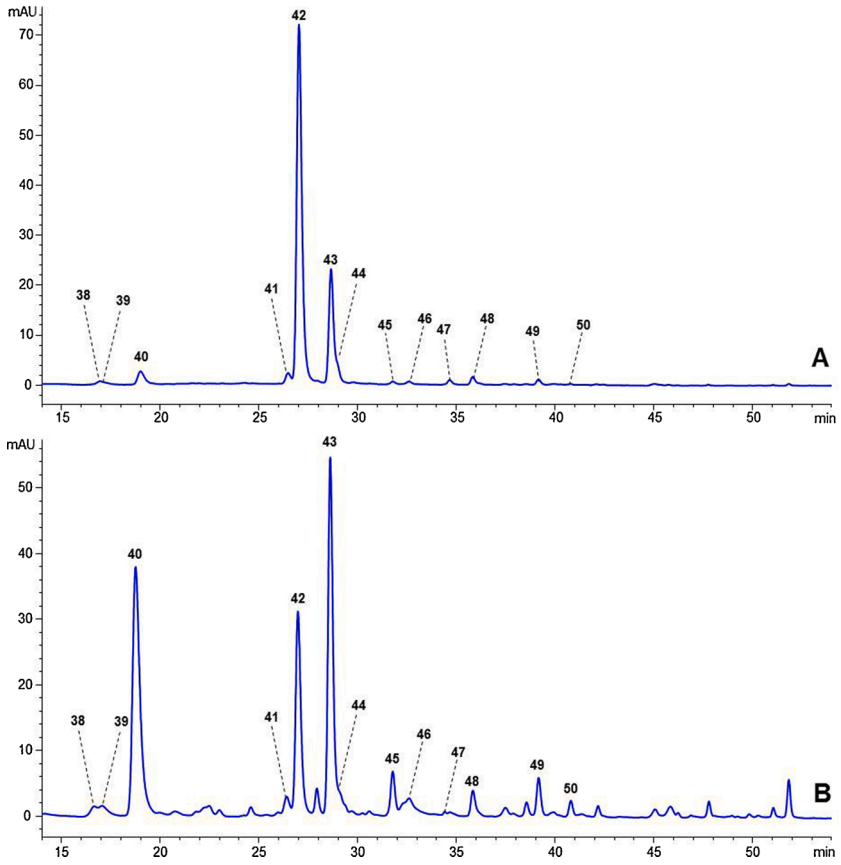

Fig. 5. HPLC–DAD chromatograms (detection at 360 nm) corresponding to flavonols detected in 'BRS Vitoria' table grape skins at different ripening stages. A: *véraison*; B: 28 days after *véraison* (full ripe or harvest). For peak assignation, see Table 2.

(Castillo-Muñoz et al., 2009a).

The flavonol 3-*O*-glycoside derivatives detected in 'BRS Vitoria' table grape belong to the 3-glucoside (3-glc), 3-galactoside (3-gal) and 3-glucuronic acid (3-glcU) series of myricetin, quercetin and kaempferol, while only 3-glc derivatives were detected for laricitrin, isorhamnetin and syringetin. Concerning quercetin, the presence of a diglucoside linked at the C-3 position, known as rutin [Q-3-rut or quercetin 3-(6″-rhamnosyl)-glucoside], was also detected and quantified as described in *V. vinifera* grape cultivars (Castillo-Muñoz et al., 2009a) and in 'Bordô' grapes (Lago-Vanzela et al., 2011b). These findings corroborate those results reported by several authors, who highlight that in red table grapes, the most common flavonols were quercetin galactoside, quercetin glucoside, quercetin glucuronide and quercetin rutinoside (Colombo et al., 2019; Colombo et al., 2020; Lago-Vanzela et al.,

Table 3
Chromatographic and spectroscopic (MS/MS spectra) characteristics of identified hydroxycinnamic acid derivatives (HCAD) and stilbenes in 'BRS Vitoria' seedless table grape at different ripening stages (days after *véraison* – DAV) by HPLC–DAD–ESI-MS/MS (negative ionization mode). Molar proportions (mean value, n = 3) and total concentration (as caftaric acid equivalents) in grape berries. Peak assignation in Fig. 6.

Peak	Assignation	Molecular ion; product ions (*m/z*)	Rt (min)	*Véraison*	7 DAV	14 DAV	21 DAV	28 DAV
				(% molar)				
51	*trans*-Caftaric acid	311; 179, 149, 135	3.94	40.97 ± 1.86	38.71 ± 4.96	56.37 ± 20.25	35.09 ± 4.55	47.83 ± 2.20
52	*trans*-Coutaric acid	295; 163, 149, 119	5.87	22.50 ± 3.92	15.68 ± 7.51	15.85 ± 0.70	18.99 ± 2.53	11.92 ± 2.39
53	*cis*-Coutaric acid	295; 163, 149, 119	6.25	10.72 ± 0.36	7.54 ± 2.50	6.98 ± 1.57	7.08 ± 0.86	5.75 ± 0.57
54	*p*-Coumaroyl-glucose	325; 163, 145	8.27	3.51 ± 0.55	7.72 ± 2.92	3.84 ± 3.48	8.62 ± 2.14	7.00 ± 0.16
55	*trans*-Fertaric acid	325; 193, 149	8.87	13.57 ± 0.69	15.69 ± 3.26	7.94 ± 6.99	15.01 ± 2.11	10.40 ± 0.75
56	*cis*-Fertaric acid	325; 193, 149	9.85	7.97 ± 1.17	11.73 ± 5.81	6.72 ± 5+95	12.06 ± 1.81	13.60 ± 0.89
57	*p*-Feruloyl-glucose	355; 193, 175	11.04	0.77 ± 0.02	2.91 ± 1.26	2.31 ± 2.06	3.16 ± 0.66	4.24 ± 3.78
	Total HCAD (mg kg^{-1} of berries, Caftaric acid)			3.67 ± 0.76	3.44 ± 1.40	5.68 ± 1.95	3.25 ± 0.39	8.17 ± 1.17
	trans-Resveratrol-glucose (mg kg^{-1} of berries)			0.04 ± 0.01	0.07 ± 0.01	0.13 ± 0.02	0.20 ± 0.05	0.63 ± 0.17
	cis-Resveratrol-glucose (mg kg^{-1} of berries)			0.05 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	0.12 ± 0.02	0.31 ± 0.06
	Total Resveratrol-glucose (mg kg^{-1} of berries)			0.09 ± 0.02	0.13 ± 0.03	0.21 ± 0.02	0.32 ± 0.07	0.94 ± 0.23

Rt: retention time (minutes). LOD and LOQ values for caftaric acid: 0.037 mg L^{-1} and 0.124 mg L^{-1}, respectively.

2011b).

When analyzing the different ripening stages evaluated, 91 % of the 'BRS Vitoria' table grape flavonol profile corresponded to the quercetin derivative sum at *véraison*, which gradually decreased until 28 DAV (46 %), especially the compound Q-glcU. On the other hand, the myricetin derivatives, essentially M-glc, increased their participation in the flavonol profile from 5% (at *véraison*) to 43 % (28 DAV). The proportion of the other compounds remained constant throughout ripening, following a slight increase in their concentrations from *véraison* to full ripening (28 DAV).

3.2.3. Hydroxycinnamic acid derivatives (HCAD) and stilbenes

Hydroxycinnamic acid derivative (HCAD) concentrations detected in 'BRS Vitoria' table grapes varied during ripening; however, it was not possible to fit a polynomial regression model to explain these results (Table 3; Fig. 6). HCADs detected for this grape cultivar are *trans*-caftaric acid, *trans*- and *cis*-coutaric acid, and *trans*- and *cis*-fertaric acid, the same HCAD found in *V. vinifera* cultivars (Castillo-Muñoz et al., 2009b) and in 'BRS Violeta' hybrid grape (Rebello et al., 2013). There was a predominance of its *trans* isomers in 'BRS Vitoria' table grapes; *trans*-caftaric acid was detected in the highest proportion and corroborated the results obtained for 'Antep Karasi' table grapes (Sen and Sonmezdag, 2020).

A compound assigned as *p*-coumaroyl-glucose was also detected, with the respective fragmentation pattern [(MS/MS), *m/z* 325; 163, 145]. This compound has already been described as an ester between an HCAD and a hexose in 'Bordô' and 'BRS Violeta' grapes (Lago-Vanzella et al., 2011b; Rebello et al., 2013). In addition, another ester derived from fertaric acid was detected and quantified, feruloyl-glucose [(MS/MS), *m/z* 355; 193, 175] (Table 3). However, the occurrence of esters between HCAD and hexose is not commonly reported in grapes, especially in *V. vinifera* and their wines (Badershneider and Winterhalter, 2001).

Regarding the total HCAD amount, the concentration detected at harvest was two times superior to those verified at *véraison*, indicating the evolution of these compounds during ripening.

For 'BRS Vitoria' table grape, the presence of resveratrol was not detected, only the *trans*- and *cis*-resveratrol-glc (*piceid*) molecules (Table 3 and Fig. 7). The total stilbene concentration was calculated from the sum of these molecules (*trans*- and *cis*-piceid) since their individual concentrations evolved similarly to the total concentration. Similar to 'Superior Seedless' (*V. vinifera*) table grapes (Hellín et al., 2010), the *trans*-piceid configuration predominated in 'BRS Vitoria' grape skins, and it was twice as high as the *cis*-configuration at harvest.

3.2.4. Flavan-3-ol monomers and dimers

From the flavan-3-ol structural analysis, seven monomers belonging to this class of compounds were detected: (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin 3-gallate, (-)-gallocatechin 3-gallate and (-)-epigallocatechin 3-gallate. (+)-Catechin was the most abundant compound found in all evaluated ripening stages; however, its concentration decreased during ripening. On the other hand, the (-)-epigallocatechin content increased from *véraison* to harvest. The other monomer concentrations remained constant during ripening evolution (Table 4; Fig. 7).

For 'Shiraz' grapes (*V. vinifera*), the major free flavan-3-ol monomer found in berry skins was (+)-catechin, which had its concentration reduced by approximately 50 %, from *véraison* to harvest (Downey et al., 2003b). These results are in agreement with those verified for 'BRS Vitoria' table grape in the present study and corroborate the results obtained for 'Superior Seedless' table grape, analyzed at different ripening stages (Hellín et al., 2010).

Concerning the flavan-3-ol B-type dimers, procyanidins B1 and B2 were detected in 'BRS Vitoria' berry skins (Table 4; Fig. 7). At *véraison*, a

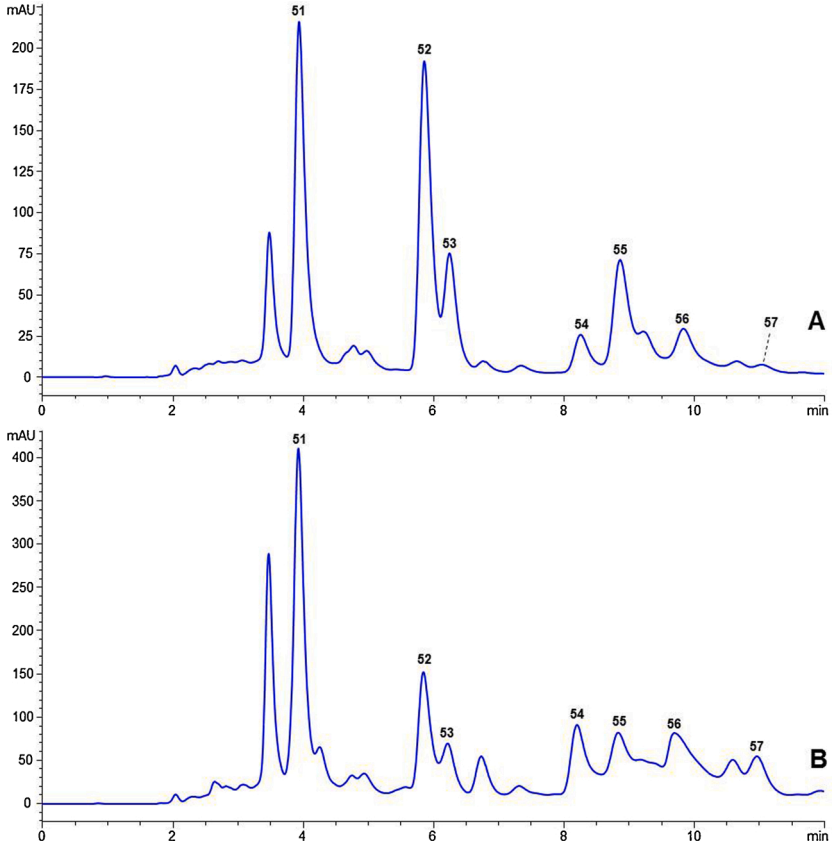

Fig. 6. HPLC–DAD chromatograms (detection at 320 nm) corresponding to hydroxycinnamic acid derivates (HCAD) detected in 'BRS Vitoria' berries at different ripening stages. A: *véraison*; B: 28 days after *véraison* (full ripe or harvest). For peak assignation, see Table 3.

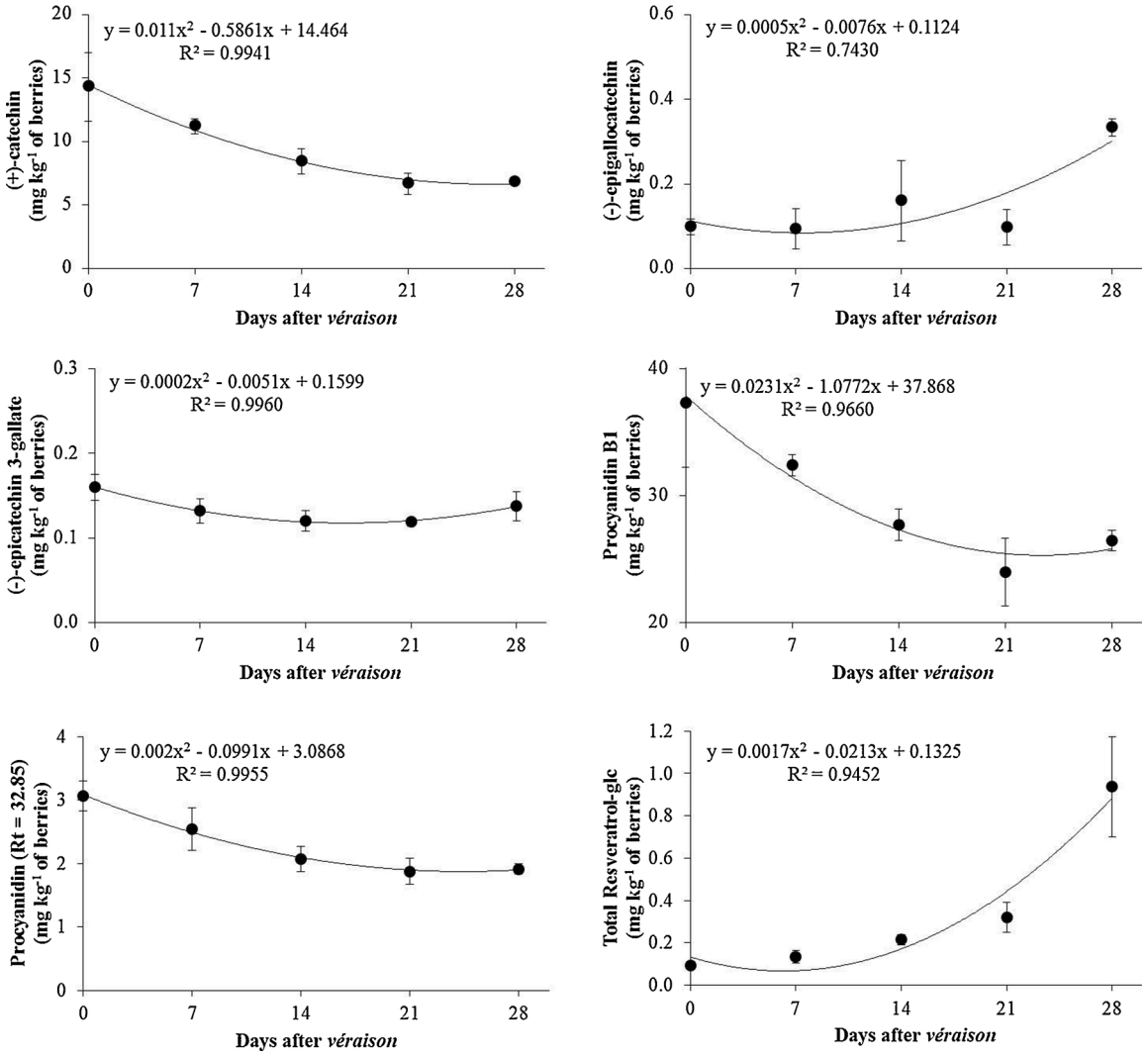

Fig. 7. Flavan-3-ol monomers and B-type dimers recorded in 'BRS Vitoria' seedless table grapes at different ripening stages.

Table 4
Structural characterization of identified proanthocyanidins, monomeric flavan-3-ol, B-type procyanidin dimer and resveratrol in 'BRS Vitoria' seedless table grapes at different ripening stages (days after *véraison* – DAV). Total content (as (+)-catechin equivalents) in berry skin.

Assignation [a]	*Véraison*	7 DAV	14 DAV	21 DAV	28 DAV
(+)-catechin	14.307 ± 2.728	11.212 ± 0.604	8.417 ± 0.999	6.674 ± 0.862	6.816 ± 0.249
(−)-epicatechin	0.935 ± 0.229	1.239 ± 0.084	0.894 ± 0.095	0.787 ± 0.039	1.411 ± 0.031
(−)-gallocatechin	0.621 ± 0.151	0.572 ± 0.135	0.638 ± 0.247	0.563 ± 0.106	0.760 ± 0.033
(−)-epigallocatechin	0.099 ± 0.018	0.094 ± 0.047	0.160 ± 0.095	0.098 ± 0.042	0.333 ± 0.021
(−)-epicatechin 3-gallate	0.160 ± 0.015	0.132 ± 0.015	0.120 ± 0.012	0.119 ± 0.005	0.138 ± 0.017
(−)-gallocatechin 3-gallate	0.011 ± 0.009	0.019 ± 0.002	0.006 ± 0.001	0.011 ± 0.009	0.006 ± 0.003
(−)-epigallocatechin 3-gallate	0.010 ± 0.009	ND	0.008 ± 0.004	0.010 ± 0.009	0.026 ± 0.015
Procyanidin B1	37.334 ± 5.138	32.398 ± 0.834	27.687 ± 1.243	23.969 ± 2.641	26.446 ± 0.811
Procyanidin B2	2.714 ± 0.428	2.783 ± 0.203	2.578 ± 0.071	2.103 ± 0.079	2.987 ± 0.053
Procyanidin (Rt = 32.85)	3.064 ± 0.236	2.546 ± 0.336	2.071 ± 0.204	1.879 ± 0.205	1.915 ± 0.084
mDP	7.801 ± 0.409	7.955 ± 0.193	8.548 ± 0.293	9.004 ± 0.111	8.894 ± 0.357
Extension-galloylation (%)	5.216 ± 0.206	4.658 ± 0.067	4.810 ± 0.366	3.143 ± 0.195	2.871 ± 0.352
Extension-prodelphinidin (%)	11.765 ± 1.951	12.723 ± 1.092	13.163 ± 1.289	11.408 ± 1.845	13.476 ± 0.701
Total PA (mg kg^{-1} of berries, eq C)	698.219 ± 121.200	732.672 ± 21.119	713.499 ± 57.285	541.108 ± 24.136	533.108 ± 43.173

[a] Total PA, total concentration of proanthocyanidins, as (+)-catechin equivalents, calculated by total sum of the concentrations of all extension and terminal subunits; mDP, mean degree of polymerization; % galloylation, % of 3-gallate subunits; % prodelphinidin, % of epigallocatechin subunits; and % of each of the flavan-3-ol monomers found as terminal and extension subunits. ND, not detectable.

higher concentration of procyanidin B1 was noticed, which decreased during ripening. On the other hand, the procyanidin B2 concentration remained constant during ripening. Procyanidin B4, commonly reported in grapes, was not detected in 'BRS Vitoria' berry skins, as well as in 'BRS Violeta' and 'Bordô' grapes (Lago-Vanzela et al., 2011b; Rebello et al., 2013), since this compound seems to be more related to grape seeds and less common in grape skins.

Furthermore, at the elution time of 32.85 min, we detected a

procyanidin pseudo-molecular ion that represents a molecular weight equal to those verified for procyanidins B1 and B2 (*m/z* 578), but we were not able to identify it correctly and elucidate its structure. This newly identified compound, as well as procyanidin B1, showed a concentration decrease during ripening.

3.2.5. Proanthocyanidins

Flavan-3-ol oligomers and polymers (proanthocyanidins, PAs) were also identified by structural analyses. The total concentration of these compounds varied according to the berry ripening stages, being higher in the first week after *véraison* and decreasing over time until full ripening. The variation observed in the total PA concentration ranged from 732.7–533.1 mg kg^{-1} at 7 DAV and harvest, respectively (Table 4 and Fig. 7).

Although there is no specific classification for hybrid grapes when compared to vinifera cultivars, the 'BRS Vitoria' can be classified as an important cultivar concerning the production of these compounds. Vinifera cultivars are classified as grapes with high PA concentrations in their skins when values reach between 280 and 720 mg kg^{-1} (Busse--Valverde et al., 2010; Travaglia et al., 2011).

From the structural analysis, the main degree of polymerization (mDP) determined varied from 7.8–9.0. According to the results obtained for mDP values, it can be inferred that the 'BRS Vitoria' PAs are mainly oligomers and short polymer chains, as described for wines by Gris et al. (2011). Similar mDP results were found in 'BRS Clara' and 'BRS Morena' hybrid seedless table grapes, 7.0 and 9.9, respectively (Lago-Vanzela et al., 2011a).

3.3. Multivariate and correlation analysis

When data were evaluated using multivariate analysis (PCA), principal components 1 and 2 represented 89.9 % of the total variation (70.39 and 19.48 %, respectively) (Fig. 8). Based on this analysis, it was observed that the groups segregated according to the ripening stages. The samplings collected at *véraison* and 7 DAV were grouped, characterized by the highest concentrations of PB1, catechin and total acidity present in the berries. These samplings were grouped based on principal component 2 (PC2, 19.48 %).

Samplings from berries collected at 14 and 21 DAV showed a trend to form another group, while those from berries collected at 28 DAV (full ripeness or harvest) comprised another group, related to most of the phenolic compounds analyzed. Principal component 1 (PC1, 70.39 %) grouped the ripening phases according to the concentration of SS, anthocyanins, mono- and diglucosides, and total flavonols. These results were expected since the grape berries should present high concentrations of sugars and phenolic compounds and lower acidity at harvest.

Fig. 9 confirms the results verified in the PCA, with the formation of three distinct groups. At *véraison* and 7 DAV, there was a predominance of proanthocyanidins and catechin, as reported in other studies. In 'Barbera' and 'Nebbiolo' grapes (*V. vinifera*), the PA content in the berry skin was high before *véraison* and decreased during ripening (Asproudi et al., 2015; Di Stefano et al., 2002). This pattern may be due to the deviation of the intermediate metabolites (cyanidin and delphinidin) towards the synthesis of anthocyanins, as they share the same biosynthetic pathway (Baranac et al., 1997) or to little-known phenomena involving PA transformation and oxidation (Asproudi et al., 2015). There was also a high association between SS, anthocyanins, flavonols, and other compounds in the last sampling period (28 DAV) when the grape berries were fully ripe.

4. Conclusion

'BRS Vitoria' hybrid table grapes can be harvested ~28 days after *véraison*, when the berries reach a soluble solids content higher than 15°Brix and low titratable acidity. At this time, the bunches are very black colored and present a total anthocyanin concentration of 596.9 mg

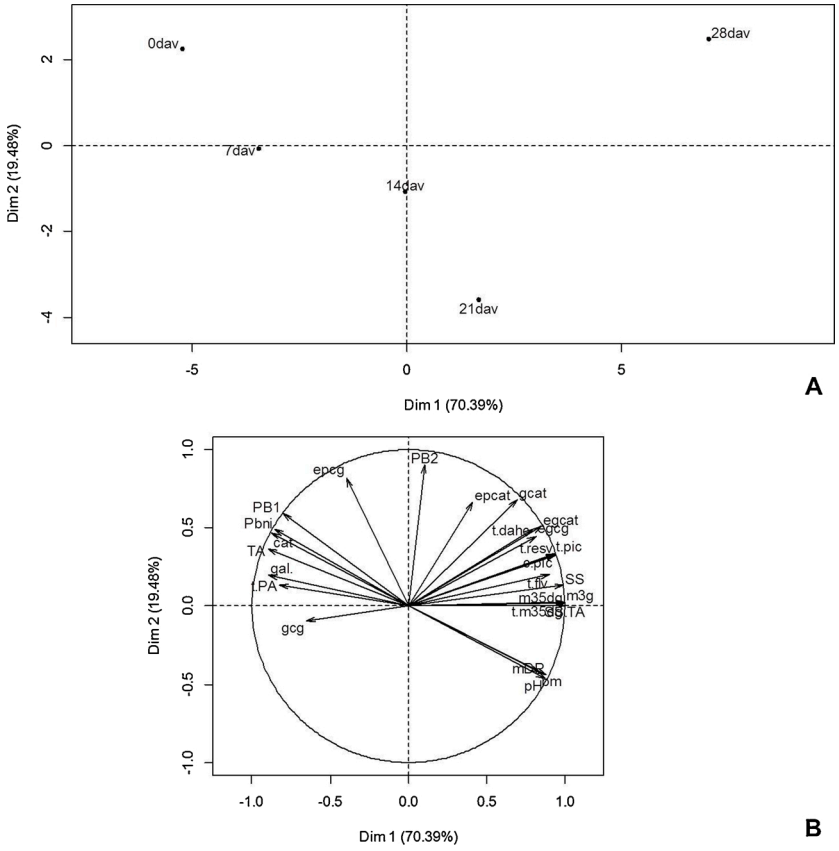

Fig. 8. Principal component analysis (PCA) for phenolic composition and ripening parameters recorded in 'BRS Vitoria' seedless table grapes during different ripening stages. A: Treatment dispersion according to the principal component scores (0, 7, 14, 21 and 28 days after *véraison* - dav). B: evaluated characteristics arrangement according to the principal component scores. pH; SS: soluble solids content; TA: total acidity; SS.TA: maturation index; bm: berry mass; m3g: anthocyanin 3-glucoside series as malvidin-3-glucoside eq; m35dg: anthocyanin 3,5-diglucoside series as malvidin-3,5-diglucoside eq; t.m35dg: total anthocyanin as malvidin-3,5-diglucoside eq; t.flv: total flavonol as Q-3-glc eq.; c.pic: *cis-piceid*; t.pic: *trans-piceid*; t.resv: total resveratrol-glucose; t.dahc: total hydroxycinnamic acid derivates content; cat: catechin; epcat: epicatechin; gcat: catechin-3-gallate; gcg: gallocatechin 3-gallate; egcat: epigallocatechin; epcg: epicatechin 3-gallate; egcg: epigallocatechin 3-gallate; PB1: procyanidin B1; PB2: procyanidin B2; Pbni: not identified procyanidin (retention time = 32.85); mDP: mean degree of polymerization; gal: percentage of galloylation; and t. PA: total proanthocyanidin content.

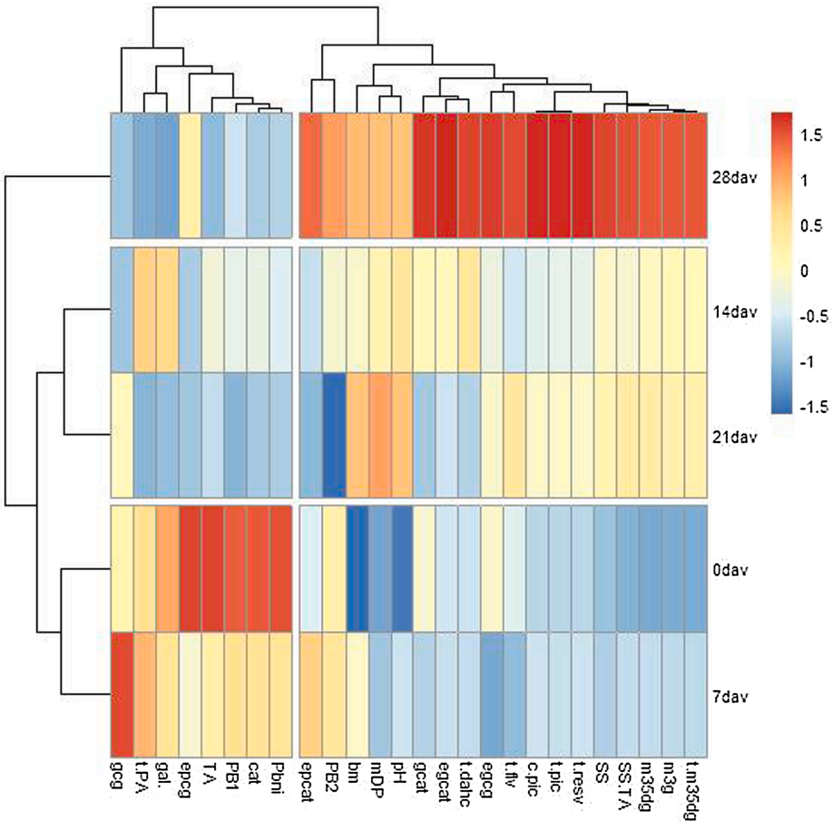

Fig. 9. Heatmap elaborated using Ward's hierarchical clustering analysis based on Euclidean distances for phenolic composition and ripening parameters recorded in 'BRS Vitoria' seedless table grape during different ripening stages: 0, 7, 14, 21 and 28 days after *véraison* - dav. pH; SS: soluble solids content; TA: total acidity; SS.TA: maturation index; bm: berry mass; m3g: anthocyanins 3-glucoside serie as malvidin-3-glucoside eq; m35dg: anthocyanins 3,5-diglucoside serie as malvidin-3,5-diglucoside eq; t.m35dg: total anthocyanins as malvidin-3,5-diglucoside eq; t.flv: total flavonols as Q-3-glc eq.; c.pic: *cis-piceid*; t.pic: *trans-piceid*; t.resv: total resveratrol-glucose; t.dahc: total hydroxycinnamic acid derivates content; cat: catechin; epcat: epicatechin; gcat: catechin-3-gallate; gcg: gallocatechin 3-gallate; egcat: epigallocatechin; epcg: epicatechin 3-gallate; egcg: epigallocatechin 3-gallate; PB1: procyanidin B1; PB2: procyanidin B2; Pbni: not identified procyanidin (retention time = 32.85); mDP: mean degree of polymerization; gal: percentage of galloylation; and t.PA: total proanthocyanidin content.

kg^{-1} (as malvidin-3,5-diglucoside equivalents). 'BRS Vitoria' table grapes present a typical anthocyanin profile reported in hybrid grapes, composed of 3-glucoside and 3,5-diglucoside derivatives. In addition, pelargonidin traces also occur, with this aglycone rarely detected in grapes. The anthocyanin 3-glucoside:3,5-diglucoside derivative ratio decreases considerably during berry ripening.

All six possible flavonol aglycones reported for grapes occur in 'BRS Vitoria' table grapes: myricetin, quercetin, laricitrin, kaempferol, isorhamnetin and syringetin, with myricetin and quercetin, which were found in greater proportions. 'BRS Vitoria' table grapes present seven different flavan-3-ol monomers, with (+)-catechin being the most abundant. Concerning proanthocyanidins (PAs), 'BRS Vitoria' PAs are mainly oligomers and short polymer chains.

CRediT authorship contribution statement

Ronan Carlos Colombo: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. **Sergio Ruffo Roberto:** Conceptualization, Funding acquisition, Investigation, Resources, Writing - original draft. **Maria Aparecida da Cruz:** Investigation, Writing - original draft, Writing - review & editing. **Deived Uilian de Carvalho:** Investigation, Writing - original draft, Writing - review & editing. **Lilian Yukari Yamamoto:** Investigation. **Suzana Lucy Nixdorf:** Investigation, Methodology. **José Pérez-Navarro:** Investigation, Methodology. **Sergio Gómez-Alonso:** Investigation, Formal analysis, Methodology. **Muhammad Shahab:** Investigation. **Saeed Ahmed:** Investigation. **Leandro Simões Azeredo Gonçalves:** Formal analysis, Investigation. **Reginaldo Teodoro de Souza:** Formal analysis, Investigation, Supervision. **Isidro Hermosín-Gutiérrez:** Conceptualization, Funding acquisition, Investigation.

Declaration of Competing Interest

None of the authors have a conflict of interest.

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