

Phenolic composition of BRS Violeta red wines produced from alternative winemaking techniques: relationship with antioxidant capacity and sensory descriptors

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Abstract The detailed phenolic composition, sensory profile and antioxidant capacity of red wines produced from the BRS Violeta grape cultivar have been studied. The alternative winemaking procedures of grape pre-drying and submerged cap have been assessed against the traditional treatment. Malvidin was the principal anthocyanidin of BRS Violeta wines, followed by delphinidin and petunidin. It was possible to detect 17 different types of pyranoanthocyanins derived from the five anthocyanidins in their non-acylated, acylated and coumaroylated forms, being vitisin A-types and hydroxyphenyl-pyranoanthocyanins the main forms detected. Pre-dried wine presented low concentrations of anthocyanins, suggesting that they were partially degraded by the thermal treatment as a result of cleavage of covalent bounds and/or by deglycosylation of the anthocyanin 3-glucosides. Submerged

cap wine presented lower anthocyanin concentration due to the limited mechanical effect caused by the constant contact between pomace and must during maceration. The 3-glucoside of the myricetin was the principal flavonol, and large amounts of coumaric and caffeic acids were observed due to the high degree of hydrolysis of their precursors, named coumaric and caffeic acids. Both alternative winemaking procedures presented no differences in the flavan-3-ol concentrations, and the antioxidant capacity of the wines did not significantly differ. The lack of differences in the main sensory descriptive attributes showed that the alternative procedures have great potential to be applied as an alternative to the traditional treatment.

Keywords Red wine · Winemaking · Antioxidant capacity · Polyphenols · Descriptive analysis

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Introduction

In general, grape is considered one of the greatest sources of phenolic compounds when compared to other fruits and vegetables [1, 2]. American grapes and their hybrids (*Vitis labrusca*) show some disadvantages when compared with European grapes (*Vitis vinifera*) concerning their low soluble solids in their optimal stage of ripening and low color potential [3]. In order to minimize these effects, the Brazilian Agro-Farming Research Agency EMBRAPA Grape and Wine developed new cultivars such as BRS Violeta, which is a result from the cross between ‘BRS Rúbea’ and ‘IAC 1398-21’ [4].

BRS Violeta grape cultivar has become a blend agent to varietal red wines with poor color intensity, since it presents high color intensity, unique flavor and rich

antioxidant properties. Few reported studies concerning the BRS Violeta wine phenolic composition have revealed a high anthocyanin content ranging from 1866 to 2173 mg L⁻¹ [5] and 1555.35 mg L⁻¹ as malvidin 3,5-diglucoside equivalents [6]. The aforementioned studies were focused only on the phenolic composition of Violeta grapes and wines and presented no data related to sensory features and, subsequently, their relationship with the phenolic composition.

Furthermore, in comparison with the several studies worldwide, which contemplate red wines produced from *Vitis vinifera* grapes, there is a lack of studies dealing with the relationship between phenolic composition and sensory descriptive attributes [6–8]. Moreover, no studies were found dealing with BRS Violeta red table wines produced following variations in winemaking procedures in order to enhance the quality of these wines.

Winemakers have employed several variations on winemaking by the application of drying process of the grapes before fermentation [9] and the use of submerged cap during the alcoholic fermentation [10]. Studies dealing with grape pre-drying showed that the heating caused an irreversible damage in the cellular structure of the grape skin increasing the extraction of the phenolic compounds to the wine during alcoholic fermentation [9].

In contrast, the thermal degradation of anthocyanins is a well-known phenomenon that could occur in parallel to the pre-drying of grapes [11]. Submerged cap winemaking procedure allows for the constant contact between the pomace and the must, increasing the extraction of anthocyanins and restricting the extraction of the flavan-3-ols from the seeds and skins to the must during alcoholic fermentation, due to the limitation of the mechanical effect caused by the pumped must on the grape pomace. All the above-mentioned studies presented relevant results; however, they handled with *Vitis vinifera* red wines and presented no relationships with sensory data.

In this context, the aim of this work was to evaluate the detailed phenolic composition, obtained by HPLC–DAD–ESI–MS/MS of BRS Violeta red wines produced from two alternative winemaking procedures, pre-drying and submerged cap wines, in comparison with the traditional winemaking procedure. The study of the quantitative and qualitative phenolic profiles covered the main grape and wine flavonoids (anthocyanins, flavonols and flavan-3-ols) and other interesting minor phenolic compounds (pyranoanthocyanins, hydroxycinnamic acid derivatives and stilbenes). Additionally to the phenolic profiles, wine sensory descriptive attributes and antioxidant capacity were measured in order to evaluate the potential of the alternative winemaking procedures.

Materials and methods

Chemicals

All solvents were of HPLC quality, all chemicals were of analytical grade (>99 %) and the water was of Milli-Q quality. The following commercial standards from Phytolab (Vestenbergsgreuth, Germany) were used for the identification of the phenolic compounds: malvidin 3-glucoside, malvidin 3,5-diglucoside, peonidin 3,5-diglucoside, *trans*-piceid, *trans*-caftaric acid, (–)-epigallocatechin and (–)-gallocatechin, as also the following commercial standards from Extrasynthese (Genay, France): 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. In addition, the following commercial standards from Sigma-Aldrich (Tres Cantos, Madrid, Spain) were used: *trans*-resveratrol, caffeic acid, (+)-catechin, (–)-epicatechin, (–)-epicatechin 3-gallate and (–)-gallocatechin 3-gallate. Other non-commercial flavonol standards such as myricetin 3-glucoside, quercetin 3-glucuronide and laricitrin 3-glucoside were previously isolated from Petit Verdot grape skins [12]. Procyanidin B4 was kindly supplied by Prof. Fernando Zamora (Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Spain). The *trans* isomers of resveratrol and its 3-glucosides (piceid) were converted into their respective *cis* isomers by UV irradiation (366 nm light for 5 min in quartz vials) of 25 % MeOH solutions of the *trans* isomers.

All the standards were used for identification and quantitation by calibration curves covering the expected concentration ranges. When a standard was not available, the quantitation was done using the calibration curve of the most similar compound: malvidin 3,5-diglucoside for 3,5-diglucoside anthocyanin type and malvidin 3-glucoside for the 3-glucoside type, quercetin 3-glucoside for flavonol 3-glycosides and their free aglycones, caffeic acid for hydroxycinnamic acid derivatives, (+)-catechin for polymeric flavan-3-ols (total proanthocyanidins), and individual flavan-3-ol monomers and dimers by their corresponding standards considering their total sum as (+)-catechin equivalents.

Winemaking

Three red wines were produced in duplicate: traditional Violeta wine (VIOT), pre-dried Violeta wine (VIOPD) and submerged cap Violeta wine (VIOSC) (2 × 7 kg × 3 treatments), totaling 42 kg of grapes were harvested in the city of Jales (20°16' 7" South and 50°32'58" West), São Paulo

state, Brazil, in 2013 vintage, at their expected maturity level and in good sanitary conditions, since they presented, at the start of the winemaking procedure, soluble solids content of 18.5 ± 0.4 °Brix, pH value of 3.33 ± 0.02 and total acidity of 4.10 ± 0.18 g L⁻¹ as tartaric acid equivalents.

All the treatments followed the standard winemaking procedure previously described by De Castilhos et al. [13]. The mixture (must + pomace) was placed into 10-L fermentation vessels and treated with sulfur dioxide (86.2 ppm), and alcoholic fermentation was induced by the inoculation of 200 ppm of dry active *Saccharomyces cerevisiae* yeasts Y904 (Amazon Group®). An aliquote of the must was removed for determination of the soluble solids in order to proceed with the chaptalization. The wines were macerated for 7 days with twice-daily punching-down, and after this time, the wines were dejuiced and chaptalized to 11 % v/v of ethanol by the direct insertion of sucrose (chaptalization). After dejuicing, the wines were properly racked three times at 10 day intervals, and between the first and second rackings, the malolactic fermentation took place by the inoculation of acid lactic bacteria *Oenococcus oeni*. The finalization of this second fermentation was followed by Thin Layer Chromatography [14]. Between the second and third rackings, the wines were placed in a refrigerated ambient (0–5 °C) for 10 days in order to stabilize the tartrate. The wines were then bottled and stabilized for 90 days.

The submerged cap treatment provided the effect of the constant maceration of the grape's solid parts by using stainless steel screens to maintain the cap at the bottom of the fermentative vessel, avoiding its rise due to the production of carbon dioxide. Submerged cap wines, as well as traditional wines, were chaptalized to 11 %v/v by the insertion of 33.5 g of sucrose per L of wine.

Pre-drying treatment consisted of drying the grapes to 22 °Brix prior to alcoholic fermentation to avoid chaptalization and obtain wines with an alcoholic content between 8.6 and 14 % v/v, as required by Brazilian legislation [15]. This winemaking process was carried out using a convective drying method with a tray dryer at 60 °C and airflow of 1.1 m s⁻¹ [13]. At the end of drying procedure, Violeta wines presented 22.6 °Brix, with 12.7 % of the water evaporated in relation to the initial weight.

The following conventional enological parameters were measured: total and volatile acidities (as g L⁻¹ tartaric and acetic acid equivalents, respectively) [15]; dry extract (g L⁻¹) by gravimetric method [16]; reducing sugars (g L⁻¹) by the Lane-Eynon method [16]; alcoholic content (ALC) (%v/v) by pycnometry [16]; and total phenolic content by spectrophotometric procedure using gallic acid as standard [17].

Analysis of the phenolic compounds

Preparation of the wine for the determination of the non-anthocyanin phenolic compounds

The flavonol fractions were isolated from diluted wine samples following the procedure described by Castillo-Muñoz et al. [18], using Bond Elute Plexa PCX solid-phase extraction cartridges (Agilent; 6 cm³, 500 mg of adsorbent). The flavan-3-ols (monomers, B-type dimers and polymeric proanthocyanidins) and stilbenes were isolated following the procedure described by Rebello et al. [19], using SPE C18 cartridges (Waters® Sep-Pak Plus, filled with 820 mg of adsorbent).

HPLC–DAD–ESI–MSⁿ analysis of the phenolic compounds

The HPLC separation, identification and quantitation of the phenolic compounds were carried out on an Agilent 1100 Series HPLC system (Agilent, Germany) equipped with DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI–MSⁿ) system, coupled to an Agilent ChemStation (version B.01.03) data-processing unit. The mass spectra data were processed using the Agilent LC/MS Trap software (version 5.3).

The anthocyanin, flavonols and hydroxycinnamic acid derivatives (HCAD) were analyzed according to a previously described method [20]. The wine samples were injected (10 µL for anthocyanin analysis and 20 µL for flavonol analysis) onto a Zorbax Eclipse XDB-C18 reversed-phase column (2.1 × 150 mm; 3.5 µm particle; Agilent, Germany) with the temperature controlled at 40 °C.

For identification, the ESI/MS–MS was used in both the positive (anthocyanins) and negative (flavonols and hydroxycinnamic acid derivatives) ionization modes set for the following parameters: dry N₂ gas with a flow of 8 L min⁻¹ at a drying temperature of 325 °C; and N₂ nebulizer at 50 psi. The ionization and fragmentation parameters were optimized by direct injection of the appropriate standard solutions (malvidin 3,5-diglucoside solution in the positive ionization mode; quercetin 3-glucoside and caftaric acid in the negative ionization mode) using a scan range of 50–1200 m/z. The anthocyanin and pyranoanthocyanin identification was based on the spectroscopic data (UV–Vis and MS/MS) obtained from the aforementioned authentic standards or using previously reported data [5–7, 18–22]. For quantitation, the DAD-chromatograms were extracted at 520 nm for anthocyanins, 360 nm for flavonols and 320 nm for the hydroxycinnamic acid derivatives (HCAD). The analyses were carried out in duplicate.

Identification and quantitation of the flavan-3-ols and stilbenes using multiple reaction monitoring (MRM) HPLC–ESI–MS/MS

The analysis was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems) with triple quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI–MS/MS). The chromatographic system was managed an Agilent ChemStation (version B.01.03) data-processing unit, and the mass spectra data were processed using the Analyst MSD software (Applied Biosystems, version 1.5).

Structural information concerning the proanthocyanidins was obtained using the pyrogallol-induced acid-catalyzed depolymerization method [23]. The reaction consisted of adding 0.50 mL of the pyrogallol solution (100 g L^{-1} pyrogallol plus 20 g L^{-1} of ascorbic acid in 0.3 N HCl) to 0.25 mL of the sample in MeOH and incubating 40 min at $30 \text{ }^\circ\text{C}$. The hydrolysis reaction was stopped by adding 2.25 mL of sodium acetate (67 mM). An aliquot of 2 mL of the reacted sample was placed in a vial and injected directly into the equipment for analysis.

The samples, before and after the acid-catalyzed depolymerization reaction, were injected ($20 \mu\text{L}$) onto an Ascenis C18 reversed-phase column ($150 \text{ mm} \times 4.6 \text{ mm}$ with $2.7 \mu\text{m}$ of particle size), with the temperature controlled at $16 \text{ }^\circ\text{C}$. The solvents and gradients used for this analysis and the two MS scan types used (enhanced MS—EMS, and multiple reaction monitoring—MRM) as well as all the mass transitions (m/z) for identification and quantitation were according to the methodology reported by Lago-Vanzela et al. [20].

Determination of the antioxidant capacity by the DPPH assay

The procedure consisted of adding $100 \mu\text{L}$ of wine diluted in methanol to 2.9 mL of a methanolic DPPH (2,2-diphenyl-1-picrylhydrazyl, Fluka Chemie) radical solution ($6 \times 10^{-5} \text{ mol L}^{-1}$) [24]. After 25 min, the decrease in the percent absorbance at 515 nm was measured. For this measurement, the range should be between 20 and 80 % of the initial DPPH absorbance and thus the dilution of the wine with methanol was adjusted in order to enter this range; for red wines, the usual dilution factors are between 1/10 and 1/20. Quantitation of the antioxidant capacity was achieved using calibration curves obtained with methanolic solutions of Trolox ($R^2 = 0.9962$) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Fluka, Chemie).

Sensory analysis

Ten panelists (Embrapa Grape and Wine, Brazil) with more than 15 years of wine tasting experience used descriptive analysis to profile the red table wines. They took part in a session using different wines among the produced samples (traditional, pre-dried and submerged cap) and reference standards. After a brief discussion among the panelists, a list of eleven attributes was established, two attributes for appearance (color intensity, violet hue) and nine for taste (sweetness, acidity, bitterness, flavor intensity/body, structure/tannins, herbaceous taste, astringency, pungency and persistence). The evaluation sessions took place in a sensory analysis room with individual booths under daylight at ambient temperature. Aliquots of 30 mL of the red wines at $18 \text{ }^\circ\text{C}$ were poured into transparent glass cups, and for each wine, the panelists evaluated each descriptor on a horizontal unstructured 9-cm scale anchored by the minimum and maximum extremes. All the samples were coded with three random digits and were presented in a monadic and randomized form. The panelists evaluated the samples in triplicate [25]. The Ethical Issues regarding the sensory analysis were approved by the Ethics in Research Committee of the Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University (process no. 15159913.3.0000.5466).

Data analysis

All the data were treated using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (when $p < 0.05$). All the statistical tests were applied at a significance level of 0.05 using the Minitab 17 software (Minitab Inc.).

Results and discussion

Conventional enological parameters

The alternative winemaking techniques, pre-drying (PD) and submerged cap (SC), have influenced all the conventional enological parameters ($p < 0.05$), except the total phenolic content (PHEN) ($p > 0.05$), suggesting that these aforementioned alternative winemaking techniques did not significantly affect these compounds, as previously reported by De Castilhos et al. [3] (Supplementary Table). It was expected that VIOPD and VIOSC presented lower total phenolic concentration due to the phenolic degradation caused by the heating and to the limited extraction promoted by the absence of pumping-over effects during maceration, respectively; however, the differences were not significant.

Table 1 Anthocyanins and pyranoanthocyanins profiles determined by HPLC/MS/MS (mean value \pm standard deviation) for BRS Violeta young red wines

Anthocyanidins and pyranoanthocyanins	Peak	R_f (min)	Molecular ion; product ions (m/z)	VIOT	VIOPD	VIOSC
<i>Anthocyanins (mg L⁻¹)</i>				818.24 \pm 8.17 a	123.72 \pm 13.06 c	335.69 \pm 0.91 b
Dp-3,5diglc	1	4.5	627; 465,303	164.30 \pm 4.94 a	16.46 \pm 2.88 c	46.41 \pm 0.02 b
Cy-3,5diglc	2	6.5	611; 449,287	56.37 \pm 1.44 a	5.43 \pm 0.65 c	27.43 \pm 0.56 b
Pt-3,5diglc	3	9.5	641; 479,317	130.84 \pm 1.62 a	25.29 \pm 4.62 c	60.73 \pm 0.05 b
Pn-3,5diglc	4	12.1	625; 463,301	72.83 \pm 0.69 a	9.52 \pm 2.28 c	34.97 \pm 0.05 b
Mv-3,5diglc	5	14.0	655; 493,331	194.18 \pm 0.73 a	38.95 \pm 2.07 c	103.23 \pm 0.31 b
Cy-3acglc-5glc	6	16.5	463; 301	3.59 \pm 0.17 a	1.95 \pm 0.25 b	2.63 \pm 0.18 b
Pt-3acglc-5glc	7	18.2	683; 521,479,317	7.50 \pm 0.06 a	2.31 \pm 0.23 c	4.25 \pm 0.23 b
Mv-3acglc-5glc	8	21.7	697; 535,493,331	1.50 \pm 0.04 b	1.29 \pm 0.02 b	2.10 \pm 0.10 a
Dp-3cmglc-5glc	10	23.9	773; 611,465,303	76.22 \pm 1.58 a	4.47 \pm 0.27 c	12.04 \pm 0.09 b
Cy-3cmglc-5glc	11	25.8	757; 595,449,287	20.33 \pm 0.57 a	1.87 \pm 0.00 c	7.26 \pm 0.21 b
Pt-3cmglc-5glc	12	27.4	801; 639,493,331	44.56 \pm 0.14 a	5.70 \pm 0.12 c	13.70 \pm 0.15 b
Pn-3cmglc-5glc	15	29.8	771; 609,463,301	8.92 \pm 0.12 a	1.98 \pm 0.23 c	3.50 \pm 0.03 b
Mv-3cmglc-5glc	16	30.5	801; 639,493,331	33.56 \pm 0.38 a	6.84 \pm 0.25 c	15.15 \pm 0.03 b
<i>Pyrananthocyanins (mg L⁻¹)</i>				46.75 \pm 0.49 a	40.75 \pm 1.48 b	40.60 \pm 1.13 b
10-Carboxy-pyrpt-3cmglc	9	23.3	503; 341	NQ	NQ	NQ
10-Carboxy-pymv-3cmglc (cm-vitisin A)	13	28.1	707; 399	5.51 \pm 0.01	NQ	4.52 \pm 0.11
10HP-pyrdp-3glc	14	28.9	581; 419	6.26 \pm 0.31 a	5.98 \pm 0.17 a	5.73 \pm 0.00 a
10DHP-pyrpt-3glc	17	31.9	611; 449	NQ	NQ	NQ
10HP-pyrcy-3glc	18	32.8	565; 403	4.27 \pm 0.01 a	4.02 \pm 0.05 a	4.40 \pm 0.18 a
10HP-pyrpt-3glc	19	34.6	595; 433	8.14 \pm 0.22 a	8.05 \pm 0.00 a	6.45 \pm 0.09 b
10HP-pyrdp-3cmglc	20	34.8	727; 419	NQ	NQ	NQ
10DHP-pyrpt-3cmglc	21	36.2	757; 449	2.26 \pm 0.35 a	2.24 \pm 0.05 a	2.06 \pm 0.09 a
10DPH-pyrmv-3glc	22	36.7	625; 463	4.17 \pm 0.95 a	3.80 \pm 0.17 a	3.00 \pm 0.87 a
10HP-pyrpt-3acglc	23	37.2	637; 433	NQ	NQ	NQ
10HP-pyrpn-3glc	24	38.2	579; 417	5.04 \pm 0.00 a	4.68 \pm 0.51 a	4.53 \pm 0.18 a
10HP-pyrcy-3cmglc	25	38.6	711; 403	NQ	NQ	NQ
10HP-pyrmv-3glc	26	39.6	609; 447	4.61 \pm 0.21 a	4.85 \pm 0.36 a	4.24 \pm 0.01 a
10HP-pyrpt-3cmglc	27	40.3	741; 433	4.71 \pm 0.38 a	5.16 \pm 0.27 a	4.55 \pm 0.00 a
10DHP-pyrmv-3cmglc	28	41.6	771; 463	0.69 \pm 0.15 a	0.62 \pm 0.00 a	0.31 \pm 0.07 a
10HP-pyrpn-3cmglc	29	42.1	725; 417	0.32 \pm 0.00 a	0.32 \pm 0.00 a	0.20 \pm 0.02 b
10HP-pyrmv-3cmglc	30	42.3	755; 447	0.76 \pm 0.03 ab	0.97 \pm 0.00 a	0.56 \pm 0.11 b

Different letters in the same row indicate significant differences (ANOVA, Tukey's post hoc test, $\alpha = 0.05$)

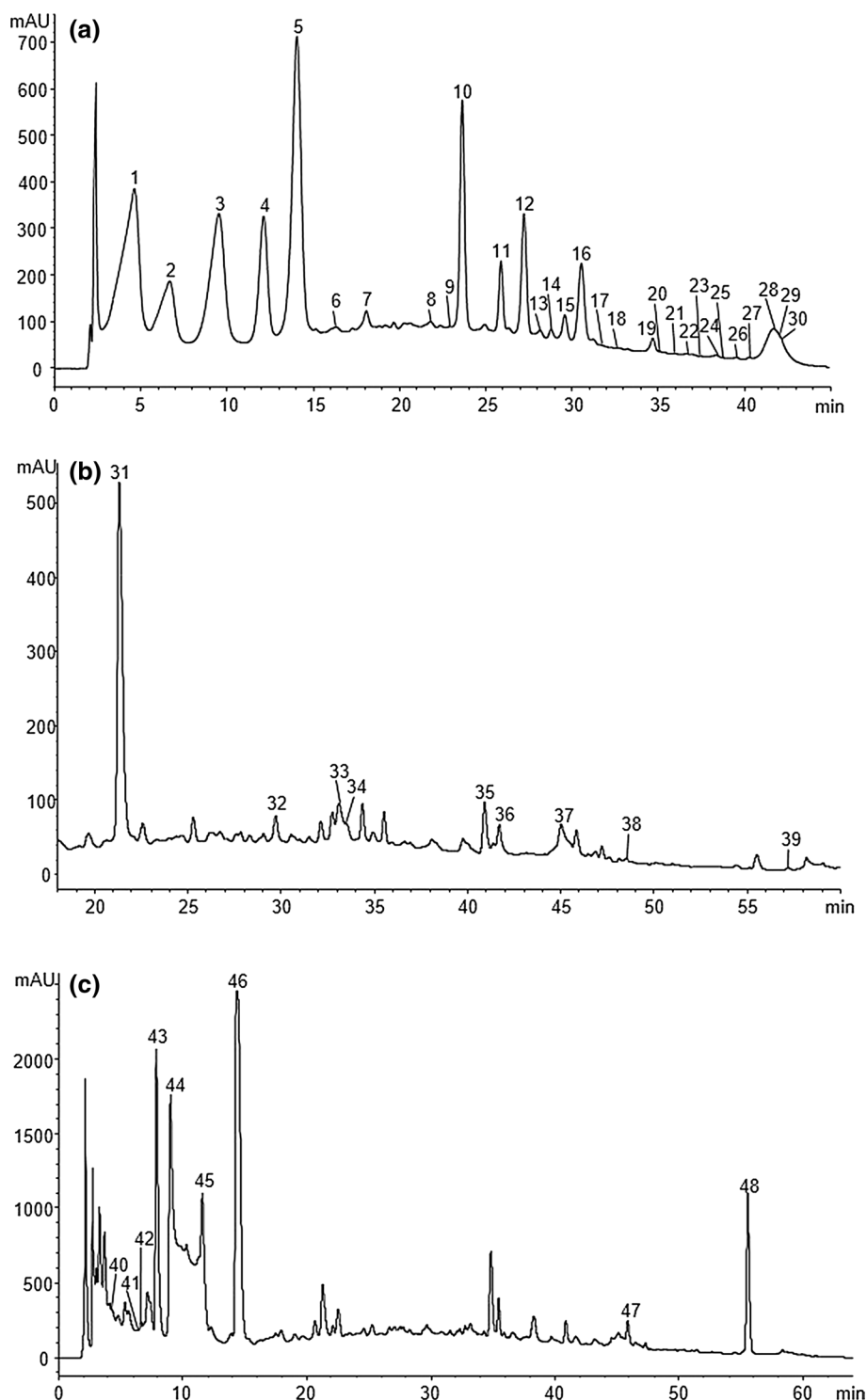
Dp delphinidin, *Cy* cyanidin, *Pt* petunidin, *Pn* peonidin, *Mv* malvidin, *3,5-diglc* 3,5-diglucosides, *3-acglc-5-glc* 3-(6''-acetyl)-glucoside-5-glucoside, *3-cmglc-5-glc* 3-(6''-p-coumaroyl)-glucoside-5-glucoside, *3-glc* 3-glucoside, *3-acglc* 3-(6''-acetyl)-glucoside, *3-cmglc* 3-(6''-p-coumaroyl)-glucoside, *10-HP* 10-*p*-hydroxyphenyl, *10-DHP* 10-*p*-dihydroxyphenyl, *VIOT* Traditional Violeta wine, *VIOPD* Pre-drying Violeta wine, *VIOSC* submerged cap Violeta wine, *ND* not detectable, *NQ* not quantifiable

Anthocyanin and pyrananthocyanin profiles

The 3,5-diglucosides of the five expected wine anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin) were identified and quantitated by DAD-chromatograms at 520 nm, with the different forms of malvidin as the principal anthocyanidin, followed by delphinidin and petunidin (Table 1; Fig. 1a). The monoglucoside anthocyanins

were not found in any Violeta red wines, and this could explain the formation of the hydroxyphenyl-pyrananthocyanins, which are resulted from the reaction between the monoglucoside anthocyanins and hydroxycinnamic acids, namely 10-(3''-hydroxyphenyl) (10-HP; reaction products with *p*-coumaric acid) or 10-(3'', 4''-dihydroxyphenyl) (10-DHP; reaction products with caffeic acid); and type-A and type-B vitisins, which are formed by the reaction between

Fig. 1 HPLC DAD-chromatogram (detection at 520 nm) of BRS Violeta young red wines anthocyanins **a** for peak assignment, see Table 1; HPLC DAD-chromatogram (detection at 360 nm) of flavonols **(b)** and HPLC DAD-chromatogram (detection at 320 nm) of hydroxycinnamic acid derivatives (HCAD) **c** for **b** and **c** peak assignment, see Table 2



monoglucoside anthocyanins and yeast metabolites such as pyruvic acid and acetaldehyde, respectively [21].

It was possible to detect 17 different pyranoanthocyanins derived from the five known anthocyanidins by means of their MS, MS/MS and UV–Vis spectral data, most of them being 10 (4'''-hydroxyphenyl) (10-HP) and 10-(3''', 4'''-dihydroxyphenyl) (10-DPH) derivatives of the five

possible pyranoanthocyanidins in their different forms of non-acylated, acylated and *p*-coumaroylated glucosides [21]. The *p*-coumaroyl derivative of vitisin A (10-carboxy-pyranomalvidin-3-*p*-coumaroylglucoside) and the similar A-type vitisin derived from petunidin were detected in all samples; however, only the *p*-coumaroylated vitisin A was possible to be quantitated in traditional and submerged cap wines.

The 3-(6''-coumaroyl)-glucoside-5-glucoside (3cmglc-5glc) derivatives of the five anthocyanidins were also detected. Traditional wine showed high concentration for all coumaroylated anthocyanins (3cmglc-5glc) followed by VIOSC and VIOPD wines. The coumaroylated derivative of delphinidin (dp-3cmglc-5glc) presented the higher concentration. The 3-(6''-acetyl)-glucoside-5-glucoside (3acglc-5glc) derivatives of all anthocyanidins, except delphinidin and peonidin, were found as minor compounds and were also quantitated. These results were in accordance with the findings of Lago-Vanzela et al. [5] who reported the higher concentration of mv-3,5diglc and dp-3-cmglc-5-glc for young Violeta red wines from different vintages. Lago-Vanzela et al. [5] also reported the detection of the 3-(6''-acetyl)-glucoside-5-glucoside forms of delphinidin and peonidin, and these compounds were not detected in BRS Violeta wines.

In all anthocyanins quantitated, when statistical differences were observed ($p < 0.05$, Table 1), the traditional wine presented higher amounts of these compounds when compared with VIOSC and VIOPD wines. A possible explanation for the lower concentration of these compounds in VIOPD wine is related to their degradation caused by the heat very likely due to the thermal degradation of these compounds caused by cleavage of the covalent bonds and/or by deglycosylation of the anthocyanin 3-glucosides [9].

In contrast, the VIOPD wines also showed significantly higher pyranoanthocyanin contents, especially the so-called hydroxyphenyl-pyranoanthocyanins, the 10-HP- and 10-DHP-pyranoanthocyanins. The results seem to suggest that the formation of hydroxyphenyl-pyranoanthocyanins already occurred during the first steps of the pre-drying treatment, before the thermal degradation of the corresponding anthocyanin precursors. The increase of the temperature could accelerate the hydrolysis of caftaric and coumaric acids, thus releasing the free caffeic and *p*-coumaric acids, respectively, that further reacted with anthocyanins [26]. The heating could modify the membrane permeability of the grape cells, thus allowing for the contact between anthocyanins and released free caffeic and *p*-coumaric acids.

In parallel, the heating could have effectively degraded tannins, which have been recognized as strong competitors of free caffeic and *p*-coumaric acid with regard to their reaction with anthocyanins [21, 27], since the tannins caused no interference in the reaction between hydroxycinnamic acids and anthocyanins. The afore-formulated hypothesis needs the final consideration that hydroxyphenyl-pyranoanthocyanins, once they were formed during the pre-drying treatment, might be more stable than anthocyanidin 3-glucosides with regard to thermal degradation. As far as we know, we have not found any study dealing with thermal stability of pyranoanthocyanins. Furthermore, it was possible to suggest that pyranoanthocyanins showed

more chemical stability [21] than anthocyanins, since the heating treatments of grape pre-drying weakly affected them.

In addition, it was expected that VIOSC presented higher anthocyanin concentration when compared to VIOT wine due to the constant contact between the pomace (skins and seeds) and the must, which led to a better dissolution of the phenolic compounds such as tannins and anthocyanins, both represented by the seeds and skins, respectively [28]; however, this aforementioned result was not possible to be observed, since VIOSC presented significantly lower concentration of these compounds when compared with VIOT. In this context, a possible explanation for this result was that the punching-down performed during maceration was responsible for the enhancement of the anthocyanin concentration in VIOT wine when compared with VIOSC wine [13].

Profile of the flavonols and hydroxycinnamic acid derivatives (HCAD)

The 3-glucosides (3-glc) of the five aglycones (Q, quercetin; M, myricetin; L, laricitrin; S, syringetin and I, isorhamnetin) were detected and quantitated in Violeta wines (Table 2; Fig. 1b). In addition, no 3-glucuronides (3-glcU) derivatives were detected and the free forms of four aglycones were detected and quantitated (M, Q, L and S). The 3-glucoside of M presented the highest concentrations in Violeta red wines, followed by the 3-glucosides of L, I and S, as well as free Q. This result was in accordance with findings of Lago-Vanzela et al. [5] who reported high concentration of myricetin 3-glucoside (M-3-glc) in Violeta red wines, however in disagreement with the findings of the same authors who reported no relevant concentrations for L-3-glc and free Q. Traditional wine showed higher concentration for free quercetin and VIOPD did not significantly differ from the VIOSC wine. In general, for all flavonols, except free Q, the lack of significant differences between the winemaking procedures suggested the weak influence of the drying process on the concentration of these compounds.

With regard to the hydroxycinnamic acid derivatives (HCAD), larger amounts of free *p*-coumaric and caffeic acids were observed (Table 2; Fig. 1c), thus indicating a high degree of hydrolysis of their grape native precursors, namely coumaric and caftaric acids, respectively, which accounted for minor concentrations. The high concentrations of free caffeic and *p*-coumaric acids in Violeta wines also explained the relevant concentrations found for their respective ethyl esters. The data concerning the HCAD showed that in almost all the comparisons, when the differences were significant ($p < 0.05$), the HCAD concentrations in VIOPD wines were higher than the traditional (VIOT) and the VIOSC wines.

Table 2 Flavonol and HCAD profile determined by HPLC/MS/MS (mean value \pm standard deviation) for BRS Violeta young red wines

Flavonols and HCAD	Peak	R_t (min)	Molecular ion; product ions (m/z)	VIOT	VIOPD	VIOSC
<i>Flavonols (mg L⁻¹)</i>				170.48 \pm 30.60 a	100.74 \pm 4.22 a	108.12 \pm 26.10 a
M-3-glc	31	21.5	479; 317	110.60 \pm 19.60 a	72.19 \pm 4.84 a	73.30 \pm 15.20 a
Q-3-glc	32	29.9	463; 301	5.86 \pm 0.37 a	5.15 \pm 1.48 a	2.33 \pm 0.14 a
L-3-glc	33	33.0	493; 331	17.40 \pm 15.20 a	4.27 \pm 1.32 a	11.77 \pm 9.80 a
Free M ¹	34	33.2	317	4.68 \pm 3.54 a	2.53 \pm 0.83 a	3.64 \pm 0.17 a
I-3-glc	35	40.1	477; 315	8.99 \pm 5.29 a	4.50 \pm 2.86 a	5.85 \pm 1.38 a
S-3-glc	36	41.6	507; 345	8.51 \pm 2.68 a	4.96 \pm 2.70 a	2.77 \pm 0.59 a
Free Q	37	45.0	301	10.72 \pm 0.41 a	4.34 \pm 1.41 b	4.73 \pm 1.78 b
Free L	38	48.7	331	2.78 \pm 0.39 a	2.11 \pm 0.74 a	2.41 \pm 0.72 a
Free S	39	57.6	345	0.86 \pm 0.45 a	0.67 \pm 0.64 a	1.33 \pm 0.82 a
<i>Hydroxycinnamic acid derivatives (HCAD) (mg.L⁻¹)</i>				400.67 \pm 25.60 a	502.03 \pm 58.10 a	374.13 \pm 16.50 a
Caftaric acid	40	4.1	311; 179,149,135	1.29 \pm 1.11 a	0.92 \pm 0.08 a	1.11 \pm 0.38 a
<i>Trans</i> -coutaric acid ¹	41	6.1	295; 163,149,119	9.89 \pm 0.03 ab	12.49 \pm 0.32 a	7.98 \pm 0.52 b
<i>Cis</i> -coutaric acid	42	6.5	295; 163,149,119	4.33 \pm 1.21 a	2.47 \pm 0.51 a	3.01 \pm 0.35 a
Caffeic acid	43	7.8	179; 135	78.81 \pm 3.71 a	69.82 \pm 2.61 ab	53.43 \pm 6.04 b
<i>p</i> -Coumaroyl-glucose-1	44	9.0	325; 163,145	44.82 \pm 3.23 b	66.32 \pm 2.25 a	51.37 \pm 4.73 b
<i>p</i> -Coumaroyl-glucose-2	45	11.6	325; 163,145	21.76 \pm 0.46 ab	27.49 \pm 1.54 a	17.21 \pm 1.87 b
<i>p</i> -Coumaric acid	46	14.4	163; 119	196.50 \pm 11.60 a	294.40 \pm 59.70 a	184.35 \pm 12.74 a
Ethyl caffeate ¹	47	46.1	207; 179,135	3.78 \pm 0.96 a	3.48 \pm 1.14 a	1.11 \pm 0.01 a
Ethyl <i>p</i> -coumarate	48	55.8	191; 163,119	39.48 \pm 12.97 a	24.63 \pm 1.55 a	54.50 \pm 17.70 a

Different letters in the same row indicate significant differences (ANOVA, Tukey's post hoc test and Games Howell post hoc test¹ when the variances were different, $\alpha = 0,05$)

M myricetin, Q quercetin, L laricitrin, K kaempferol, S syringetin, I isorhamnetin, *glcU* glucuronide, *gal* galactoside, *glc* glucoside, VIOT traditional violeta wine, VIOPD pre-drying violeta wine, VIOSC submerged cap violeta wine

This result corroborates with the findings of Marquez et al. [9] who reported that the HCAD amounts of Tempranillo and Merlot red wines submitted to chamber-drying process at 40 °C presented significant differences when the initial and the final processes of drying were compared, and in almost all HCAD the concentration was higher after the drying. These authors also stated that the drying process allowed for the concentration of the HCAD by the water evaporation of the grapes before the winemaking procedure and this is a possible explanation for the increase of these compounds in pre-dried Violeta wines. The concentration of the caftaric, *cis*-coutaric, *p*-coumaric acids and ethyl esters presented no significant differences when the winemaking procedures were compared ($p > 0.05$). In almost all cases, when $p < 0.05$, the submerged cap wine presented the same behavior as seen for the traditional treatment.

Profile of the flavan-3-ols and stilbenes

Catechin (C), epicatechin (EC), epicatechin 3-gallate (ECG), proanthocyanidin B1 (PB1) and proanthocyanidin B2 (PB2) were detected and quantitated in Violeta red

wines, except PB4 which could not be quantitated for all treatments and VIOPD wine in which ECG was not found (Table 3). The lack of ECG in VIOPD wine could be caused by its accelerated hydrolysis under heating conditions of the pre-drying treatment, similarly to that hypothesized for the above-mentioned discussion of the results dealing with the higher content of hydroxyphenyl-pyranoanthocyanins in VIOPD wine. There were no significant differences on the flavan-3-ol contents when the winemaking procedures were compared, and this result suggests that both alternative winemaking procedures did not influence the amounts of these aforementioned compounds.

A possible explanation for this aforementioned result is due to a balance between flavan-3-ol losses and gains. On the one hand, the grapes lost their physiological integrity during dehydration, thus favoring the diffusion of phenolic compounds and flavan-3-ols, from the grape skin to the pulp, which could be transferred to wine during alcoholic fermentation, increasing their concentration [27]. On the other hand, the higher expected content of flavan-3-ols in VIOPD wine due to the latter reason seems to be counteracted by the also expected thermal degradation

Table 3 Flavan-3-ol/stilbenes profiles determined by HPLC–ESI–MS/MS (MRM) and antioxidant capacity (mean value \pm standard deviation) for BRS Violeta young red wines

Flavan-3-ols and stilbenes	VIOT	VIOPD	VIOSC
<i>Flavan-3-ol monomers and dimers (mg L⁻¹)</i>	42.44 \pm 30.20 a	29.54 \pm 13.54 a	6.98 \pm 2.32 a
C	13.98 \pm 7.58 a	12.40 \pm 4.59 a	3.38 \pm 1.16 a
EC	2.02 \pm 0.77 a	2.02 \pm 0.51 a	0.56 \pm 0.08 a
ECG	0.03 \pm 0.05	NQ	0.16 \pm 0.23
PB1 ¹	19.70 \pm 16.30 a	10.17 \pm 5.71 a	2.16 \pm 0.75 a
PB2 ¹	6.75 \pm 5.59 a	4.78 \pm 2.50 a	0.87 \pm 0.31 a
PB4	NQ	NQ	NQ
<i>Proanthocyanidin total content (mg L⁻¹)</i>	91.26 \pm 4.22 a	68.40 \pm 16.80 ab	25.45 \pm 6.94 b
mDP	2.51 \pm 0.11 a	1.99 \pm 0.13 ab	1.63 \pm 0.19 b
% Galloylation	5.90 \pm 0.64 ab	7.97 \pm 0.19 a	2.66 \pm 1.17 b
% Prodelphinidin	7.53 \pm 0.93 a	4.90 \pm 0.49 a	4.57 \pm 3.19 a
<i>Stilbenes (mg L⁻¹)</i>	0.97 \pm 0.70 a	0.66 \pm 0.18 a	0.20 \pm 0.01 a
<i>cis-resveratrol</i>	0.24 \pm 0.20 a	0.08 \pm 0.06 a	0.13 \pm 0.02 a
<i>Cis-piceid</i>	0.47 \pm 0.66 a	0.43 \pm 0.11 a	0.006 \pm 0.00 a
<i>Trans-piceid</i>	0.26 \pm 0.24 a	0.14 \pm 0.01 a	0.05 \pm 0.01 a
<i>Antioxidant capacity (mmol L⁻¹ of Trolox equivalents)</i>	21.96 \pm 1.33 a	18.51 \pm 1.90 a	15.65 \pm 1.81 a

Different letters in the same row indicate significant differences (ANOVA, Tukey's post hoc test and Games Howell post hoc test¹ when the variances were different, $\alpha = 0,05$)

C catechin, EC epicatechin, ECG epicatechin gallate, PB1 proanthocyanidin B1, PB2 proanthocyanidin B2, PB4 proanthocyanidin B4, mDP mean degree of polymerization, VIOT traditional violeta wine, VIOPD pre-drying violeta wine, VIOSC submerged cap violeta wine, NQ not quantifiable

of these flavonoids, decreasing their concentration. The apparent balance between these two opposite effects could explain the lack of significant differences in the content of flavan-3-ols in VIOPD wines compared to the other wines. In addition, according to Ribéreau-Gayon et al. [29], the flavan-3-ols configuration affects their reactivity, and this fact could be related to their high stability to heat, being an additional explanation for this result. The level of proanthocyanidins found in all Violeta wines were lower than those usually found in wines elaborated from *Vitis vinifera* grape cultivars, they were in agreement with the low content of proanthocyanidins reported for Violeta grapes [19].

With respect to stilbenes, *cis-resveratrol*, *trans-piceid* and *cis-piceid* were detected and quantitated for all wines. In all wines, the total and individual contents of each stilbene were low and confirmed previous findings, suggesting that Violeta grape is a low resveratrol producer [19]. The content of resveratrol, its glycoside forms (piceids) and the global content of phenolic compounds have been suggested to be significantly correlated with the antioxidant capacity of grapes [7]. However, wines presenting high global amounts of stilbenes or phenolic concentration not always show the greatest antioxidant capacity, because this property depends more of the types of the phenolic compounds than their global amounts [7, 30, 31].

The values found for the antioxidant capacity (AC) of BRS Violeta wines according to the winemaking procedures did not significantly differ. The pre-drying process of the Violeta grapes was carried out using 60 °C and probably induced Maillard reaction (non-enzymatic browning) that could take importance on the formation of compounds such as melanoidins with suggested antioxidant capacity, as reported by Tagliazucchi et al. [31] and Marquez et al. [9], and this could be a possible explanation for the absence of AC significant differences between VIOPD and VIOT/VIOSC.

Sensory assessment

The comparison of the winemaking treatments only provided significant differences with respect to the violet hue of the red wine color and its sweetness (Table 4). Pre-dried wine (VIOPD) showed intermediate values for both descriptive sensory attributes, the traditional wine (VIOT) high scores for sweetness and submerged cap wine (VIOSC) high scores for violet hue. The other sensory descriptors presented similar scores for all the three winemaking procedures. The lack of significant differences in the main descriptive sensory attributes showed that the pre-drying and submerged cap winemaking procedures presented potential to be applied as an alternative to traditional

Table 4 Descriptive sensory profile (mean \pm standard deviation) for BRS Violeta red wines

Sensory attributes	VIOT	VIOPD	VIOSC
<i>Appearance</i>			
Color intensity	8.40 \pm 0.59 a	8.41 \pm 0.60 a	8.05 \pm 0.75 a
Violet hue	5.26 \pm 2.85 b	6.33 \pm 2.15 ab	6.76 \pm 1.73 a
<i>Taste</i>			
Sweetness	4.63 \pm 1.57 a	3.78 \pm 1.84 ab	3.30 \pm 1.54 b
Acidity	3.70 \pm 1.12 a	3.86 \pm 1.63 a	4.13 \pm 1.39 a
Bitterness	2.51 \pm 1.98 a	3.30 \pm 2.50 a	2.95 \pm 2.04 a
Flavor intensity/body	5.70 \pm 1.47 a	6.00 \pm 1.21 a	5.78 \pm 1.05 a
Structure/tannins	5.56 \pm 1.90 a	5.90 \pm 1.44 a	5.26 \pm 1.57 a
Herbaceous taste	2.58 \pm 1.56 a	3.35 \pm 1.62 a	3.43 \pm 1.85 a
Astringency	3.06 \pm 1.84 a	2.95 \pm 1.57 a	2.85 \pm 1.45 a
Pungency	5.86 \pm 1.58 a	5.95 \pm 1.16 a	5.73 \pm 1.25 a
Persistence	5.90 \pm 1.32 a	5.86 \pm 1.19 a	5.81 \pm 1.12 a

Different letters in the same row indicate significant differences (ANOVA, Tukey's post hoc test, $\alpha = 0.05$)

VIOT traditional violeta wine, VIOPD pre-drying violeta wine, VIOSC submerged cap violeta wine

winemaking, since the scores obtained for most descriptive attributes were similar. In addition, the high violet hue for VIOSC is a strong feature that can be considered as a sensory driver for acceptance of these red wines as reported by De Castilhos et al. [3].

Conclusion

The chemical and sensory profiles provided essential information about the Violeta red wines submitted to alternative winemaking procedures. Pre-drying winemaking led to significantly different wines regarding the anthocyanin content when compared to traditional and submerged cap wines. Despite the inherent thermal degradation of the phenolic compounds during the pre-drying treatment, the heating may also have induced the formation of products by Maillard reactions, giving rise to the restitution of part of the lost antioxidant capacity and making the wines not significantly different according to the winemaking procedures. The univariate results showed the lack of significant differences in the descriptive sensory profile for the main attributes, showing that the submerged cap and pre-drying winemaking procedures could be applied as an alternative to the traditional winemaking. In fact, the submerged cap red wines presented higher scores for violet hue and this sensory feature could be considered as a sensory acceptance driver. Finally, this study provided relevant results regarding the potential of the alternative winemaking procedures and their application in order to improve the Brazilian wine quality.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements The ethics were provided for sensory evaluation by the process number linked with the Ethic Politics of the São Paulo State University.

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