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Individual phenolic compounds in grape juice prepared using different extraction systems

Compostos fenólicos individuais em sucos de uva elaborados por diferentes sistemas de extração

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Highlights

3'BRS Violeta' juices stand out for their high 4-hydroxybenzoic and catechin content.
'Concord' produced juices with a lower catechin content in both extraction systems.
Juices prepared with the 'Bordô' grape exhibited a higher resveratrol content.

Abstract

The aim of this study was to assess the influence of steam (S1) and enzyme distillation (S2) processes on the polyphenolic composition of 'Bordô', 'Concord' and 'BRS Violeta' grape juices produced in the Vale do Rio region of Peixe, Santa Catarina state (SC). Twelve phenolic compounds were identified and quantified by high-performance liquid chromatography diode array detection (HPLC-DAD), whose concentration changed according to the variety and distillation system. Principal component analysis (PCA) showed that 'BRS Violeta' exhibited a strong correlation with caffeic and 4-hydroxybenzoic acid, as well as catechin and tyrosol and the sum of phenolic compounds. 'Bordô' S1 and S2 displayed the highest concentration of t-resveratrol and vanillic acid, and 'Concord' S1 and S2 the lowest phenol concentrations. Variety was a decisive factor in the final concentration of each phenolic compound, due to the genetic determinants and technological properties. The distillation systems influenced the phenolic composition of the juices, but the impact on each compound was different for each variety.

Key words: Polyphenols. Varieties. HPLC-DAD. Extracting pan. Pectinase.

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Resumo

Objetivou-se com este estudo avaliar a influência dos processos de extração arraste de vapor (S1) e enzimático (S2) sobre a composição polifenólica de sucos de uva das variedades Bordô, Concord e BRS Violeta produzidos na região do Vale do Rio do Peixe-SC. Foram identificados e quantificados 12 compostos fenólicos por cromatografia líquida de alta eficiência com detector de arranjo de diodos (CLAE-DAD), que diferiram sua concentração de acordo com a variedade e sistema de extração. A partir de análises de componentes principais observou-se que 'BRS Violeta' apresentou forte correlação com os ácidos cafeico e 4-hidroxibenzóico, bem como, com catequina e tirosol e a soma dos compostos fenólicos. 'Bordô' no S1 e S2 apresentaram a maior concentração para t-resveratrol e ácido vanílico. 'Concord' S1 e S2, apresentaram as menores concentrações de fenóis. A variedade foi determinante para a concentração final de cada composto fenólico, devido aos determinantes genéticos e propriedades tecnológicas. Os sistemas de extração influenciam na composição fenólica dos sucos, no entanto, o impacto sobre cada composto específico foi diferente em cada variedade.

Palavras-chave: Polifenóis. Variedades. CLAE-DAD. Panela extratora. Pectinase.

Introduction

Brazilian viticulture exhibits unique characteristics linked to historic, environmental and territorial values, and is an important income source in most of the grape producing regions, particularly where small family farming predominates. However, the activities of several major table grape producing and processing ventures on small, medium and large winegrowing properties have contributed to sustainable viniculture/viticulture, thereby creating jobs and generating income (Mello, 2018; Mello & Machado, 2019, 2020).

Grape juice is an economic alternative for traditional wine producing companies, cooperatives, small producers and agribusinesses (Bender et al., 2021), which exhibit quite different profiles in Brazil, including large high technology companies that use heat exchange distillation known as "tube in tube", and small family undertakings that produce so-called "artisanal", "homemade", "colonial" or "pan" juice via the steam distillation extracting pan system (Frölech et al., 2019; Guerra et al., 2016; Marcon et al., 2016).

Grape juice extraction by steam distillation is an alternative for small growers and makes small farms economically feasible (Costa et al., 2019; Marcon et al., 2013), since the extraction pan needed to produce this juice is relatively cheap and easy-to-use apparatus, and "pan juice" is sold primarily at small farm markets and to consumers living near the producing properties, adding value to the raw material and increasing family income (Canossa et al., 2017; Guerra et al., 2016). However, the steam condensation used to extract the juice may cause exogenous water addition (Bender et al., 2019; Bresolin et al., 2013; Marcon et al., 2016).

Adding water to juice poses no health risk to consumers, but the product must be clearly labeled to adhere to legislation (IN nº 14, 2018) and could exhibit distinct physicochemical characteristics and phenolic compounds when compared to other extraction systems (Bender et al., 2019, 2021; Bresolin et al., 2013; Guerra et al., 2016; Marcon et al., 2013, 2016). Bioactive content and profile are influenced by the juice processing techniques, primarily the time and temperature used (Lambri et al., 2015; Lima et al., 2015; Paranjpe et al., 2012; Toaldo et al., 2014). One factor that drives grape juice commercialization is its polyphenolic composition, due to the benefits related to consumers' health. Different studies show beneficial evidences of grape juice consumption, such as preventing platelet aggregation, endothelial function, antioxidant action and helping the body against the action of free radicals as well. (Dalponte & Medeiros, 2020; Honisch et al., 2020; M. J. da R. Silva et al., 2019).

Given the importance of grape juice for sustainable domestic viticulture, as well as its colonial and artisanal counterparts for family farms, especially in Southern Brazil, and the impasse between the composition of these products and Brazilian law, which reinforces the need to establish a category for these juices that does not depreciate the product (Bender et al., 2021; Bresolin et al., 2013), the present study aimed at assessing the influence of extraction processes on the polyphenol composition of juices produced in the Vale do Rio region of Peixe, Santa Caterina state (SC).

Material and Methods

Reagents

The analytical standards of the phenolic compounds studied were obtained from Sigma-Aldrich (St. Louis, MO, USA), with purity greater than or equal to 98%: caffeic acid (331-39-5), p-coumaric acid (501-98-4), ferulic acid (1135-24-6), vanillic acid (121-34-6), syringic acid (530-57-4), 4-hydroxybenzoic acid (99-50-3), gallic acid (149-91-7), quercetin (6151-25-3), kaempferol (520-18-3), (+) - catechin (154-23-4), (-) - epicatechin (490-46-0) , tyrosol (501-94-0), and trans-resveratrol (501-36-0). Ethanol, acetonitrile, tartaric acid and acetic acid were acquired from Synth (Diadema, São Paulo state (SP), Brazil). Ultrapure water was obtained from a Gehaka purification system (Gehaka, São Paulo, SP, Brazil). Standard solutions were prepared in synthetic wine (5 g L^{-1} of tartaric acid, 11% ethanol, pH 3.5).

Experimental location, grape varieties and growing conditions

The following varieties were used: Bordô, Concord and BRS Violeta, produced in an experimental vineyard established in 2008 using a using a mixed pruning and Y-shaped training system on a VR 043-43 rootstock, with 3.0 x 2.0 m spacing between rows and plants, respectively. The physicochemical aspects of the grapes at harvest are listed in Table 1. The grapes were collected from the 2018 crop at the Videira Experimental Vineyard of the Santa Catarina Agricultural Research Company and Rural Extension (Videira, SC, Brazil) at 27º02'27,59'' S, 51º08'04,73'' W, altitude of 830 meters above sea level. The climate in the region is classified as humid mesothermal with mild summers (Cfb), according to Köppen's classification. The average temperature and accumulated rainfall during grape ripening (December to March) were 21.4 °C and 252.20 mm, respectively (EPAGRI/CIRAM).

Table 1
Average soluble solid and total acidity values in grapes used in juice production in the 2018 growing season

	Soluble Solids (°Brix)	Total Acidity ($mEq.L^{-1}$)
Cultivars	2018	2018
Bordô	14.90	51.20
Concord	13.50	82.60
BRS Violeta	17.42	72.52

Grape juice processing

After harvesting, the grapes were taken to the experimental vineyard where the juices were prepared in the following and the berries soaked for one hour (±50°C). They stages:

Steam distillation (S1): Must was extracted using the juice extractor or extracting pan with a capacity of 20 kg of fruit. Initially, the berries were destemmed using a manual destemmer, placed in a perforated container within another receptacle, both of which were attached to a water vessel and positioned on a gas stove to generate steam. After approximately 40 minutes, the must started to flow through the outlet tube and was collected in a pan to homogenize the juice, and kept on the stove to keep the juice heated (± 80°C). Samples were bottled as soon as the sold mass was devoid of liquid. Packaging occurred at a temperature of 86°C to ensure microbiological stability.

Enzyme (S2): The grapes were destemmed using a mechanical destemmer and heated to 50°C, where they remained for around 20 minutes under constant homogenization, until the must reached the desired temperature (±50°C). Thermoresistant commercial pectolytic enzymes (®Pectinex Ultra SP-L) were added at a concentration of 3 $g.hL^{-1}$, were then pressed to separate the liquid and taken to the cooling chamber, at an average temperature of 1ºC, to decant the solid particles for 24 hours. The following day, the juice was racked, pasteurized and bottled at a temperature of 86°C, then rapidly cooled to a temperature of ±30°C.

Physicochemical analyses of grapes and juices

Solid soluble content was determined with a digital benchtop refractometer and automatic temperature compensation (QUIMIS®), and the result expressed in °Brix. The pH was determined using an AD1030 pH meter and total acidity by sample titration, with a standardized solution of 0.1N NaOH, adopting pH = 8.2 as final titration point and the result expressed in mEq L^{-1} (International Organisation of Vine and Wine [OIV], 2009). Gente Three 500 mL glass bottles were used for each treatment, each bottle representing one repetition. The physicochemical aspects of the grapes and juices are listed in Tables 1 and 2.

Table 2
Physicochemical aspects (soluble solids, titratable acidity and pH) of grape juices produced in the 2018 growing season

	Variables					
	Soluble Solids (°Brix)		Titratable Acidity (meq.L^{-1})		pH	
Cultivars	S1	S2	S1	S2	S1	S2
Bordô	11.60	14.30	105.73	131.03	3.27	3.25
Concord	10.93	13.10	123.60	155.50	3.19	3.17
BRS Violeta	14.07	17.03	96.70	107.37	3.45	3.48

S1- Steam distillation; S2- Enzyme extraction.

Quantitative analysis of phenolic compounds

To prepare the sample, a 5 mL aliquot of grape juice was extracted twice with 10 mL of ethyl acetate under 5 min agitation (vortex mixer). The organic phases of the two extractions were combined and evaporated in a rotary evaporator with controlled temperature (28 ± 1 ºC). The remaining residue was redissolved in 2 mL of methanol: water solution (1: 1 v / v) (Burin et al., 2014).

Phenolic compounds were determined according to the methodology proposed by Arcari et al., (2020), in a high-performance liquid chromatograph (Agilent Technologies, St. Clara, CA, USA) equipped with a quaternary pump (G1311C), diode array detector - DAD (G1316A), automatic sampler (G7167A) and Agilent Lab Advisor Software. The stationary phase consisted of a pre-column and C18 reverse phase column (particle size of 4.6 mm x 250 mm x 5 µm) (Phenomenex, Torrance, CA, USA). Mobile phase A consisted of ultrapure water and acetic acid (98: 2 v / v) and mobile phase B of ultrapure water, acetic acid and acetonitrile (58: 2: 40 v / v / v). Linear gradient elution was performed as follows: 0-80% of solvent B for 55 min, 80-100% B for 15 min, and 100-0% B for 10 min. A flow rate of 0.9 mL/min^{-1} was used. Detection was carried out at 280 nm (gallic acid, tyrosol, protocatechuic acid, (+) - catechin, vanillic acid, syringic acid, (-) - epicatechin), 306 nm (*trans*-resveratrol), 320 nm (caffeic acid, *p*-coumaric acid, ferulic acid) and 360 nm (quercetin). Phenolic compounds were identified by comparing the peak retention times of the samples with those of the standards and quantified by external standardization.

Statistical analysis

Statistical analysis was conducted using R statistics software (R Core Team [R], 2020) and RStudio interface (RStudio Team [RStudio], 2020). The data were submitted to ANOVA and Tukey's test ($p < 0.05$). Principal component analysis (PCA) was carried out using the average polyphenol content in each treatment. The principal components (PCs) selection criterion used was recommended by Jolliffe (2002), establishing that a number of PCs exhibit between 70 and 90% of total variance. A heat map chart was created from normalized phenolic compound concentrations in the juice samples obtained by different extraction methods, using GraphPad Prism software, version 9.4.1 (GraphPad, 2022).

Results and Discussion

Phenolic acids

The results for phenolic compounds quantified in the grape juices are presented in Table 3. P-coumaric acid values were higher for 'Bordô' (4.81 $mg.L^{-1}$) and 'Concord' (4.89 $mg.L^{-1}$) juices prepared in S2, while the opposite behavior was observed in 'BRS Violeta' juices (1.81 $mg.L^{-1}$), where the highest result was found in S1. 'Concord' in S1 (0.31 $mg.L^{-1}$) exhibited the highest p-coumaric acid concentration across the experiment, followed by 'BRS Violeta' with 1.81 $mg.L^{-1}$ and 1.03 $mg.L^{-1}$ for S1 and S2, respectively. The values obtained for 'BRS Violeta' were similar to those of 1.32 $mg.L^{-1}$ reported by J. K. da Silva et al. (2016) for 'BRS Cora' juices and the 1.10 $mg.L^{-1}$ to 1.70 $mg.L^{-1}$ range obtained by Lima et al. (2014) in juice varieties and cuts between varieties. However, all the results of the present study were lower than the average values of 11.23 $mg.L^{-1}$ and 10.37 $mg.L^{-1}$ reported by Toaldo et al. (2015) for organic and conventional juices, respectively.

Gallic acid was quantified in higher concentrations for 'Bordô' (3.67 $mg.L^{-1}$) and 'BRS Violeta' (21.16 $mg.L^{-1}$) juices in S1, while the highest 'Concord' concentration was in S2 (9.70 $mg.L^{-1}$). It is important to note that 'BRS Violeta' juices obtained better results that the other varieties in S1, a value significantly higher than that recorded in S2 (3.75 $mg.L^{-1}$). The gallic acid values of 'BRS Violeta' in S1 were higher than the 10.50 $mg.L^{-1}$ observed by Lima et al. (2014), while concentrations in the other juices in the present study remained between the 1.8 $mg.L^{-1}$ and 13.06 $mg.L^{-1}$ obtained by these authors for 'Isabel Precoce' and 'BRS Cora' juices, respectively. Toaldo et al. (2015) found that organic juices obtained 16.96 $mg.L^{-1}$ and their conventional counterparts 11.51 $mg.L^{-1}$ of gallic acid, Lima et al. (2015) reported between 2.2 $mg.L^{-1}$ and 3.4 $mg.L^{-1}$ for juices extracted with different enzyme doses at different temperatures and M. J. da R. Silva et al. (2019) obtained values between 4.27 $mg.L^{-1}$ and 5.97 $mg.L^{-1}$.

Table 3
Individual phenolic compounds of Bordô, Concord and BRS Violeta grape juices produced by enzyme extraction and steam distillation extraction systems in Southern Brazil

	Bordô		Concord		BRS Violeta	
Phenolic Acids ($mg.L^{-1}$)	S1	S2	S1	S2	S1	S2
p-Coumaric	2.63±0.35 Ab	4.81±0.19 Aa	0.31±0.65 Cb	4.89±0.11 Aa	1.81±0.13 Ba	1.03±0.15 Bb
Gallic	3.67±0.23 Ca	1.86±0.57 Cb	8.22±0.09 Ba	9.70±0.46 Ab	21.16±0.21 Aa	3.75±0.23 Bb
Caffeic	0.39±0.02 B^{ns}	0.52±0.04 C	0.16±0.05 Bb	5.46±1.51 Ba	25.13±0.01 A^{ns}	23.12±2.52 A
Syringic	0.54±0.03 NS^{ns}	1.08±0.01 B	0.74±0.12 ^{ns}	1.40±0.12 B	1.73±0.03 b	28.91±2.71 Aa
4-Hydroxybenzoic	1.15±0.16 Cb	4.36±0.01 Ca	6.08±0.18 Bb	12.03±1.76 Ba	58.80±0.31 Aa	45.84±4.01 Ab
Ferulic	1.06±0.10 Ab	1.79±0.12 Aa	0.06±0.17 Bb	1.11±0.00 Ba	0.22±0.00 Bb	0.28±0.05 Ca
Vanillic	4.43±0.29 Aa	3.73±0.61 Ab	0.31±0.14 Cb	0.65±0.10 Ca	1.60±0.02 Bb	2.01±0.18 Ba
Flavanols ($mg.L^{-1}$)						
(+) Catechin	35.2±1.93 Bb	75.23±0.16 Ba	1.18±2.72 Cb	16.48±5.56 Ca	147.84±0.14 Aa	118.06±7.96 Ab
(-) Epicatechin	13.80±1.50 C^{ns}	19.47±0.17 A	32.78±0.87 Ba	8.31±9.21 Bb	155.14±2.63 Aa	0.77±0.08 Bb
Flavonol ($mg.L^{-1}$)						
Quercetin	4.61±0.06 Aa	2.17±0.02 Ab	1.20±0.13 Ca	0.77±0.39 Bb	2.78±0.17 Ba	1.05±0.07 Bb
Tyrosol ($mg.L^{-1}$)	9.43±0.69 Ba	0.62±2.24 Bb	0.89±0.01 Cns	1.11±0.45 B	14.99±0.04 A^{ns}	15.01±2.22 A
t-resveratrol ($mg.L^{-1}$)	1.03±0.12 A^{ns}	0.98±0.01 A	0.52±0.04 Ba	0.25±0.04 Bb	0.41±0.02 Ba	0.25±0.15 Bb

*Means followed by the same uppercase letter differ for the cultivar within the system. **Means followed by the same lowercase letter do not differ within the cultivar. ns Not significant (Tukey´s test, p <0.05). S1- Steam distillation; S2- Enzyme extraction.

Caffeic acid showed no significant system differences for 'Bordô' (S1 0.39 mg.L^{-1} and S2 0.52 mg.L^{-1}) and 'BRS Violeta' (S1 25.13 mg.L^{-1} and S2 23.12 mg.L^{-1}), whereas 'Concord' values were higher when the juices were prepared by S2 (5.46 mg.L^{-1}). Caffeic acid was the most concentrated phenolic acid in 'BRS Violeta' juices for both preparation methods, with values significantly higher than those found in the other varieties assessed, results similar to those of Lima et al. (2014) (28.90 mg.L^{-1}) for this variety and Toaldo et al. (2015) for organic (25.63 mg.L^{-1}) and conventional grapes (28.15 mg.L^{-1}). The findings of Lima et al. (2015) (15.03 mg.L^{-1} to 17.9 mg.L^{-1}) and M. J. da R. Silva et al. (2019) (10.2 mg.L^{-1}) were lower than those of 'BRS Violeta', but higher than the other juices of the present study.

Syringic variety concentrations did not differ significantly between varieties in S1. Bordô and Concord showed no statistical differences between preparation systems. For 'BRS Violeta' juices prepared in S2 (28.91 mg.L^{-1}), syringic acid concentration was higher than that of S1 (1.73 mg.L^{-1}), and those of the other varieties, regardless of the extraction system used. In general, syringic acid concentrations were lower than those reported by Toaldo et al. (2015) and J. K. da Silva et al. (2016), except for 'BRS Violeta' in S2.

4-hydroxybenzoic acid was detected at higher concentrations in 'Bordô' (S1 1.15 mg.L^{-1} and S2 4.36 mg.L^{-1}) and 'Concord' (S1 6.08 mg.L^{-1} and S2 12.03 mg.L^{-1}) juices when compared to those prepared in S2. A higher 4-hydroxybenzoic acid concentration in was found in 'BRS Violeta' in the S1 juices when compared to S2 (S1 58.80 mg.L^{-1} and S2 45.84 mg.L^{-1}). 4-hydroxybenzoic acid was the phenolic acid with the highest concentration in 'BRS Violeta' juices in both extraction systems, and in the entire experiment.

The juices prepared in S2 exhibited the highest ferulic acid concentrations in the three varieties analyzed. 'Bordô' juices obtained the highest ferulic acid values in both extraction systems (S1 1.06 mg.L^{-1} and S2 1.79 mg.L^{-1}). 'Bordô' organic juice contained 5.20 mg.L^{-1}, higher than that found here; however, the conventional 'Isabel' and 'Bordô' values were similar (1.59 mg.L^{-1}) (Toaldo et al., 2015).

Like ferulic acid, vanillic acid concentration was higher in 'Bordô' juices when compared to the other varieties (S1 4.43 mg.L^{-1} and S2 3.73 mg.L^{-1}) , regardless of extraction system, a behavior opposite to that observed for 'Concord' juices, which obtained the lowest vanillic acid values in both systems (S1 0.31mg.L^{-1} and S2 0.65 mg.L^{-1}). A comparison of extraction methods among the varieties, 'BRS Violeta' (S1 1.60 mg.L^{-1} and S2 2.01 mg.L^{-1}) and 'Concord' juices in S2 contained the highest vanillic concentrations, while for 'Bordô' the highest value was for S1 juices. All the experimental values were lower than those reported by Toaldo et al. (2015) for organic 'Bordô' (444.92 mg.L^{-1}) juices and cuts of conventional 'Isabel' and 'Bordô' (108.47 mg.L^{-1}).

The different results between varieties and the influence of extraction system on phenolic acids (Figure 1) may be due to the genetic factor, the primary determinant of the phenolic compound content in grapes (Dalponte & Medeiros, 2020; Gris et al., 2013, 2010), as well as the location of these compounds in the grapes, given that phenolic compounds are present mainly in the skin, pulp and seeds, resveratrol ionomers in the skin, flavonoids, mainly catechin and epicatechin, in the seeds, and phenolic acids in the pulp (Granato et al., 2016). Different authors report that skin and seed fractions may contain (quantitatively and qualitatively) more phenolic compounds than their pulp counterparts; however, concentrations can vary among varieties (Xu et al., 2010; Lago-Vanzela et al., 2011; Santos et al., 2011; Moreno-Montoro et al., 2015; Margraf et al., 2016). The extraction capacity of phenolic compounds may also influence the final composition, especially in relation to extraction methods, given that the structure and composition of cell walls vary significantly among varieties (Ortega-Regules et al., 2006; Bindon et al., 2012; Zoccatelli et al., 2013).

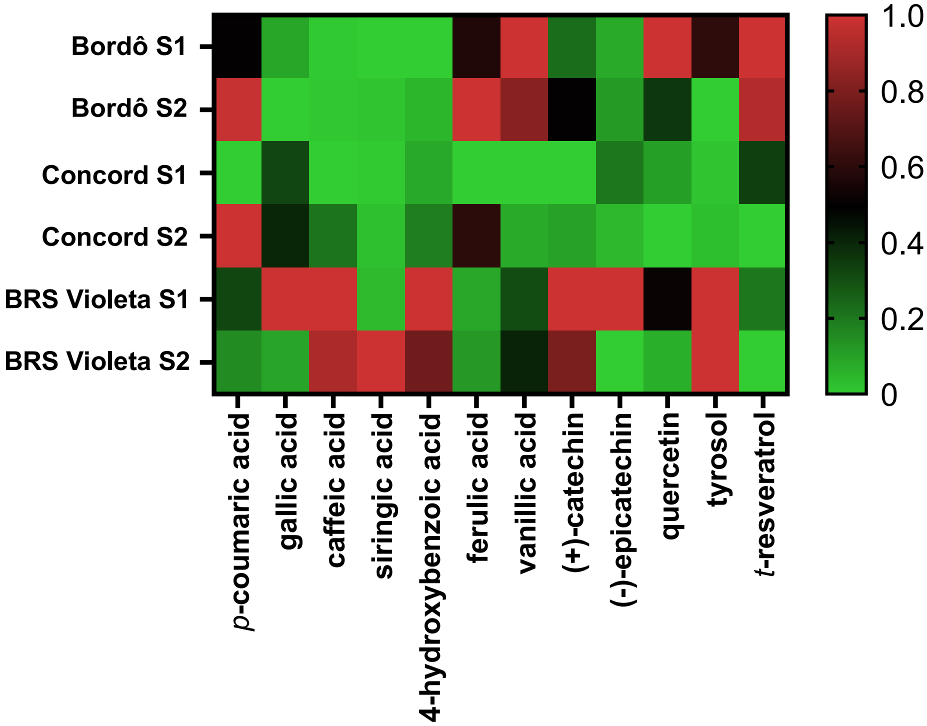

Figure 1. Heatmap created from the normalized phenolic compound concentration data of Bordô, Concord and BRS Violeta grape juices obtained by different extraction systems (S1 = steam distillation; S2 = enzyme extraction).

Different technological behavior among varieties was expected given their distinct berry characteristics. BRS Violeta has strong thick skin, 'Concord' a thin highly sensitive skin, and Bordô displays intermediate strength (Borges et al., 2012; Camargo et al., 2005); in addition, the extraction systems are based on different techniques. Steam distillation involves heating the whole grape, thereby softening or partially dissolving the solid parts of the berries (pulp and skin), in order to release the juice (Guerra et al., 2016). In the enzyme system, the grape is previously crushed to better expose the cells, and enzymes are added to help degrade the pectin and break the cell walls, thereby allowing easier extraction of vacuole content (Guerra et al., 2016; Lambri et al., 2015; Ortega-Regulares et al., 2008; Ortega-Regules et al., 2006; Toaldo et al., 2014).

Flavanols and flavonol

The highest catechin concentrations were observed for 'BRS Violeta' juices in the two extraction systems (S1 147.84 $mg.L^{-1}$ and S2 118.06 $mg.L^{-1}$) and the lowest for 'Concord' (S1 1.18 $mg.L^{-1}$ and S2 16.48 $mg.L^{-1}$). The catechin concentrations in the Bordô and Concord varieties were higher in S2, and those of 'BRS Violeta' in S1 (Figure 1). In the juices produced by Lima et al. (2014) with 'BRS Violeta' cultivar in a cut of 'Isabel Precoce', catechin values were between 19.80 $mg.L^{-1}$ and 21.00 $mg.L^{-1}$, respectively. The highest concentration of epicatechin was found in 'BRS Violeta' juices in S1 (155.14 $mg.L^{-1}$) when compared to the other varieties; but when prepared by S2, they obtained the lowest value (0.77 $mg.L^{-1}$) in the entire experiment (Figure 1). When 'Concord' juices were prepared by S1 (32.78 $mg.L^{-1}$), epicatechin concentrations were higher than those in S2 (8.31 $mg.L^{-1}$), and for 'Bordô', the behavior was the opposite, namely, a higher concentration in S2 (19.47 $mg.L^{-1}$) than in S1 (13.80 $mg.L^{-1}$), albeit with no significant difference. The higher epicatechin content in S1 may be explained by its greater solubility at higher temperatures. Souza (2016) explains that high temperatures increase extraction efficiency, since heat makes the cell walls permeable, raising the diffusion of the compounds to be extracted.

Quercetin concentration was greater in S1 than S2 for the three cultivars assessed (Figure 1). 'Bordô' juices (S1 4.61 $mg.L^{-1}$ and S2 2.17 $mg.L^{-1}$) obtained higher values than those of 'Concord' (S1 1.20 $mg.L^{-1}$ and S2 0.77 $mg.L^{-1}$) and 'BRS Violeta' (S1 2.78 $mg.L^{-1}$ and S2 1.05 $mg.L^{-1}$) in the two extraction systems. Lima et al. (2015) assessed 'Isabel'/'BRS Cora' juices submitted to different temperatures and enzyme doses and found higher quercetin content in juices added with higher doses. Based on different study revisions, Cosme et al. (2018) found higher quercetin contents when higher soaking temperatures were used, reinforcing the influence of the preparation system on the phenolic composition of the juices. The differences in the juices composition made using different systems can be explained by the different cell wall composition between grape cultivars, as well as by the technological differences used during preparation (Ortega-Regulares et al., 2008). As previously described, the grapes have different berry compositions. The varieties used in the present study are used in juice production due to the color intensity in the case of 'Bordo' and 'BRS Violeta' and the pleasant aroma and flavor of 'Concord' (Cosme et al., 2018). Extraction systems are based on different techniques. For the steam extraction, the intact grapre is heated and in the enzymatic system, the grape is previously crushed, providing greater exposure of the cells. However, both extraction systems are used in juice industries, steam for small and family-sized industries and enzymatic for medium and large scale industries (Guerra et al., 2016).

(E)- Stilbene and tyrosol

No significant concentration differences were found for 'Concord' and 'BRS Violeta' juices between extraction systems, while for 'Bordô', the highest content occurred in S1. The 'BRS Violeta' juices obtained higher tyrosol concentrations when compared to the other varieties in both extraction systems (Figure 1). Gatto et al. (2008) and Gris et al. (2010) concluded that the variety factor and variety associated with the growing season influenced the tyrosol concentration of grapes. Toaldo et al. (2015) detected no tyrosol in organic and conventional juices produced in Southern Brazil.

The *t*-resveratrol content was higher in S1 for the three varieties when compared to S2; however, 'Bordô' showed no significant difference between extraction systems. This variety exhibited the highest t-resveratrol concentration, irrespective of extraction system. Burin et al. (2014) assessed the phenolic composition of *Vitis labrusca* and *Vitis vinifera* grapes produced in the Vale do Rio region of Peixe, SC, and observed the highest t-resveratrol concentration for 'Bordô'. The authors suggest that this variety could be classified as a high resveratrol producer.

In addition to variety, the juice preparation system influenced the final phenolic composition, and incorporating pectinases during grape soaking may result in important changes in the chemical composition of the grape juice, primarily in terms of phenolic compounds (Lima et al., 2014). Different studies revealed that a combination of temperature and enzyme doses resulted in greater phenolic compound extraction (Cabrera et al., 2009; Leblanc et al., 2008; Lima et al., 2015, 2014). In artisanal preparation in a steam pan, grapes were aggressively heated at extraction temperatures of up to 80 °C, which may degrade some compounds (Lima et al., 2014). This did not occur in the present study for t-resveratrol, since the results obtained showed different behavior for this compound when compared to that reported in the literature, whereby the highest values were obtained in S1 juices, which received no enzyme dose, suggesting that the extraction behavior of this compound differs from that of the other phenolic components of the juices.

Figures 1, 2 and 3 show that phenolic compound levels in the juices behaved differently depending on the extraction system (Figure 2). Trends can be more clearly observed within each variety (Figure 3), where 'BRS Violeta' had the highest phenolic compound levels, influenced by high catechin, epicatechin, 4-hydroxybenzoic and syringic acid concentrations. However, there is significant discrepancy between extraction methods for some of these compounds, especially catechin and syringic acid, with lower values in S2 and S1, respectively. For the other varieties, the discrepancies were even lower between the systems, highlighting catechin for 'Bordô' and epicatechin for 'Concord'. In terms of the systems (Figure 2), the behavior of compounds was different for each variety, where 4-hydroxybenzoic, catechin and caffeic acid showed a similar trend for 'BRS Violeta'. It is known that the content and bioactive compound profile in juices are influenced by the processing techniques used (Lambri et al., 2015; Lima et al., 2015; Margraf et al., 2016; Toaldo et al., 2014).

Based on the literature, it is expected that S2 would obtain higher phenolic compound concentrations due to the addition of enzymes that help degrade the cell wall, thereby improving polyphenol release (Zouid et al., 2013). However, the variety had more influence than the system, because the genetic factor increases the presence of certain compounds in different genotypes, and the technological adversities of each variety (J. K. da Silva et al., 2016; Keller, 2020; Ortega-Regulares et al., 2008). On the other hand, the differences between the systems may be attributed to the degradation of some compounds caused by high temperatures (Toaldo et al., 2014) of around 80°C in the steam system (Lima et al., 2014), as well as a possible destabilization of some polyphenols in the enzyme system as a function of deglycosylation, leading to the precipitation of a number of compounds due to the use of commercial enzymes (Paranjpe et al., 2012). Cosme et al. (2018) explain that the extraction of compounds varies according to time and temperature, with the maximum extraction of anthocyanins occurring at 63ºC, after 20 minutes of extraction. However, 25 to 30 g/100g of this compound can be lost during juice processing. Wang and Xu (2007) evaluated blackberry juices and concluded that anthocyanin degradation rates increased as temperatures increased from 60ºC to 90ºC.

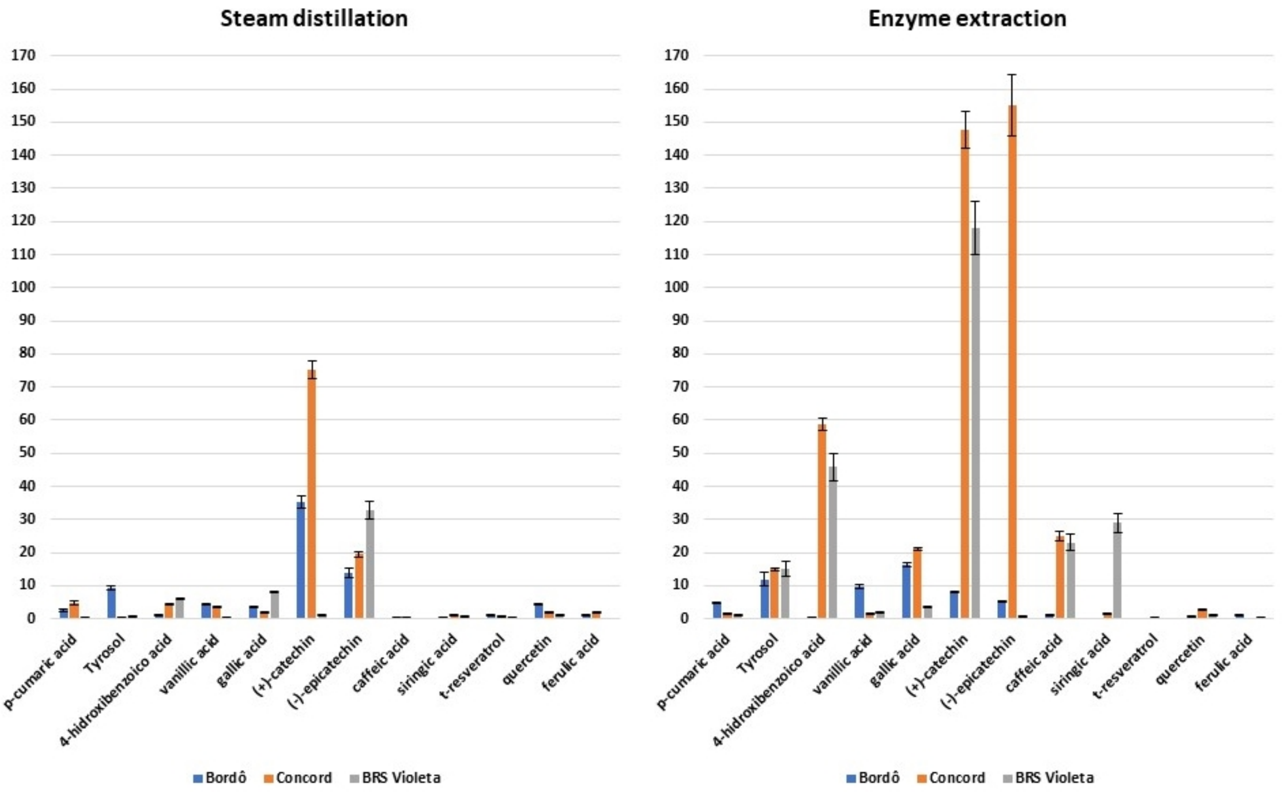

Figure 2. Phenolic compound levels in the treatments grouped by extraction system. S1-steam distillation; S2-enzyme extraction.

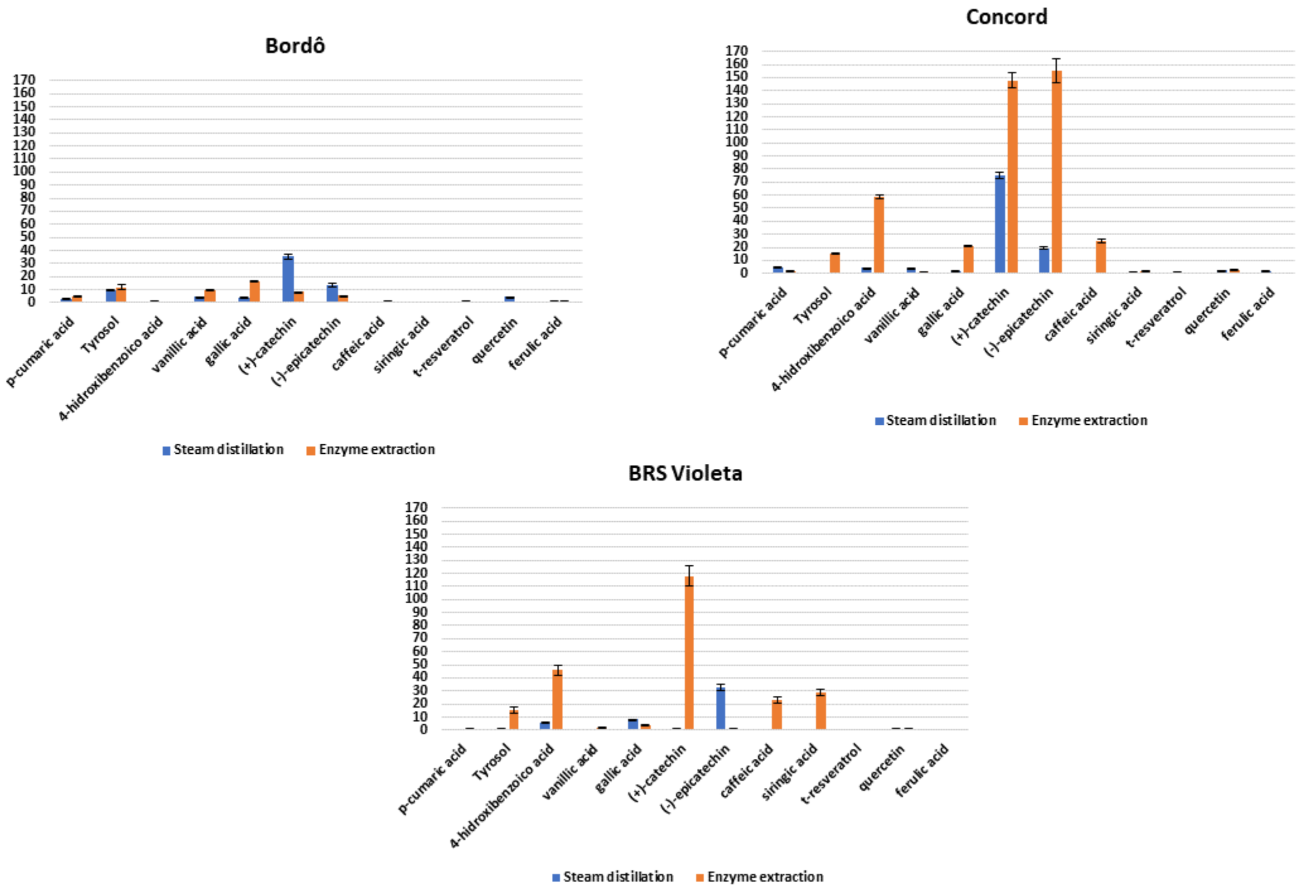

Figure 3. Phenolic compound levels in the treatments grouped by cultivar. S1-steam distillation; S2-enzyme extraction.

Principal component analysis

The first two principal components (CP1 and CP2) explained 70.41% of the dataset variance. The variables 4-hydroxybenzoic (0.93) and 'BRS Violeta' (0.95) can summarize the first dimension, because of their high correlation. Positive correlations were observed between catechin, tyrosol and the sum of phenolic compounds (Figure 4a), as well as between quercetin, vanillic acid and t-resveratrol. Component 1 (Figure 4b) shows 'BRS Violeta' in S1, placed to the right, characterized by a strongly positive coordinate in relation to 'Bordô' in S1 and S2, to the left of the graph, characterized by a negative coordinate on the axis.

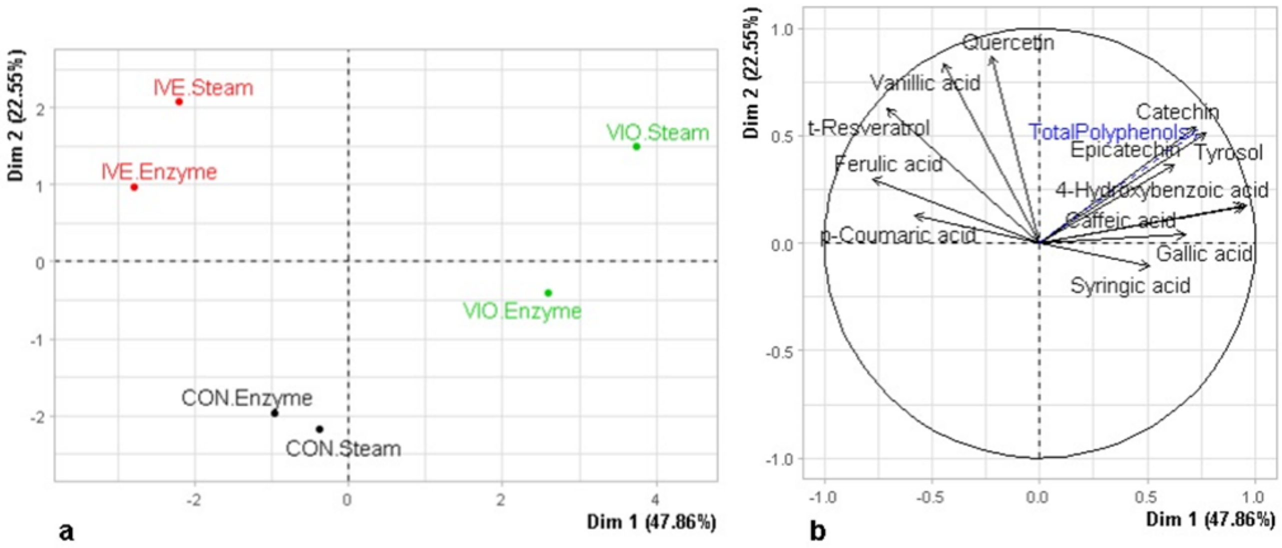

Figure 4. Principal component analysis of phenolic compound data from grape juices prepared by enzyme extraction and steam distillation systems produced in Southern Brazil.
PC1 / PC2 scores (a) and load chart (b) represented 70.41% of total variation. BOR.steam-'Bordô' S1; BOR.enzyme-'Bordô' S2; VIO.steam- 'BRS Violeta' S1; VIO.enzyme-'BRS Violeta' S2; CON.steam-'Concord' S1; CON.enzyme- 'Concord' S2.

The score graph (Figure 3b) demonstrates a clear difference between the three varieties, but not the systems. Within each variety, only S1 and S2 'BRS Violeta' exhibited a significant difference between extraction systems. The group formed by 'BRS Violeta' in S1 was characterized by higher caffeic and 4-hydroxybenzoic acid, catechin and tyrosol concentrations, as well as a strong correlation with the sum of phenolic compounds. Lima et al. (2014) found that the number of phenolic compounds quantified in juices from different cultivars was higher in 'BRS Violeta'. Bender et al. (2021) assessed the juices from eleven cultivars prepared by the steam and enzyme systems, where the 'BRS Violeta' variety showed the highest total polyphenol concentration.

The 'Bordô' sample in S1 and S2 exhibited the highest values for t-resveratrol and vanillic acid, while 'Concord' S1 and S2 juices was characterized by low vanillic acid, catechin and tyrosol values. Burin et al. (2014) reported that the 'Bordô' cultivar showed the highest t-resveratrol concentration when compared to the other Vitis labrusca and Vitis viniferas varieties, demonstrating the antioxidant potential of this variety. Lago-Vanzela et al. (2011) also considered the 'Bordô' variety a high resveratrol producer after assessing the compounds contained in the pulp and skin of this grape. Gris et al. (2013) explains that the nature of phenolic compounds, and the different relative amounts, are the consequences of a genetic determinant, specific to each variety.

The Concord variety showed no association with the variables analyzed, possibly due to their very weak skin, which makes the berries highly susceptible to splitting between harvest and processing, causing a substantial loss of must between the field and the winery. For the steam distillation system, this factor may be harmful, since the grapes should be intact when placed in the pan for intracellular soaking before splitting, and for enzyme preparation where grapes are destemmed and crushed, their significant vulnerability to mechanical damage decreases the solid/liquid relationship, thereby reducing compound extraction, in contrast to the BRS Violeta behavior, which exhibits high grape skin strength.

Conclusion

Variety was a determining factor for the concentration of each phenolic compound assessed in the juices, due to the different genetic determinants of compound synthesis, technological properties and different preparation protocols.

The extraction systems caused variations in the phenolic composition of the juices. However, the impact of the extraction system on individual phenolic compounds differed for each variety. Only 4-hydroxybenzoic, catechin and caffeic acid showed a similar trend for 'BRS Violeta' in the two extraction systems.

The varieties formed similar groups from different phenolic compounds, indicating that the Concord variety showed the lowest concentrations, and 'BRS Violeta' the highest.

4-hydroxybenzoic and catechin were the main phenolic compounds in 'BRS Violeta' juices and 'Bordô' contained the largest t-resveratrol concentration.

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