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ORIGINAL PAPER

Fast quantitative determination of phenolic compounds in grape juice by UPLC-MS: method validation and characterization of juices produced with different grape varieties

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Abstract

A fast method for the simultaneous quantitative determination of 16 phenolic compounds in grape juice by UPLC-MS was developed and validated. Run time was 4.5 min and the method proved to be specific, linear ($r > 0.9961$), precise (RSD < 5%), accurate (recovery range was under ± 5%) and sensitive with a limit of detection ranging from 0.45 to 35.34 μg L^{-1} and limit of quantification ranging from 1.35 to 107.08 μg L^{-1}. The validated method was used to characterize 49 grape juice samples which were produced with different grape varieties. Anthocyanins were the compounds present in the highest amounts on the analyzed samples and BRS-Violeta was the cultivar that presented the highest quantity of phenolic compounds in its juice. Exploratory analysis of the obtained results from the characterization of grape juice samples was performed and a tendency to form groups according to the grape variety used in the elaboration of each juice was observed. Results confirmed that the UPLC-MS method is effective and suitable for the determination of phenolic compounds in grape juice.

Keywords Food composition · Grape juice · *Vitis labrusca* L. · Phenolic compounds · Quantification analysis · Method validation

Introduction

Oxidative stress corresponds to an unbalance between production and degradation of oxidative agents such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1, 2]. Oxidative stress can generate many deleterious consequences related especially to lipid oxidation, protein modification and DNA damage [1]. Therefore, oxidative stress is involved in many pathologies such as ischemia and reperfusion, atherosclerosis, excitotoxicity, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, diabetes complications, premature aging, among others [2–4].

Phenolic compounds are secondary metabolites biosynthesized in plants as a defense mechanism against biotic and abiotic stress [5–7]. They are important antioxidant substances that fight against oxidative stress and reduce its consequences in the human body. These compounds act against oxidative stress by suppressing free radical formation, inhibiting the initiation of chain reactions, intercepting chain propagations and repairing oxidative processes [1]. They are largely present in grapes and grape-derived products, such as wine and grape juice [8–14].

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Grape juice is mainly produced from *Vitis labrusca* L. grapes and their hybrids [15]. These grapes are commonly called rustic grapes and among the most important varieties used in the elaboration of grape juice are Bordo, Concord, Isabel, Isabel Precoce (*Vitis labrusca*) and BRS-Magna and BRS-Violeta (hybrids of *Vitis vinifera* L. × *Vitis labrusca*) [15–19]. The absence of alcohol in grape juice makes its consumption adequate for the great majority of people, including children and those who have alcohol-related problems. The nutraceutical value of grape juice has been studied with greater interest in the past few years [20–23]. Grape juice consumption has grown over the years and Brazil is among the countries with the greatest amount of producers and consumers [24–26]. The extreme southern region of Brazil is responsible for over 60% of the country's grape and juice productions [25]. The growing availability of grape juice and an increased stimulus to its consumption especially due to the nutraceutical value of this drink arouses interest in studying more carefully the phenolic profile of grape juice.

Folin–Ciocalteu is a traditional method commonly used in enology for the determination of phenolic compounds. This method expresses the result of phenolic compounds in a sample in terms of total phenolic content and, therefore, it is not a specific method of quantification [27]. Specific methods for the quantification of phenolic compounds in wine have been developed [11]. However, although wine and grape juice are both grape-derived products, they are significantly different. Wines are generally elaborated with *V. vinifera* grapes, while grape juices are, as mentioned before, elaborated with *V. labrusca* grapes and their hybrids, which generates a different phenolic profile. Moreover, the differences between the elaboration processes of these two drinks give them completely distinct characteristics. In literature, information is available only on few methods developed and validated for the quantification of phenolic compounds specifically in grape juice. In most cases, those methods have a long time of analysis, are not very sensitive and/or determine only a few compounds [8–10, 13, 28, 29]. Ultra-performance liquid chromatography coupled to a mass spectrometer (UPLC-MS) is an accurate, sensitive and fast method of analysis [30, 31]. Therefore UPLC-MS is an interesting tool to be used for phenolic compound determination in grape juice.

In this context, this study aimed to develop and validate a fast quantitative UPLC-MS method for the determination of phenolic compounds in grape juice and then to apply the validated methodology in the characterization of different grape juice samples.

Materials and methods

Chemicals and standards

Formic acid for analysis and methanol LC–MS grade were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from Fluka Analytical (Munich, Germany) and was used to prepare all solutions. Kaempferol, (+)-catechin, myricetin, quercetin, *trans*-resveratrol, rutin and taxifolin analytical standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Procyanidin B1, procyanidin B2, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, cyanidin-3,5-diglucoside, malvidin-3-*O*-glucoside, malvidin-3,5-diglucoside and peonidin-3,5-diglucoside were obtained from Extrasynthese (Genay, France).

Grape juice samples

Grape juice samples were obtained from Embrapa (Brazilian Agricultural Research Corporation—Grape and Wine Research Center) in Bento Gonçalves, Rio Grande do Sul, Brazil. To validate the methodology, integral grape juice produced from the 2018 harvest using BRS-Magna variety was employed. The validated method was applied in the quantitative determination of phenolic compounds in 49 integral grape juice samples (characterization of grape juice samples). The samples studied belong to four different agronomical experiments conducted by researchers from Embrapa Grape and Wine Research Center: juices elaborated with grapes cultivated in the conventional farming system, juices elaborated with grapes cultivated in the organic farming system, juices elaborated with grapes cultivated under different levels of soil fertilization and juices elaborated with grapes from different harvests. This study focused on the phenolic profile of the evaluated samples; thus, specific agronomical aspects of the experiments were not assessed.

Grape varieties used in each juice elaboration (individually) were either traditional cultivars (Bordo, Concord, Isabel, Isabel Precoce and Niagara Rosada) or cultivars developed by Embrapa Grape and Wine's Genetic Improvement Program (BRS-Carmem, BRS-Cora, BRS-Magna, BRS-Rubea, BRS-Violeta and Seleção 13). All grape juices were prepared in an innovative system denominated Integral Juicer. The Integral Juicer was developed and patented by researchers from Embrapa Grape and Wine Research Center with the purpose of offering an appropriate production system for small-scale grape juice producers. It consists of an inclined rotating tank where grapes, already destemmed and crushed, are put in. The Integral Juicer

applies thermal processing to extract the juice from the grapes and guarantee pasteurization [15, 32, 33]. On the outside wall of the tank, it has a liquid that serves as a heater for the inside part. The grapes inside the tank are heated to 80 °C, a process that takes about 2 h to reach that temperature. When the estimated temperature is reached, the grape juice is collected on the bottom valve of the tank [32].

Instruments and conditions

Analyses were performed on a Waters Acquity UPLC System (Milford, MA, USA) equipped with a quaternary solvent pump, an autosampler, a column oven and a single mass quadrupole detector (MS). The data analysis was carried out using Empower 3 software. A Waters Acquity UPLC BEH C18 column (50 × 2.1 mm, 5 µm) was used and it was protected with a guard column of the same material (5 × 2.1 mm, 5 µm). Mobile phase A (aqueous) consisted of formic acid and water (2:98 v/v) and mobile phase B (organic) consisted of methanol, formic acid and water (90:2:8 v/v). The linear gradient used was: 0 min (min), 15% of B; 1.35 min, 40% of B; 2.65 min, 65% of B; 3.55 min, 90% of B; 3.90 min, 90% of B; 4.25 min, 30% of B; 4.50 min, 15% of B. The chromatograms were recorded for 4.5 min. At the end of each injection, the column was equilibrated with the mobile phase in its initial condition (15% of B) for 3 min. The flow rate was 0.45 mL/min and the injection volume was 5 µL.

The MS detector (Waters QDa) was equipped with an electrospray ionization (ESI) source. The detection was performed based on the molecular weight (monoisotopic mass) of each compound on monitoring mode Single Ion Recording (SIR). ESI mode (positive or negative) and cone voltage were selected to obtain a high selectivity for each compound. Mass-to-charge (*m/z*) ratio, ESI mode and cone voltage for each compound are shown in Table 1. The probe temperature was adjusted to 600 °C, the capillary voltage was − 0.8 kV in negative mode and + 1.5 kV in positive mode. Smoothing was programmed in the processing method (Smoothing Type: Mean, Smoothing Level: 5) in order to obtain the best peak format.

Standards and sample preparation

Stock and intermediate solutions of non-anthocyanic compounds (flavanols, flavanonol, flavonols and stilbene) were prepared individually diluting the standards in water and methanol (50:50 v/v). Anthocyanic standards were prepared individually diluting the standards in formic acid, water and methanol (0.05:49.95:50 v/v). The final concentration of stock solutions was 200 mg L^{-1}. The final concentration of intermediate solutions was 10 mg L^{-1}, except for procyanidin B1 e B2 that had an intermediate concentration of 40 mg L^{-1}. All stock and intermediate solutions were stored in a freezer (− 20 °C), except for kaempferol's and quercetin's solutions which were stored in a refrigerator because precipitation was observed in lower temperatures. Working standard solutions were prepared freshly at the moment of use in three separate groups: anthocyanins, procyanidins and non-anthocyanic compounds (except for procyanidins). They were diluted to the intended concentration with a solution that mimics the mobile phase's initial condition, which is formic acid, water and methanol (2:84.5:13.5 v/v).

Table 1 MS detector conditions

Compound	Cone voltage (V)	ESI	*m/z*
Anthocyanins			
Cyanidin-3,5-diglucoside	2	+	611.16
Malvidin-3-*O*-glucoside	2	+	493.13
Malvidin-3,5-diglucoside	2	+	655.19
Peonidin-3,5-diglucoside	2	+	625.18
Flavanols			
(+)-Catechin	15	−	289.08
(−)-Epicatechin	15	−	289.08
(−)-Epicatechin gallate	15	−	441.09
(−)-Epigallocatechin gallate	15	−	457.08
Procyanidin B1	10	−	577.14
Procyanidin B2	10	−	577.14
Flavanonol			
Taxifolin	10	+	305.06
Flavonols			
Kaempferol	10	−	285.05
Myricetin	10	−	317.04
Quercetin	10	−	301.04
Rutin	10	−	609.15
Stilbene			
trans-Resveratrol	15	+	229.08

For the method validation, each working standard solution was prepared at a concentration according to the parameter evaluated. For the characterization of grape juice samples, working standard solutions were prepared with a concentration at the center of the calibration curve. Grape juices were centrifuged at 10,000 rotations per min for 10 min and the supernatant was then diluted at least five times (to reach a concentration within the calibration range of each compound) in formic acid, water and methanol (2:84.5:13.5 v/v). The diluted sample solutions were centrifuged again as mentioned before and the supernatant injected. Prior to use, working standard solutions were also centrifuged and the supernatant injected. The chromatograms obtained for the standard solutions of the 16 studied phenolic compounds and their respective retention times are shown in Fig. 1. For better visualization, chromatograms are divided into two

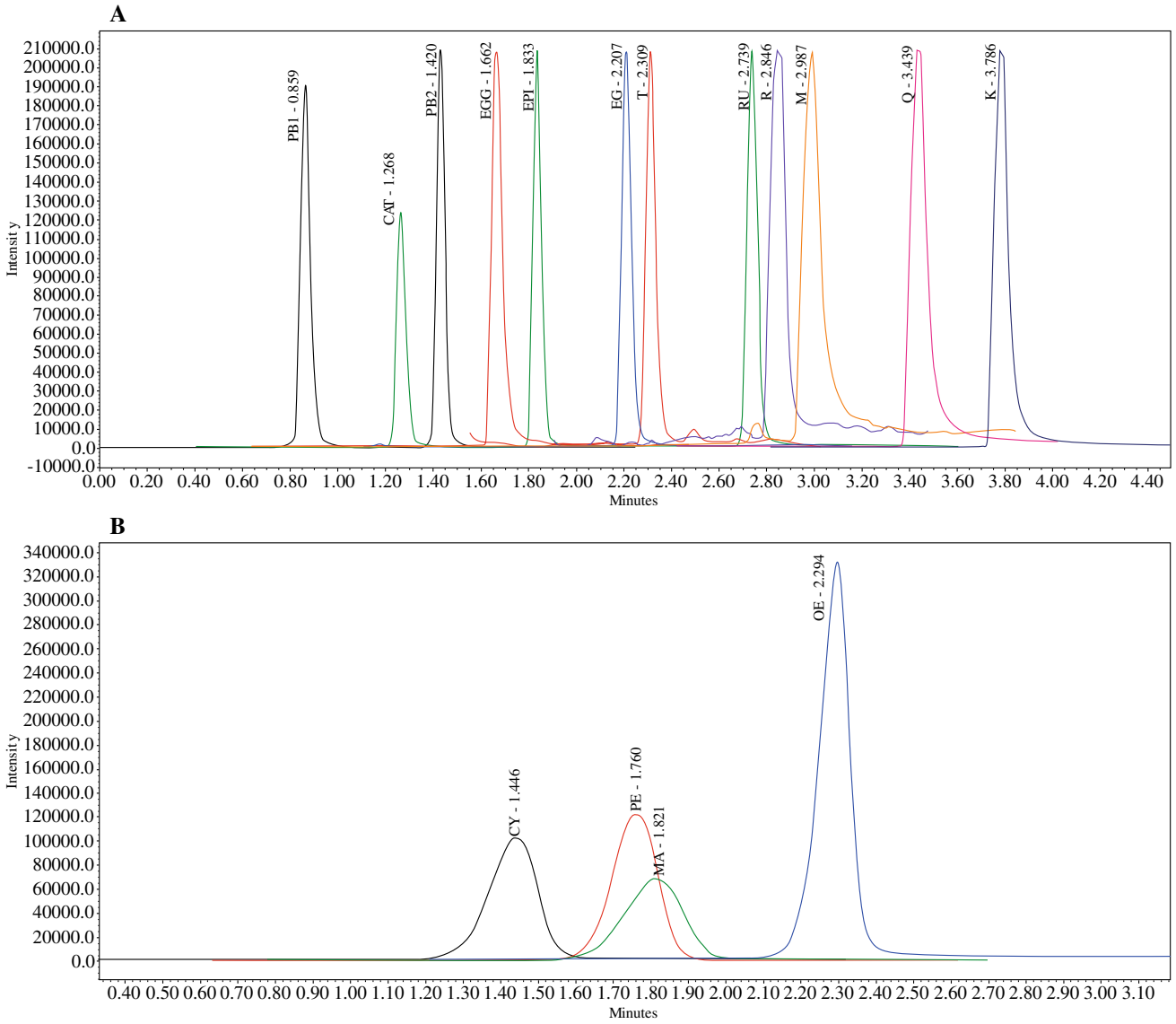

Fig. 1 Chromatograms of the studied phenolic compounds and their corresponding retention time (RT) in minutes (non-anthocyanic compounds are shown in image **a** and anthocyanins in image **b**). In order of elution: procyanidin B1 (PB1): 0.86; (+)-catechin (CAT): 1.27; procyanidin B2 (PB2): 1.42; cyanidin-3,5-diglucoside (CY): 1.45; (−)-epigallocatechin gallate (EGG): 1.66; peonidin-3,5-diglucoside (PE): 1.76; malvidin-3,5-diglucoside (MA): 1.82; (−)-epicatechin (EPI): 1.83; (−)-epicatechin gallate (EG): 2.21; malvidin-3-*O*-glucoside (OE): 2.29; taxifolin (T): 2.31; rutin (RU): 2.74; *trans*-resveratrol (R): 2.85; myricetin (M): 2.99; quercetin (Q): 3.44; kaempferol (K): 3.79

groups, non-anthocyanic compounds (A) and anthocyanins (B).

Method validation

The International Conference on Harmonization (ICH) guideline for Validation of Analytical Procedures: Text and Methodology Q2(R1) [34] and the Brazilian Health Surveillance Agency (ANVISA) resolution for validation of analytical methods [35] were used as guidance for method validation. The evaluated parameters were specificity, linearity, range, precision (repeatability and intermediate precision), accuracy, detection and quantification limits. Robustness was evaluated during the development of the analytical method.

Specificity

MS detector is capable of providing a high degree of specificity in the identification of compounds of interest. To assess the specificity of the method, peak purity was evaluated in terms of its base peak mass on scan mode. Base

peak mass is the most abundant mass corresponding to the peak of interest and this evaluation can be performed at the beginning of the peak (leading), on the peak's apex and on the final part of the peak (trailing), ensuring that there are no coelutions. Phenolic compounds not present in the grape juice sample used for the method validation were evaluated through spiking the grape juice with standards of those compounds. The base peak mass for each peak of interest was determined in the grape juice sample.

Linearity

Linearity was evaluated through the preparation of three calibration curves for each compound with five concentration levels and prepared on three different days. Concentrations versus response ratio were plotted for each analyte and linear regression analysis was performed using the least-squares method.

Precision

Precision was determined by intra-day (repeatability) and inter-day (intermediate precision) tests. Repeatability was conducted through the preparation, on the same day, of three curves of each compound with three concentration levels (low, medium and high from the linearity calibration curve). Intermediate precision was conducted through the preparation of three curves of each compound with three concentration levels (low, medium and high from the linearity calibration curve), prepared twice on two different days and by two different analysts. The precision results were expressed by the relative standard deviation (% RSD).

Accuracy

Accuracy was assessed by a recovery study. Three curves of grape juice spiked with low, medium and high concentration levels within the calibration range (nine replicates) of each standard compound were prepared. The native concentrations of each phenolic compound in the grape juice samples (if present) were considered for the definition of the spiking levels, avoiding final concentrations out of the linear range. Recovery was determined comparing the theoretical concentration of the added standard with the measured concentration on the spiked sample.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were obtained based on the standard deviation of the intercepts and slope from the calibration curve. LOD was calculated as 3.3 times the standard deviation of the intercepts divided by the slope of the calibration curve and LOQ as 10 times the standard deviation of the intercepts divided by the slope of the calibration curve.

Exploratory analysis

Exploratory analysis (hierarchical cluster analysis (HCA) and principal component analysis (PCA)) of the obtained results in the characterization of grape juice samples was conducted on Chemostat® free software, available at www.chemostat.com.br [36]. Forty-nine grape juice samples and 15 variables (phenolic compounds except for kaempferol that wasn't found in any sample) were evaluated. For multivariate analysis, the data were initially autoscaled. Singular Value Decomposition (SVD) was the algorithm used in PCA. In HCA, Euclidean distance and complete linkage method were used.

Results and discussion

Method validation

The obtained values for each parameter studied in the method validation are shown in Tables 2 and 3.

Specificity and linearity

Apex base peak mass found for each compound is presented in Table 2. The values found corresponded to those of the expected ions in question. To guarantee specificity in all determinations, compounds with the same *m/z* ratio must be separated. Isomers (+)-catechin and (−)-epicatechin (*m/z* 289.08) and procyanidin B1 and B2 (*m/z* 577.14) were completely separated as presented in Fig. 1. Specificity results found in this study were considered suitable for the intended purpose. The developed method is fast and able to specifically determine 16 phenolic compounds in only 4.5 min. Similar studies presented runtimes varying from 25 to 55 min [9, 10].

Calibration ranges and calibration curves equations for each analyte are presented in Table 2. The calibration range of each compound was selected taking into consideration the quantitative results found in previous studies [8–10, 13, 28, 29]. Regarding the correlation coefficient, the closest it is to unity ($r = 1$), the more evident that the calibration curve is linear. However, the value of r also depends on the number of data points used to calculate it. For instance, if only two points are used, r will be 1, although it could not necessarily indicate a statistically significant correlation at an adequate confidence level. That is why at least three points should be used. In this case, each calibration curve was prepared with five concentration levels (points). Values obtained for the correlation coefficient (r) of the 16 phenolic compounds

Table 2 Specificity, linearity, LOD and LOQ values obtained during method validation

Compound	Apex base peak mass	Calibration range (μg L^{-1})	Calibration curve (equation)	Correlation coefficient (*r*)	LOD (μg L^{-1})	LOQ (μg L^{-1})
Anthocyanins						
Cyanidin-3,5-diglucoside	611.16	100–450	y = 2983.52x − 30,666.88	0.9993	12.29	37.24
Malvidin-3-*O*-glucoside	493.14	40–450	y = 8017.51x + 3913.45	0.9994	0.45	1.35
Malvidin-3,5-diglucoside	655.17	100–450	y = 2932.62x − 36,247.47	0.9988	14.87	45.06
Peonidin-3,5-diglucoside	625.16	100–500	y = 3926.09x − 27,356.79	0.9995	15.58	47.22
Flavanols						
(+)-Catechin	289.04	50–450	y = 1082.85x − 10,780.03	0.9991	6.72	20.35
(−)-Epicatechin	289.04	80–470	y = 1626.70x − 43,841.96	0.9964	25.87	78.40
(−)-Epicatechin gallate	441.07	50–450	y = 1236.38x − 27,721.99	0.9977	10.61	32.15
(−)-Epigallocatechin gallate	457.07	75–450	y = 1224.40x − 44,863.44	0.9990	22.21	67.32
Procyanidin B1	577.15	120–1600	y = 287.93x − 14,723.85	0.9995	16.42	49.77
Procyanidin B2	577.15	120–1600	y = 238.38x − 18,243.71	0.9985	34.15	103.48
Flavanonol						
Taxifolin	304.98	250–450	y = 2082.00x + 50,600.99	0.9976	35.34	107.08
Flavonols						
Kaempferol	284.98	25–200	y = 5872.05x – 30,032.02	0.9983	4.01	12.16
Myricetin	317.05	75–450	y = 1885.70x – 74,961.90	0.9987	12.02	36.41
Quercetin	301.04	50–400	y = 3391.52x – 34,913.14	0.9996	7.52	22.80
Rutin	609.22	50–450	y = 1576.98x – 7306.98	0.9994	4.77	14.47
Stilbene						
trans-Resveratrol	229.09	250–450	y = 1864.18x + 33,167.27	0.9961	10.72	32.49

Table 3 Repeatability, intermediate precision and accuracy values obtained during method validation

Compound	Repeatability (% RSD)			Intermediate precision (% RSD)			Accuracy (% recovery)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Anthocyanins									
Cyanidin-3,5-diglucoside	3.62	2.48	1.17	2.64	0.43	0.81	101.64	103.23	102.16
Malvidin-3-*O*-glucoside	1.13	1.91	1.30	2.38	4.65	2.42	103.05	99.32	102.53
Malvidin-3,5-diglucoside	4.09	3.27	1.19	3.86	1.86	0.14	100.22	98.74	99.60
Peonidin-3,5-diglucoside	3.60	0.39	0.22	0.24	0.32	1.75	103.68	103.99	104.55
Flavanols									
(+)-Catechin	1.48	0.90	1.22	1.18	3.85	2.02	100.87	99.04	103.63
(−)-Epicatechin	0.62	4.87	4.34	3.43	4.53	4.36	103.14	98.42	97.32
(−)-Epicatechin gallate	0.63	3.18	2.96	3.72	2.49	1.78	103.36	102.65	100.03
(−)-Epigallocatechin gallate	2.32	3.66	0.82	3.39	3.12	2.60	99.15	103.40	103.65
Procyanidin B1	2.52	0.84	0.28	1.09	3.55	4.26	98.82	96.61	99.53
Procyanidin B2	2.43	1.65	1.77	4.88	1.47	4.36	96.59	100.12	101.50
Flavanonol									
Taxifolin	0.35	0.52	1.49	3.69	2.97	2.37	97.73	95.33	96.47
Flavonols									
Kaempferol	3.93	1.96	0.83	2.61	0.74	3.75	99.17	98.99	99.10
Myricetin	0.18	2.39	1.20	2.56	0.16	0.76	103.05	101.31	103.78
Quercetin	3.11	2.51	1.52	2.53	4.62	0.41	103.65	95.44	95.13
Rutin	3.84	1.22	1.68	1.69	3.21	4.44	97.46	101.12	97.71
Stilbene									
trans-Resveratrol	0.16	0.65	1.26	4.42	3.75	4.65	98.11	97.97	102.45

evaluated ranged from 0.9961 to 0.9996, being all higher than 0.990 and, therefore, demonstrating adequate linearity.

Precision and accuracy

The relative standard deviation (% RSD) values obtained for the precision (repeatability and intermediate precision) of the 16 phenolic compounds evaluated ranged from 0.14 to 4.88% demonstrating that the method is precise for all the analytes studied.

Accuracy study showed a recovery range from 95.33 to 104.55% of the spiked standards in the grape juice sample. These results show that the validated method is accurate for all 16 phenolic compounds evaluated and are similar to those obtained in other studies of the same type. For instance, Padilha et al. [10] obtained recovery values ranging from 94.8 to 105.1%, similar to what was obtained in the present study, although a little higher than ± 5%.

Relative standard deviation (% RSD) and recovery (%) values obtained for each analyte are presented in Table 3.

Limit of detection and limit of quantification

LOD values varied from 0.45 μg L^{-1} (malvidin-3-*O*-glucoside) to 35.34 μg L^{-1} (taxifolin). LOQ ranged from 1.35 to 107.08 μg L^{-1} for the same compounds. The obtained results are presented in Table 2 and they show that the method validated presents a significant sensitivity for the determination of very low concentrations of the evaluated compounds. Furthermore, the method can be considered more sensitive than previously reported studies that quantify phenolic compounds in grape juice with LOD ranging from 40 to 850 μg L^{-1} and LOQ ranging from 50 to 1410 μg L^{-1} [10].

Characterization of grape juice samples

The quantitative results obtained in the characterization of grape juice samples are shown on the heatmap presented in Fig. 2. The heatmap color scale varies, in this case, according to the concentration of phenolic compounds, from green, if not detected, to red with the highest concentrations found.

Nine conventional juices were elaborated with Bordo, BRS-Cora, BRS-Carmem, Concord, Isabel, Isabel Precoce, BRS-Magna, BRS-Rubea and BRS-Violeta grape varieties. Organic juices were produced using the same cultivars except for Isabel (only conventional juice) and Seleção 13 (only organic juice). Seleção 13 is a temporary name given to a new selection of grape cultivar that was developed by the Embrapa Grape and Wine's Genetic Improvement Program and is in the final stages of development. Grape

	Grape cultivar	CY	OE	MA	PE	CAT	EPI	EG	EGG	PB1	PB2	T	K	M	Q	RU	R	Σanthocyanins	Σnon-anthocyanic	TOTAL
GJ1	BRS-Cora	30.31	0.11	0.50	0.73	1.31	1.06	ND	ND	2.88	1.49	ND	ND	0.59	0.15	0.25	<LOQ	31.65	7.73	39.38
GJ2	Isabel	2.17	35.59	8.04	4.11	5.40	2.51	<LOQ	ND	4.02	2.05	<LOQ	ND	<LOQ	0.28	0.42	0.44	49.91	15.11	65.01
GJ3	BRS-Violeta	86.06	3.19	328.59	140.43	74.64	1.77	ND	ND	7.32	2.03	ND	ND	2.76	0.26	ND	<LOQ	564.26	88.78	653.05
GJ4	BRS-Magna	19.40	1.12	89.31	24.81	14.89	0.86	ND	ND	3.81	1.73	ND	ND	0.76	0.20	ND	ND	134.63	22.25	156.88
GJ5	Bordo	2.79	18.91	165.86	33.87	6.92	1.67	<LOQ	ND	4.82	2.94	ND	ND	1.93	0.22	0.21	<LOQ	221.43	18.72	240.15
GJ6	Concord	13.81	4.33	6.17	8.84	7.00	3.38	<LOQ	ND	6.64	3.59	ND	ND	<LOQ	0.20	0.16	ND	33.15	20.97	54.13
GJ7	BRS-Rubea	25.00	0.26	4.27	0.88	11.61	2.19	<LOQ	ND	5.10	3.65	<LOQ	ND	0.73	0.29	0.45	<LOQ	30.41	24.02	54.43
GJ8	Isabel Precoce	<LOQ	54.39	9.65	6.68	5.40	2.04	ND	ND	3.07	1.52	0.81	ND	ND	<LOQ	<LOQ	0.50	70.72	13.36	84.08
GJ9	BRS-Carmem	3.03	8.86	80.71	24.47	15.32	3.93	ND	ND	6.06	2.10	ND	ND	0.56	<LOQ	<LOQ	<LOQ	117.07	27.97	145.03
GJ10	Bordo 043-43 uncovered	4.89	13.49	90.71	18.67	19.45	9.76	0.31	ND	17.44	6.10	<LOQ	ND	1.44	0.28	0.32	0.25	127.74	55.34	183.08
GJ11	Bordo 043-43 covered	0.81	1.56	134.83	4.23	1.69	0.79	ND	<LOQ	2.47	1.56	ND	ND	0.45	0.20	0.16	<LOQ	141.43	7.32	148.76
GJ12	Bordo P1103 uncovered	5.60	26.91	198.39	42.65	4.24	0.65	ND	ND	4.27	1.31	<LOQ	ND	1.68	<LOQ	0.16	ND	273.55	12.31	285.86
GJ13	Bordo P1103 covered	2.51	6.31	168.95	16.39	10.75	1.74	0.30	0.68	17.32	4.95	<LOQ	ND	1.00	0.23	0.35	0.36	194.16	37.68	231.84
GJ14	Concord 043-43 uncovered	9.43	6.99	6.01	6.70	11.19	2.18	<LOQ	ND	14.88	3.79	<LOQ	ND	1.52	0.42	0.18	<LOQ	29.13	34.16	63.29
GJ15	Concord 043-43 covered	12.65	2.18	28.40	12.22	9.09	1.80	0.17	ND	17.68	3.72	<LOQ	ND	0.30	0.25	0.17	<LOQ	55.44	33.17	88.61
GJ16	Concord P1103 uncovered	12.30	9.87	14.91	10.64	10.70	4.99	0.19	ND	13.85	4.65	<LOQ	ND	1.69	0.26	0.09	<LOQ	47.72	36.42	84.14
GJ17	Concord P1103 covered	10.96	5.14	23.60	8.91	10.10	2.00	0.28	ND	14.25	3.95	<LOQ	ND	0.44	0.20	0.09	<LOQ	48.62	31.33	79.94
GJ18	BRS-Magna 043-43 covered	19.52	0.60	106.12	17.10	7.07	0.56	ND	ND	3.48	1.32	ND	ND	0.43	0.20	ND	ND	143.34	13.06	156.41
GJ19	BRS-Magna P1103 covered	26.32	0.66	117.24	25.93	6.03	0.55	ND	ND	2.73	1.20	ND	ND	0.38	0.16	ND	ND	170.15	11.06	181.21
GJ20	Isabel Precoce 043-43 uncovered	1.30	70.84	7.64	7.13	26.41	10.99	0.22	ND	16.73	6.12	0.54	ND	0.44	0.32	0.23	0.78	86.91	62.78	149.69
GJ21	Isabel Precoce 043-43 covered	0.94	22.46	13.45	8.92	11.11	4.60	<LOQ	ND	7.72	2.46	<LOQ	ND	<LOQ	0.17	0.14	0.56	45.77	26.76	72.52
GJ22	Isabel Precoce P1103 uncovered	1.42	107.25	10.42	9.03	85.72	32.21	0.34	ND	23.24	9.04	0.66	ND	0.88	0.46	0.21	1.53	128.13	154.28	282.41
GJ23	Isabel Precoce P1103 covered	0.81	22.22	17.49	7.64	7.53	3.64	ND	ND	9.14	2.64	<LOQ	ND	0.19	0.20	0.14	0.27	48.16	23.76	71.92
GJ24	BRS-Violeta 043-43 covered	95.55	1.16	124.21	62.76	6.05	<LOQ	ND	0.65	4.72	0.95	ND	ND	0.79	0.16	ND	1.14	283.68	14.45	298.13
GJ25	BRS-Violeta P1103 covered	86.89	0.76	77.34	57.25	7.87	0.47	ND	0.51	4.10	0.87	ND	ND	0.52	<LOQ	ND	1.38	222.24	15.74	237.98
GJ26	BRS-Cora 043-43 covered	18.20	0.44	2.25	1.09	1.06	0.57	ND	<LOQ	3.53	1.25	ND	ND	<LOQ	<LOQ	0.08	0,25	21.97	6.74	28.71
GJ27	BRS-Cora P1103 covered	11.54	9.29	17.10	16.24	1.78	0.79	ND	ND	4.55	1.40	ND	ND	0.45	0.17	0.20	<LOQ	54.16	9.34	63.51
GJ28	Seleção 13 043-43 covered	24.60	36.55	111.83	35.16	8.61	0.83	ND	ND	6.11	1.15	<LOQ	ND	ND	0.16	0.10	0.50	208.13	17.46	225.60
GJ29	Seleção 13 P1103 uncovered	7.46	72.36	111.34	111.38	18.89	4.78	ND	ND	11.34	2.21	ND	ND	1.20	0.43	0.42	ND	302.55	39.27	341.81
GJ30	Seleção 13 P1103 covered	7.87	56.89	112.06	134.25	10.21	1.00	ND	ND	6.03	1.17	<LOQ	ND	<LOQ	0.14	0.07	<LOQ	311.07	18.62	329.70
GJ31	BRS-Rubea 043-43 covered	0.91	3.86	25.24	19.52	2.03	0.75	<LOQ	0.50	6.41	1.79	<LOQ	ND	0.44	0.26	0.38	<LOQ	49.53	12.55	62.09
GJ32	BRS-Rubea P1103 uncovered	32.83	3.04	2.52	1.39	8.04	1.09	0.16	ND	6.80	2.72	<LOQ	ND	0.88	0.26	0.54	<LOQ	39.78	20.49	60.28
GJ33	BRS-Rubea P1103 covered	44.22	1.35	15.95	2.03	1.97	0.76	ND	ND	5.60	1.72	<LOQ	ND	0.39	0.14	0.24	<LOQ	63.55	10.82	74.36
GJ34	BRS-Carmem 043-43 uncovered	2.15	7.06	105.29	22.15	12.22	1.55	ND	ND	7.07	1.78	<LOQ	ND	0.41	<LOQ	0.11	<LOQ	136.65	23.13	159.78
GJ35	BRS-Carmem 043-43 covered	1.51	1.57	28.72	11.75	1.79	<LOQ	ND	ND	1.38	0.58	ND	ND	ND	ND	ND	0.31	43.55	4.07	47.62
GJ36	BRS-Carmem P1103 uncovered	2.13	5.43	81.07	21.71	6.91	0.95	ND	ND	4.91	1.09	ND	ND	ND	ND	ND	0.47	110.34	14.34	124.68
GJ37	BRS-Carmem P1103 covered	1.70	4.12	89.71	29.86	1.90	<LOQ	ND	ND	1.57	<LOQ	ND	ND	ND	ND	ND	0.28	125.39	3.75	129.14
GJ38	Niagara Rosada fertilization 1	4.36	0.09	ND	ND	11.08	5.29	ND	ND	11.43	3.12	ND	ND	ND	0.17	ND	ND	4.45	31.09	35.54
GJ39	Niagara Rosada fertilization 2	3.53	0.05	ND	ND	10.81	2.75	ND	ND	7.58	2.79	ND	ND	ND	0.12	ND	ND	3.58	24.05	27.63
GJ40	Niagara Rosada fertilization 3	4.06	0.06	ND	ND	12.11	6.41	ND	ND	10.74	3.15	ND	ND	ND	0.17	ND	ND	4.12	32.58	36.70
GJ41	Niagara Rosada fertilization 4	3.69	0.08	ND	ND	16.25	10.68	ND	ND	12.71	4.34	ND	ND	ND	0.34	ND	<LOQ	3.77	44.32	48.10
GJ42	Niagara Rosada fertilization 5	3.51	0.06	ND	ND	15.00	9.84	ND	ND	10.93	3.79	<LOQ	ND	ND	0.20	ND	<LOQ	3.57	39.76	43.33
GJ43	Niagara Rosada fertilization 6	3.58	0.06	ND	ND	9.52	5.17	ND	ND	7.16	2.26	ND	ND	ND	0.17	ND	<LOQ	3.64	24.28	27.91
GJ44	Bordo 2017 harvest	2.61	6.84	115.26	22.24	0.57	<LOQ	<LOQ	ND	2.62	0.62	ND	ND	2.03	0.26	0.20	ND	146.94	6.30	153.24
GJ45	BRS-Magna 2017 harvest	10.89	0.29	52.69	7.84	1.53	0.47	ND	ND	3.94	2.41	ND	ND	1.82	0.46	ND	<LOQ	71.71	10.64	82.34
GJ46	Bordo 2018 harvest	5.46	8.02	158.25	28.85	1.49	0.48	ND	<LOQ	4.16	1.88	ND	ND	1.82	0.31	0.24	<LOQ	200.59	10.39	210.98
GJ47	BRS-Magna 2018 harvest	17.36	0.60	83.67	16.19	16.72	1.25	<LOQ	<LOQ	14.99	4.83	ND	ND	1.71	0.46	ND	0.49	117.83	40.45	158.27
GJ48	Bordo 2019 harvest	3.71	14.87	215.90	43.28	19.74	13.93	0.28	ND	5.29	4.96	ND	ND	1.74	0.16	0.08	0.54	277.75	46.72	324.47
GJ49	BRS-Magna 2019 harvest	21.51	1.07	112.63	22.45	76.04	14.57	ND	ND	16.04	5.21	ND	ND	1.65	0.25	ND	1.42	157.85	115.17	272.82

ND — 653.05 mg L^{-1}

Fig. 2 Heat map of the concentration of phenolic compounds, in mg L^{-1}, in grape juice (GJ) samples. <LOQ: under limit of quantification. ND: not detected. Each value represents mean (n = 3). Compounds: cyanidin-3,5-diglucoside (CY); malvidin-3-*O*-glucoside (OE); malvidin-3,5-diglucoside (MA); peonidin-3,5-diglucoside (PE); (+)-catechin (CAT); (−)-epicatechin (EPI); (−)-epicatechin gallate (EG); (−)-epigallocatechin gallate (EGG); procyanidin B1 (PB1); procyanidin B2 (PB2); taxifolin (T); kaempferol (K); myricetin (M); quercetin (Q); rutin (RU); *trans*-resveratrol (R). Online Resource 1 presents complete information including standard deviation for each concentration result (Color figure online)

varieties cultivated for the organic juices experiment were graft in two different rootstocks (Paulsen 1103 and VR 043-43), half of the area was conducted under a plastic cover and the other half without it. Some of the treatments in this experiment were not apt for grape juice elaboration and, in the end, 28 juices were produced.

Seleção 13 juice presented the highest quantities of phenolic compounds among organic juices, especially due to its elevated amounts of anthocyanins (malvidin-3,5-diglucoside and peonidin-3,5-diglucoside). Comparing conventional and organic juices elaborated with the same grape varieties, organic juices presented higher quantities of phenolic compounds in Bordo, BRS-Carmem, BRS-Cora, BRS-Magna, BRS-Rubea, Concord and Isabel Precoce juices. The exception was the conventional BRS-Violeta juice that showed the highest concentration of phenolic compounds among all 49 samples analyzed. Phenolic compounds are biosynthesized in plants when those are under biotic and abiotic stress situations. Organic agriculture does not use pesticides during cultivation. Organic grapes are, consequently, more susceptible to the action of pathogens and, therefore, biosynthesize greater amounts of phenolic compounds [37–39].

In both conventional and organic juices, those elaborated with BRS-Cora (GJ1 and GJ26) presented the smallest quantities of phenolic compounds. GJ27 (organic BRS-Cora) presented double the quantity of phenolics compared to the other two juices elaborated with the same variety, but that amount is still not very significant when compared to the other samples of organic juices. Niagara Rosada juices (elaborated with grapes cultivated under six different levels of soil fertilization) also showed small quantities of phenolic compounds, especially anthocyanins. Cyanidin-3,5-diglucoside is responsible for the pinkish coloration of this cultivar [40] and is present in higher quantities when compared to other anthocyanins. Considering non-anthocyanic compounds, catechin, epicatechin and procyanidins B1 and B2 were found in higher quantities. GJ41 (intermediate level of soil fertilization) showed the greatest amounts of phenolics of the group.

Regarding juices elaborated with grapes from different harvests, the samples were prepared at the time of each harvest (2017, 2018 and 2019) with Bordo and BRS-Magna cultivars. Juices elaborated with Bordo grape showed higher quantities of anthocyanins in all three harvests, while BRS-Magna juices were richer in non-anthocyanic compounds. Of all anthocyanins analyzed, maldivin-3,5-diglucoside was found in greatest levels. Catechin, epicatechin and procyanidins B1 and B2 were the non-anthocyanic compounds found in highest amounts. All samples were analyzed at the same time (juices had different ages at the time of analysis), limiting the understanding about the influence of each harvest season on the phenolic profile of the grape juice samples studied. Nevertheless, it was possible to observe that the older the juice, the less phenolic compounds it has. That is the case found for all phenolics except for myricetin that presented higher amounts with older juices. A possible explanation for the increase in myricetin concentration over time is that the phenolic compounds involved in its biosynthetic pathway, as kaempferol, quercetin and taxifolin, can go through degradative reactions generating more myricetin with time [41–43]. Concerning anthocyanins, even though they represent the final step in the flavonoid biosynthetic pathway, which yields flavonols and flavan-3-ols at intermediate steps, they are very reactive molecules and may, with time, generate different anthocyanins not analyzed in the present study [41–44]. A more specific stability study should be conducted to better understand the behavior over time of phenolic compounds in grape juice.

Anthocyanins were the phenolic compounds present in greatest amount in grape juice samples analyzed. Malvidin-3,5-diglucoside was the major compound encountered, with values varying from non-detectable range (Niagara Rosada juices) to 328.59 mg L^{-1} (BRS-Violeta conventional juice—GJ3). Peonidin-3,5-diglucoside was the second most abundant anthocyanin with values varying from non-detectable range (Niagara Rosada juices) to 146.43 mg L^{-1} (BRS-Violeta conventional juice—GJ3). Malvidin-3-*O*-glucoside and cyanidin-3,5-diglucoside were found in smaller quantities. The malvidin-3-*O*-glucoside concentration ranged from 0.05 (Niagara Rosada juice—GJ39) to 107.25 mg L^{-1} (Isabel Precoce organic juice—GJ22) while cyanidin-3,5-diglucoside ranged from under LOQ (Isabel Precoce conventional juice—GJ8) to 95.55 mg L^{-1} (BRS-Violeta organic juice—GJ24). These results are in accordance with the values found in other studies regarding grape juice samples from *V. labrusca* and its hybrids [9, 10].

Anthocyanic results also confirm what has been stated about anthocyanins glycosylation, that in *V. vinifera* the glucose molecules are linked to the anthocyanidin to form 3-*O*-monoglucosides. Conversely, in non *V. vinifera*, like *V. labrusca* and its hybrids analyzed in this study, the glycosylation produces mostly 3,5-diglucosides [45]. This corroborates with the results encountered in this study where 3,5-diglucosides anthocyanins are mostly in higher concentrations than malvidin-3-*O*-glucoside. The thermal processing during grape juice elaboration, may also have some influence on anthocyanins content. Specific studies have shown that higher temperatures can be responsible for phenolic degradation (anthocyanins and total phenolic content), although a temperature of 80 °C for a short period of time (1 min) does not seem to have a significant effect on quantitative results [46, 47].

Flavanols or flavan-3-ols were the second most abundant class of phenolic compounds present in grape juice samples analyzed. The most abundant flavanol was (+)-catechin ranging from 0.57 (Bordo 2017 harvest juice—GJ44)

to 85.72 mg L^{-1} (Isabel Precoce organic juice—GJ22). (+)-catechin was also found in higher levels in BRS-Violeta juice (GJ3) and BRS-Magna juice (GJ49). The (−)-epicatechin concentration ranged from under LOQ (BRS-Violeta organic juice—GJ24, BRS-Carmem organic juice—GJ35 and GJ37, Bordo 2017 harvest juice—GJ44) to 32.21 mg L^{-1} (Isabel Precoce organic juice—GJ22). On the other hand, (−)-epicatechin gallate and (−)-epigallocatechin gallate were not detected or were under LOQ in most of the samples analyzed. The highest concentration of (−)-epicatechin gallate found was 0.34 mg L^{-1} (Isabel Precoce organic juice—GJ22). The highest concentration of (−)-epigallocatechin gallate found was 0.68 mg L^{-1} (Bordo organic juice—GJ13). Procyanidin B1 ((+)-catechin and (−)-epicatechin conjugates) was found in higher concentrations than Procyanidin B2 ((−)-epicatechin conjugates), which makes sense since (+)-catechin was more abundant than (−)-epicatechin. Procyanidin B1 ranged from 1.38 (BRS-Carmem organic juice—GJ35) to 23.24 mg L^{-1} (Isabel Precoce organic juice—GJ22) while procyanidin B2 ranged from under LOQ (BRS-Carmem organic juice—GJ37) to 9.04 mg L^{-1} (Isabel Precoce organic juice—GJ22).

In general, (+)-catechin values found in this study were in accordance with those presented by other similar studies [10, 13, 28, 48]. Regarding the concentration of (−)-epicatechin gallate in grape juice samples, Natividade et al. [9] and Padilha et al. [10] also found low or none. In terms of procyanidin B1 and B2, the results are in agreement with other studies that also encountered greater values for procyanidin B1 [9, 28, 48]. In most cases, the results presented in this study agree with other studies previously reported for *V. labrusca* and its hybrids.

Myricetin was the flavonol found in greatest quantity in grape juice samples analyzed. The highest value of myricetin encountered was 2.76 mg L^{-1} in BRS-Violeta conventional juice (GJ3). Quercetin was found in more samples than myricetin, but in smaller quantities. Quercetin's concentration was very similar in most of the samples studied, the highest quantity of quercetin encountered was 0.46 mg L^{-1} in three samples elaborated with two grape varieties: Isabel Precoce organic juice (GJ22) and BRS-Magna 2017 and 2018 harvests (GJ45 and GJ47, respectively). Rutin was not detected or was under LOQ in almost half of the analyzed samples. Conventional and organic BRS-Rubea juices (GJ7 and GJ32) presented the highest concentrations of rutin: 0.45 and 0.54 mg L^{-1}, respectively. Kaempferol was not detected in any of the analyzed samples. All of the studied flavonols were either not detected or under LOQ in conventional Isabel Precoce juice (GJ8) and in three of four of the organic BRS-Carmem juices (GJ35, GJ36 and GJ37).

Myricetin, quercetin and rutin values found in this study were in agreement with those reported by Natividade et al. [9] and Dutra et al. [39]. No other studies were found regarding the quantification of kaempferol in *V. labrusca* grape juice for comparison.

Taxifolin was only found in Isabel Precoce juices (GJ8, GJ20 and GJ22) with a maximum concentration of 0.81 mg L^{-1} (GJ8). In all the other samples, taxifolin was either under LOQ or not detected. No other studies were found regarding the quantification of taxifolin in *V. labrusca* grape juice for comparison.

Trans-resveratrol was not detected or was under LOQ in BRS-Rubea, Concord and Niagara Rosada juices. It was found in greatest amount in organic Isabel Precoce juice (GJ22) with 1.53 mg L^{-1}. These results are in accordance with other studies that also quantified *trans*-resveratrol in grape juices produced with *V. labrusca* grapes and its hybrids [9, 13, 39].

Exploratory analysis

The quantitative results obtained in the characterization of grape juice samples were analyzed by HCA and PCA. HCA and PCA are unsupervised methods of pattern recognition. HCA objectives to concentrate on the same cluster samples that are more similar. PCA, on the other hand, shows how much each variable used contributes to the principal components which differentiate the samples from each other [49]. The dendrogram obtained is shown in Fig. 3. PC1 versus PC2 score biplot (Fig. 4) accounted for 50.85% data variance (PC1 = 32.22% and PC2 = 18.63%). The PCA loadings are shown in Fig. 5.

Dendrogram analysis shows a tendency to form groups according to the grape variety used in the elaboration of each juice. However, Isabel Precoce organic juice (GJ22) is distinct and, for that reason, it was completely separated from all the rest. That separation was also evident in PCA score biplot. Evaluating the influence of each phenolic compound (Figs. 4, 5), it is possible to observe that GJ22 was different from other juices especially for its content of (−)-epicatechin and procyanidins B1 and B2. In fact, GJ22 presented the highest amounts of those compounds.

Another group of samples was also separated from the rest, but with a smaller Euclidean distance. BRS-Violeta juices (GJ3, GJ24 and GJ25), distinct by their elevated anthocyanic content, and also Bordo and BRS-Magna 2019 harvest juices (GJ48 and GJ49) showing in common a significant quantity of malvidin-3,5-diglucoside and/or (+)-catechin and myricetin.

The other samples were primarily separated into two clusters. All Concord organic juices (GJ14, GJ15, GJ16 and GJ17) appeared together in the smaller of the two clusters. Concord conventional juice (GJ6), poorer in phenolic compounds than the others of the same type, especially procyanidin B1, was grouped with Niagara Rosada juices. GJ38 and GJ40 (both Niagara Rosada) were found to be more

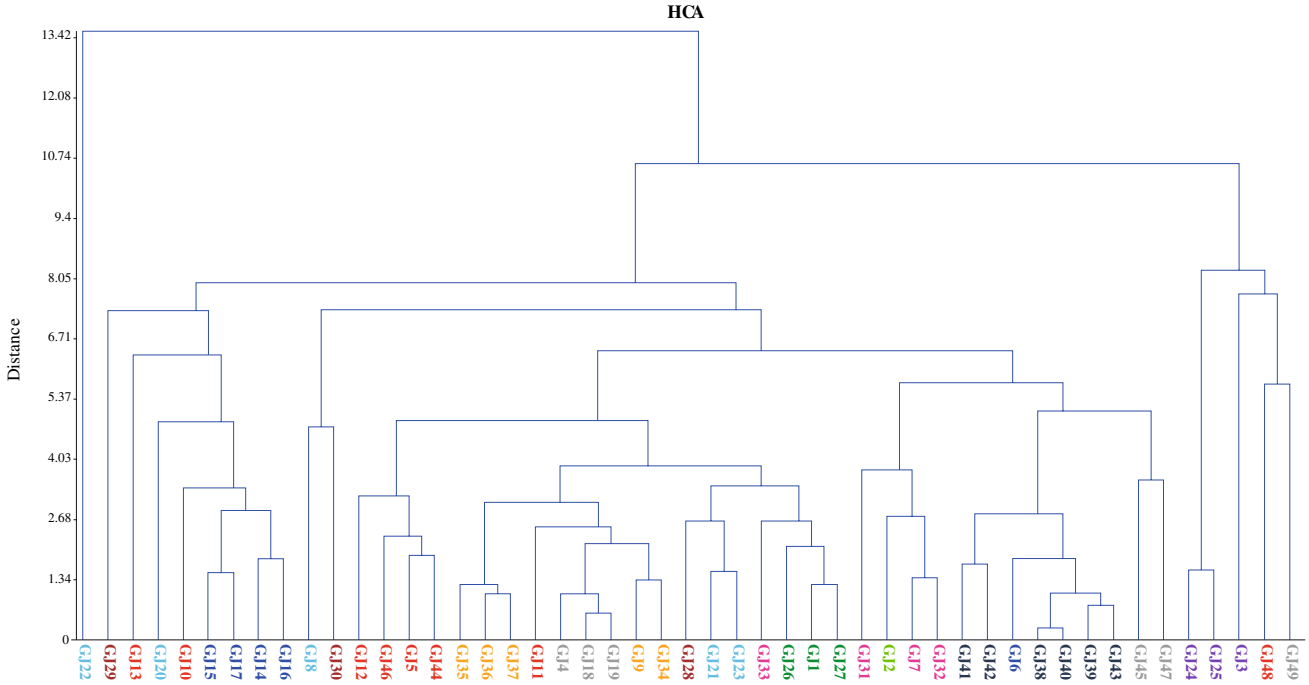

Fig. 3 Dendrogram of the grape juice samples studied. Each grape juice label was colored according to the grape cultivar used in its elaboration: BRS-Cora (dark green): GJ1, GJ26, GJ27; Isabel (light green): GJ2; BRS-Violeta (purple): GJ3, GJ24, GJ25; BRS-Magna (light gray): GJ4, GJ18, GJ19, GJ45, GJ47, GJ49; Bordo (red): GJ5, GJ10, GJ11, GJ12, GJ13, GJ44, GJ46, GJ48; Concord (blue): GJ6, GJ14, GJ15, GJ16, GJ17; BRS-Rubea (pink): GJ7, GJ31, GJ32, GJ33; Isabel Precoce (light blue): GJ8, GJ20, GJ21, GJ22, GJ23; BRS-Carmem (orange): GJ9, GJ34, GJ35, GJ36, GJ37; Seleção 13 (brown): GJ28, GJ29, GJ30; Niagara Rosada (dark gray): GJ38, GJ39, GJ40, GJ41, GJ42, GJ43 (Color figure online)

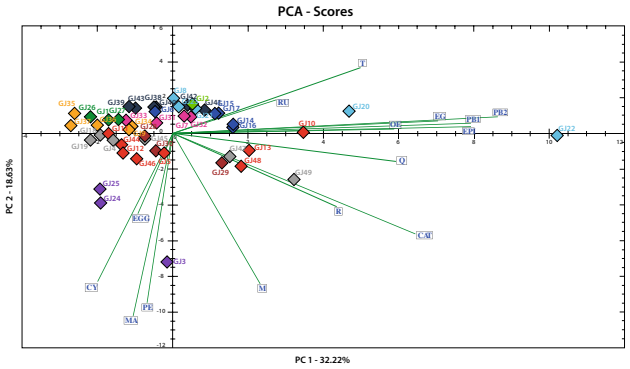

Fig. 4 PC1 versus PC2 score biplot for the grape juice samples studied. Loading vectors (phenolic compounds): cyanidin-3,5-diglucoside (CY); malvidin-3-*O*-glucoside (OE); malvidin-3,5-diglucoside (MA); peonidin-3,5-diglucoside (PE); (+)-catechin (CAT); (−)-epicatechin (EPI); (−)-epicatechin gallate (EG); (−)-epigallocatechin gallate (EGG); procyanidin B1 (PB1); procyanidin B2 (PB2); taxifolin (T); myricetin (M); quercetin (Q); rutin (RU); *trans*-resveratrol (R). Each grape juice label was colored according to the grape cultivar used in its elaboration as described in Fig. 3. Online Resource 2 presents PC1 and PC2 scores for each sample (Color figure online)

similar to each other (smallest Euclidean distance) than all other juices studied.

Bordo juices (GJ5, GJ12, GJ44 and GJ46), influenced mostly by cyanidin-3,5-diglucoside, malvidin-3,5-diglucoside and peonidin-3,5-diglucoside, showed significant similarity. On the other hand, GJ11 (Bordo organic juice) was more similar to BRS-Magna (GJ4, GJ18 and GJ19) and BRS-Carmem juices, all of them presented intermediate concentration of most compounds. GJ35, GJ36, GJ37 are slightly different from the rest of this group especially due to the absence of myricetin and the presence of *trans*-resveratrol.

Finally, BRS-Cora juices (GJ1, GJ26 and GJ27), very poor in most compounds, particularly flavanols, were clustered with BRS-Rubea organic juice (GJ33). The other three BRS-Rubea juices (GJ7, GJ31 and GJ32) were more similar to Isabel juice (GJ2) and seem to be influenced mostly by rutin and taxifolin.

PCA loadings (Fig. 5) indicate how much each variable contributes to differentiate the samples studied. For instance, positive PC1 scores are associated to samples

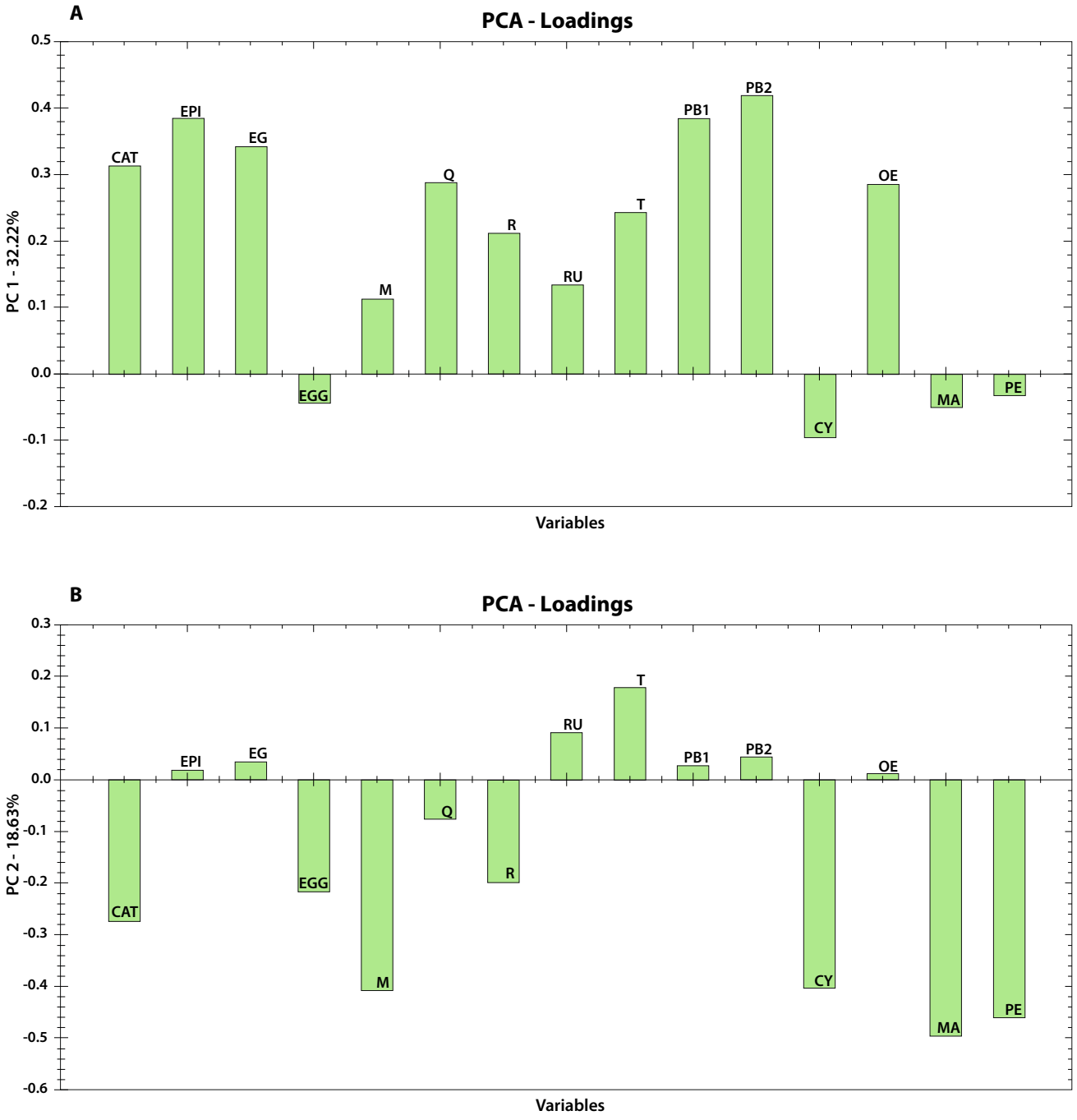

Fig. 5 PC1 (**a**) and PC2 (**b**) loadings for the phenolic compounds studied. Compounds' labels are described in Fig. 4

containing higher amounts of flavanols, quercetin and/or malvidin-3-*O*-glucoside, such as Concord organic juices and Isabel Precoce juices. While positive PC2 scores are not very significant, negative PC2 scores, on the other hand, can be associated to grape varieties that are differentiated from the rest especially because of their lower content of diglucosilated anthocyanins, such as BRS-Cora juices, BRS-Carmem juices, BRS-Rubea juices and Niagara Rosada juices.

Conclusion

The validation parameters used to evaluate the proposed method showed that the method is specific, linear, precise, accurate and very sensitive with LOD and LOQ in the order of μg L^{-1} and even ng L^{-1}. The validated method is fast and determinates, simultaneously, 16 phenolic compounds belonging to five different classes, including anthocyanins, in only 4.5 min and does not require complex sample preparation or previous purification.

The applicability of the validated method was verified through the characterization of 49 samples of grape juice produced with different grape varieties. Traditional cultivar Bordo and two varieties (BRS-Violeta and Seleção 13) developed by Embrapa Grape and Wine's Genetic Improvement Program generated the richest juices in terms of phenolic compounds, especially anthocyanins. The anthocyanin found in greatest amounts was malvidin-3,5-diglucoside while malvidin-3-*O*-glucoside was found in smallest concentrations.

Isabel Precoce juice presented the most significant quantity of non-anthocyanic compounds, especially (+)-catechin and procyanidin B1. Kaempferol, taxifolin, (−)-epicatechin gallate and (−)-epigallocatechin gallate were the phenolic compounds less found in the samples analyzed. BRS-Cora and Niagara Rosada juices presented the smallest concentration of the compounds studied.

The validated method proved to be able to evaluate quantitatively grape juices from the most different natures. It is a useful tool for studying the phenolic profile of grape juices, as well as helping to assure the quality of this drink with important nutraceutical value.

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Data availability The authors declare that the data supporting the findings of this study are available within the article and its supplementary information files. Additional raw data are available from the corresponding author on request.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval Not applicable.

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