

Nota applicativa

Rapid Quantification of Grape Juice Phenolics Using UPLC-QDa Mass Detection

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Abstract

Phenolic compounds, abundant in grapes and grape products, act as antioxidants, combating oxidative stress in the body. We employed a rapid UPLC-MS method to quantify 16 phenolic compounds in 49 grape juice samples of varying grape varieties. Anthocyanins predominated among the compounds analyzed. Our findings validate UPLC-QDa Mass Detector as a reliable method for routine analysis of phenolic compound in grape juice.

Benefits

- Efficiently determine 16 phenolic compounds in just 4.5 minutes per sample
- Reduced solvent use by 93%, aligning with the green chemistry principle of waste reduction
- High sensitivity phenolic compounds detection with the Waters ACQUITY™ UPLC™ H-Class System paired with the Waters ACQUITY™ QDa™ Mass Detector, this method enables precise mass confirmation and accurate quantification of phenolic compounds at concentrations as low as 1.35 µg/L (e.g.: malvidin-3-O-glucoside)

Introduction

Phenolic compounds are largely present in grapes and grape-derived products, and they are important antioxidant substances that fight against oxidative stress and reduce its consequences in the human body.^{4,5,6} Oxidative stress is involved in many pathologies (*e.g.*: ischemia, atherosclerosis, Alzheimer's disease), hence the nutraceutical value of grapes arouses interest in studying more carefully their phenolic profile.

Grape juice is the simplest product obtained from grapes and it is mainly produced from *Vitis labrusca* L. grapes and their hybrids such as Bordo, Concord, Isabel, Isabel Precoce (*Vitis labrusca*) and BRS-Magna and BRS-Violeta (hybrids of *Vitis vinifera* L. x *Vitis labrusca*).

Phenolic compounds are commonly determined through a non-specific method of quantification that expresses the results in terms of total phenolic content. Very few specific methods for the quantification of phenolic compounds in grape juice are cited in literature, although, in most cases, those methods have a long time of analysis, are not very sensitive and/or determine only a few compounds.^{2,4,5} Ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) is a promising tool for phenolic compound determination in grape juice as it allows for accurate, sensitive, and fast analysis of these compounds.

In this study, the objective was to quantitatively analyze 16 phenolic compounds present in 49 integral grape juice samples derived from various grape varieties. A rapid and validated UPLC-MS method was employed, as detailed in, to facilitate accurate measurements and robust analysis.¹

Experimental

Peonidin-3,5-diglucoside, malvidin-3,5-diglucoside, malvidin-3-*O*-glucoside, cyanidin-3,5-diglucoside, (-)-epigallocatechin gallate, (-)-epicatechin gallate, (-)-epicatechin, procyanidin B1 and procyanidin B2 were obtained from Extrasynthese (Genay, France). Taxifolin, rutin, *trans*-resveratrol, quercetin, myricetin, (+)-catechin, and kaempferol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was supplied from Fluka Analytical (Munich, Germany). Methanol LC-MS and formic acid for analysis were supplied by Merck (Darmstadt, Germany).

Embrapa (Brazilian Agricultural Research Corporation – Grape and Wine Research Center) in Bento Gonçalves, Rio Grande do Sul, Brazil supplied all grape juice samples belonging to four different agronomical experiments

(specific agronomical aspects of the experiments were not assessed in this study): juices from grapes cultivated in the conventional farming system, juices from grapes cultivated in the organic farming system, juices from grapes cultivated under different levels of soil fertilization and juices from grapes from different harvests.

Grape juices were prepared in an innovative system denominated Integral Juicer (developed and patented by researchers from Embrapa Grape and Wine Research Center). Peonidin-3,5-diglucoside, malvidin-3,5-diglucoside, malvidin-3-O-glucoside, cyanidin-3,5-diglucoside, (-)-epigallocatechin gallate, (-)-epicatechin gallate, (-)-epicatechin, procyanidin B1 and procyanidin B2 were obtained from Extrasynthese (Genay, France). Taxifolin, rutin, *trans*-resveratrol, quercetin, myricetin, (+)-catechin, and kaempferol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was supplied from Fluka Analytical (Munich, Germany). Methanol LC-MS and formic acid for analysis were supplied by Merck (Darmstadt, Germany).

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LC Parameters

Instrument:	ACQUITY UPLC H-Class System
Column:	Waters™ ACQUITY UPLC BEH™ C ₁₈ Column (50 mm x 2.1 mm, 5 μm) (p/n: 186003108) with a guard

column of the same material (5 mm x 2.1 mm, 5 µm) (p/n: 186007769)

Mobile phase A: formic acid and water (2:98 v/v)

Mobile phase B: methanol, formic acid, and water (90:2:8 v/v)

Injection volume: 5 µL

Flow rate: 0.45 mL/min

Gradient: Table 1

LC Gradient Settings

Time (min)	%A	%B
0.0	85	15
1.35	60	40
2.65	35	65
3.55	10	90
3.90	10	90
4.25	70	30
4.50	85	15

The chromatograms were acquired over a duration of 4.5 minutes using the Empower™ Software, which handled system control, data acquisition, and processing. Following each injection, the column was then re-equilibrated with the initial mobile phase condition (15% B) for three minutes.

MS Parameters

Instrument:	ACQUITY QDa Mass Detector*
Acquisition mode:	Selected Ion Recording (SIR)
Capillary voltage:	-0.8 kV (ESI-) and +1.5 kV (ESI+)
Probe temperature:	600 °C

**equivalent or better performance is achieved on ACQUITY QDa II Mass Detector*

Individual mass to charge (m/z) for target analytes used in the SIR method, ionization mode and specific cone voltage for each compound are shown in Table 2.

Compound	Cone voltage (V)	ESI	SIR (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
Non-anthocyanic			
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	-	285.05
Flavonols			
kaempferol	10	-	285.05
myricetin	10	-	317.04
quercetin	10	-	301.04
rutin	10	-	609.15
Stilbene			
trans-resveratrol	15	+	229.08

Table 2. Mass detector conditions.

Fresh working standard solutions were prepared from intermediate solutions in three groups: anthocyanins, procyanidins, and other non-anthocyanic compounds. These were diluted to the midpoint of the calibration curve using a solution of formic acid, water, and methanol (2:84.5:13.5 v/v). Before use, the solutions were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was injected. Chromatograms for the 16 phenolic compounds studied are shown in Figure 1.

For sample preparation, grape juice samples were centrifuged similarly, the supernatant was diluted at least fivefold (to fit within the calibration range) with the same solution, and then centrifuged again. The supernatant was injected. Figure 2 illustrates an example of grape juice dilution.

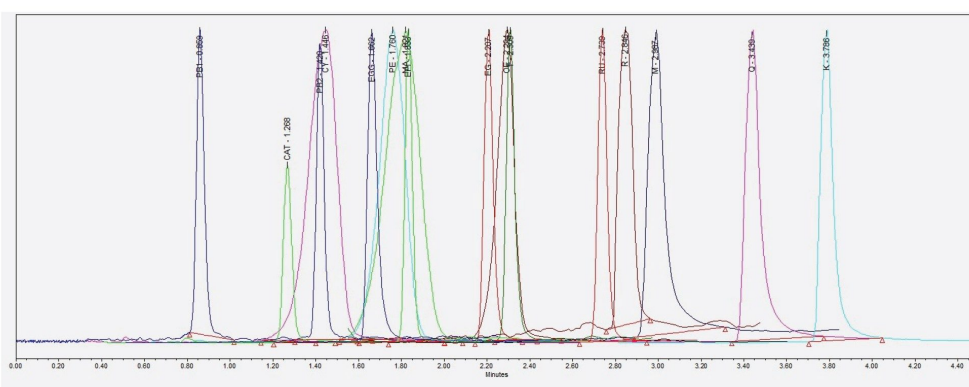


Figure 1. Retention time (RT) in minutes of target phenolic compounds: B1 (PB1): 0.86; (+)-catechin (CAT): 1.27; procyanidin B2 (PB2): 1.42; cyanidin-3,5-diglucoside (CY): 1.45; (-)-epigallocatechin gallate (EGG): 1.66; peonidin-3,5-diglucoside (PE): 1.76; malvidin-3,5-diglucoside (MA): 1.82; (-)-epicatechin (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.

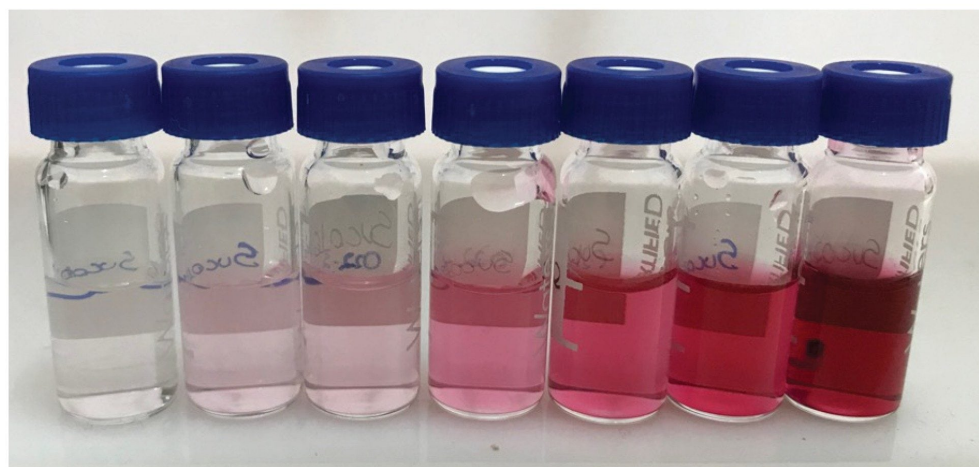


Figure 2. Grape juice dilution to reach a concentration within the calibration range of each compound.

Results and Discussion

Few methods exist for quantifying phenolic compounds in grape juice, and those that do are often insensitive, time-consuming, and limited in scope. Chromatographic techniques like UPLC have advanced sensitivity and reduced analysis time, enabling the detection of low-concentration molecules while minimizing solvent use and waste. Mass detection, highly selective and effective in complex matrices like grape juice, complements UPLC. Table 3 presents the quantitative results of phenolic compounds in 49 grape juice samples analyzed by UPLC-MS.

Grape cultivar		CY	OE	MA	PE	CAT	EPI	EG	EGG	PBI	PB2	T	K	M	Q	RU	R	ΣA	ΣNA
Juices elaborated with grapes cultivated in conventional farming system																			
GJ1	BRS-Cora	30.3	0.1	0.5	0.7	1.3	1.1	ND	ND	2.9	1.5	ND	ND	0.6	0.2	0.3	<LOQ	31.7	7.7
GJ2	Isabel	2.2	35.6	8.0	4.11	5.4	2.5	<LOQ	ND	4.0	2.0	<LOQ	ND	<LOQ	0.3	0.4	0.44	49.9	15.1
GJ3	BRS-Violeta	86.1	3.2	328.6	146.4	74.6	1.8	ND	ND	7.3	2.0	ND	ND	2.8	0.3	ND	<LOQ	564.3	88.8
GJ4	BRS-Magna	19.4	1.1	89.3	24.8	14.9	0.9	ND	ND	3.8	1.7	ND	ND	0.8	0.2	ND	ND	134.6	22.3
GJ5	Bordo	2.8	18.9	165.9	33.9	6.9	1.7	<LOQ	ND	4.8	2.9	ND	ND	1.9	0.2	0.2	<LOQ	221.4	18.7
GJ6	Concord	13.8	4.3	6.2	8.8	7.0	3.4	<LOQ	ND	6.6	3.6	ND	ND	<LOQ	0.2	0.2	ND	33.2	20.9
GJ7	BRS-Rubea	25.0	0.3	4.3	0.9	11.6	2.2	<LOQ	ND	5.1	3.7	<LOQ	ND	0.7	0.3	0.5	<LOQ	30.4	24.0
GJ8	Isabel Precoce	<LOQ	54.4	9.7	6.7	5.4	2.0	ND	ND	3.1	1.5	0.81	ND	ND	<LOQ	<LOQ	0.5	70.7	13.4
GJ9	BRS-Carmem	3.0	8.9	80.7	24.5	15.3	3.9	ND	ND	6.1	2.1	ND	ND	0.6	<LOQ	<LOQ	<LOQ	117.1	27.9
Juices elaborated with grapes cultivated in organic farming system																			
GJ10	Bordo 043-43 uncovered	4.9	13.5	90.7	18.7	19.5	9.8	0.31	ND	17.4	6.1	<LOQ	ND	1.4	0.3	0.3	0.3	127.7	55.3
GJ11	Bordo 043-43 covered	0.8	1.6	134.8	4.2	1.7	0.8	ND	<LOQ	2.5	1.6	ND	ND	0.5	0.2	0.7	<LOQ	141.4	7.3
GJ12	Bordo P1103 uncovered	5.6	26.9	198.4	42.7	4.2	0.7	ND	ND	4.3	1.3	<LOQ	ND	1.7	<LOQ	0.7	ND	273.6	12.3
GJ13	Bordo P1103 covered	2.5	6.3	168.9	16.4	10.8	1.7	0.30	0.7	17.3	4.9	<LOQ	ND	1.0	0.2	0.4	0.36	194.2	37.7
GJ14	Concord 043-43 uncovered	9.4	6.9	6.0	6.7	11.2	2.2	<LOQ	ND	14.9	3.8	<LOQ	ND	1.5	0.4	0.2	<LOQ	29.1	34.2
GJ15	Concord 043-43 covered	12.7	2.2	28.4	12.2	9.1	1.8	0.17	ND	17.7	3.7	<LOQ	ND	0.3	0.3	0.2	<LOQ	55.4	33.2
GJ16	Concord P1103 uncovered	12.3	9.9	14.9	10.6	10.7	4.9	0.19	ND	13.9	4.7	<LOQ	ND	1.7	0.3	0.1	<LOQ	47.7	36.4
GJ17	Concord P1103 covered	10.9	5.1	23.6	8.9	10.1	2.0	0.28	ND	14.3	3.9	<LOQ	ND	0.4	0.2	0.1	<LOQ	48.6	31.3
GJ18	BRS-Magna 043-43 covered	19.5	0.6	106.1	17.1	7.1	0.6	ND	ND	3.5	1.3	ND	ND	0.4	0.2	ND	ND	143.3	13.1
GJ19	BRS-Magna P1103 covered	26.3	0.7	117.2	25.9	6.0	0.6	ND	ND	2.7	1.2	ND	ND	0.4	0.2	ND	ND	170.2	11.1
GJ20	Isabel Precoce 043-43 uncovered	1.3	70.8	7.6	7.1	26.4	10.9	0.22	ND	16.7	6.1	0.54	ND	0.4	0.3	0.2	0.8	86.9	62.8
GJ21	Isabel Precoce 043-43 covered	0.9	22.5	13.5	8.9	11.1	4.6	<LOQ	ND	7.7	2.5	<LOQ	ND	<LOQ	0.2	0.1	0.6	45.8	26.8
GJ22	Isabel Precoce P1103 uncovered	1.4	107.3	10.4	9.0	85.7	32.2	0.34	ND	23.2	9.0	0.66	ND	0.9	0.5	0.2	1.5	128.1	154.3
GJ23	Isabel Precoce P1103 covered	0.8	22.2	17.5	7.6	7.5	3.6	ND	ND	9.1	2.6	<LOQ	ND	0.2	0.2	0.1	0.3	48.2	23.8
GJ24	BRS-Violeta 043-43 covered	95.6	1.2	124.2	62.8	6.1	<LOQ	ND	0.7	4.7	0.9	ND	ND	0.8	0.2	ND	1.1	283.7	14.5
GJ25	BRS-Violeta P1103 covered	86.9	0.8	77.3	57.3	7.9	0.5	ND	0.5	4.1	0.9	ND	ND	0.5	<LOQ	ND	1.4	222.2	15.7
GJ26	BRS-Cora 043-43 covered	18.2	0.4	2.3	1.1	1.1	0.6	ND	<LOQ	3.5	1.3	ND	ND	<LOQ	<LOQ	0.1	0.3	21.9	6.7
GJ27	BRS-Cora P1103 covered	11.5	9.3	17.1	16.2	1.8	0.8	ND	ND	4.6	1.4	ND	ND	0.5	0.2	0.2	<LOQ	54.2	9.3
GJ28	Seleção 13 043-43 covered	24.6	36.6	111.8	35.2	8.6	0.8	ND	ND	6.1	1.2	<LOQ	ND	ND	0.2	0.1	0.5	208.1	17.5
GJ29	Seleção 13 P1103 uncovered	7.5	72.4	111.3	111.4	18.9	4.8	ND	ND	11.3	2.2	ND	ND	1.2	0.4	0.4	ND	302.6	39.3
GJ30	Seleção 13 P1103 covered	7.9	56.9	112.1	134.3	10.2	1.0	ND	ND	6.0	1.2	<LOQ	ND	<LOQ	0.1	0.1	<LOQ	311.1	18.6
GJ31	BRS-Rubea 043-43 covered	0.9	3.9	25.2	19.5	2.0	0.8	<LOQ	0.5	6.4	1.8	<LOQ	ND	0.4	0.3	0.4	<LOQ	49.5	12.6
GJ32	BRS-Rubea P1103 uncovered	32.8	3.0	2.5	1.4	8.0	1.1	0.16	ND	6.8	2.7	<LOQ	ND	0.9	0.3	0.5	<LOQ	39.8	20.5
GJ33	BRS-Rubea P1103 covered	44.2	1.4	15.9	2.0	1.9	0.8	ND	ND	5.6	1.7	<LOQ	ND	0.4	0.1	0.2	<LOQ	63.6	10.8
GJ34	BRS-Carmem 043-43 uncovered	2.2	7.1	105.3	22.2	12.2	1.6	ND	ND	7.1	1.8	<LOQ	ND	0.4	<LOQ	0.1	<LOQ	136.6	23.1
GJ35	BRS-Carmem 043-43 covered	1.5	1.6	28.7	11.8	1.8	<LOQ	ND	ND	1.4	0.6	ND	ND	ND	ND	ND	0.31	43.6	4.1
GJ36	BRS-Carmem P1103 uncovered	2.1	5.4	81.1	21.7	6.9	0.9	ND	ND	4.9	1.1	ND	ND	ND	ND	ND	0.5	110.3	14.3
GJ37	BRS-Carmem P1103 covered	1.7	4.1	89.7	29.9	1.9	<LOQ	ND	ND	1.6	<LOQ	ND	ND	ND	ND	ND	0.3	125.4	3.8
Juices elaborated with grapes cultivated under different levels of soil fertilization																			
GJ38	Niagara Rosada fertilization 1	4.4	0.1	ND	ND	11.1	5.3	ND	ND	11.4	3.1	ND	ND	ND	0.2	ND	ND	4.5	31.1
GJ39	Niagara Rosada fertilization 2	3.5	0.05	ND	ND	10.8	2.8	ND	ND	7.6	2.8	ND	ND	ND	0.1	ND	ND	3.6	24.1
GJ40	Niagara Rosada fertilization 3	4.1	0.06	ND	ND	12.1	6.4	ND	ND	10.7	3.2	ND	ND	ND	0.2	ND	ND	4.1	32.6
GJ41	Niagara Rosada fertilization 4	3.7	0.1	ND	ND	16.3	10.7	ND	ND	12.7	4.3	ND	ND	ND	0.3	ND	<LOQ	3.8	44.3
GJ42	Niagara Rosada fertilization 5	3.5	0.06	ND	ND	15.0	9.8	ND	ND	10.9	3.8	<LOQ	ND	ND	0.2	ND	<LOQ	3.6	39.8
GJ43	Niagara Rosada fertilization 6	3.6	0.06	ND	ND	9.5	5.2	ND	ND	7.2	2.3	ND	ND	ND	0.2	ND	<LOQ	3.6	24.3
Juices elaborated with grapes from different harvests																			
GJ44	Bordo 2017 harvest	2.6	6.8	115.3	22.2	0.6	<LOQ	<LOQ	ND	2.6	0.6	ND	ND	2.0	0.3	0.2	ND	146.9	6.3
GJ45	BRS-Magna 2017 harvest	10.9	0.3	52.7	7.8	1.5	0.5	ND	ND	3.9	2.4	ND	ND	1.8	0.5	ND	<LOQ	71.7	10.6
GJ46	Bordo 2018 harvest	5.5	8.0	158.3	28.9	1.5	0.5	ND	<LOQ	4.2	1.9	ND	ND	1.8	0.3	0.2	<LOQ	200.6	10.4
GJ47	BRS-Magna 2018 harvest	17.4	0.6	83.7	16.2	16.7	1.3	<LOQ	<LOQ	14.9	4.8	ND	ND	1.7	0.5	ND	0.5	117.8	40.5
GJ48	Bordo 2019 harvest	3.7	14.9	215.9	43.3	19.7	13.9	0.28	ND	5.3	4.9	ND	ND	1.7	0.2	0.1	0.5	277.8	46.7
GJ49	BRS-Magna 2019 harvest	21.5	1.1	112.6	22.5	76.0	14.6	ND	ND	16.0	5.2	ND	ND	1.7	0.3	ND	1.42	157.7	115.2

Table 3. Concentration of phenolic compounds (in $\mu\text{g/L}$), determined in the grape juice samples. <LOQ: below the limit of quantification. ND: not detected. ΣA: Σanthocyanins. ΣNA: Σnon-anthocyanic.

Figure 3 presents total phenols concentrations of the 49 grape juice samples studied.

Organic juices were made with Bordo, BRS-Cora, BRS-Carmem, Concord, Isabel Precoce, BRS-Magna, BRS-Rubea, BRS-Violeta, and Seleção 13 (an experimental variety). Conventional juices used Bordo, BRS-Cora, BRS-Carmem, Concord, Isabel, Isabel Precoce, BRS-Magna, BRS-Rubea, and BRS-Violeta. Isabel was exclusive to

conventional juices, and Seleção 13 was exclusive to organic juices, while other varieties were common to both. Organic juices had higher phenolic compounds in most varieties: Bordo, BRS-Carmem, BRS-Cora, BRS-Magna, BRS-Rubea, Concord, and Isabel Precoce. Seleção 13 had the highest phenolic content among organics, particularly malvidin-3,5-diglucoside and peonidin-3,5-diglucoside. However, conventional BRS-Violeta had the highest phenolic concentration overall. BRS-Cora had the lowest phenolic content in both groups.

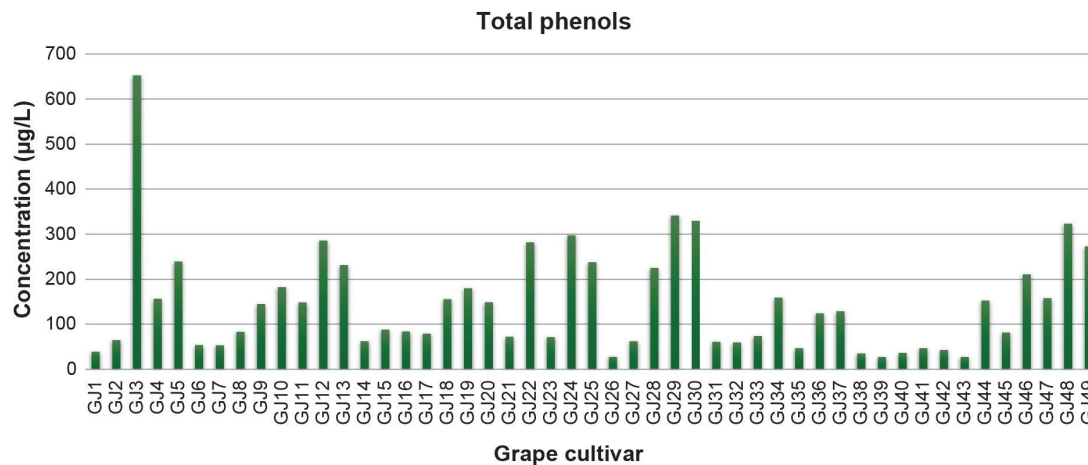


Figure 3. Total phenols concentrations (in µg/L) determined in 49 grape juices.

Bordo and BRS-Magna juices from different harvests (2017, 2018, and 2019) were studied. Samples were prepared at each harvest and analyzed simultaneously, revealing that older juices had fewer phenolic compounds, except for myricetin, which increased with age. Bordo juices consistently had higher anthocyanin levels, while BRS-Magna juices were richer in non-anthocyanin compounds.

The impact of soil fertilization on phenolic content was examined using Niagara Rosada juices. Grapes grown with intermediate fertilization produced juices with the highest phenolic levels.

Across the 49 grape juice samples, anthocyanins were the most abundant phenolics in most of them, with malvidin-3,5-diglucoside being the predominant compound, especially in BRS-Violeta conventional juice. Peonidin-3,5-diglucoside was the second most abundant anthocyanin. Flavanols were the second most prevalent phenolics, with (+)-catechin most abundant in Isabel Precoce organic juice. Procyanidin B1 was more prevalent than Procyanidin B2, and myricetin was the most abundant flavonol, especially in BRS-Violeta conventional juice. Quercetin appeared in more samples than myricetin but in smaller quantities. Taxifolin was

only found in Isabel Precoce juices, and trans-resveratrol was most abundant in organic Isabel Precoce juice. These findings align with previous grape juice studies.^{2,4,5}

Conclusion

The ACQUITY UPLC System coupled with the ACQUITY QDa Mass Detector provided several key benefits for the analysis of phenolic compounds in grape juice:

- Simultaneous detection and quantification of 16 phenolic compounds at µg/L levels.
- Simplified sample processing by eliminated the need for complex sample preparation or prior purification of the grape juice, streamlining the analytical workflow.
- Mass identification of target compounds, revealed that anthocyanins were the most abundant phenolic compounds in the samples analyzed.
- High phenolic content in specific varieties such as Bordo, BRS-Violeta, and Seleção 13 grape juices, highlighting their potential health benefits and quality.

Acknowledgement

We would like to acknowledge the contributions of Celito Crivellaro Guerra, Letícia Flores da Silva, Luísa Carolina Wetzstein, Carlos Henrique Junges, Marco Flôres Ferrão, and Ana Maria Bergold from their respective institutions: the Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul; LACEM – Laboratório de Cromatografia e Espectrometria de Massas, Embrapa Uva e Vinho; Tecnologia em Viticultura e Enologia, Instituto Federal do Rio Grande do Sul; and the Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal do Rio Grande do Sul.

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