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ORIGINAL PAPER

Targeted metabolomics reveals the storage potential of grape juice produced using different vine training systems and rootstocks

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Abstract

Training system and rootstock influence grape quality by regulating vegetative vigor, canopy structure, and metabolite biosynthesis, which may impact the juice shelf life. This study applied a targeted metabolomics approach to evaluate how combinations of training system and rootstock affect the physicochemical, phenolic, antioxidant, and volatile profiles of ‘BRS Magna’ grape juices during 24 months of storage. The metabolomic evaluation was conducted using LC-DAD-qTOF-MS for phenolic profiling, HS-SPME-GC-qMS for volatile characterization, and spectrophotometric assays to determine antioxidant activity and color. All juices met regulatory standards of total acidity (61.77–75.31 mEq L^{-1}), volatile acidity (1.13 and 1.75 mEq L^{-1}) and soluble solids (16.3–19.2 °Brix) during 24 months of storage. Total acidity and phenolic compounds, especially anthocyanins, declined during storage, causing orange color shifts and reduced antioxidant activity. These changes were observed for all combinations of training systems and rootstocks. However, the espalier × IAC 572 combination resulted in juices with the highest phenolic content, which contributed to greater antioxidant activity and enhanced color stability even after 24 months of storage. These juices also contained higher levels of esters and furaneol, enhancing pleasant aromas compared to other combinations. The enhanced performance of this combination was attributed to greater sunlight exposure and vegetative vigor, which stimulate the biosynthesis of metabolites linked to stability. This study demonstrated that the storage potential of grape juices can be optimized through agronomic practices that promote the accumulation of quality- and stability-related metabolites.

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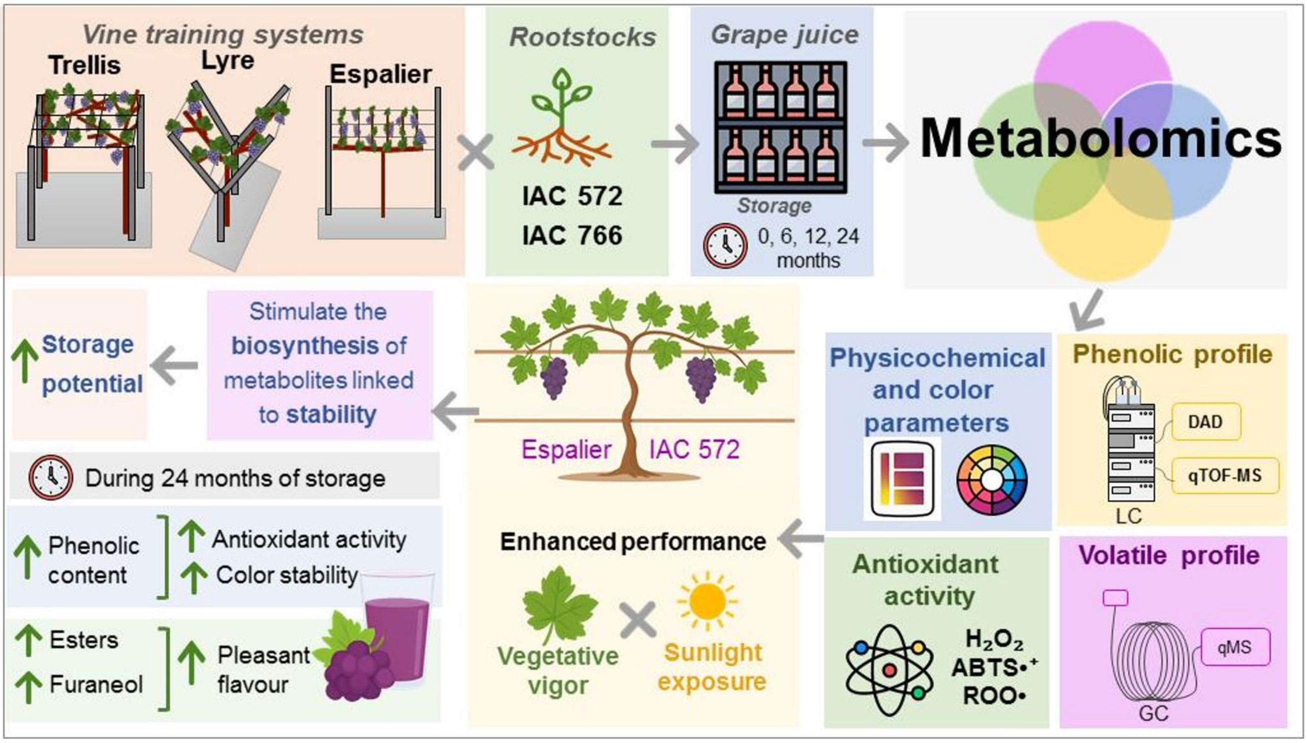
Graphical abstract



Keywords Phenolic compounds · Anthocyanin · Volatile compounds · Aroma · Shelf life · Tropical viticulture · BRS Magna

Introduction

Tropical viticulture is becoming more prominent globally, contrasting with the traditional cultivation models prevalent in temperate-climate areas and introducing new paradigms for grape production. Brazil, India, Thailand, and Venezuela are among the top producers of tropical grapes worldwide. In addition, Bolivia, Colombia, Madagascar, Tanzania, Vietnam, and China have allocated areas to the cultivation of tropical grapes [1].

The characteristics of tropical viticulture differ from those found in temperate regions. In temperate-climate vineyards, the vines undergo a dormancy period due to low winter temperatures. Techniques to break dormancy, such as the application of plant growth regulators or specific pruning practices (bending branches, late pruning, or spur pruning), are essential for promoting budding and enhancing productivity. Conversely, tropical viticulture regions do not experience sufficiently low temperatures to induce dormancy in the vines. Consequently, the vines grow continuously, enabling two or more harvests each year in the same vineyard [2].

Identifying suitable management practices for tropical viticulture, including rootstock selection and grapevine training systems, is crucial for achieving high-quality grape-derived products. Furthermore, determining the best combination of training system and rootstock can serve as a climate change adaptation strategy to overcome the harmful effects of global warming on viticulture [3, 4].

The rootstock provides the vine with a root system, which plays a crucial role in absorbing water and nutrients [2]. The choice of rootstock is determined by its adaptability to environmental conditions, resistance to pests, and, consequently, the characteristics intended to be transmitted to the grapes [5].

The grapevine training system defines the spatial organization of the canopy, trunk, cordons, and shoots. Besides modulating the canopy architecture, it plays a fundamental role in regulating the exposure of leaves and berries to solar radiation, wind, and humidity, influencing the vineyard microclimate. The most prevalent training systems in the world include the trellis, lyre, and espalier [3]. The trellis system guides the growth of vine branches horizontally, while the lyre system involves growing vines along a Y-shaped structure. In contrast, espalier is a vertical training method in which shoots are arranged along parallel wires supported by a vertical trellis [6].

Cultivation conditions and practices modulate the physicochemical properties, phenolic profile, and volatile composition of grape-derived products, which may directly influence their shelf life [7]. Phenolic compounds are associated with health-promoting properties and play a key role in the quality and stability of grape juice due to their antioxidant potential. Furthermore, they contribute to sensory attributes such as color, astringency, body and flavor. The sensory profile is also strongly influenced by the volatile compounds present in the juice, which are related to the acceptability and commercialization potential [8].

The shelf life of grape juice produced in traditional temperate regions is generally set at 24 months, a standard that producers from tropical areas have also adopted. However, empirical evidence indicates that grape juices produced under different climatic conditions may exhibit divergent stability profiles, underscoring the importance of accurately defining the storage potential of tropical grape juices. Appropriate agronomic practices tailored to the region/climate that enable the production of juices with low pH, high acidity, and increased phenolic compound content, among other factors, may help maintain stability during storage [8].

Targeted metabolomics is an approach focused on the comprehensive assessment of key metabolites, including sugars, organic acids, phenolics, and volatile compounds. This tool has been employed to determine the best cultivation strategies [9], harvesting, and/or processing [10], enabling decisions based on the chemical and sensory composition of the products [11].

The aim of this study was, for the first time, to evaluate the storage potential of grape juice produced from different combinations of vine training systems and rootstocks, using a targeted metabolomics approach. The physicochemical properties, color, phenolic profile, and volatile compounds of the juices were monitored throughout storage to identify agronomic practices that enhance the production of higher-quality and more stable juices.

Materials and methods

Agronomic experiment

The experiment was conducted with ‘BRS Magna’ grapevines, developed by Brazilian Agricultural Research Corporation (Embrapa) as a result of crossing ‘BRS Rúbea’ and IAC 1398-21 (Traviú). This cultivar exhibits broad climatic adaptability, high sugar content, and color potential, making it a promising option for juice grape production in tropical regions [12].

The grapes were cultivated in Petrolina (9°08’03"S, 40°18’28"W), at an altitude of 370 m, in the Sub-Middle San Francisco Valley, Brazil. The climate of this tropical region is BSh, according to the Köppen classification, which is characteristic of hot semi-arid regions [13]. The main characteristics of the grape-growing area are summarized in Fig. 1A.

Vineyard management was conducted under drip irrigation, using two emitters per plant spaced 50 cm apart, each with an average flow rate of 2.1 L h^{-1}. The vineyard experiment was designed using a randomized block experimental layout, featuring four repetitions, as depicted in Fig. 1B. Treatments were arranged in a 3 × 2 factorial scheme comprising three training systems (trellis, lyre, and espalier; Fig. 1C) and two rootstocks (IAC 572 and IAC 766). Each experimental plot consisted of five plants, which constituted the experimental unit.

The spacing between the plants was 3 × 1 m (density of 3.3 plants per hectare). The grapes were grown under three distinct training systems (trellis, lyre, and espalier), as illustrated in Fig. 1C. The training system influences the distribution and orientation of the canopy leaves, resulting in the following order of solar radiation incidence: espalier > lyre > trellis.

Two rootstocks were used (‘IAC 572’ and ‘IAC 766’), which were developed by the Agronomic Institute of Campinas (IAC, Brazil). ‘IAC 572’ (Jales) is the result of crossing 101 − 14 MGT (*Vitis riparia* × *Vitis rupestris*) with *Vitis caribaea* and provides high vigor. The rootstock ‘IAC 766’ (Campinas) is of medium vigor and results from the cross between Riparia do Traviú and *V. caribaea* [14]. Previous studies have consistently identified ‘IAC 572’ and ‘IAC 766’ as among the most promising rootstocks for ‘BRS Magna’ under tropical viticulture conditions, particularly due to their ability to promote higher yield and enhanced total phenolic accumulation [15–18].

Juice production

The steam extraction method was employed to produce BRS Magna grape juices. For each treatment conducted in the vineyard in triplicate (Fig. 1B), 20 kg of grapes were manually harvested. Clusters presenting visible rot or physical damage were discarded, and no washing or sanitization procedures were performed in order to reproduce standard industrial practices commonly adopted by grape juice producers. The grapes were destemmed and subjected to a temperature of 80 ± 5 °C for 60 min in a stainless steel extractor (Recifer, Brazil). Steam extraction was selected because it is widely used by small- and medium-scale producers due to its operational simplicity, low equipment cost, and ability to ensure efficient juice recovery and microbiological safety

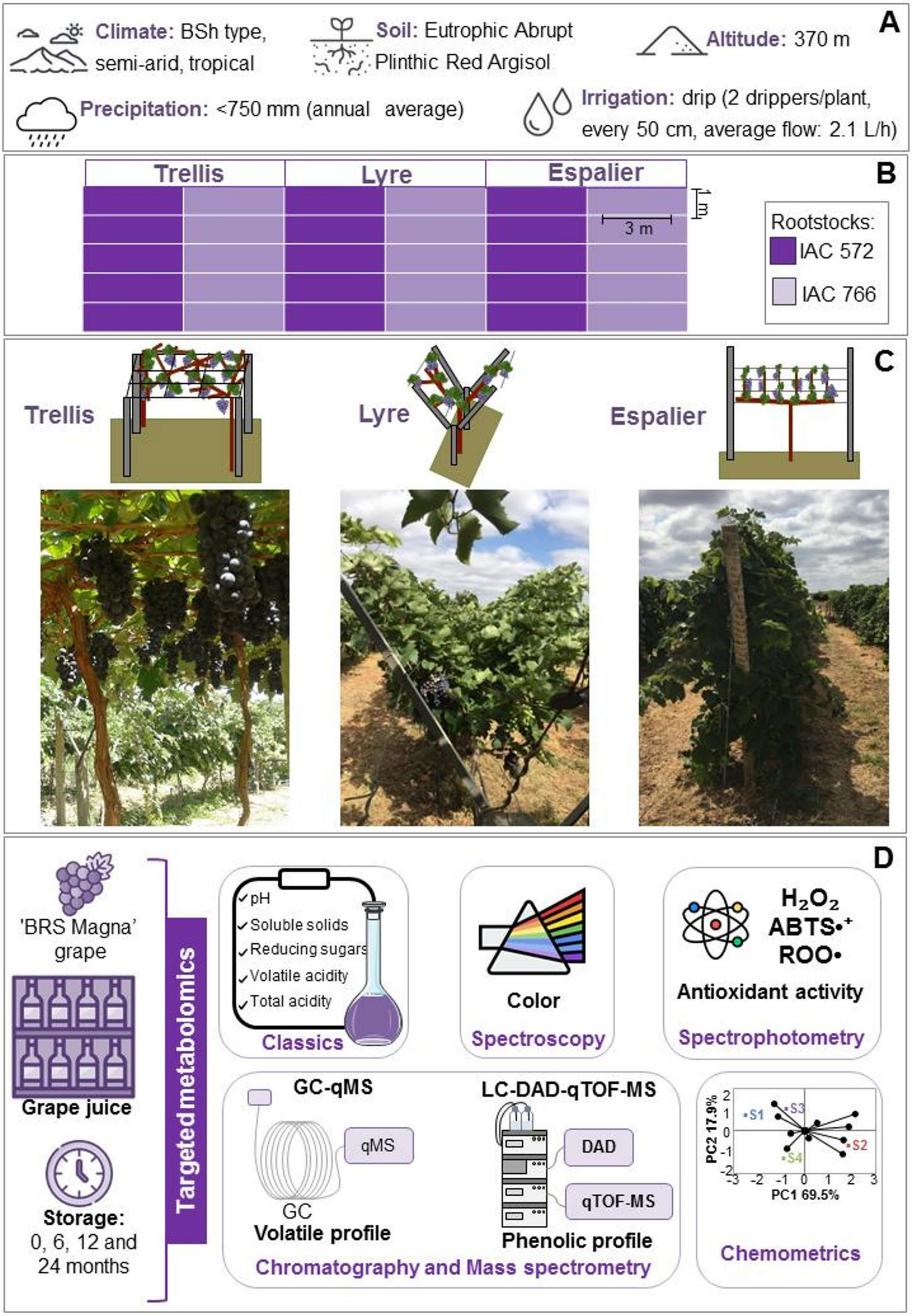

A
Climate: BSh type, semi-arid, tropical
Soil: Eutrophic Abrupt Plinthic Red Argisol
Altitude: 370 m
Precipitation: <750 mm (annual average)
Irrigation: drip (2 drippers/plant, every 50 cm, average flow: 2.1 L/h)
B
Trellis
Lyre
Espalier
1 m
3 m
Rootstocks:
IAC 572
IAC 766
C
Trellis
Lyre
Espalier
D
'BRS Magna' grape
Grape juice
Storage: 0, 6, 12 and 24 months
Targeted metabolomics
pH
Soluble solids
Reducing sugars
Volatile acidity
Total acidity
Classics
Color
Spectroscopy
H_2O_2
$ABTS^{•+}$
ROO•
Antioxidant activity
Spectrophotometry
GC-qMS
qMS
GC
Volatile profile
LC-DAD-qTOF-MS
DAD
qTOF-MS
Phenolic profile
Chromatography and Mass spectrometry
PC2 17.9%
PC1 69.5%
S1
S2
S3
S4
Chemometrics

Fig. 1 Illustration of the experiment conducted to evaluate the storage potential of 'BRS Magna' grape juices produced in a tropical climate region in the Sub-Middle Valley of the São Francisco River, Brazil. **A** Characteristics of the grape-growing area **B** Vineyard treatments were carried out in plots represented by three training systems (trellis, lyre and espalier) and two rootstocks (IAC 572 and IAC 766). The experiment was carried out using a randomized block design with four repetitions, in which each plot consisted of five plants **C** Images of the three vine training systems. **D** An overview of the metabolomic approach employed to assess juices during storage through classical, spectrophotometric, chromatographic, spectrometric, and chemometric analyses

[19]. The juice was hot-filled into 500 mL transparent glass bottles and sealed with screw caps.

Assessment of juice storage potential

Typical retail storage conditions for grape juice were simulated by keeping the bottles in an upright position at controlled room temperature (approximately 22 °C) and 40–50% relative humidity. The storage room was air-conditioned from 7:00 a.m. to 10:00 p.m., and the air conditioning was turned off overnight. These conditions reflect common retail practices in the market, where products are maintained under air-conditioned environments during business hours and without active cooling during the night. Analyses were performed 20 days after bottling, which corresponds to the average time required for the juices to reach the final consumer (defined as time zero). In addition, samples stored for 6, 12, and 24 months were also evaluated.

The metabolomic evaluation of the juices was carried out as summarized in Fig. 1D, employing a range of analytical methods: (i) classical physicochemical analyses, (ii) spectroscopic assessment of color, (iii) determination of phenolic compounds through liquid chromatography coupled with diode array detection and quadrupole-time of flight mass spectrometry (LC-DAD-qTOF-MS), (iv) analysis of the volatile profile via gas chromatography coupled with quadrupole mass spectrometry (GC-qMS), (v) assessment of antioxidant activity against hydrogen peroxide (H_2O_2), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS\cdot^{+}$) and peroxyl ($ROO\cdot$) through spectrophotometric analyses.

Physicochemical analysis

The physicochemical characterization of the juices was conducted following the methods described by the International Organisation of Vine and Wine [4], and the specific method codes are indicated as follows. The pH was measured using a pH meter (Hanna Instruments, São Paulo, Brazil) (method OIV-MA-AS313-15) and the soluble solids (SS) content was determined using a hydrostatic electronic balance (Super Alcomat, Gibertini, Milan, Italy) (method OIV-MA-AS2-02). The alcohol content was determined by distilling the sample and quantifying by densimetry (OIV-MA-AS312-01 A). The total acidity was assessed by titration with 0.1 N NaOH until the pH reached 8.2 (method OIV-MA-AS313-01). Volatile acidity was determined by steam distillation in a digital enological distiller (SuperDee, Gibertini, Milan, Italy), followed by titration with 0.1 N NaOH using phenolphthalein as the indicator (method OIV-MA-AS313-02). The reducing sugars were evaluated using the Fehling method and titration under boiling conditions with methylene blue as the indicator (method OIV-MA-AS311-01 A).

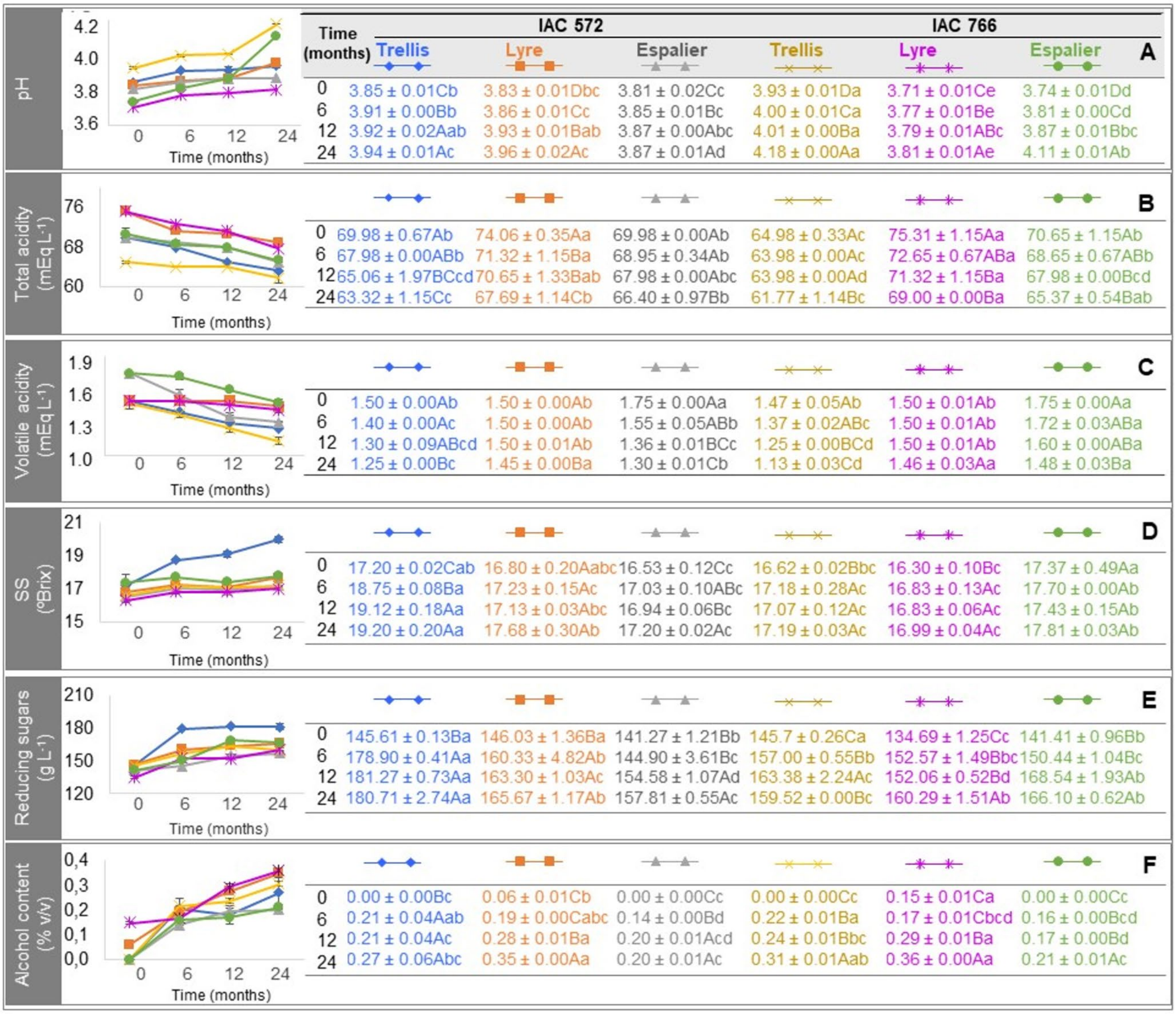
Phenolic profile

Juice samples were prepared using the 'Dilute and Shoot' approach [20]. Briefly, 1 mL of juice was diluted with 9 mL of ultrapure water containing 0.05% formic acid and filtered through a hydrophilic PTFE membrane (0.22 µm, Fitrilo, Brazil). Samples were analyzed using a liquid

Table 1 Effects of storage time, training system, rootstock, and their interactions on the physicochemical parameters of grape juices, as determined by three-way ANOVA

Parameter/Source		Storage time (S)	Training system (T)	Rootstock (*R*)	S × T	S × *R*	T × *R*	S × T × *R*
pH	*F*	142.3007	177.6753	6.1985	5.7927	29.0785	120.8337	16.2208
	p	**<0.0001**	**<0.0001**	**0.0163**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Total acidity	*F*	84.4202	218.5026	8.7795	1.1203	1.2193	20.9967	2.6078
	p	**<0.0001**	**<0.0001**	**0.0047**	0.3648	0.3129	**<0.0001**	**0.0287**
Volatile acidity	*F*	196.9245	346.3009	4.7876	46.1678	14.0778	132.5854	23.6297
	p	**<0.0001**	**<0.0001**	**0.0336**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Soluble solids	*F*	37.5940	62.9498	54.2788	4.4881	5.0518	111.6142	3.2602
	p	**<0.0001**	**<0.0001**	**<0.0001**	**0.0011**	**0.0040**	**<0.0001**	**0.0091**
Reducing sugars	*F*	339.4933	128.9495	70.1646	17.2558	2.1825	120.2022	22.5626
	p	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1023	**<0.0001**	**<0.0001**
Alcohol content	*F*	671.1282	166.0544	13.6366	20.0004	2.5392	3.9115	5.8939
	p	**<0.0001**	**0.0001**	**0.0006**	**0.0001**	0.0675	0.0267	0.0001

S × *T* Storage time and Training system, *S* × *R* Storage time × Rootstock, *T* × *R* Training system × Rootstock, *S* × *T* × *R* Storage time, Training system, and Rootstock, Significant effects at $p<0.05$ are shown in bold



A

Time (months)	IAC 572			IAC 766		
	Trellis ◆	Lyre ■	Espalier ▲	Trellis ×	Lyre ✱	Espalier ●
0	3.85 ± 0.01Cb	3.83 ± 0.01Dbc	3.81 ± 0.02Cc	3.93 ± 0.01Da	3.71 ± 0.01Ce	3.74 ± 0.01Dd
6	3.91 ± 0.00Bb	3.86 ± 0.01Cc	3.85 ± 0.01Bc	4.00 ± 0.01Ca	3.77 ± 0.01Be	3.81 ± 0.00Cd
12	3.92 ± 0.02Aab	3.93 ± 0.01Bab	3.87 ± 0.00Abc	4.01 ± 0.00Ba	3.79 ± 0.01ABc	3.87 ± 0.01Bbc
24	3.94 ± 0.01Ac	3.96 ± 0.02Ac	3.87 ± 0.01Ad	4.18 ± 0.00Aa	3.81 ± 0.01Ae	4.11 ± 0.01Ab

B

	◆	■	▲	×	✱	●
0	69.98 ± 0.67Ab	74.06 ± 0.35Aa	69.98 ± 0.00Ab	64.98 ± 0.33Ac	75.31 ± 1.15Aa	70.65 ± 1.15Ab
6	67.98 ± 0.00ABb	71.32 ± 1.15Ba	68.95 ± 0.34Ab	63.98 ± 0.00Ac	72.65 ± 0.67ABa	68.65 ± 0.67ABb
12	65.06 ± 1.97BCcd	70.65 ± 1.33Bab	67.98 ± 0.00Abc	63.98 ± 0.00Ad	71.32 ± 1.15Ba	67.98 ± 0.00Bcd
24	63.32 ± 1.15Cc	67.69 ± 1.14Cb	66.40 ± 0.97Bb	61.77 ± 1.14Bc	69.00 ± 0.00Ba	65.37 ± 0.54Bab

C

	◆	■	▲	×	✱	●
0	1.50 ± 0.00Ab	1.50 ± 0.00Ab	1.75 ± 0.00Aa	1.47 ± 0.05Ab	1.50 ± 0.01Ab	1.75 ± 0.00Aa
6	1.40 ± 0.00Ac	1.50 ± 0.00Ab	1.55 ± 0.05ABb	1.37 ± 0.02ABc	1.50 ± 0.01Ab	1.72 ± 0.03ABa
12	1.30 ± 0.09ABcd	1.50 ± 0.01Ab	1.36 ± 0.01BCc	1.25 ± 0.00BCd	1.50 ± 0.01Ab	1.60 ± 0.00ABa
24	1.25 ± 0.00Bc	1.45 ± 0.00Ba	1.30 ± 0.01Cb	1.13 ± 0.03Cd	1.46 ± 0.03Aa	1.48 ± 0.03Ba

D

	◆	■	▲	×	✱	●
0	17.20 ± 0.02Cab	16.80 ± 0.20Aabc	16.53 ± 0.12Cc	16.62 ± 0.02Bbc	16.30 ± 0.10Bc	17.37 ± 0.49Aa
6	18.75 ± 0.08Ba	17.23 ± 0.15Ac	17.03 ± 0.10ABc	17.18 ± 0.28Ac	16.83 ± 0.13Ac	17.70 ± 0.00Ab
12	19.12 ± 0.18Aa	17.13 ± 0.03Abc	16.94 ± 0.06Bc	17.07 ± 0.12Ac	16.83 ± 0.06Ac	17.43 ± 0.15Ab
24	19.20 ± 0.20Aa	17.68 ± 0.30Ab	17.20 ± 0.02Ac	17.19 ± 0.03Ac	16.99 ± 0.04Ac	17.81 ± 0.03Ab

E

	◆	■	▲	×	✱	●
0	145.61 ± 0.13Ba	146.03 ± 1.36Ba	141.27 ± 1.21Bb	145.7 ± 0.26Ca	134.69 ± 1.25Cc	141.41 ± 0.96Bb
6	178.90 ± 0.41Aa	160.33 ± 4.82Ab	144.90 ± 3.61Bc	157.00 ± 0.55Bb	152.57 ± 1.49Bbc	150.44 ± 1.04Bc
12	181.27 ± 0.73Aa	163.30 ± 1.03Ac	154.58 ± 1.07Ad	163.38 ± 2.24Ac	152.06 ± 0.52Bd	168.54 ± 1.93Ab
24	180.71 ± 2.74Aa	165.67 ± 1.17Ab	157.81 ± 0.55Ac	159.52 ± 0.00Bc	160.29 ± 1.51Ab	166.10 ± 0.62Ab

F

	◆	■	▲	×	✱	●
0	0.00 ± 0.00Bc	0.06 ± 0.01Cb	0.00 ± 0.00Cc	0.00 ± 0.00Cc	0.15 ± 0.01Ca	0.00 ± 0.00Cc
6	0.21 ± 0.04Aab	0.19 ± 0.00Cabc	0.14 ± 0.00Bd	0.22 ± 0.01Ba	0.17 ± 0.01Cbcd	0.16 ± 0.00Bcd
12	0.21 ± 0.04Ac	0.28 ± 0.01Ba	0.20 ± 0.01Acd	0.24 ± 0.01Bbc	0.29 ± 0.01Ba	0.17 ± 0.00Bd
24	0.27 ± 0.06Abc	0.35 ± 0.00Aa	0.20 ± 0.01Ac	0.31 ± 0.01Aab	0.36 ± 0.00Aa	0.21 ± 0.01Ac

Fig. 2 Changes in **A** pH, **B** total acidity, **C** volatile acidity, **D** soluble solids **SS**, **E** reducing sugars, and **F** alcohol content during storage (0, 6, 12, and 24 months) of juices produced from 'BRS Magna' grapes grown under three training systems (trellis, lyre, and espalier) and two rootstocks (IAC 572 and IAC 766). Line graphs illustrate trends over storage time, while the accompanying tables present mean values ± standard deviation (SD) and statistical comparisons. Due to the low variability among replicates ($n = 3$), the error bars may not be visually distinguishable in the graphs. The physicochemical characteristics of the juices were evaluated by ANOVA followed by Tukey's test ($p = 0.05$). Different uppercase letters in the same column and lowercase letters in the same row indicate statistically significant differences

chromatograph equipped with a diode array detector (LC-DAD, SPD-M20A, Shimadzu, Japan) and an electrospray ionization quadrupole time-of-flight mass spectrometer (ESI-QToF-MS, micrOTOF-Q III, Bruker Daltonics, Germany). Mass spectrometry conditions were applied as previously described by Nievierowski [21].

Phenolic compounds were identified by comparing retention times, UV–Vis spectra, and mass spectra with those of analytical standards and literature data. Detailed information on compound identification is provided in Table S1 of the Supplementary Material.

For quantification, calibration curves were constructed using standard solutions of phenolic compounds at concentrations ranging from 0.10 to 30 µg mL^{-1}, following the validated protocol of Natividad [22]. Briefly, all calibration curves showed excellent linearity ($R^2 > 0.99$). Procyanidin B2 exhibited the lowest LOD (0.001 µg mL^{-1}) and LOQ (0.003 µg mL^{-1}), whereas (−)-epigallocatechin gallate showed the highest LOD (0.19 µg mL^{-1}). The highest LOQ values (0.37 µg mL^{-1}) were observed for cyanidin-3-glucoside-chloride and cinnamic acid.

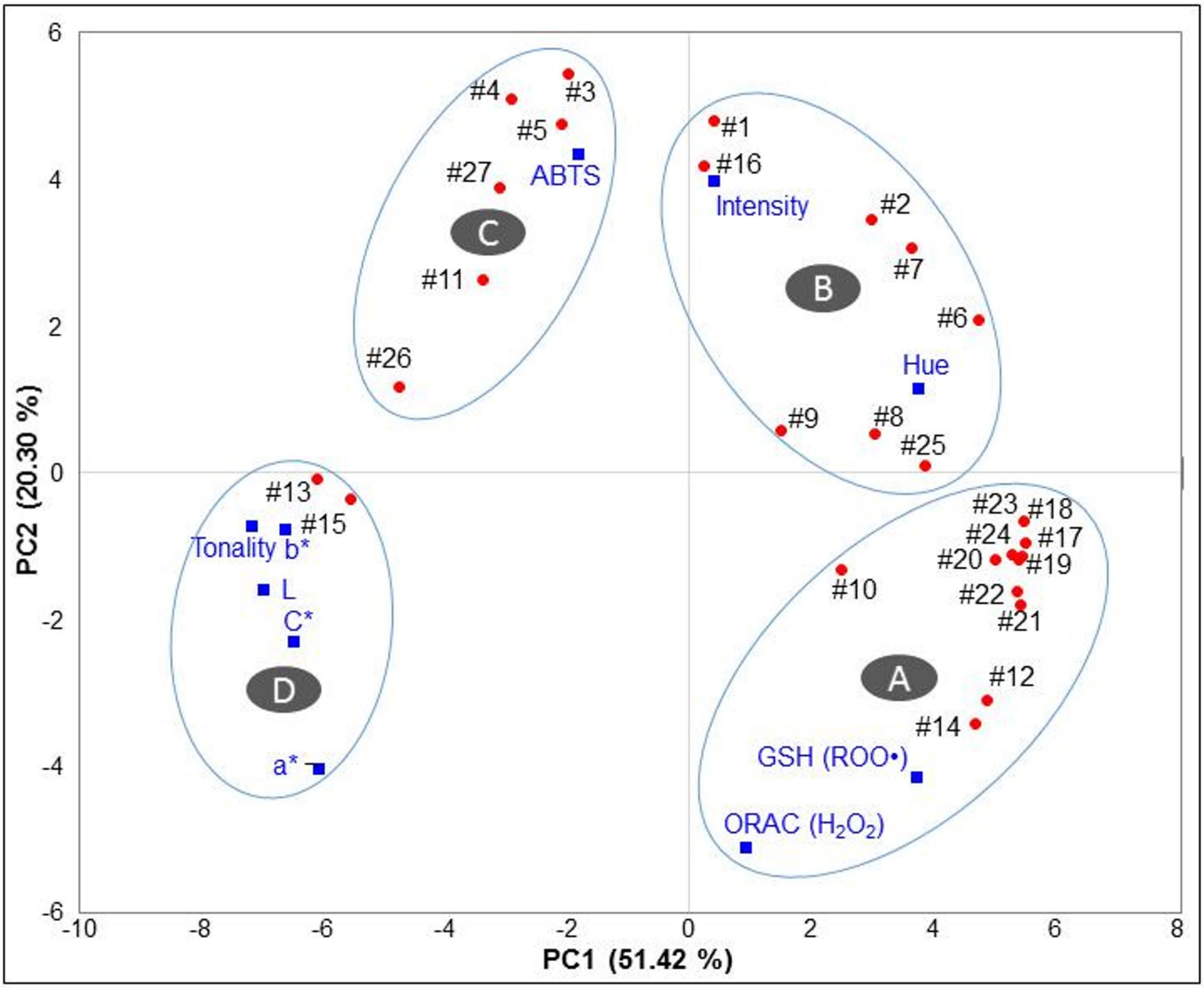


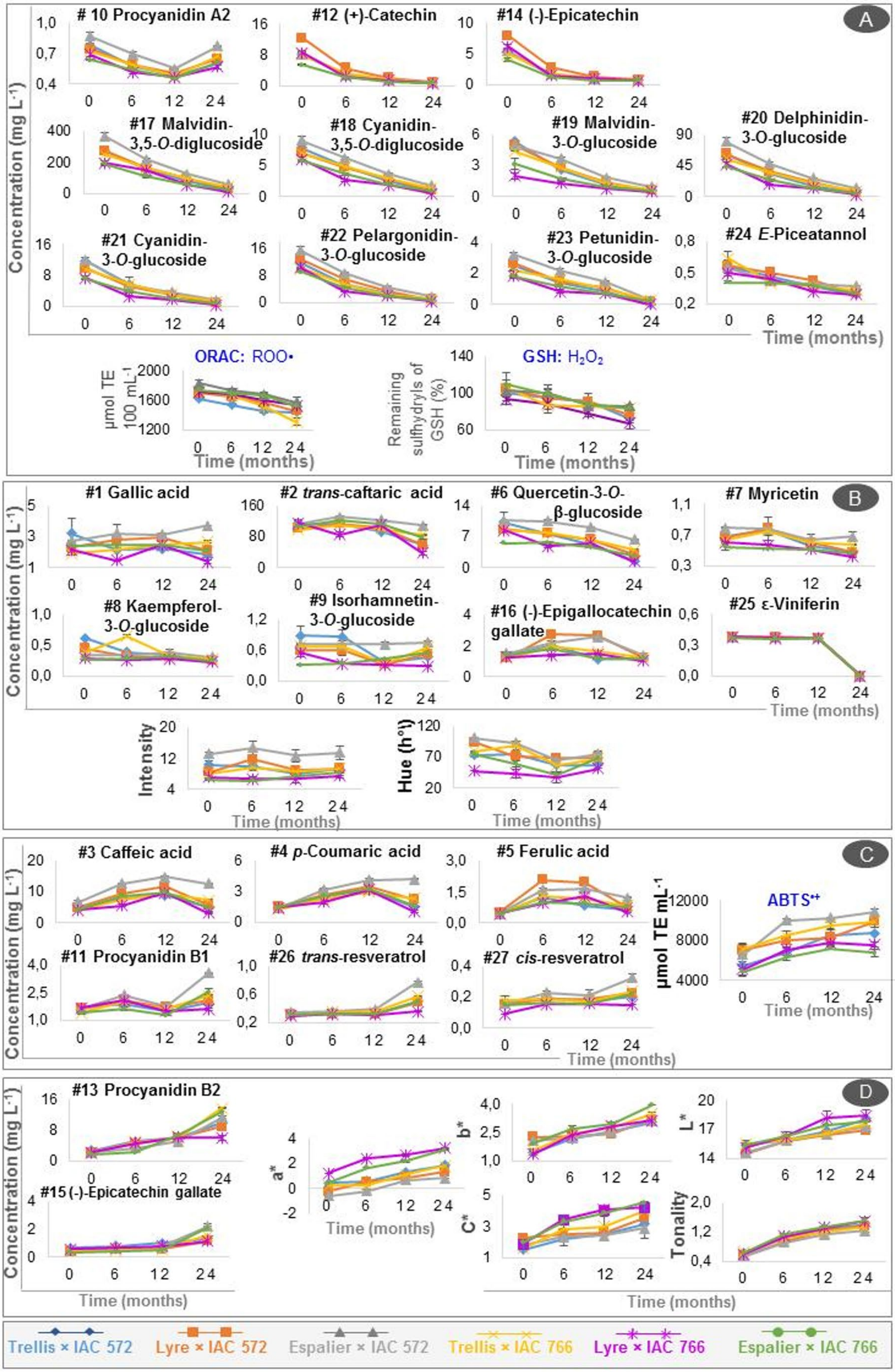
Fig. 3 Principal Components Analysis (PCA) based on the concentration of phenolic compounds (#1 to #27, Table S1), color data (L, a*, b*, C*, tonality, intensity, and Hue, Table S2), and antioxidant capacity (ABTS·+, ORAC, and GSH, Table S3) of the 'BRS Magna' grape juices stored for 6, 12, and 24 months. Grapes were cultivated using three training systems (trellis, lyre, and espalier) and two rootstocks (IAC 572 and IAC 766)

Antioxidant activity

The antioxidant activity of grape juices against peroxyl radicals (ROO·) and hydrogen peroxide (H_2O_2) was assessed using the oxygen radical absorbance capacity (ORAC) [23] and the reduced glutathione (GSH) protection [24] assays, respectively. Measurements were performed using a microplate reader (EnSpire 2300, Multimode Plate Reader, Perkin Elmer, Waltham, MA, USA). ABTS·+ radical scavenging was evaluated at 734 nm using a spectrophotometer (Shimadzu UV-1800, Japan) [25]. Detailed procedures are described in a previous study [26].

Color evaluation

For color evaluation, the CIELAB and CIELCh coordinate systems were used. The luminosity measurements (L*), color coordinates (a* and b*), and chromaticity (C*) were obtained using a portable colorimeter (Delta Color, São Leopoldo, Brazil), calibrated for the D65 illuminant and an observation angle of 10°. Color intensity was quantified as the sum of absorbances measured on a UV-vis spectrophotometer (Biospectro, SP-220, Brazil) at wavelengths of 420, 520, and 620 nm, corresponding to the yellow, red, and violet regions of the visible spectrum, respectively. Hue was determined by the ratio between absorbances at 420 and 520 nm [27].


A
10 Procyanidin A2
#12 (+)-Catechin
#14 (-)-Epicatechin
#17 Malvidin-3,5-O-diglucoside
#18 Cyanidin-3,5-O-diglucoside
#19 Malvidin-3-O-glucoside
#20 Delphinidin-3-O-glucoside
#21 Cyanidin-3-O-glucoside
#22 Pelargonidin-3-O-glucoside
#23 Petunidin-3-O-glucoside
#24 E-Piceatannol
Concentration (mg L-1)
Time (months)
0 6 12 24
ORAC: ROO•
μmol TE 100 mL-1
2000 1600 1200
GSH: H2O2
Remaining sulfhydryls of GSH (%)
140 100 60
B
#1 Gallic acid
#2 trans-caftaric acid
#6 Quercetin-3-O-β-glucoside
#7 Myricetin
#8 Kaempferol-3-O-glucoside
#9 Isorhamnetin-3-O-glucoside
#16 (-)-Epigallocatechin gallate
#25 ε-Viniferin
Intensity
Hue (h°)
C
#3 Caffeic acid
#4 p-Coumaric acid
#5 Ferulic acid
#11 Procyanidin B1
#26 trans-resveratrol
#27 cis-resveratrol
ABTS•+
μmol TE mL-1
12000 8000 4000
D
#13 Procyanidin B2
#15 (-)-Epicatechin gallate
a*
b*
C*
L*
Tonality
Trellis × IAC 572
Lyre × IAC 572
Espalier × IAC 572
Trellis × IAC 766
Lyre × IAC 766
Espalier × IAC 766

◀ **Fig. 4** Changes in the concentration of phenolic compounds, color parameters (intensity, Hue, a*, b*, L*, C* and tonality) and antioxidant capacity against hydrogen peroxide (H_2O_2), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot +}$), and peroxyl ($ROO\cdot$) radicals, during storage of juices produced with 'BRS Magna' grapes grown under three training systems (trellis, lyre and espalier) and two rootstocks (IAC 572 and IAC 766). Variables correspond to groups (**A**), (**B**), (**C**), and (**D**) of the PCA shown in Fig. 3

The total color difference (ΔE) between freshly processed juices (time 0) and those stored for 6, 12, and 24 months was calculated according to Eq. 1. Variations in color were classified as slightly noticeable (ΔE < 1.5), moderate (1.5 ≤ ΔE* < 3.0), or large (ΔE* ≥ 3.0), based on the visual perception criteria proposed by Pathare [28].

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad (1)$$

Where:

- L^*_1, a^*_1, and b^*_1 correspond to the freshly processed juices (time 0);
- L^*_2, a^*_2, and b^*_2 correspond to the stored juices.

Volatile profile

The volatile compounds of grape juices were extracted according to Welke [29], using 1 mL of sample and 0.30 g of NaCl (w/v, Nuclear, São Paulo, Brazil) at 55 °C for 45 min. Headspace solid-phase microextraction (HS-SPME) was conducted using a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) and a divinylbenzene/carboxene/polydimethylsiloxane fiber (DVB/Car/PDMS, 2 cm, 50/30 µm, Supelco, USA).

Volatile compounds were analyzed using a gas chromatograph coupled to a quadrupole mass spectrometer (GC/qMS; Shimadzu QP 2010 S, Japan) equipped with a polar column (DB-WAX, 30 m × 0.25 mm × 0.25 µm, J&W Scientific Inc., USA). Volatile compounds were identified following the procedures described by Hernandes [30]. Compounds were identified by coinjection of authentic standards (Sigma, USA). Additional identification criteria were considered: (i) a similarity score ≥ 80% between experimental and library mass spectra, and (ii) a difference of less than 10 units between the experimental retention index (RIexp) and the corresponding literature value (RIlit).

The quantification was carried out using calibration curves prepared in a model solution of grape juice [1 L of ultrapure water (Millipore, Bedford, MA, USA), 6 g of tartaric acid (Synth, São Paulo, Brazil), 80 g of glucose, and 70 g of fructose (Êxodo Científica, São Paulo, Brazil), with the pH adjusted to 3.8 using NaOH (Nuclear, São Paulo, Brazil)]. Internal standards (IS) were used to normalize the peak areas of volatile compounds according to their chemical class: 1,4-cineole (C13-norisoprenoids and terpenes), isobutyric acid (acids found in pulp), nonanoic acid (acids), 3-octanol (alcohols), dodecane (aldehydes and ketones), methyl nonanoate (ethyl and methyl esters), and phenyl acetate (acetate esters). Preliminary tests were conducted to confirm the absence of these compounds in the samples. A mixed IS solution (10 mg L^{-1}) was prepared in double-distilled ethanol, and 10 µL were added to each sample prior to HS-SPME extraction. The entire quantification procedure and validation of the HS-SPME-GC/qMS method (detection and quantification limits, repeatability, reproducibility, and linearity) were detailed in a previous study [31].

Statistical analysis

Statistical analysis was conducted using XLSTAT software (Addinsoft, Nova York, EUA, 2017). A three-way analysis of variance (ANOVA) was conducted for the physical-chemical data considering storage time, training system, and rootstock as fixed factors, including their interaction terms. This approach was used to determine the relative contribution of each factor and their interactions to the variability in juice composition during storage. When significant effects were observed ($p < 0.05$), mean comparisons were performed using Tukey's test. Volatile profile, phenolic composition, and antioxidant capacity were analysed using one-way ANOVA followed by Tukey's test ($p < 0.05$) to compare mean values among storage times and vineyard factor combinations. Principal Component Analysis (PCA) was employed to examine multivariate relationships and similarities among samples during storage.

Results and discussion

Physicochemical analysis

Table 1 presents the results of the three-way ANOVA assessing the effects of storage time, training system, rootstock, and their interactions on the physicochemical parameters of grape juices. Storage time and training system significantly affected all variables ($p < 0.05$). Rootstock also showed a significant effect on all parameters, although its contribution to the total variance was generally smaller for some variables. Significant interaction terms were observed for most parameters, indicating that storage-related changes depended on the specific combination of training system and rootstock. In particular, pH, volatile acidity, soluble solids, reducing sugars, and alcohol content showed significant interactions involving storage time and its combination with training system and/or rootstock. In contrast, total acidity exhibited fewer interactions, suggesting that its variation during storage was less dependent on factor combinations and followed a more uniform pattern across treatments. Overall, these findings confirm that storage time, training system, and rootstock interacted to modulate juice composition.

Figure 2 shows the changes in physicochemical parameters during storage of juices produced from ‘BRS Magna’ grapes cultivated under three training systems (trellis, lyre, and espalier) and two rootstocks (IAC 572 and IAC 766).

During 24 months of storage, juice pH increased, whereas total acidity decreased. In freshly processed juices (time 0), the lowest pH (3.71) was observed in the lyre × IAC 766 combination (Fig. 2A). Throughout 6, 12, and 24 months of storage, these juices also exhibited the lowest pH values and the highest total acidity compared to the other training system and rootstock combinations (Fig. 2B), which is favorable for grape juice stability. These juices were also notable for maintaining similar volatile acidity up to 24 months of storage (Fig. 2C). In contrast, juices from the trellis × IAC 766 combination presented the highest pH values and the lowest total acidity, which may result in lower storage stability than the other samples. Higher pH values reduce the microbiological and oxidative stability of grape-derived beverages, favoring pigment degradation and accelerating browning reactions [32].

An increase in the SS content was observed during storage (Fig. 2D), except for the juices derived from the lyre × IAC 572 and espalier × IAC 766 combinations, whose levels remained unchanged over the 24 months of storage, suggesting lower rates of sugar degradation. Reducing sugar content also increased over time, although in juices from the espalier system with both rootstocks, this increase was observed only after 12 months of storage (Fig. 2E). The increase in reducing sugars during storage may result from the hydrolysis of polysaccharides by residual enzymatic activity. In the absence of undesired fermentation or sensory alterations, this change is considered normal and does not compromise product quality [33]. Alcohol content was experimentally determined throughout storage and remained below 0.5% in all samples after 24 months (Table 1). This value is below the maximum limit established by Brazilian

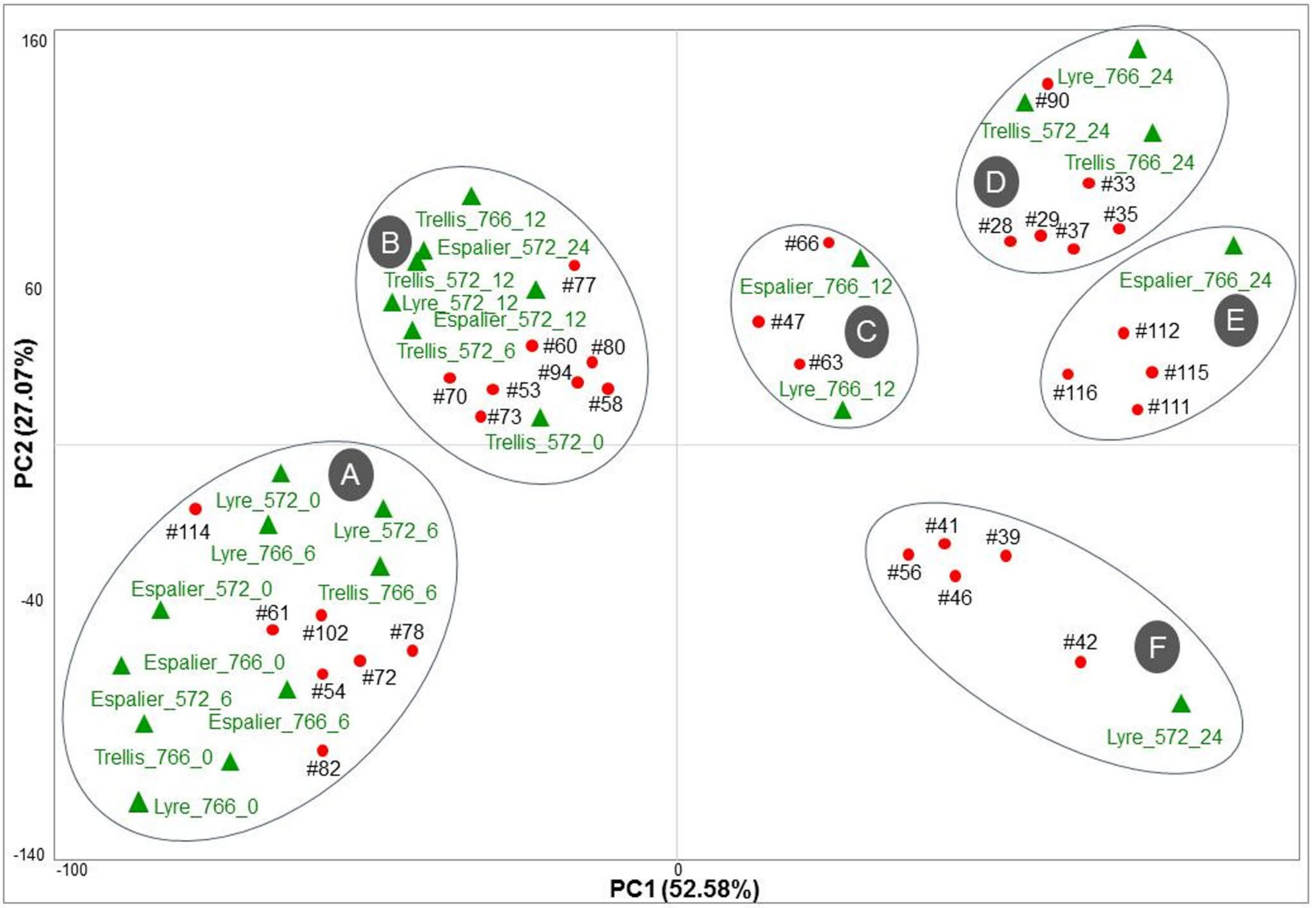


Fig. 5 Principal Component Analysis (PCA) performed using the concentration data of the 89 volatile compounds indicated by the Fisher ratio (Table S4) as the main factors responsible for differentiating the juices from ‘BRS Magna’ grapes. The grapes were grown using three training systems (trellis, lyre, and espalier) and two rootstocks (IAC 572 and IAC 766) and stored for 0, 6, 12, and 24 months

legislation [34] and the Codex Alimentarius [35] for grape juice classification, indicating negligible sugar consumption due to unwanted fermentation.

All samples complied with regulations for whole grape juice even after 24 months of storage [34, 35]. Total acidity exceeded 55.0 mEq L^{-1} in all samples (61.77–75.31 mEq L^{-1}), meeting Brazilian standards [34]. Volatile acidity ranged from 1.13 to 1.75 mEq L^{-1}, remaining below the maximum limits established by Brazilian legislation (10 mEq L^{-1}) [34] and Codex Alimentarius (6.7 mEq L^{-1}) [35]. SS varied between 16.3 and 19.2 °Brix, complying with the minimum requirements of Brazilian (≥ 14 °Brix) [34] and Codex Alimentarius (≥ 15 °Brix) [35] standards, regardless of storage time.

Phenolic profile, color parameters, and antioxidant activity

Table S1 presents the identification and quantification data of the 27 phenolic compounds determined in the grape juices by LC-DAD-ESI-QToF-MS. Tables S2 and S3 display the changes in antioxidant activity and color parameters, respectively, during storage. Given the well-established relationship between phenolic compounds and both color parameters and antioxidant capacity in fruit-derived products, the role of juice storage on these variables was assessed using PCA, as shown in Fig. 3.

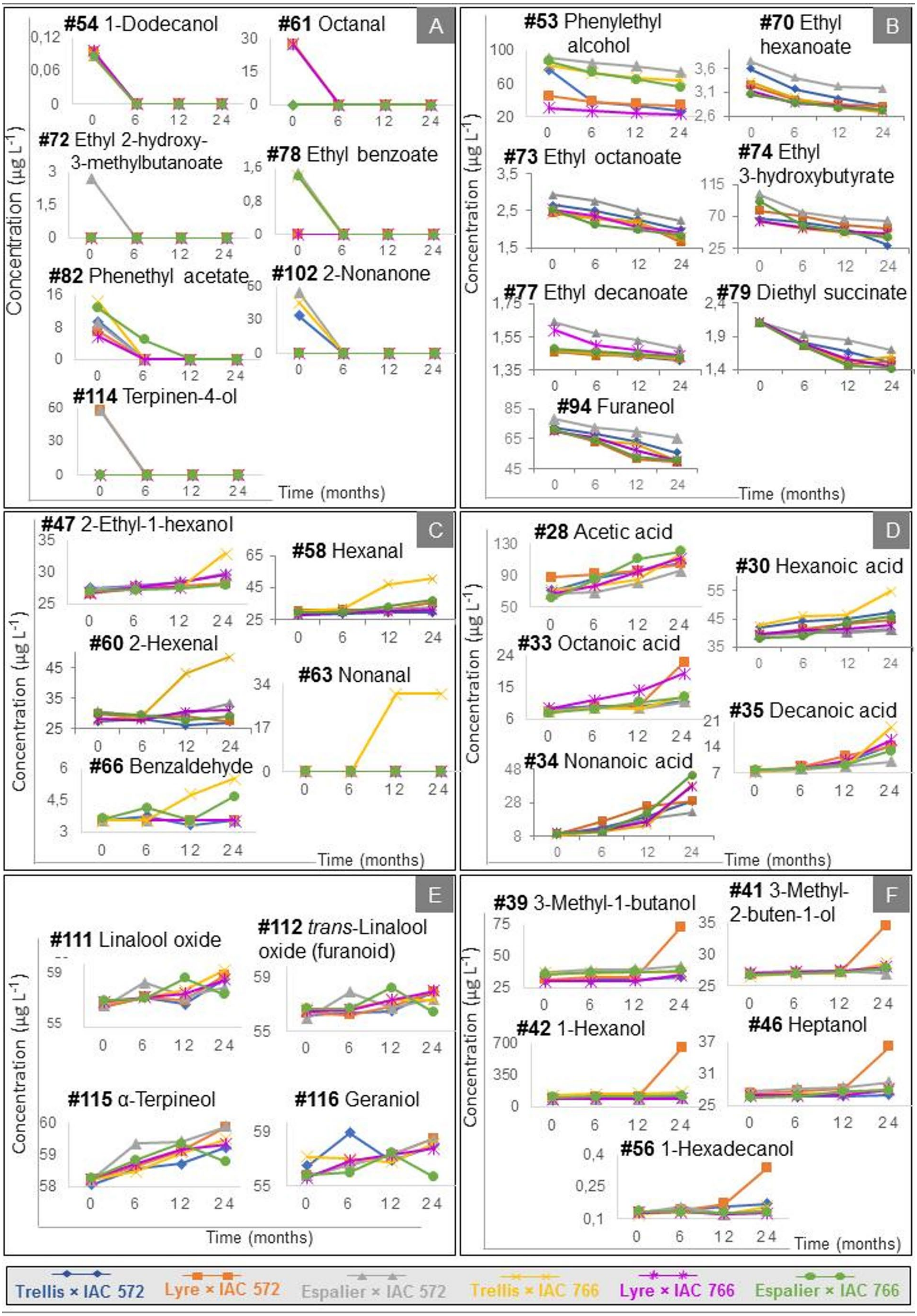
The first two principal components, PC1 and PC2, accounted for 71.72% of the variability in the data. Figure 3 shows the projection of PC1 and PC2, revealing four distinct clusters (A–D) based on the loadings. In group A, seven anthocyanins [malvidin-3,5-*O*-diglucoside (#17), cyanidin-3,5-*O*-diglucoside (#18), malvidin-3-*O*-glucoside (#19), delphinidin-3-*O*-glucoside (#20), cyanidin-3-*O*-glucoside (#21), pelargonidin-3-*O*-glucoside (#22), and petunidin-3-*O*-glucoside (#23)], three flavan-3-ols [procyanidin A2 (#10), (+)-catechin (#12), and (-)-epicatechin (#14)] and the stilbene *E*-piceatannol (#24) were associated with the antioxidant capacity against ROO· and H_2O_2 of the juices.

Figure 4 shows the behavior of the variables associated with each PCA cluster. Reductions in the concentration of phenolic compounds from group A of the PCA were observed throughout the 24 months of storage, except procyanidin A2 (#10), which decreased up to 12 months and subsequently increased at 24 months (Fig. 4A and Table S1). The increase in concentration of this flavan-3-ol may be attributed to the polymerization of (−)-epicatechin [36], whose levels decrease during storage. The reduction of anthocyanins (#17, #18, #19, #20, #21, #22, and #23) as storage time progresses is attributed to their structural instability under light exposure and fluctuations in pH and temperature [37]. Petunidin-3-O-glucoside (#23) was identified as the anthocyanin with the highest susceptibility to degradation, resulting in its absence in the juices of the trellis x IAC 572 and lyre x IAC 766 combinations after 24 months of storage. The progressive decline group A compounds during storage was accompanied by a significant reduction in antioxidant capacity against ROO· and H_2O_2 (Table S2). This pattern indicates that the loss of these phenolic compounds directly contributed to the decreased radical scavenging activity of the juices over time.

Despite reductions over time, juices from the espalier × IAC 572 combination consistently exhibited the highest anthocyanin concentrations throughout storage (Table S1), which was reflected in their superior antioxidant capacity (Table S2). Notably, antioxidant activity in this combination remained stable for up to 12 months and declined only thereafter, suggesting greater oxidative stability compared with the other treatments.

Cluster B (Fig. 3) encompassed gallic acid (#1), *trans*-caftaric acid (#2), quercetin-3-O-β-glucoside (#6), myricetin (#7), kaempferol-3-O-glucoside (#8), isorhamnetin-3-O-glucoside (#9), (−)-epigallocatechin gallate (#16), and ε-viniferin (#25), as well as with color parameters related to intensity and Hue. Fluctuations in these phenolic compounds (Fig. 4B) suggest the occurrence of concurrent formation and degradation reactions during storage. As shown in Table S1, juices from the espalier × IAC 572 combination exhibited the highest levels of seven (#1, #2, #6, #7, #8, #9, and #16) of the eight phenolic compounds associated with cluster B. Moreover, this was the only combination in which the levels of flavonols (#6, #7, #8, and #9) remained stable throughout the 24 months of storage. ε-Viniferin (#25) was the only compound that exhibited similar behavior during storage across all samples. This stilbene maintained its constant levels after up to 12 months of storage and was completely degraded in the juices stored for 24 months. Although ε-viniferin is formed through oxidative dimerization of resveratrol, its potential depolymerization back to monomeric stilbenes under storage conditions cannot be excluded [38]. In the present study, the disappearance of ε-viniferin after 24 months coincided with an increase in *trans*- and *cis*-resveratrol between 12 and 24 months (cluster C of the PCA), which may suggest a possible relationship between these compounds. Although the cleavage of ε-viniferin into resveratrol is a plausible hypothesis, further targeted kinetic studies are needed to demonstrate whether this pathway occurs under grape juice storage conditions.

Color parameters associated with group B (Fig. 3) exhibited variation during juice storage (Fig. 4B). Juices from the espalier × IAC 572 combination exhibited the most intense coloration and the highest Hue values both when freshly processed and during 24 months of storage (Table S3). This finding suggests that the combination of this training system


A
#54 1-Dodecanol
0,12
0,06
0
#61 Octanal
30
15
#72 Ethyl 2-hydroxy-3-methylbutanoate
3
1,5
#78 Ethyl benzoate
1,6
0,8
#82 Phenethyl acetate
16
8
#102 2-Nonanone
60
30
#114 Terpinen-4-ol
0
6
12
24
Concentration (µg L-1)
Time (months)
B
#53 Phenylethyl alcohol
100
60
20
#70 Ethyl hexanoate
3,6
3,1
2,6
#73 Ethyl octanoate
3,5
2,5
1,5
#74 Ethyl 3-hydroxybutyrate
115
70
25
#77 Ethyl decanoate
1,75
1,55
1,35
#79 Diethyl succinate
2,4
1,9
1,4
#94 Furaneol
85
65
45
C
#47 2-Ethyl-1-hexanol
35
30
25
#58 Hexanal
65
45
#60 2-Hexenal
45
35
#63 Nonanal
34
17
#66 Benzaldehyde
6
4,5
3
D
#28 Acetic acid
130
90
50
#30 Hexanoic acid
55
45
35
#33 Octanoic acid
24
15
6
#35 Decanoic acid
21
14
7
#34 Nonanoic acid
48
28
8
E
#111 Linalool oxide
59
55
#112 trans-Linalool oxide (furanoid)
#115 α-Terpineol
60
58
#116 Geraniol
F
#39 3-Methyl-1-butanol
75
50
25
#41 3-Methyl-2-buten-1-ol
35
30
#42 1-Hexanol
700
350
#46 Heptanol
37
31
#56 1-Hexadecanol
0,4
0,25
0,1
Trellis × IAC 572
Lyre × IAC 572
Espalier × IAC 572
Trellis × IAC 766
Lyre × IAC 766
Espalier × IAC 766

◀ **Fig. 6** Changes in volatile compounds during storage (0, 6, 12, and 24 months) of juices produced with 'BRS Magna' grapes grown under three training systems (trellis, lyre, and espalier) and two rootstocks (IAC 572 and IAC 766). Effect of juice storage on volatile compounds corresponding to PCA groups **A**, **B**, **C**, **D**, **E**, and **F** shown in Fig. 5. Values represent mean ± standard deviation (SD) ($n = 3$). Due to the low variability among replicates, error bars may not be visually distinguishable. SD values and ANOVA results are provided in Table S4 (Supplementary Material)

and the rootstock favored pigment preservation mechanisms, contributing to the maintenance of the visual quality of the juices in the long term.

Group C (Fig. 3) was associated with the compounds that demonstrated the most notable modifications between the 12th and 24th months of storage, which are related to the antioxidant capacity measured by the ABTS•+ method. In general, regardless of the training system and rootstock combination, caffeic acid (#3), *p*-coumaric acid (#4) and ferulic acid (#5) showed a decrease in concentration at 24 months of storage (Fig. 4). Conversely, procyanidin B1 (#11), *trans*-resveratrol (#26), and *cis*-resveratrol (#27) exhibited an increase in their levels between the 12th and 24th month of storage. These trends suggest that storage promotes differential metabolic transformations, in which hydroxycinnamic acids (#3, #4, and #5) may undergo degradation or conversion reactions. The accumulation of stilbenes (#26 and #27) and procyanidins (#11) could be associated with polymerization processes or stress-related metabolic responses triggered over time. Juices from the espalier × IAC 572 combination presented the highest concentrations of these compounds (Table S1), which was reflected in their superior antioxidant capacity against ABTS•+ compared with the other juices (TableS2), even after 24 months of storage.

Group D (Fig. 3) encompassed procyanidin B2 (#13), (-)-epicatechin gallate (#15), and the color parameters (a, b, C*, L*, and tonality), which reached their highest values after 24 months of storage across all samples (Fig. 4). The progressive increase in a* (red), b* (yellow), and tonality indicated a gradual color shift toward yellowish and orange tones. L (lightness) and C (chroma, an indicator of color intensity) also increased during storage, resulting in a lighter coloration and an enhancement of color saturation, respectively. The findings suggest that the degradation of anthocyanins (Table S1), as well as the development of pigments from phenolic compounds, results in a modification of the juice color during storage, as illustrated in group D. Although these differences might not be readily distinguishable by the naked eye, the instrumental measurements confirm progressive chromatic modification over time.

In Table S3, ΔE values are presented as indicators of the magnitude of chromatic alterations between freshly processed and stored samples. This parameter integrates the combined variations in the L, a*, and b* coordinates, providing an objective assessment of color perception. At 6 months of storage, juices from the trellis × IAC 572, trellis × IAC 766, and espalier × IAC 572 combinations exhibited minor color variation ($\Delta E < 1.5$). The other training system and rootstock combinations resulted in juices with color differences classified as 'medium' ($1.5 \le \Delta E^* < 3.0$), which persisted until the 12th month of storage. Juices from the lyre × IAC 766 combination deviated from this trend, exhibiting a large color change ($\Delta E = 3.70$) at the 12th month. At 24 months, notable color changes were detected in the juices ($\Delta E^* > 3.0$), resulting in a shift toward more yellowish tones, whereas juices from the espalier × IAC 572 combination maintained moderate color variation ($\Delta E^* = 2.75$). This finding suggests that prolonged storage has a reduced impact on the coloration of juices from this specific combination.

Volatile profile

Figure 5 illustrates the distribution of grape juices and volatile compounds along the first two principal components, which together explain 79.5% of the total variability in the dataset, indicating a robust model for sample discrimination. The PCA projection revealed six distinct clusters, labeled A, B, C, D, E, and F. Table S4 presents the concentrations of the volatile compounds, and their changes during storage is depicted in Fig. 6.

Group A of the PCA (Fig. 5) encompassed 1-dodecanol (#54), octanal (#61), ethyl 2-hydroxy-3-methylbutanoate (#72), ethyl benzoate (#78), phenethyl acetate (#82), 2-nonanone (#102), and terpinen-4-ol (#114). These compounds become non-detectable (<LD) within the initial six months of storage (Table S4and Fig. 6), which may reduce the pleasant perceptions of floral (#54, #82, #102), fruity (#78), citrus (#61), and herbaceous (#114) notes in all juices, regardless of the training system and rootstock.

Group B (Fig. 5) included compounds that progressively declined during storage (Fig. 6), potentially contributing to a reduction in the floral (phenylethyl alcohol #53) and fruity (ethyl# 77 and diethyl succinate #79) aroma notes. Furthermore, it is important to highlight the presence of **furaneol (#94)**, which is associated with strawberry-like notes and linked to the sensory perception known as "foxy." This descriptor is highly appreciated by consumers in grape juice and is characteristic of *Vitis labrusca* [39], *Vitis rotundifolia* [40], and hybrid grapes such as 'BRS Magna' [41]. Figure 6 and Table S4 show that the juices from espalier × IAC 572 presented higher levels of furaneol and other compounds from group B of the PCA, even after 24 months of storage. These results suggest that the combination of this conduction system and the rootstock favors the retention of these

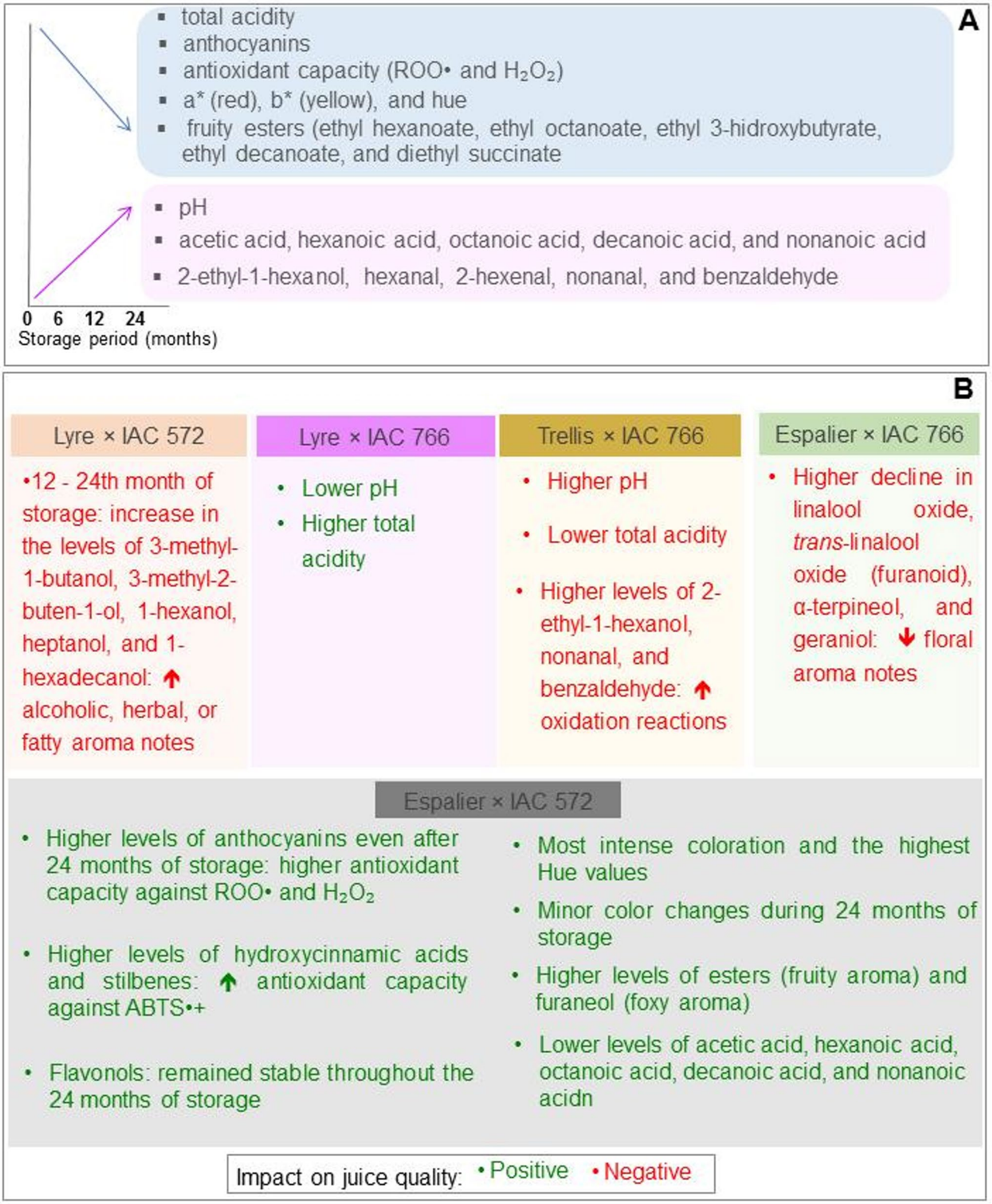


Fig. 7 Overview of the results from the metabolomic evaluation of grape juices **A** during storage and **B** according to the training system and rootstock. Positive and negative characteristics for stability/quality are indicated in green and red font, respectively

key metabolites, which may help preserve the characteristic sensory attributes of the grape juice during storage.

Cluster C (Fig. 5) comprised compounds whose concentrations increased from the 6th and/or 12th month of storage, with particular prominence in juices from the trellis × IAC 766 combination (Fig. 6), which showed marked increases in 2-ethyl-1-hexanol (#47), hexanal (#58), 2-hexenal (#60), nonanal (#63), and benzaldehyde (#66). The accumulation of these compounds results from oxidative reactions involving fatty acids and phenolic compounds, which may negatively impact juice quality by reducing freshness and promoting the development of less desirable sensory attributes [8].

Group D (Fig. 5) comprised compounds that exhibited a progressive increase during storage, including acetic acid (#28), hexanoic acid (#30), octanoic acid (#33), nonanoic acid (#34) and decanoic acid (#35), with the highest concentrations detected after 24 months (Fig. 6). The rancid and pungent odors associated with these fatty acids may become more pronounced in long-term stored juices, potentially compromising sensory acceptance [42]. According to Table S4, the juices from the espalier × IAC572 combination had the lowest concentrations of these compounds, even after 24 months of storage.

Group E included the compounds that exhibited oscillations in concentration during storage, such as linalool oxide (#111), *trans*-linalool oxide (furanoid) (#112), α-terpineol (#115), and geraniol (#116). These fluctuations indicate a dynamic balance between the release of glycosylated precursors and the rearrangement of monoterpenes, in contrast to losses from volatilization and degradation [8]. According to Fig. 6E, the juices derived from the espalier × IAC 766 combination showed the greatest decline in the levels of these compounds from the 12th to the 24th month of storage, resulting in a reduction in floral notes compared to the other juices.

Group F comprised compounds whose concentration increased between the 12th and 24th months of storage in juices from the lyre × IAC 572 combination. The increased concentration of 3-methyl-1-butanol (#39), 3-methyl-2-buten-1-ol (#41), 1-hexanol (#42), heptanol (#46), and 1-hexadecanol (#56) can impart aroma notes defined as alcoholic, herbal, or fatty. Furthermore, these higher alcohols are known to mask fresh and floral notes in beverages, which can negatively impact consumer acceptance [43].

Overview of the effect of juice storage according to the training system and rootstock used in grapevine cultivation

Figure 7 provides an integrative overview of the metabolomic evaluation of grape juices, including physicochemical parameters, color attributes, phenolic profile, and volatile composition throughout storage for the training system and rootstock combinations. This approach highlighted the main changes that occur during storage (Fig. 7A) and the impact of combinations of training systems and rootstocks on juice quality (Fig. 7B), thereby guiding the identification of grape production management strategies that favor the stability and preservation of sensory and functional characteristics during beverage storage.

Metabolomic analysis demonstrated that the storage of grape juices was associated with reductions in total acidity and anthocyanin levels, accompanied by color shifts toward orange hues and a decline in esters responsible for fruity aroma (Fig. 7A). Conversely, increases in pH, fatty acid levels, and certain aldehydes were observed throughout storage. These modifications were observed in all juices, irrespective of the training system or rootstock employed. Nevertheless, the extent of such alterations was dependent on the specific combination of training system and rootstock. In Fig. 7B, the parameters that differentiated each juice in the metabolomic analysis are presented in green and red font, indicating positive and negative impacts on juice quality, respectively. Juices from the trellis × IAC 572 combination showed no distinct positive or negative features, whereas lyre × IAC 572, trellis × IAC 766, and espalier × IAC 766 were characterized by traits associated with reduced stability and quality.

Juices from the lyre × IAC 766 combination were distinguished by lower pH and higher volatile acidity compared to those from the other training system and rootstock combinations, a profile that may favor stability during storage. In contrast, juice from the espalier × IAC 572 combination stood out for the greater number of parameters associated with enhanced quality and stability. These juices exhibited the highest levels of phenolic compounds, particularly anthocyanins, flavonols, stilbenes, and hydroxycinnamic acids, which translated into higher antioxidant activity, greater color intensity, and lower color changes during 24 months of storage. Moreover, this combination resulted in juices with the highest concentrations of ethyl esters imparting fruity notes, as well as furaneol, a key aroma marker described as raspberry, characteristic of the studied grapes. Conversely, the lowest levels of acids associated with rancid and pungent odors were detected compared to juices from the other training systems and rootstock combinations.

The espalier system promotes greater solar exposure on grapes than other training systems, while the IAC 572 rootstock exhibits higher vegetative vigor than IAC 766. Yin [44] reported that sunlight exposure modulates the biosynthesis of different subclasses of phenolic compounds by activating the phenylpropanoid pathway, which is primarily regulated by UV-responsive transcription factors (e.g., MYB, bHLH, bZIP). This regulation results in specific metabolic responses for each compound class. Enzymes such as chalcone synthase (CHS), dihydroflavonol reductase (DFR), and flavonol 3-O-glycosyltransferase (UFGT) are upregulated, leading to increased accumulation of anthocyanins in the berry skins. Similarly, the expression of flavonol synthase (FLS) and stilbene synthase (STS) is associated with the synthesis of flavonols and stilbenes, respectively. In the case of hydroxycinnamic acids, sunlight exposure induces regulatory enzymes such as phenylalanine ammonia-lyase (PAL) and cinnamate-4-hydroxylase (C4H), resulting in their accumulation in the berries. Furthermore, phenolic compound synthesis represents an adaptive mechanism to radiation, as these compounds function as photoprotective barriers against oxidative stress. Enhanced antioxidant activity mitigates oxidative processes that would otherwise promote fatty acid accumulation linked to off-flavors. Solar radiation can also modulate the activity of enzymes, such as alcohol acyltransferases, which catalyze esterification, leading to the accumulation of esters, which are responsible for fruity aromas [45].

Regarding the role of rootstock, higher vegetative vigor enhances photosynthetic capacity and the supply of metabolic precursors to the phenylpropanoid pathway, which drives the synthesis of phenolic compounds [46] reported that the increase in photosynthetic activity also enhances the availability of important precursors, such as pyruvate and acetyl-CoA, which are essential for the biosynthesis of esters.

Conclusions

This study assessed for the first time the storage potential of tropical grape juices produced from various combinations of training systems (espalier, trellis, and lyre) and rootstocks (IAC 572 and IAC 766). Over a 24-month storage period, changes in physicochemical characteristics, color, phenolic profile, and volatile compounds were observed regardless of the type of rootstock and training system used. Nevertheless, all juices exhibited total acidity, volatile acidity, and soluble solids content in accordance with the standards set by the Codex Alimentarius and Brazilian legislation. Therefore, the traditionally used shelf life of 24 months for grape juices from temperate climate regions is also suitable for tropical juices.

The metabolomic assessment revealed that the greater solar exposure and vegetative vigor associated with the espalier × IAC 572 combination resulted in enhanced juice stability throughout storage. These juices exhibited higher levels of anthocyanins, flavonols, stilbenes, and hydroxycinnamic acids, resulting in greater antioxidant activity and color stability compared to the other combinations of training system and rootstock. In addition, the espalier × IAC 572 juices showed higher concentrations of fruity esters and furaneol, a characteristic marker of the studied grapes that imparts a strawberry-like aroma. These findings highlight the role of vineyard management practices in the postharvest behavior of grape-derived products, providing valuable insights for optimizing juice production to ensure greater stability during storage.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-026-05125-5.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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