


## Research

# Exploring grape juice volatile compounds after inactive yeast extract foliar treatment using SPME and GC–MS

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## Abstract

This research aims to investigate the effects of foliar application of selected inactivated yeast on grape varieties of juice ('Isabel', 'Isabel Precoce', 'Ives', 'Concord', and 'BRS Violeta') cultivated at the *Centro Avançado de Pesquisa e Desenvolvimento de Frutas* of the *Instituto Agrônômico (IAC)*, using vertical shoot-positioning and trellis systems. The experimental design utilized was a randomized block layout with four replicates. The study evaluated plant productivity and physical characteristics of clusters and berries. Furthermore, the grape juice extracted from these grape varieties underwent analysis for volatile compounds in a two-year study period. The volatile profile of the grape juice was determined using headspace solid phase microextraction (SPME) coupled with gas chromatography combined with mass spectrometry (GC–MS). At the end of this research, despite the application of yeast extract, no significant impact on vine productivity or the physicochemical parameters of the berries was observed. The varieties exhibited distinct responses to the treatment, with 'Isabel' showing the least responsiveness while 'Ives' demonstrated the most significant response. Overall, the treatment was most effective in influencing esters, followed by ketones. This indicates that the impact of the treatment varied across different grape varieties and volatile compositions, highlighting the importance of considering varietal differences in grape cultivation practices and product quality.

**Keywords** Elicitors · Preharvest · *Saccharomyces cerevisiae* · Training systems · *Vitis labrusca*

## 1 Introduction

In viticulture, environmental factors such as soil composition and climate play a crucial role in shaping the characteristics of grapes and their byproducts. In recent years, significant progress has been made in grapevine management, production cycles, and the development of new grape varieties adapted to the conditions of the Southeast Region of Brazil [1]. In grape juice production, the primary varieties utilized are *Vitis labrusca* L. and its hybrids. These grapes are typically cultivated using the vertical shoot-positioning system (VSP) or the trellis system (TS). The choice of training system,

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coupled with canopy management practices, can significantly influence the microclimate surrounding the clusters [2]. Harvesting grapes for juice production in this region predominantly occurs during the summer months. However, the climate conditions during ripening pose challenges, particularly due to high levels of rainfall. These conditions increase the susceptibility of grapes to fungal diseases, impacting their sanitary quality. Additionally, the grapes' phenolic composition and sugar content can be affected [3].

Steam extraction is a cost-effective technique commonly used for juice production, although it may lead to a reduction in soluble solids content. Several studies have reported a decrease in this parameter compared to the content found in grapes [4–7]. Grape juice holds significant importance for the Brazilian population as it contributes to the intake of beneficial polyphenols such as stilbenes, anthocyanins, and proanthocyanidins [8–10]. The main cultivars utilized for grape juice production in Brazil are of American origin, including 'Isabel', 'Bordo/Ives', and 'Concord' (*Vitis labrusca* L. and its hybrids, *Vitis labrusca* x *Vitis vinifera*). These cultivars are preferred due to their high productivity, disease resistance, nutritional properties, and unique flavor [11, 12].

Several agronomic techniques have been studied to mitigate the potential negative impacts of climate change on grapes and their juice. These include the use of elicitors such as chitosan, methyl jasmonate, yeast extract, and UV-C radiation, which have shown promising results [5, 13–15]. Yeast extract (YE) contains several compounds that function as elicitors, including  $\beta$ -1,3 and  $\beta$ -1,6 glucanases, chitin from yeast cell walls, lipids from membranes composed of sterols, and proteins. LalVigne® MATURE is a specific formulation developed for foliar application, with the patented technology (WO/2014/024039) by Lallemand Inc., Canada [13, 15]. Research conducted with 'Nebbiolo' grapes (*Vitis vinifera* L.) in Italy aimed at wine production observed an increase in berry skin thickness, enhanced accumulation of anthocyanins, and the wines were richer in free volatile esters [16]. The application of YE on 'Agiorgitiko' (*Vitis vinifera* L.) grapes cultivated in the semiarid climate of Southern Greece did not show any significant effect on wine parameters such as color intensity, pH, alcohol content, or titratable acidity. Additionally, it resulted in the lowest concentration of anthocyanins. However, when YE was associated with irrigation practices, it led to higher concentrations of anthocyanins and total phenols in the wines [17].

The association of YE with canopy management techniques, when applied to grapes cultivated in the Southeast Region of Brazil with the aim of grape juice production, holds significant potential for quality enhancement. It is hypothesized that this treatment may have a positive impact on volatile compounds, thereby improving the aromatic profile of the juices. However, it's important to note that during the juice extraction process, various factors such as thermal degradation, water vapor drag, and volatilization can potentially diminish the aromatic typicality of the juices [18]. Therefore, the objective of this research is to assess the combined effect of foliar application of YE LalVigne® Mature and vertical shoot-positioning (VSP) and trellis system (TS) as training systems on grape physicochemical parameters and the volatile compounds present in the juice.

## 2 Material and methods

### 2.1 Plant material and experimental design

Treatments were conducted over 2021/2022 (harvest 2022) and 2022/2023 (harvest 2023) seasons with 'Isabel', 'Isabel Precoce', 'Ives', 'Concord', and 'BRS Violeta' grape varieties grafted onto 'IAC 572' rootstock (101–14 MGT (*Vitis Riparia* x *Vitis rupestris*) x *Vitis caribaea*). According to *Vitis* International Variety Catalogue (VIVC—vivc.de) 'Isabel' are a *Vitis* Interspecific crossing among *Vitis labrusca* x *Vitis vinifera*. 'Isabel Precoce' is a spontaneous somatic mutation of 'Isabel'. 'Ives' is a *Vitis* Interspecific crossing created by Ives Henry, in the United States of America. 'Concord' was also created in the United States of America, is a *Vitis* Interspecific crossing between *Catawba* x *Vitis labrusca* linne. 'BRS Violeta' was created in Brazil by Brazilian Agricultural Research Agency EMBRAPA, is a *Vitis* Interspecific crossing between *Traviu* x *Rubea*. The plant materials used in our study complied with local and national guidelines.

The experiments took place in Jundiaí, São Paulo State, Brazil, located at 23°17'S and 46°9'W, with an altitude ranging from 730 m. The vines were grown in an eleven-year-old vineyard trained on the vertical shoot-positioning system (VSP) and the overhead trellis system (TS), with row spacing of 2 m and within-row spacing of 1 m. Standard vineyard management practices for the region, including pruning, pest and disease control and nutrient management, were applied to all vines. Vine pruning was conducted in August, while flowering happened at the end of October. The treatments were applied during the *veraison*, which occurred in November and December, with harvest taking place in January for all varieties. Weather conditions were recorded and collected using an Automatic Meteorological

Station (EMA, Davis, USA), and data were gathered through the GroWeather software (GroWeatherlink®, Version 1.2, Davis Instruments Corp. 1999).

For each training system/cultivar, two blocks were used, control (CT) versus treatment (YE). The experimental design utilized was a randomized block layout with four replicates. Each block consisted of twelve vines, further divided into two rows with six vines per row. The treatment with inactive dry yeast (YE) was prepared in accordance with the manufacturer's instructions (LalVigne® Mature, Lallemand Inc., Montreal, Canada) at a concentration of 1 kg ha<sup>-1</sup>. The application was carried out twice during ripening: first at 5% of *veraison*, BBCH code 81, and approximately 10 days after the first application.

The solution was uniformly sprayed over the entire canopy using a spray irrigator Yamaha model FT-16S, with 16 L of capacity, without causing dripping. Water was used for the control (CT) group. The grapes were harvested at commercial maturity, according to the viticultural parameters of each region.

## 2.2 Grape juice processing

The juice extraction process utilized a steam juice extractor (Stamp Inox, Caxias do Sul, Rio Grande do Sul State, Brazil). Grapes from the YE and CT groups harvested in 2022 and 2023 were hand-destemming, and 10 kg of berries for each treatment were placed in the extraction chamber. Juice extraction was conducted using steam for 75 min [5]. Subsequently, the extracted juice was pasteurized at 80 °C and bottled without the addition of preservatives in glass bottles previously sterilized and closed with screw caps. Three replicates of the processing were performed for each treatment, resulting in three lots for each block.

## 2.3 Grape physicochemical characteristics and juice basic parameters

For these evaluations, six representative clusters per experimental plot were selected. The chemical characteristics of the grapes were assessed by measuring soluble solids (SS, expressed in °Brix), titratable acidity (TA, expressed as g tartaric acid. L<sup>-1</sup>), and pH of the must analyses. The physical characteristics of the clusters, rachis, and berries were evaluated by determining the mass (g) of clusters (FMC), berries (FMB), and rachis (RM). Additionally, berry length (BL) and width (BW), as well as rachis length (RL) and width (RW) following the guidelines provided by IPGRI—International Plant Genetic Resources Institute. [19]. Yield productivity (t ha<sup>-1</sup>) was estimated as a function of the yield per vine and the spacing between them, considering a planting density of 4000 plants ha<sup>-1</sup>.

Juice basic parameters, such as soluble solids (SS), density, pH, titratable acidity and volatile acidity were measured using WineScan™ SO2—FOSS [20].

## 2.4 Free volatile compound extraction and GC–MS analysis

The sample preparation occurred with 5 mL of grape juice, which was added to a 20 mL solid-phase microextraction (SPME) glass vial, along with 1.5 g of saturated NaCl solution and 5 µl of the internal standard (2-octanoate at 44.8 mg L<sup>-1</sup>). A quality control (QC) sample was prepared by pooling a mixture of all samples. The vials were tightly capped with a Teflon/silicone septum and stored at –20 °C until analysis. The analysis of free volatile compounds was conducted following the method described by Moro et al. [21] with some modifications. SPME was performed using a 100-µm fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, Pennsylvania, USA), which was thermally conditioned for 1 h at 270 °C prior to use. For a sampling of volatiles, the vials were incubated at 40 °C for 10 min. The SPME fiber was then exposed to the sample for 40 min at 40 °C under agitation with a magnetic stir bar [21].

The GC analysis was conducted using an HP6890 chromatograph coupled with an HP5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA), fitted with a Sulpelcowax 10 column (60 m × 0.25 mm I.D. × 0.25 µm). The SPME fiber was inserted into the inlet at 250 °C in splitless mode. The purge was activated at 2 min, and helium was used as the carrier gas in constant flow mode at a rate of 1 mL min<sup>-1</sup>. The oven temperature was held at 35 °C for 3 min, programmed at 6 °C min<sup>-1</sup> to 250 °C, and held for 5 min isothermally [21]. Mass spectra were acquired over m/z 33–250 [21].

## 2.5 Data processing and compound identification

For data acquisition Chemstation software version G1701EA E.02.00.493.33 was used, while for data processing, Agilent MassHunter Qualitative Analysis software (v. B.07.00) was used. Compound identification was performed by several assignments: to determine retention indices, a C7- C30 n-alkane standard was run under the same conditions, and linear retention indices were calculated using standard approaches. The mass spectra of volatile compounds were compared to those in the NIST library, version 2011 [21]. The internal standard response was used for normalization and to make a relative estimation of the concentrations of identified compounds.

## 2.6 Statistical analysis

For the Principal Component Analysis—PCA, the data were expressed as the normalized chromatographic area (area of the volatile compound by the area of internal standard), log transformation, and Pareto scaling, in addition to other multivariate analyses, applying MetaboAnalyst 6.0 software (<https://www.metaboanalyst.ca/>).

# 3 Results and Discussion

## 3.1 Climatic conditions and yield parameters

The climatic conditions during this period are shown in Fig. 1. Although there were fluctuations in mean temperatures, the thermal amplitude (difference between minimum and maximum temperatures) did not significantly vary

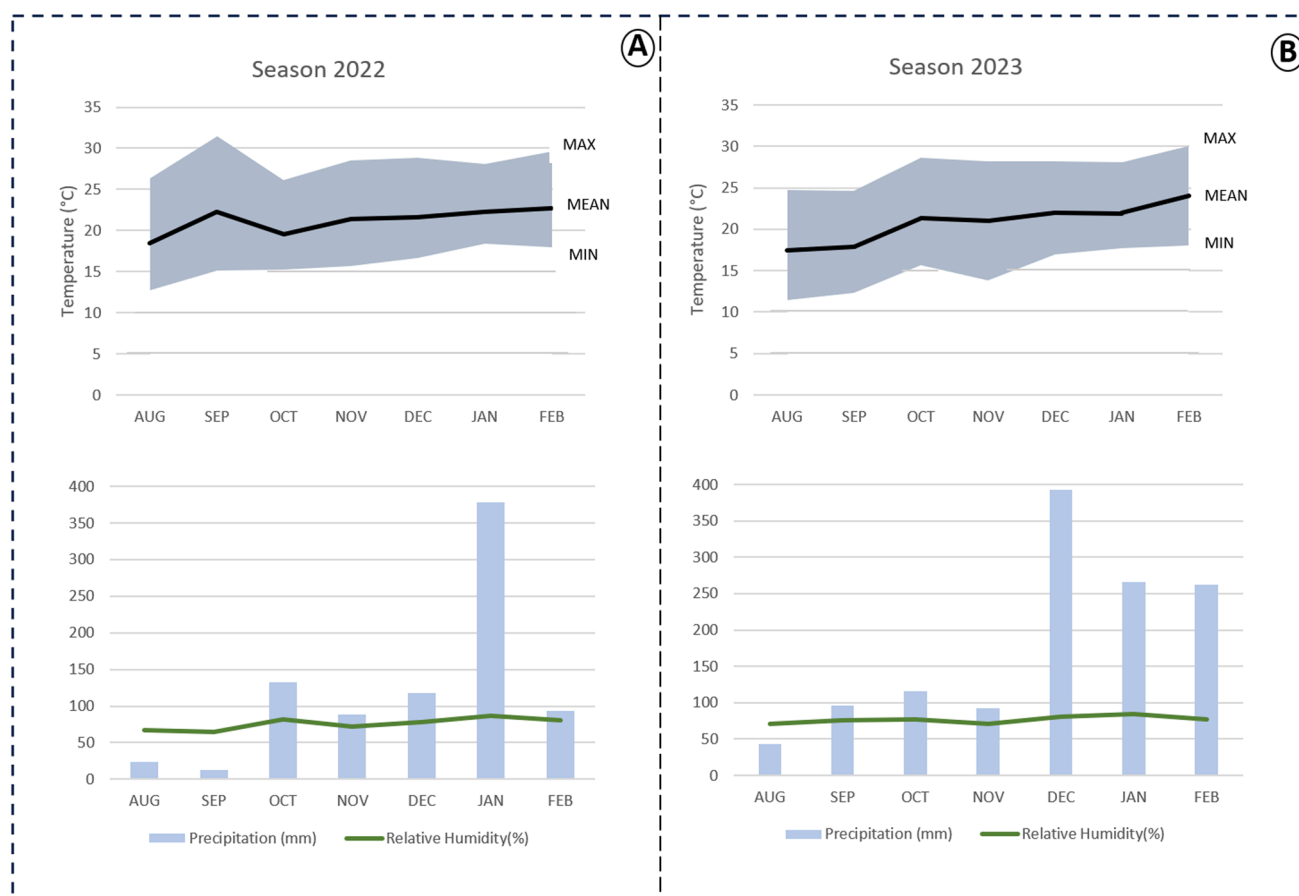


Fig. 1 Climatic conditions of grapes harvested in 2022 (A) and 2023 (B)

across the years. The highest rainfall was recorded in January during the 2022 season, while in the 2023 season, these levels were reached in December, followed by January.

Temperature is indeed one of the key factors influencing grapevine phenology. Variations in conditions such as radiation, grape cultivar, and terroir can significantly impact the biosynthesis of volatile compounds and their precursors. Concentrations of these compounds typically increase during berry development. However, it's important to note that different classes of compounds exhibit distinct chemical characteristics and organoleptic properties [2, 22, 23]

Analyzing the cultivars individually, 'Concord' was the most productive cultivar in 2022, while 'Isabel Precoce' was in 2023. Interestingly, for both years, 'Isabel Precoce' had the highest number of clusters (Supplementary Table S1). In both consecutive years, 'BRS Violeta' was the least productive variety regardless of the training system, with yields ranging from 2.86 to 11.86 tons ha<sup>-1</sup> (Supplementary Table S1).

Regarding the training system and YE effect, higher productivity was mostly observed in the CT group for the VSP system. For TS, similar behavior was detected, with the exception of the 'Isabel' variety in both seasons (Supplementary Figure S2). This finding is particularly promising as the 'Isabel' variety is among the most cultivated and processed grapes for juice production [7, 12]. The five varieties studied in our research accounted for 69% of all red grapes processed in the Rio Grande do Sul State during the 2021 season, with 'Ives' and 'Isabel' being responsible for 29.84% and 31.88%, respectively. Due to its flavor characteristics and especially anthocyanin content, its demand in wineries is still high in many Brazilian regions. These findings are consistent with observations made for YE treatments in 'Tempranillo' grapes cultivated in La Rioja, Spain, where no significant effect was observed on yield per vine (kg vine<sup>-1</sup>) [15]. Similarly, in 'Sangiovese' grapes cultivated in Bologna area, Italy, no differences were observed in terms of the number of bunches, yield per vine, cluster weight, and compactness after YE treatment [13].

### 3.2 Grape physicochemical characteristics

Due to the interaction between the training system and variety, we chose to evaluate the treatment impact on grape chemical parameters individually for each variety and training system, using data from each year. Acidity levels were elevated in 'Concord' grapes treated in both training systems, while for 'Isabel Precoce', this result was only observed in the VSP system (Supplementary Table S2). Acidity is crucial for grape byproducts as it helps maintain a lower pH, ensuring product safety and anthocyanin stabilization. Moreover, acidity plays a significant role in sensory perception, contributing to freshness and balance the soluble solids (SS). Additionally, an increase in acidity is generally well-received by the viticultural community. This is particularly important, given the observed decrease in acidity, especially malate, on warm growing seasons due to respiration rates associated with higher temperatures [2, 24].

Despite no significant difference being detected for SS content, a trend was observed with higher content in the CT group (Supplementary Table S2). These results agree with previous report by Kogkou et al. [17] in cv. Agiorgitiko (*Vitis vinifera* L.) cultivated in Greece, where yeast extract alone didn't promote any effect on the berries regarding physicochemical parameters. Similarly, Pastore et al. [25] did not detect significant changes in 'Sangiovese' (*Vitis vinifera* L.) cultivated in Italy.

Physical characteristics of the bunches (Supplementary Table S3) showed no significant effect of YE treatment for most of the parameters for clusters and rachis. Only the berry length of 'Isabel' berries from the VSP training system harvested in 2022 showed a significant difference. Berry size is an important variable not only regarding volatile compounds but also secondary metabolism in general, as it is where biosynthesis takes place and most of the secondary compounds are concentrated. Previous studies have detected significant changes in skin mechanical properties induced by YE treatment [16]. In the context described, the fresh mass of clusters (FMC) emerged as an important feature for all grapes from the TS system, while for the VC system, 'Isabel' variety was the only exception. Independent of training system, and harvest, 'Ives' variety presented the lightest grapes clusters.

In general, in 2022 season, heaviest clusters were observed in the CT group, whereas the opposite was observed for the 2023 season. These findings align with those reported by Kogkou et al. [17], where berry weight was significantly influenced by water status, with higher values observed in CT vines. The effect of climatic conditions during growing conditions, can also be accounted for, but not fully responsible for treatment effectiveness. The highest amount of rain that was detected on January on 2022 season, were achieved in December followed by January on 2023.

### 3.3 Grape juice characteristics

Chemical attributes of grape juices treated with YE conducted under different training systems (Table 1) shows that most of the juices produced in the study did not comply with the current regulation. According to Brazilian regulatory instructions, integral grape juices should have a minimum soluble solids (SS) content of 14 °Brix [26]. Furthermore, when compared to the SS content of the grapes ( Supplementary Table S2), a decrease was observed in the juices (Table, 1). This result was previously described by others who also used steam extraction for juice production [4, 5, 7]. Although the SS content in the juices from the YE treatment was statistically higher than those from the CT group, particularly in 'Concord' juices from TS system (2022 season) and 'BRS Violeta' from both training systems (2023 season). But, no treatment, managed to reach the minimum required by Brazilian regulatory instructions. It's important to highlight, as observed on Fig. 1, the heavy rain during ripening favored the development of fungal diseases, in addition to insect attacks, leading to advance the harvest date. However, pH and total acidity values were within the range described for juices extracted using the steam method in previous studies [5, 7]. Despite the rainy period close to the harvest time, all the juices contained less than 0.6 g L<sup>-1</sup> of maximum volatile acidity, thus complying with Brazilian regulations for integral juice [26].

### 3.4 Effect of YE treatment on volatile composition

The aroma is formed by low molecular weight volatile compounds belonging to several chemical classes. Its importance as an attribute for acceptance and quality parameter for grape juice it is undeniable. In this study, we identified a total of 87 compounds across both harvests (Supplementary Table S5 and Fig. 2). The majority of the identified compounds belonged to the terpenoid family, totaling 24 compounds. Followed by esters (20 compounds), alcohols (11 compounds), ketones (10 compounds), and aldehydes (7 compounds). It was also identified: ethers, benzenoids, carbonyls, higher alcohols, volatile fatty acids, and volatile phenols.

Principal Component Analysis (PCA) plots demonstrated clear separation among most grape varieties during Harvest 2022. However, for Harvest 2023, 'Isabel', 'Ives', and 'Isabel Precoce' formed a large cluster (purple, cyan, and pink, respectively). Additionally, in both harvests, QC samples (yellow) were positioned in the middle of all samples in the PCA plots, demonstrating the data set quality.

The compounds identified in our study were similar to those observed by Viana et al. [27], that determined the volatile profile of 'Bordo/Ives', 'Concord', and 'Isabel' (*V. labrusca*) musts. Their study identified several chemical classes including carboxylic acids, alcohols, aldehydes, ketones, esters, furans, and terpenoids. Similarly, the volatile profile for grape juices from 'Ives', 'Isabel Precoce', 'Concord Clone 30', 'BRS Rúbea', 'BRS Cora', 'BRS Carmen', 'BRS Violeta', and 'BRS Magna', were described by five chemical classes: esters, aldehydes, alcohols, ketones, alkenes, acids, and monoterpenes [11]. The grapes used in our study, also known as 'American grapes,' are often described with the distinct "foxy" flavor attributed to their *Vitis labrusca* parentage. The term "foxy" aroma encompasses a complex set of characteristics, including a unique, earthy, and sweet muskiness that is typically present in most *V. labrusca* and some derived hybrid grapes [28, 29]. These findings suggest consistency in the volatile profiles across different studies and provide valuable insights into the aroma composition of grape juices from different cultivars.

'Concord' grapes are very popular for the production of non-fermented juice and jellies due to their desirable fruity aromas, which are associated with compounds such as methyl anthranilate, o-aminoacetophenone, furaneol, and β-damascenone [29, 30]. On the other hand, 'BRS Violeta', grape juices are characterized by vegetal notes, primarily attributed to the higher concentration of terpenes, methoxyphenols, and C6 alcohols [31]. 'Isabel' juices are characterized by their volatile esters, which promote characteristics associated with fresh fruit flavors. These qualities are particularly appreciated by Brazilian juice consumers [32]. Similarly, juices from 'Ives' grapes exhibit notes described as sweet, grape, blackberry, and roses, highlighting their aromatic complexity and fruity characteristics [33].

As described, each grape variety possesses unique characteristics that contribute to its distinct flavor and aroma profile. While juices available on the market are often composed of blends among different grape varieties, allowing the complementation of the different profiles (such as chromatic and aromatic). Our study focused on detecting the specific impact of YE treatment. Therefore, juice extraction was performed individually for each grape variety and training system, enabling to assess the effects of the treatment on each variety's characteristics independently.

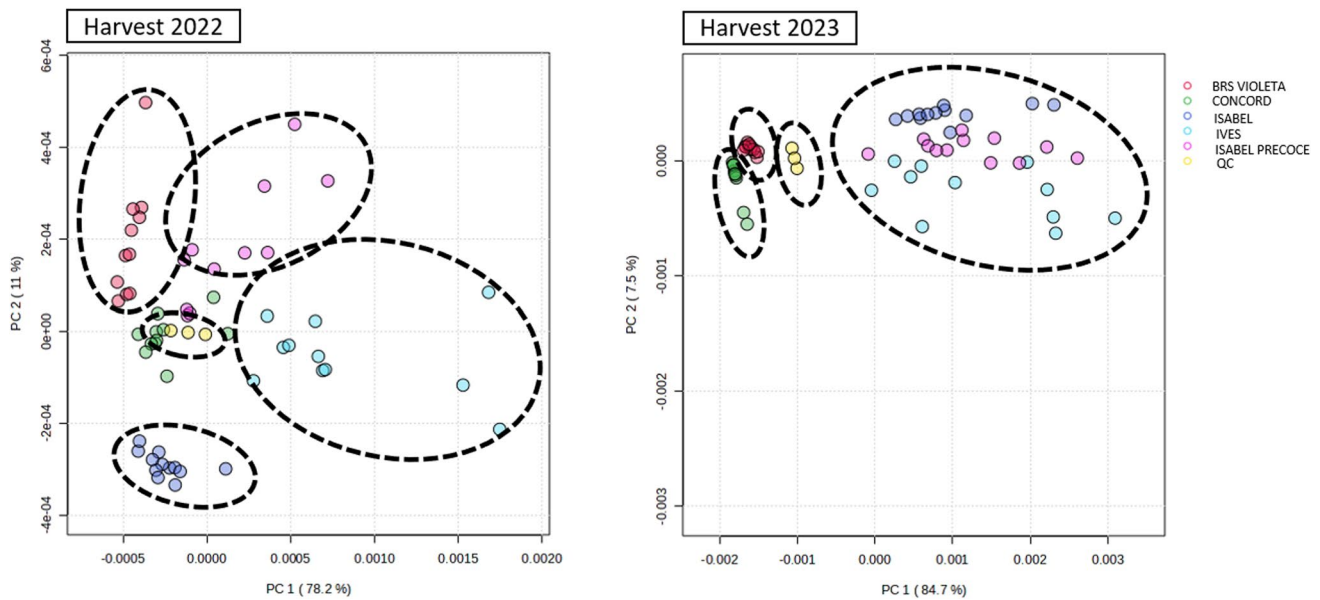
Previous studies have demonstrated significant differences in the accumulation and evolution of volatile compounds during grape ripening, influenced by variety and growing region in *Vitis vinifera* grapes cultivated in Italy

**Table 1** Chemical attributes of grape juices treated with YE conducted under different training systems

Varieties	T.S	Treatment	SS (°Brix)	Density (g ml <sup>-1</sup> )	pH	Total Acidity (g L <sup>-1</sup> )	Volatile Acidity (g L <sup>-1</sup> )
<b>Harvest 2022</b>							
<b>A</b>							
BRS Violeta	VS	CT	14.2±0	1.059±0	3.53±0.1	7.25±0.1	0.1±0
BRS Violeta	VS	YE	13.9±0	1.058±0	3.52±0	7.00±0	0.0±0
BRS Violeta	TS	CT	13.9±0	1.058±0	3.65±0	6.80±0	0.0±0
BRS Violeta	TS	YE	13.9±0	1.058±0	3.67±0	6.80±0	0.0±0
Concord	VS	CT	14.4±0	1.059±0*	3.33±0	7.50±0	0.1±0
Concord	VS	YE	13.5±0	1.055±0	3.36±0	7.55±0.1	0.1±0
Concord	TS	CT	12.7±0 *	1.052±0*	3.37±0	7.40±0	0.2±0
Concord	TS	YE	13.5±0	1.055±0	3.37±0	7.30±0	0.2±0
Isabel	VS	CT	14.5±0	1.059±0	3.33±0.1	7.85±0	0.15±0
Isabel	VS	YE	15.0±0	1.061±0	3.36±0.1	7.90±0	0.2±0
Isabel	TS	CT	13.2±0	1.054±0	3.37±0	7.60±0*	0.2±0
Isabel	TS	YE	13.5±0	1.055±0	3.34±0	7.85±0	0.2±0
Isabel Precoce	VS	CT	15.1±0	1.062±0*	3.34±0	7.20±0*	0.2±0
Isabel Precoce	VS	YE	13.8±0	1.057±0	3.32±0	7.30±0	0.2±0
Isabel Precoce	TS	CT	14.1±0	1.058±0*	3.40±0*	7.10±0	0.2±0
Isabel Precoce	TS	YE	14.5±0	1.059±0	3.34±0	7.40±0	0.2±0
Ives	VS	CT	13.1±0	1.053±0*	3.50±0	7.25±0*	0.1±0
Ives	VS	YE	13.1±0	1.054±0	3.50±0	7.40±0	0.1±0
Ives	TS	CT	13.2±0 *	1.054±0*	3.58±0	7.10±0	0.1±0
Ives	TS	YE	12.9±0	1.053±0	3.53±0	7.00±0	0.1±0
<b>Harvest 2023</b>							
<b>B</b>							
BRS Violeta	VS	CT	13.3±0*	1.015±0*	3.27±0.1*	3.40±0*	0.01±0
BRS Violeta	VS	YE	13.9±0	1.017±0	3.38±0.1	3.20±0	0.0±0
BRS Violeta	TS	CT	13.6±0*	1.016±0	3.61±0	2.80±0	0.01±0
BRS Violeta	TS	YE	13.8±0	1.017±0	3.59±0.1	2.80±0	0.0±0
Concord	VS	CT	13.0±0*	1.055±0*	3.07±0*	8.40±0*	0.3±0
Concord	VS	YE	12.5±0	1.053±0	3.11±0.1	8.80±0	0.3±0
Concord	TS	CT	11.8±0	1.050±0*	3.18±0*	8.20±0	0.3±0
Concord	TS	YE	12.2±0	1.052±0	3.22±0	8.00±0	0.3±0
Isabel	VS	CT	13.1±0*	1.056±0*	3.14±0*	9.20±0	0.3±0
Isabel	VS	YE	12.6±0	1.053±0	3.10±0	9.20±0	0.3±0
Isabel	TS	CT	11.9±0	1.050±0	3.18±0	8.50±0	0.4±0
Isabel	TS	YE	11.9±0	1.051±0	3.16±0	9.00±0	0.4±0
Isabel Precoce	VS	CT	14.5±0	1.019±0	3.08±0*	4.10±0	0.0±0
Isabel Precoce	VS	YE	14.2±0	1.018±0	3.15±0	4.40±0	0.0±0
Isabel Precoce	TS	CT	13.3±0	1.057±0	3.25±0	8.10±0	0.4±0
Isabel Precoce	TS	YE	13.3±0.2	1.056±0	3.25±0.3	7.60±0.1	0.4±0
Ives	VS	CT	12.6±0	1.054±0	3.32±0*	8.20±0	0.2±0
Ives	VS	YE	12.2±0	1.052±0	3.38±0	8.10±0	0.2±0
Ives	TS	CT	11.5±0	1.049±0	3.36±0	7.80±0	0.2±0
Ives	TS	YE	11.5±0	1.049±0	3.37±0	7.80±0	0.2±0

T.S., Training System; VS, vertical shoot positioning system; TS, trellis system; CT, Control; YE, Yeast Extract; SS, Soluble solids expressed as °Brix; Total acidity expressed as sulphoric acid, volatile acidity expressed as g L<sup>-1</sup> as acetic acid. Values are expressed as mean ± standard deviation (*n* = 6)

\* *p* < 0.05, in comparison with CT group



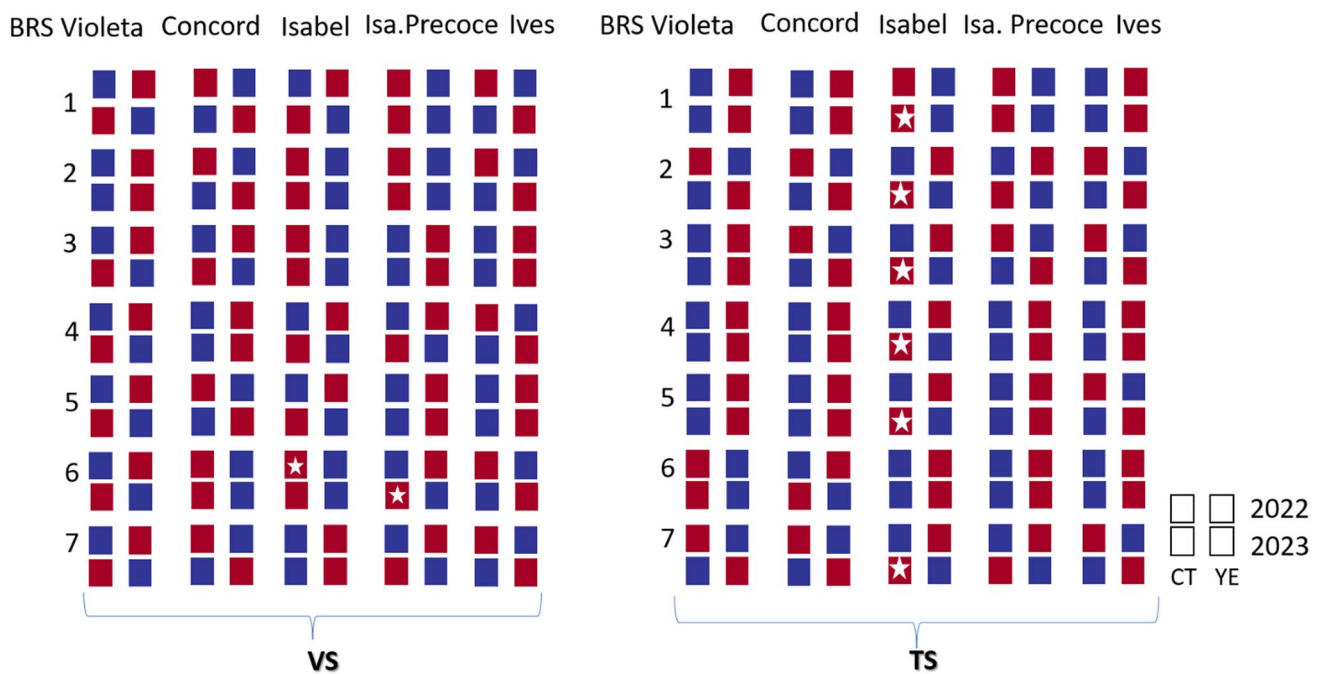
**Fig. 2** PCA of identified compound of harvest 2022 and harvest 2023. Varieties were identified by color: red for 'BRS Violeta', green for 'Concord', purple for 'Isabel'; cyan for 'Ives' and pink for 'Isabel Precoce'; Quality control samples (QC) are yellow in the middle of the groups

[34, 35]. In cv Nebbiolo (*Vitis vinifera*) berries, Ferrandino et al. [34] observed that the biosynthesis of terpenes started before *véraison*, while the maximum accumulation was dependent of the growing area (due to substantial meteorological difference among the areas). Ester compounds were important indicative of the growing area. Regarding aliphatic alcohols, with some exception, they were mostly already present. They behavior vary, increasing in some regions while declining in others. Total aldehydes were accumulated through the ripening. Also, post-*véraison* Carlo-magno et al., [35] observed in (*Vitis vinifera*) cv Barbera, cv Dolcetto and cv Nebbiollo, that a carotenoid degradation product, at *véraison*, was already present in high concentrations, highlighting early metabolic changes. The varietal factors play a crucial role in the biosynthesis of these compounds. The esters concentrations and profiles, differed across these grape varieties, with cv Barbera exhibiting unique ester compositions. Regarding alcohols, varietal differences also influenced biosynthesis: cv Nebbiolo and cv Barbera had more stable alcohol levels throughout ripening, while cv Dolcetto had the highest concentrations. Aldehyde concentrations consistently increased, especially in cv Nebbiolo, with smaller variations in the other varieties.

Given that our treatment was applied in the beginning of the *véraison*, it was expected that among these compounds we were able to see this pattern of behavior. Among the detected compounds, we focused on identifying those consistently present in all samples, narrowing down our findings to seven key compounds (Fig. 3). These compounds were identified as follows: (1) 1,6-Octadien-3-ol, 3,7-dimethyl, (linalool); (2) 1-Nonanol; (3) 2-Buten-1-one,1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, ( $\beta$ -damascenone); (4) Benzeneacetic acid, ethyl ester; (5) Butanoic acid, 3-hydroxy-, ethyl ester; (6) Methyl anthranilate; (7) Nonanal. They are composed of terpenes (1), esters (4, 5, 6), alcohols (2), ketones (3) and aldehydes (7) (Fig. 3).

Briefly, 1,6-Octadien-3-ol, 3,7-dimethyl Fig. 3 – (1), also known as linalool, is recognized for its pleasant floral odor, free from camphoraceous and terpenic notes. Biosynthesis of linalool can be detected in both the exocarp and mesocarp of grape berries. In 'Riesling' berries, on its free and glycosylated forms, was mainly accumulated in the skin (80% per berry) [36–38]. Esters are among the main compounds detected on grape juice, contributing to the distinctive strawberry-like odor, as well as certain "foxy" aromatic compounds such as methyl anthranilate. 2-Aminoacetophenone has also been described among the "foxy" aromas, characterized by its sweet and grape-like odor. These compounds expression is coordinately regulated coinciding with the initiation of berry ripening [28, 37, 39].

We chose to closely observe the behavior of the selected compounds (Fig. 3). Among them, certain patterns were observed between both training systems, as well as for some compounds across both seasons. The treatment with YE has been demonstrated to be particularly effective on esters, followed by ketones. Among the esters, methyl anthranilate, Fig. 3 – (6), stands out due to its significance and numerous studies demonstrating its particular impact on sensorial analysis. For 'Concord' grapes, it has been suggested that the biosynthesis of methyl anthranilate involves catalyzation through



**Fig. 3** Effect of the YE foliar application on grape juice volatile content over the two consecutive studied seasons. Compounds: (1) 1,6-Octadien-3-ol, 3,7-dimethyl; (2) 1-Nonanol; (3) 2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl); (4) Benzenoacetic acid, ethyl ester; (5) Butanoic acid, 3-hydroxy-, ethyl ester; (6) Methyl anthranilate; (7) Nonanal. VS: vertical shoot positioning system TS: trellis system; CT: Control; YE: Yeast Extract; \*  $p < 0.05$  in comparison with CT group. Red represents higher presence of this compound, while blue represents lower concentration

alcohol acyltransferase, from anthraniloyl-coenzyme A (CoA) and methanol as substrates. Additionally, the hydrolysis of pectin polymers, releasing methyl groups, occurs through the action of pectin methyl esterase, in conjunction with tryptophan biosynthesis and breakdown. These processes are correlated with grape ripening and the accumulation of anthranilic acid and methanol, which are associated with the accumulation of methyl anthranilate [39, 40].

The observed increase in esters content in early-harvest varieties such as 'BRS Violeta', 'Isabel Precoce', and 'Ives' juices may be attributed to an increase in the availability of precursors. However, this specific profile was not consistently detected across both harvests, particularly in the VSP training system. Literature suggests evidence that aroma expression in grapes and their subsequent by products is positively associated with radiation. This association leads to an increase in fruity flavors induced by compounds such as monoterpenes, volatile thiols, esters, and C13-norisoprenoids [40]. Under the conditions of our experiments, early-harvest varieties exhibited a more consistent response to YE treatment in TS training system. The company recommends the use of LalVigne Aroma to improve aroma compounds. However, according to the manufacturer's technical sheet, it is possible, for some varieties, to increase the wine aroma intensity in the mouth with the use of LalVigne Mature. It has also been described that the use of LalVigne Mature resulted in a reduction of vegetable hints and methoxypyrazines in 'Merlot' grapes [41]. Indeed, Biasoto et al. [33] described that the majority of Brazilian consumers in their study did not favor notes described as earthy/mushroom, yeast, and vegetative/green beans. Evaluating the compounds detected during our experiment, out of the 87 compounds identified, the odor description was found for 68 compounds (Supplementary Table S5). Among them, 16 compounds were described with these characteristics.

Reducing vegetable notes is highly desirable for 'BRS Violeta' grape juices. This hybrid variety has a complex genealogy between *V. labrusca* and *V. vinifera*. This variety produces intensely colored juices with low acidity and very pronounced sweetness, but they can also exhibit an unbalanced and unpleasant odor. It is recommended to be used in blends due to the efficient improvement in color [42]. A relationship between terpenes, methoxyphenols, volatile phenols, and the aroma descriptor of vegetal intensity has been described in 'BRS Violeta' wines. It was also suggested the presence of C6 alcohols [31]. In Concord grape juices, the "vegetative" or "green" aromas have been associated with six-carbon (C6) alcohols and aldehydes [30].

1-Nonanol, Fig. 3 – (2), is an alcohol described with herbaceous notes. In 'BRS Violeta' juices, those treated with YE showed responsiveness during both seasons in the VSP system, whereas in TS system, this only occurred in the last season. In table grapes like 'Jingya', 1-Nonanol decreased one week after *véraison* and then remained relatively stable

during ripening. Most alcohols and carbonyls tended to decrease during ripening [43]. Additionally, it has been reported that the concentrations of some alcohol compounds decrease with pasteurization and thermosonication treatments during grape juice processing [44]. Regarding C6 compounds and methoxypyrazines, often associated with green notes, a tendency of lower concentration was described in sun-exposed bunches compared to shaded bunches [40]. Despite the evidence favoring the reduction of these compounds in 'BRS Violeta', they continue to be elevated in YE-treated grapes from the VSP training system. Furthermore, additional studies would be necessary to confirm the impact of this concentration, considering olfactory detection thresholds.

The primary objective of using YE (LaVigne® MATURE) on grapevines is to improve wine quality by enhancing phenolic maturity, tannin quality, structure, and mouthfeel. After application, YE are recognized by the vine, which in response, stimulates the natural biosynthetic pathway of these compounds [13, 41]. Unfortunately, there are relatively few studies that have investigated the response of volatile composition to this treatment.

The findings from each season revealed that in 2023, 73 compounds were detected, while 71 compounds were detected in the 2022 harvest. Despite this numerical difference, the 2022 harvest showed more compounds that were responsive to the YE treatment (Fig. 3). Among the varieties, 'Ives' exhibited a positive response to the YE treatment in both training systems, followed by 'BRS Violeta' and 'Concord'. Conversely, 'Isabel' showed the least responsiveness in both seasons.

Statistical analysis revealed greater detection of compounds in CT samples from 'Isabel' than TS and for methyl anthranilate from the VSP system (Fig. 3). However, despite these findings, our results indicate that the TS system showed a better response to the YE treatment compared to the VSP system. Additionally, based on the yield per vine of grapes (Supplementary Table S1), the TS system was more productive than the VSP system.

It's known that volatile compounds belong to the secondary metabolism in plants, and their concentration and profile can be influenced by various factors including genotype, growth stage, and environmental conditions such as temperature, sunlight exposure, and soil characteristics [36–38, 45]. For instance, evidence suggests that UV light plays a crucial role in the biosynthesis of compounds like linalool in grape berries. Researches have shown that UV light is essential for the expression of enzymes involved in both the mevalonate (MVA) pathway, which occurs in the cytosol, and the methylerythritol phosphate (MEP) pathway in the plastids [36]. Restriction of UV light or shading can lead to a decrease in the expression levels of these enzymes, thereby affecting the biosynthesis of linalool and other volatile compounds.

The present results involving the interaction with YE treatment doesn't favor this theory. In fact, for 'Isabel Precoce' juices higher levels of linalool (Fig. 3 – (1)) on CT juices, on both training systems. Besides, for some varieties, such as 'BRS Violeta' and 'Ives', TS presented a more consistent response involving both harvests.

It's essential to thoroughly analyze the compounds detected exclusively in either the treated or non-treated groups to understand the specific effects of the yeast extract treatment on grape juice composition. We now examine in detail those compounds that were detected on only one group, treated or non-treated (Supplementary Table S6).

Among these compounds we focus on those that were detected on both training system and harvest, disregarding a possible vintage effect. The identification of  $\gamma$ -Terpinene in the 'Concord' grape juices YE treated is particularly noteworthy due to its potential contribution to the aroma profile of the juice.  $\gamma$ -Terpinene is a monoterpene known for its lemon-like odor, accompanied by subtle bitter and herbaceous notes [36]. Interestingly, previous studies have identified  $\gamma$ -Terpinene as one of the main free monoterpenes in other grape varieties such as 'Summer Black' and 'Hutai 8', (hybrids of *V. labrusca* and *V. vinifera* L.) varieties described with characteristic strawberry aroma [46].

UV attenuation film, increased concentrations of  $\gamma$ -terpinene in 'Shiraz' (*Vitis vinifera* L.) grapes when applied from pre-*véraison* to harvest, compared to naturally exposed grape bunches [45]. It appears that in 'Concord' grapes, YE application was able to mitigate the response among the training systems. Considering that TS promote shading into the clusters, the presence of this compound on VSP system is interesting. This is particularly significant given the growing interest in the impacts of training systems, especially in shading clusters to mitigate extreme temperatures and modify the microclimate around the clusters [2, 24]. The impact of UV radiation on promoting the biosynthesis of monoterpenes may depend on various factors, including the type of monoterpenes, the intensity of radiation, besides vintage effects [45]. This could explain why  $\gamma$ -terpinene was also detected in 'BRS Violeta' juices in the CT group.

The variety's sensorial attributes, especially the fruity notes associated with the aroma and flavor of grape were sensory drivers for the acceptance to the Brazilian population, as showed by research of Biasoto et al. [33]. These notes are commonly found in wines from *V. labrusca*, such as 'Ives' and 'Isabel', which were preferred by the majority of consumers due to their intense aromas described as sweet, grape, grape juice and roses. Moreover, in exploratory analyses conducted with grape juices (100% and blends) of the varieties 'Niagara Rosada', 'Ives', 'Isabel', and 'BRS Nubia', greater acceptance

was observed for 'Ives' and its blends with 'Isabel'. The varieties 'Ives' and 'Isabel' consistently ranked highest in aroma acceptability [47].

Furaneol contributes to the characteristic strawberry notes described for 'Isabel' variety, and it has been detected in both free and bound forms. Additionally, it has been found in cv Niagara [48, 49]. In our study, furaneol was detected in 'Ives' grape juice from both training systems in the CT group, but only in the 2023 harvest (Supplementary Table S5). Despite the singularity of this compound, its absence in 'Isabel' and its early-ripening variety may be justified by the general reduction of volatile compounds during juice extraction processing, including thermal degradation, water vapor drag, and volatilization, among others [18, 44]. Dutra et al. [50], described a significant decrease in furaneol content during the concentration process.

## 4 Conclusions

In general, YE application is an eco-friendly approach, very easy to manipulate, since doesn't use OGM, can be even use in farmers with production on organic system. To our knowledge, it's first study, that evaluate its application on *Vitis labrusca* L. and its hybrids aiming grape juice production. Regarding the juice itself, the increase in acidity and lower sugar content can be welcome to many consumers, especially those concerned regarding the caloric value. However, further sensorial analysis will show, the preference and the real impact on aroma, since the perception of a volatile molecule is involved with the perception threshold.

Results presented, which are, to our knowledge, new for yeast extract (YE) application on different training systems, have enabled us to associate a positive response to the main volatile compounds detected in grape juice with the YE application and the TS training system. This is a promising finding, particularly considering the TS training system's high productivity compared to VSP. The treatment is most effective on esters, followed by ketones.

The 'Ives' variety exhibited a positive response to YE treatment on both training systems, followed by 'BRS Violeta' and 'Concord'. 'Isabel' showed the least responsiveness regarding volatile composition."

The YE treatment did not affect yield/vine, or berries physicochemical parameters.

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**Data availability** The data used to support the findings of this study are included within the article and its Supplementary files. The raw data files are openly available in available in MendeleyData repository (Moro, Lais (2024), "Unlocking grape juice's aroma: exploring volatile compounds after inactive yeast extract foliar treatment using SPME and GC-MS", Mendeley Data, V1, <https://doi.org/10.17632/w3mvs78956.1>).

## Declarations

**Competing interests** The authors declare no competing interests.

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