Elevating grape juice excellence: unveiling the impact of pre-harvest yeast extract foliar application on quality parameters

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ABSTRACT

This research investigates the effects of foliar application of selected inactivated yeast on juice grape varieties ('Isabel,' 'Isabel Precoce,' 'Ives,' 'Concord,' and 'BRS Violeta') cultivated at the Centro Avançado de Pesquisa e Desenvolvimento de Frutas of the Instituto Agronômico (IAC), São Paulo, Brazil. The vines were trained on vertical shoot-positioning (VSP) and overhead trellis systems (TOS), with a randomized block design applied in four replicates. The study assessed plant productivity and the physical characteristics of grape clusters and berries. Additionally, grape juice extracted from these varieties in the 2022 season underwent sensory analysis in a laboratory at São Paulo State University (UNESP), with approval from the Research Ethics Committee of the Botucatu School of Medicine (FMB-UNESP, CAAE: 63416122.0.0000.5411). Stilbene compounds were quantified post-processing using high-performance liquid chromatography (HPLC) with a diode array detector (DAD). Conducted over three years, the study found no significant impact of yeast extract application on vine productivity or berry physicochemical parameters. However, different training systems responded uniquely to the treatment, with VSP showing the most notable effects in stilbene content. Among the varieties, 'Ives' and 'BRS Violeta' received the highest sensory scores, while 'Concord' received the lowest overall impression scores. Results varied by grape variety and training system, emphasizing the importance of varietal selection and suggesting the potential for creating blends that highlight each variety's strengths. The initial part of the study is freely available at Discover Food journal within the title: Exploring grape juice volatile compounds after inactive yeast extract foliar treatment using SPME and GC–MS.

1. Introduction

In the last five years, there has been a 12.7% increase in grape juice commercialization within Brazil. Data from the grapes, wine, and derivatives database of the main productive state (Rio Grande do Sul), presented that natural grape juice has shown a tendency to increase over the years, as well as organic and reconstituted juice. The amount exported also increased in the last five years by 209%, reaching an additional 22 countries. The main importers of Brazilian grape juice are Japan and the United States.

Red wine and grapes are among the main foods that contribute to anthocyanidins in Europe and the United States. In Chinese adults, grapes were identified as one of the top three fruit sources of flavonols. In Brazil, grapes and wine are among the main food sources of anthocyanins and stilbenes, while grape juice is highlighted as a major source of proanthocyanidins (Anacleto et al., 2019; Carnauba et al., 2020; Li et al., 2013). This increase in grape juice consumption might be due to the absence of alcohol, allowing grape juice to be appreciated by a wide range of consumers and reaching regions where fresh grapes are unavailable, all at a very competitive price. Additionally, numerous studies have demonstrated the importance of grapes and their derivatives, such as wine, as sources of phenolic compounds. There is significant interest within the scientific community regarding the potential benefits of flavonoids in reducing the risk of chronic diseases, among other health benefits (Barbalho et al., 2020).

The use of yeast extract has been documented as an elicitor in *Vitis vinifera* L. grapes cultivated in Europe for wine production. This treatment resulted in an increase in the concentration of extractable anthocyanins and the accumulation of tannins in the grape skin. Additionally, it increased the degree of polymerization of these tannins, benefiting the products made from these grapes by improving wine longevity, increasing the thickness of the grape skin, and reducing herbaceous aromatic notes, which are aggressive to the palate. Grape juice biofortification with yeast extract emerges as an eco-friendly alternative for enhancing bioactive compounds. Composed of 100% natural, non-pathogenic, and food-grade ingredients, LalVigne® MATURE is a specific formulation developed for foliar application, patented by Lallemand Inc., Canada (WO/2014/024039) (Gil-Muñoz et al., 2017; Pastore et al., 2019; Portu et al., 2016).

As a pre-harvest application, the efficiency of yeast extract treatment and grapevine production is strongly dependent on climate, which interacts with factors such as grape variety and training system. Additionally, thermal processing during juice extraction might also affect the final content of bioactive compounds. Therefore, this study aimed to investigate whether pre-harvest yeast extract treatment impacts the physicochemical characteristics of *Vitis labrusca* and its hybrid grapes. The study was performed in three seasons.

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 (*V*IVC - 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 meters. The vines were grown in an eleven-year-old vineyard trained on the vertical shoot-positioning system (VSP) and the overhead trellis system (TOS), with row spacing of 2 meters and within-row spacing of 1 meter. 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 (Supplementary Table ST1). 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^{-1}. 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 Yamaho model FT-16S, with 16L 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 minutes [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. Basic enological analysis

The titratable acidity (AT), pH, density and total soluble solids (SS) of juices were measured with WineScan (Gutiérrez et al., 2021).

2.4. Stilbene Content

The extraction was performed according to Valéria da Silva Padilha et al., (2019) with some modifications, 500μL of grape juice was added 500μL of mobile phase A, then centrifuge at 7000g for 3 minutes. Then filter with milifiltro 0.22 μm (Filtrilo) into identified vial. The quantitative analysis of phenolic compounds in grape extracts and grape juices followed the method of Padilha et al. (2019). The column and pre-column used were a Zorbax Eclipse Plus RP-C18 (100×4.6 mm, 3.5 μm) and a Zorbax C18 (12.6×4.6 mm, 5 μm), respectively (Agilent Technologies). The column temperature was set at 35 °C. The mobile phase consisted of a solution of phosphoric acid 0.1 mol L−1, pH=2.0 (A) and methanol acidified with 0.5% H3PO4 (B). The flow rate was 0.8 mL min−1 and 20 μL was the injection volume of the sample. Before sample injection, grape peel extracts were diluted with the mobile phase A, and filtered through a 0.45 μm membrane (Millex Millipore, Barueri, SP, Brazil). The elution gradient used was 0–5 min: 5% B; 5–14 min: 23% B; 14–30 min: 50% B; 30–33 min: 80% B. Stilbenes were identified according to the retention times and UV−vis data obtained from the authentic standards of trans-resveratrol and piceid (Extrasynthese, Genay, France). The results were expressed as mg/L juice.

2.5.Sensory analysis

The sensory analysis was carried out in a sensory analysis laboratory at São Paulo State University (UNESP). The analysis was performed with the approval (CAAE: 63416122.0.0000.5411) of the Research Ethics Committee of the Botucatu School of Medicine (FMB- UNESP).

For the analysis, 15 mL of grape juice was individually served at 20°C in plastic cups by a random order of a 3-number code, with one glass of water and a soda cracker, served to the subject to rinse the mouth between the samples The tests were performed, using a sensory analysis sheet. First, one consumer profile assessment test to explore basic information about their consumer profile. Second, the acceptance test using a hedonic 5-point scale (where 1 represents "dislike extremely" and 5 represents "like extremely") for the characteristics of color, aroma, flavor, body, and global impression. There were performed 250 evaluations by 150 consumers: 46% female and 54% male. Additionally, to the sensorial test, the consumers answered a questionnaire devoted to the habit of consumption of fruit juices, including the frequency and form of grape juice intake.

2.6. Statistical analysis

The results were expressed as average ± standard deviation, followed by the analysis of variance (ANOVA). For the Principal Component Analysis - PCA, and other multivariate analyses were performed in the MetaboAnalyst 6.0 software (https://www.metaboanalyst.ca/).

3. Results and discussion

3.1. Climatic conditions and yield parameters

Climatic conditions throughout this period are showed in Figure 1. Despite fluctuations in mean temperature, the thermal amplitude (difference between minimum and maximum temperatures) remained relatively consistent across the years. The highest amount of rain was detected near the harvest, on January on 2022 season, while for 2023 these levels were achieved in December followed by January. On 2024 the highest amount of rain was detected on October near the flowering period.

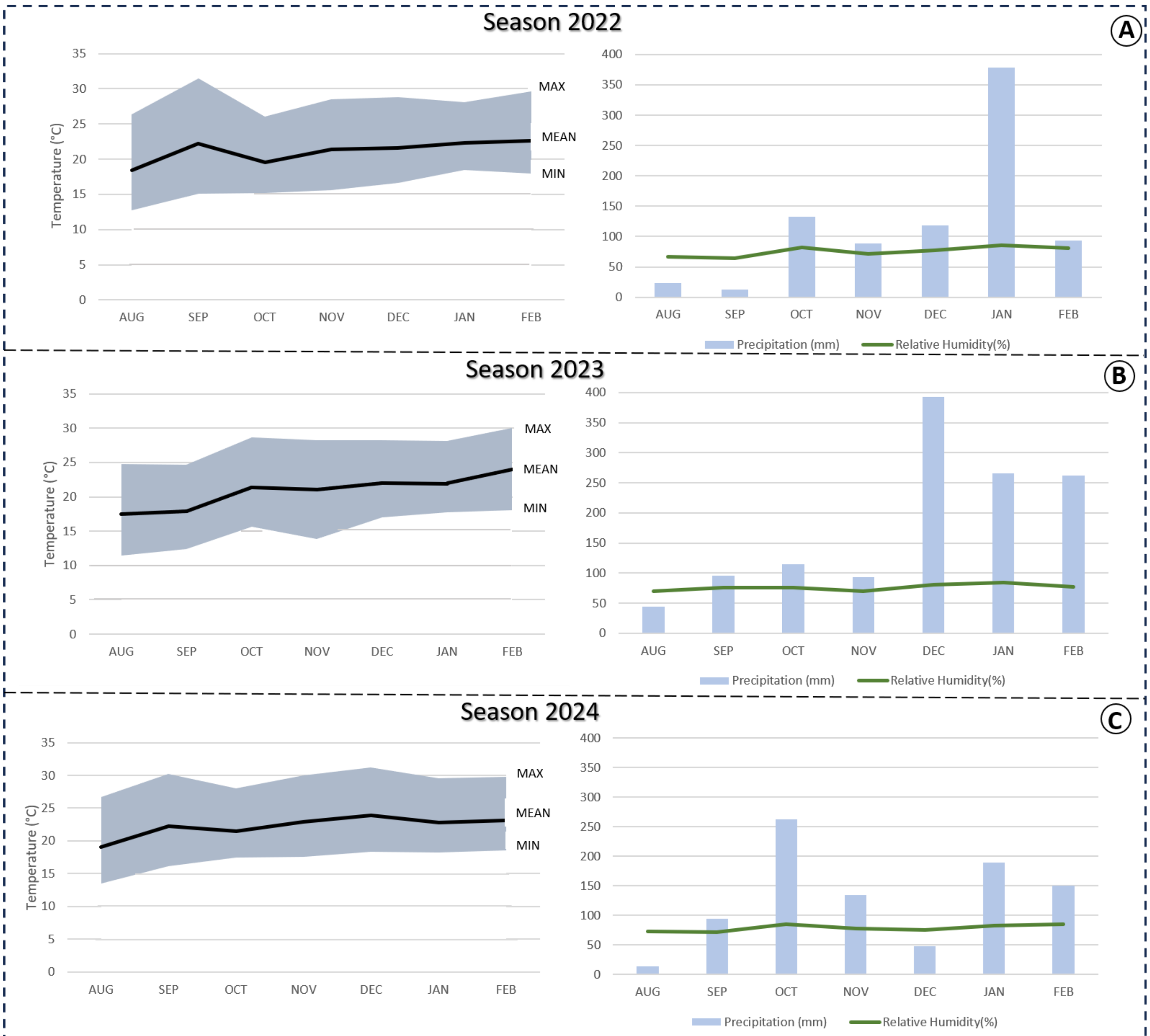

Figure 1. Climatic conditions during grape development till harvest in 2022 (A); 2023 (B) and 2024 (C).

3.2.Grape physicochemical characteristics

In consideration of the interaction between the conductive system and grape variety, we chose to evaluate the treatment impact on grape chemical parameters individually (Supplementary Table ST2). Some patterns were observed among the research. We consider those, that presented the same behavior among the years, with statistical difference at minimum on two seasons.

For VSP system it was observed for Isabel Precoce higher total acidity levels on YE treated grapes. While for TOS system, higher acidity were detected on CT grapes. Interestingly, for this variety the opposite is observed for VSP system. Also, in VSP system, Concord presented a similar answer into the two first seasons, with higher levels on YE. Suggesting that the interaction with the environment hardly effect acidity content, and the interaction between the treatment and the grapes. Since the first two season present similar behavior regarding heavy rain, during ripening period.

The SS content on VSP system was expected to be higher in relation to grapes from TS system, as can be observed on Ives on 2023 harvest and Concord on 2024 harvest.

Regarding the physical parameters of the bunches (Supplementary Table ST3), there was no significant effect of YE treatment on most of the parameters for clusters and rachis. However, an exception was observed for berry length in YE treated berries. There was an increase observed in 'Isabel' berries from the VSP conductive system (season 2022) and 'Ives' berries from the TOS conductive system (season 2024). Throughout the experiment, the Ives variety consistently presented smaller berries in terms of both berry length and width, compared to the other studied varieties. A similar pattern was detected in Isabel berries only during the first two years of the study. A correlation of this observation could be made with the pluviometric data (Figure 1), as heavy rainfall occurred during ripening in these first two seasons.

3.3.Basic enological analysis

In comparison with grapes SS content (Supplementary Table ST3), a diminish in soluble solids was observed (Supplementary Table ST5). However, this result was previously described by others who also used steam extraction for juice production (Kaltbach et al., 2022; Moro et al., 2022; Mota et al., 2017).

According to Brazilian regulatory instruction whole grape juices should have 14 ºBrix as minimum SS content (Brasil, 2018). The juices produced with grapes from 2022 and 2023 season doesn't comply with the current regulation. SS content on juices from YE treatment were statistically higher from those from CT, on Concord juices from TS system (2022 season) and BRS Violeta from both conductive system (2023 season). But only in 2024 for BRS Bioleta from TS system, the treatment it was able to reach the minimum required by Brazilian regulatory instruction. It important to emphasize that 2024 was a dry year during veraison and harvest, in comparison with the previously seasons (Figure1). Our results demonstrate how vintage effect promote a higher impact, that the treatment and juice extraction, regarding SS content.

Despite the rainy period close to the harvest time considering 2023 and 2023 season, all the juices contained less than 0.6 $g.L^{-1}$ of maximum volatile acidity, in compliance with Brazilian regulations for integral juice (Brasil, 2018).

pH and total acidity values were in accordance with mean values described by juices extracted using the steam method (Moro et al., 2022; Mota et al., 2017).

3.4. Stilbene Content

Most published studies have reported an increase in grape stilbenes, particularly in wines, following the application of elicitors (Gil-Muñoz et al., 2017; Portu et al., 2018). Due to its wide-ranging effects, resveratrol can impact various cells and tissues, aiding in the maintenance or restoration of health. This influence is especially significant in relation to chronic degenerative diseases, which are often associated with inflammation and oxidative stress(Barbalho et al., 2020). By modulating these pathological processes, resveratrol shows promising therapeutic potential and is a key focus of our study.

The blue bars (CT) and gray bars (YE) represent the concentration levels for *trans*-resveratrol (Fig 2) and piceid (Fig 3) over the season 2022, 2023 and 2024. The year-by-year comparison enables the identification of any temporal trends or variations in concentration levels that may be attributable to climatic or environmental factors affecting that year's harvest. Allowing to compare to treatment effect across different vintages and trellising systems.

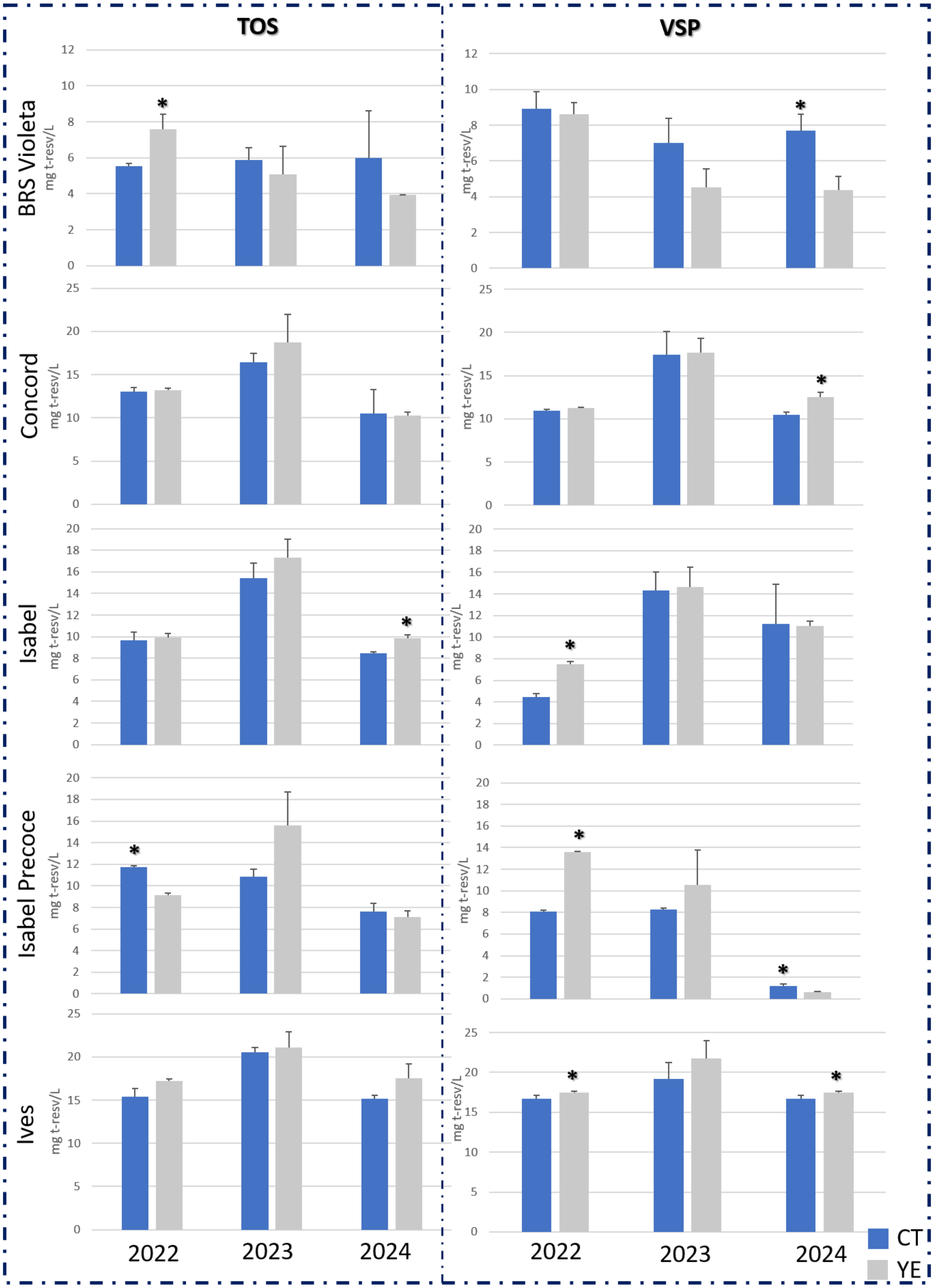

Figure 2. *Trans*-resveratrol quantification in grape juices from grapes under VSP: vertical shoot positioning system and TOS: tendon overhead system; CT: control (blue); YE: yeast extract (gray) ($n = 6$); '*' represents significant difference between samples ($p < 0.05$) in comparison with the CT group. During 2022, 2023 and 2024 season

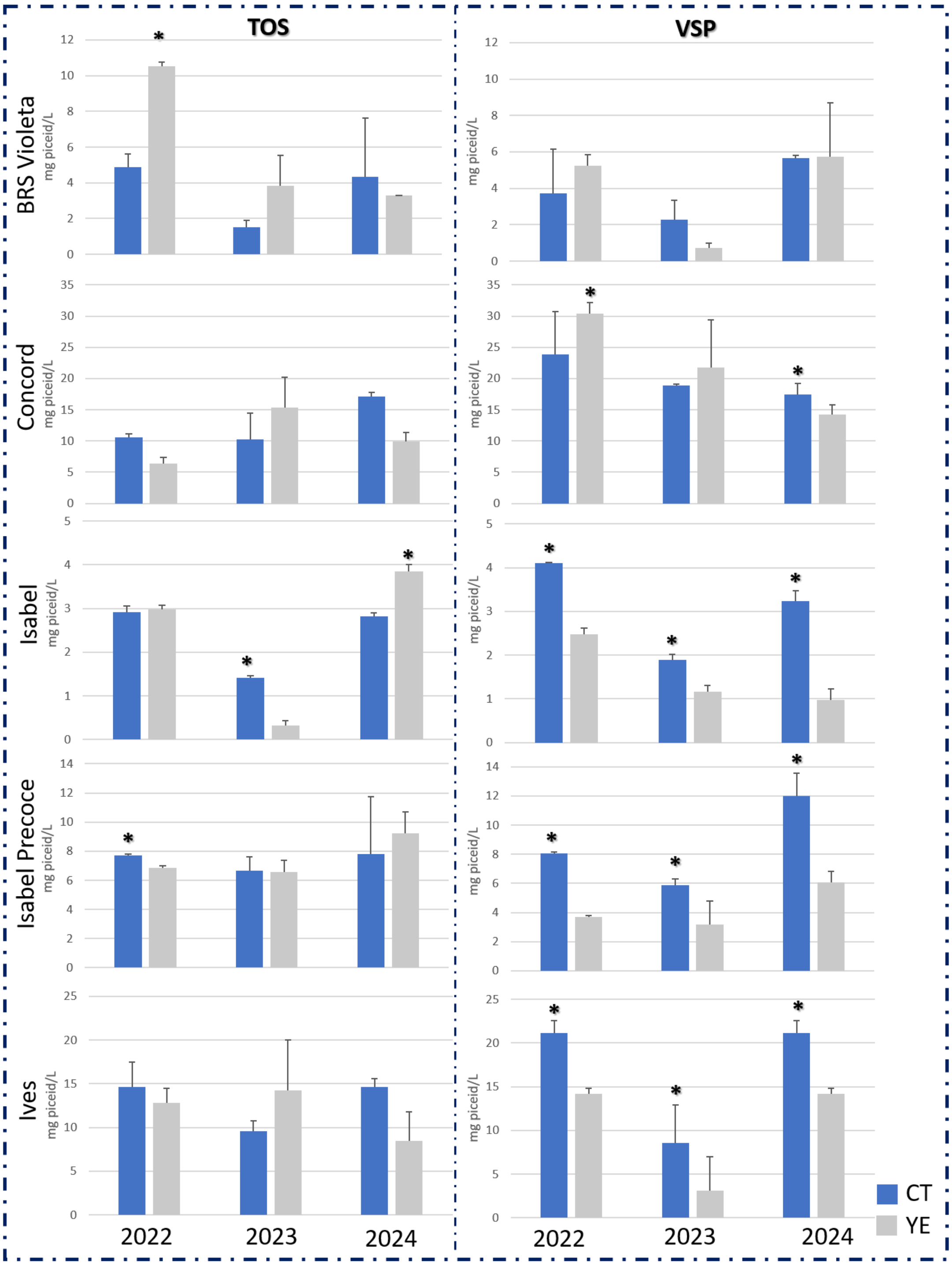

Figure 3. Piceid quantification in grape juices from grapes under VSP: vertical shoot positioning system and TOS: tendon overhead system; CT: control (blue); YE: yeast extract (gray) (n = 6); '*' represents significant difference between samples ($p < 0.05$) in comparison with the CT group. During 2022, 2023 and 2024 season.

Regarding the conductive system, in some years, TOS appears to yield higher concentrations in both CT and YE treatments, indicating that trellis orientation might affect concentration levels regardless of the treatment. Since VSP seems to be more responsive to the treatment, where higher concentrations were found on grape juice produce from these plants. An interesting pattern was observed in VSP juices from 'Ives' and 'Isabel Precoce', both early gravest varieties. They presented opposite behavior regarding 'piceid' and 'resveratrol' during all the seasons. Since *cis*- and *trans*-resveratrol and piceid isomers are the primary stilbenes found in grapes, similar or complementary behavior among them was expected, as they share most of their metabolic pathways. Notably, piceid is derived from the glycosylation of resveratrol. (Flamini et al., 2013)

An increase in total stilbenes content was expected, since research with Sangiovese (*Vitis vinifera* L.) variety, in Bologna (Italy), correlated the increase in secondary metabolites after the YE treatment with the upregulation of gene expression such as PAL1 (phenylalanine ammonia-lyase 1), which catalyzes the biosynthetic pathway of phenylpropanoids, namely compounds like stilbenes and other flavonoids. It was emphasized that environmental conditions and vine management practices can influence the effectiveness of the treatment, as leaf receptors will signal to the plant to carry out physiological and biosynthetic reactions (Lallemand, 2018; (Pastore et al., 2019)). Also, 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, in fact, it resulted in 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 (Kogkou et al., 2017).

The results of our study demonstrate that effectiveness of the treatment is indeed affected by the edaphoclimatic conditions. On 2023 season, heavy rain was observed (Figure 1) during the *veraison*, where the treatments were applied, and higher level of stilbenes were detected on CT group.

Considering the three years of the study, a positive pattern was detected on *trans-r*esveratrol content for 'Concord', 'Isabel' and 'Ives' grape juice. Highlighting the potential of a blend composed of these varieties, allowing the complementation of the different profiles (such as chromatic and aromatic) and increased stilbene content.

However, further analysis is needed to evaluate the behavior of these compounds after a storage period.

3.5. Sensory analysis

The consumer profile showed that the majority of the costumers have the habit to consume grape juice more than once a week (40.54%) followed by once a month (32.43%), once a week (17.57%). The most common form of grape juice consumption is whole/natural (75%), followed by concentrated (9.46%), nectar/beverage (5.42%) and powder (4.05%).

This information is in agreement with the data collected by the main productive state (Rio Grande do Sul) of Brazil, that natural grape juice has shown a tendency to increase its commercialization over the years. It is noteworthy to say that children, elderly, pregnant and others, might prefer grape juice over wine due to the lack of alcohol content. Despite the observed tendency of juice to present lower phenolic level than wine. It was described that the consumption of grape juice or wine produced similar antioxidant effects as they increased the total antioxidant capacity and reduced lipid oxidation (Copetti et al., 2018)

Figure 4, demonstrates that grape juices from 'Ives' grapes lead the color preference among the consumers (YE group) from TOS conductive system, followed by VSP. The samples 'Ives' (VSP) and 'BRS Violeta' TOS from CT group had the highest aroma attribute. While flavor was led by 'BRS Violeta' and 'Isabel' VSP from YE and CT group respectively. Body is an important attribute; the highest average scores were in 'Ives' and 'Isabel Precoce' from VSP with YE treatment and TOS from CT group. The overall impression scores were lowest for 'Concord' juice, while the highest were detected in 'Isabel' and 'Ives' VSP, from CT and YE group respectively.

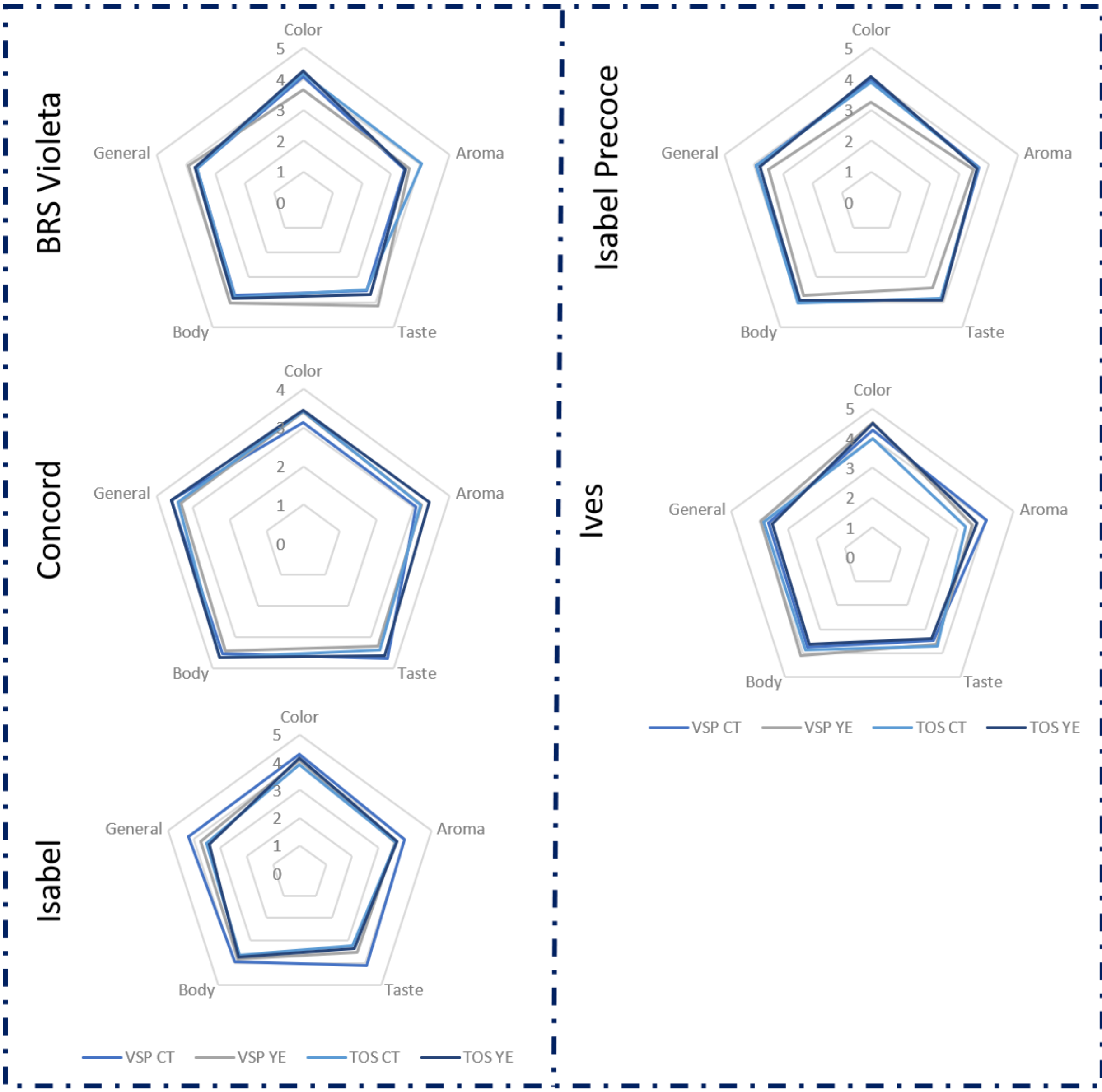

Figure 4. Sensory analysis of the attributes color, aroma, taste, body and general impression of grape juices from grapes under VSP: vertical shoot positioning system and TOS: tendon overhead system; CT: control; YE: yeast extract.

These results indicate 'Ives', 'BRS Violeta and 'Isabel' juices as the favorite by the consumers. With exception of aroma and general impression, all the highest scores were in YE juices. This is in agreement with the technical sheet description of Merlot and Syrah wine produced in Languedoc (2012 season) after YE treatment. Sensorial panel described a better quality in the mouth, included volume, softness, roundness, and quality of tannins. It would appear that the treatment is effective also in *Vitis labrusca* L. and its hybrids, even after a thermic process, such as the steam extraction.

Additionally, these 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 (Biasoto et al., 2014). These notes are commonly found in 'Ives' and 'Isabel', which were preferred by the majority of consumers due to their intense aromas described as sweet, grape, grape juice and roses.

4. Conclusion

In summary, this study investigated the potential of pre-harvest yeast extract (YE) application in grapevines to enhance grape juice quality. Results indicate that YE treatment can be effectively applied to *Vitis labrusca* and its hybrids for juice production. The treatment did not significantly affect productivity or physicochemical parameters, suggesting that vintage and training system had a greater influence than the treatment itself. Over the three-year study, initial stilbene content analysis showed a positive trend in 'Concord,' 'Isabel,' and 'Ives' grape juices. Sensorial analysis further supported the potential of YE treatment, with consumers favoring juices from 'Ives,' 'BRS Violeta,' and 'Isabel' varieties. Future studies should examine the stability of stilbenes following a storage period.

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Conflicts of interest

The authors declare no conflict of interest.

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