









Physicochemical, aromatic and sensory properties of the 'Riesling Itálico' wines fermented with *Saccharomyces* and non-*Saccharomyces* yeasts

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ABSTRACT: *The sensory quality of wines is affected by the compounds produced by the action of the yeasts used in alcoholic fermentation. This work aimed at assessing the physicochemical, aromatic and sensory properties of the 'Riesling Itálico' white wines produced by using the Saccharomyces and non-Saccharomyces yeasts. The yeasts S. cerevisiae, S. cerevisiae cerevisiae, S. bayanus, Torulaspora delbrueckii and Metschnikowia pulcherrima were inoculated individually into the must. For 10 days, the alcohol was left to ferment in 7 L carboys in triplicate, at 15° C temperature. The standard physicochemical analyses were done and the quality of the wines was determined. The volatile compounds were identified and quantitative descriptive sensory analyses were performed. All the yeasts produced wines with the characteristic alcohol, volatile acidity and residual sugar content as stipulated by the Brazilian legislation. The T. delbrueckii yeast produced high-content phenylethyl acetate, isoamyl acetate and ethyl decanoate wines, of superior olfactory quality and sensory rating.*

Key words: white wines, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, volatile compounds, sensory analysis.

Propriedades físico-químicas, aromáticas e sensoriais do vinho 'Riesling itálico' fermentado com leveduras *Saccharomyces* e não-*Saccharomyces*

RESUMO: *Durante a fermentação alcoólica as leveduras produzem compostos que influenciam a qualidade sensorial dos vinhos. O objetivo do trabalho foi avaliar as propriedades físico-químicas, aromáticas e sensoriais do vinho branco da variedade Riesling Itálico elaborado a partir de leveduras Saccharomyces e "não-Saccharomyces". Foram inoculadas separadamente no mosto as leveduras S. cerevisiae, S. cerevisiae cerevisiae, S. bayanus, Torulaspora delbrueckii e Metschnikowia pulcherrima. A fermentação alcoólica ocorreu em garrações com 7 L de mosto, em triplicata, por 10 dias a 15 °C. Foram realizadas análises físico-químicas padrão e de identificação da qualidade dos vinhos, além das análises de compostos voláteis e sensorial descritiva quantitativa. Todas as leveduras produziram vinhos com teores de álcool, acidez volátil e açúcar residual de acordo com a legislação. A levedura T. delbrueckii produz vinhos com concentrações elevadas de acetato de feniletíla, acetato de isoamila e decanoato de etila, resultando em boa qualidade olfativa e avaliação sensorial.*

Palavras-chave: vinhos brancos, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, compostos voláteis, análise sensorial.

INTRODUCTION

Yeast plays a crucial role in alcoholic fermentation by facilitating the sugar content to get converted into ethanol, speedily and completely, as well as to restrain the fermentation of the acetic acid (JIMÉNEZ-MARTÍ et al., 2011). Besides, in the course of alcoholic fermentation, the yeasts release secondary metabolites including alcohols, esters

and other compounds, all of which affect the wine aroma (SABERI et al., 2012). Thus, the yeast-action generated compounds strongly impact the chemical composition of the wines and their sensory traits (CSOMA et al., 2010).

In the alcoholic fermentation process, the commonest ones used are the *Saccharomyces* yeasts, particularly those belonging to species *S. cerevisiae*. However, several non-*Saccharomyces* yeasts too

can enhance the complexity of the wines and impart some desirable qualities (BREDA et al., 2013; HU et al., 2018). The following species *Torulaspora delbrueckii*, *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Starterella bacillaris*, *Lachancea thermotolerans*, *Pichia kluyveri*, *Wickerhamomyces anomalus*, *ZygoSaccharomyces bailii*, besides a few others, are outstanding in this capacity (VARELA, 2016). In fact, in the initial stages of alcoholic fermentation, it is the “non-*Saccharomyces*” yeasts which are dominant (CONTRERAS et al., 2014); however, when the *Saccharomyces* yeasts are inoculated along with them, their growth rate slows down and their influence on the wines becomes negligible (COMITINI et al., 2011). Besides, the non-*Saccharomyces* yeasts may not complete the fermentation process, as their tolerance for ethanol, sulfur dioxide and other wine components is very low (CHEN et al., 2018).

However, when the non-*Saccharomyces* yeasts alone are inoculated, they impart significant features to the wines (VARELA, 2016). The addition of *Torulaspora delbrueckii* yeast affects the aromatic profile, and the fresh fruit descriptors are enhanced (RENAULT et al., 2015; RAMÍREZ et al., 2016). When *Metschnikowia pulcherrima*, which shows high tolerance to ethanol, is added the higher alcohols and terpenes increase, resulting in the wine becoming imbued with the aromas of tropical and floral fruits (SADOUDI et al., 2012). From these studies it becomes evident that the addition of the non-*Saccharomyces* yeasts has the potential to boost the wine quality. However, to our knowledge, no studies have been done to show such influences on the Brazilian wines. Therefore, this goal of this work is to study the Riesling Itálico wines produced in the Serra Gaúcha area and identify their physicochemical, aromatic and sensory characteristics using the *Saccharomyces* and non-*Saccharomyces* yeasts.

MATERIALS AND METHODS

For the experiment we used 105 L of Riesling Itálico grape must from Serra Gaúcha, Brazil (latitude 29°S, longitude 51°W, and altitude 700 m). The grapes were destemmed and pressed to acquire the must. Next, sulfitation of the must (75 mg L⁻¹ of SO₂) with potassium metabisulfite (BASF SE, Ludwigshafen, Germany) was done, followed by transfer to a stainless-steel tank for the *débourbage* (decanting of solid particles). It was left undisturbed for 24 hours, after which 7 L of the must was transferred to each of the 15 carboys, of 14-L

capacity each; samples were then drawn and sent for physicochemical analysis.

The alcoholic fermentation experiments were done by adopting a completely randomized design, which included five treatments and three replications. The treatments involved the addition of the *Saccharomyces cerevisiae* (Uoa Maxithiol[®], AB Biotek, Australia), *Saccharomyces cerevisiae cerevisiae* (Lalvin ICV D47[®], Lallemand Inc., Canada); *Saccharomyces bayanus* (Lalvin QA23[®], Lallemand Inc., Canada); *Torulaspora delbrueckii* (Zymaflore AlphaTD[®], Laffort, France) and *Metschnikowia pulcherrima* (*Levulia pulcherrima*[®], AEB Group, Italy), and the inoculations were given in a dose of 30 g hL⁻¹. To the must was added a combination of yeast nutrients and vitamins (Gesferm Plus[®], Amazon Group, Brazil) in a dose of 25 g hL⁻¹. The must was left undisturbed for 10 days for alcoholic fermentation to take place at 15 °C, and density measurements were recorded every day. The nutrients were added, in a dose of 10 g hL⁻¹, on days 3 and 5 of the fermentation (ActimaxVit[®], Amazon Group, Brazil). Next, 18 g L⁻¹ sucrose was added on day 4 of fermentation to raise the alcohol content of the wines by 1.0 % (v v⁻¹), a process termed chaptalization.

Once the fermentation was complete, the lees or coarse sediment was decanted. The wine was then transferred to fully fill up the 4.6 L carboys. Next, 40 mg L⁻¹ of SO₂ was added to deter malolactic fermentation, followed by 10 g hL⁻¹ of bentonite-based additive to clarify the wines (La Elcha[®], La Elcha Minera Industrial SA, Argentina). After ten more days, another transfer was done using flasks of the same volume for sediment removal. The carboys were retained at 0° C for 30 days to stabilize the tartaric acid, and preventing crystal formation. Next, the wine was filled and samples were drawn for analysis.

Filtration of the must and wine samples was done by passing them through a 7.5 µm mesh, packed in conical bottom centrifuge tubes. After centrifugation, further analysis was performed employing Fourier Transform Infrared (FTIR) spectroscopy (WineScan™ SO₂, Foss, Denmark) and the FOSS Integrator software version 1.6.0. The must was analyzed with respect to the following components: reducing sugars (167.6 g L⁻¹); total soluble solids (17.1 ° Brix); total acidity (6.1 g L⁻¹HAT - expressed as tartaric acid); malic acid (4.0 g L⁻¹); tartaric acid (5.1 g L⁻¹) and pH 3.5. The wine was analyzed, with respect to the following constituents: alcohol (% v v⁻¹); residual sugar (g L⁻¹); glycerol (g L⁻¹); and volatile acidity (g L⁻¹ Hac) (Table 1); total acidity (6.6 to 6.7 g L⁻¹ HAT); malic acid (2.5 to 2.6

g L⁻¹); lactic acid (0.1 to 0.2 g L⁻¹); and pH (3.34 to 3.37), with confidence interval, where $\alpha = 0.05$.

The volatile compounds chosen from the literature were ascertained using gas chromatography-mass spectrometry (HP 6890, Agilent Technologies, USA) with an HP Innowax capillary column (30 m × 250 μm × 0.25 μm) in a flame ionization detector. By comparing the retention times with the analytical standards, the final results were acquired. A calibration curve was prepared for each compound, by taking the readings of seven concentrations (from 1 to 1,300 μg L⁻¹) in triplicate, and the straight regression lines, respectively, were calculated, applying the acceptance criteria of R² > 0.99. (WEBBER et al., 2014). The Sigma-Aldrich (Steinheim, Germany) analytical standards were used, identified via the NIST library (National Institute of Standards and Technology). In this analysis, we mixed 50 mL of wine, 500 μL of 3-octanol (of 250 mg L⁻¹ concentration and utilized as an internal standard for not being found in wines) and 70 μL of phosphoric acid (1: 3). The samples were treated with three liquid / liquid (4: 2: 2) extractions using a mixture of diethyl ether / n-hexane (1: 1). Maintaining the temperature at 240 °C, one microliter was injected into the split mode chromatograph having 60 mL min⁻¹ divisions (30: 1). Hydrogen was chosen as the carrier gas (at 2.0 mL min⁻¹) and nitrogen as the auxiliary gas at 37 mL min⁻¹. The temperature of the oven was held at 40 °C for 5 min, then increased 3 °C min⁻¹ up to 230 °C and held for 20 min at 230 °C. Synthetic air flow at 350 mL min⁻¹ and hydrogen at 35 mL min⁻¹ were used to maintain the combustion, while the temperature of the detector was set at 230 °C.

Seventeen experienced evaluators (having above three years of experience), conducted sensory analyses of the wines through quantitative descriptive analyses (QDA), as stated by JACKSON (2002). Standard wine glasses were used to serve the 50 mL

samples (ISO 3591: 1977) to determine the olfactory and taste sensations. The sensory terms describing the organoleptic sensations perceived were noted. By common consensus the following seven attributes that described the wine aromas were selected: aromas of floral, citrus and white flesh fruit, besides aromatic intensity, olfactory character, taste quality and overall impression. Using an unstructured 10 cm scale, the evaluators assessed the strength of each attribute. While the left end of the scale implied zero descriptor intensity, the right indicated the maximum value.

The physicochemical analyses, volatile and sensory compositions-generated data were analyzed using the ANOVA method (analysis of variance). The Tukey test was employed to compare the statistically significant values, with $p < 0.05$. The SISVAR 5.6 software was used.

RESULTS AND DISCUSSION

All the yeasts resulted in the release of ethanol in percentages that complied with the Brazilian legislation for dry fine white wines (from 8.6 to 14% v v⁻¹) and none of them crossed the stipulated maximum residual sugars limit of 4 g L⁻¹ (BRASIL, 2018) (Table 1). When the *Saccharomyces cerevisiae cerevisiae* yeast was used, the resultant wine showed high alcohol content, as well as the least residual sugar content. However, when the yeast *Torulasporea delbrueckii* was used as a fermenting agent, the wine displayed the lowest alcohol content and the highest residual sugar content. These results concurred with the findings of BELDA et al. (2015) who also reported the low capacity of this yeast for sugar-ethanol conversion.

The *S. bayanus* yeast produced wine containing the highest glycerol concentration (6.34 g L⁻¹). According to MARTINHO (2008) *Saccharomyces* yeasts produce between 2 and 10 g

Table 1 - Physicochemical properties of 'Riesling Itálico' wines fermented by the yeasts *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Torulasporea delbrueckii* and *Metschnikowia pulcherrima*.

Properties	<i>S. cerevisiae</i>	<i>S. cerevisiae cerevisiae</i>	<i>S. bayanus</i>	<i>T. delbrueckii</i>	<i>M. pulcherrima</i>	CV (%)
Alcohol (% v v ⁻¹)	10.63b± 0.06	10.69a± 0.00	10.54c± 0.06	10.49d± 0.10	10.61b± 0.00	0.35
Residual sugar (g L ⁻¹)	1.02bc± 0.06	0.93c± 0.06	1.03bc± 0.06	3.50a± 0.17	1.11b± 0.10	7.93
Glycerol (g L ⁻¹)	6.01c± 0.06	5.09d± 0.10	6.34a± 0.12	6.24b± 0.06	5.99c± 0.10	1.23
Volatile acidity (g L ⁻¹ HAc)	0.40c± 0.00	0.10d± 0.00	0.40c± 0.00	0.42b± 0.06	0.50a± 0.00	4.86

Different letters on the same line indicate a significant difference statistically by the Tukey test, with $P < 0.05$.

L⁻¹ glycerol, the cryotolerant species, like *S. bayanus* (*S. uvarum*), induce higher glycerol concentrations. The *M. pulcherrima* and *T. delbrueckii* yeasts on the other hand, liberated glycerol only in intermediate quantities. Glycerol production ranks among the desirable traits of the yeast because it enhances the taste of the wine, with sensations of viscosity, smoothness and roundness (ZHAO et al., 2015).

The *S. cerevisiae cerevisiae* produced the lowest volatile acidity in the wines, among the many yeasts tested (Table 1). All the remaining yeasts fermented the wines to have higher acidity (0.4 to 0.5 g L⁻¹) and showed similarity to each other. Therefore, as these non-*Saccharomyces* yeasts yield high levels of volatile acidity their use is limited (BELDA et al., 2015). However, in the current study, the volatile acidity was similar to that of *Saccharomyces* yeasts (0.44 and 0.42 g L⁻¹), as reported by GOBBI et al. (2013) and AZZOLINI et al. (2015), respectively. As these concentrations are well within the maximum limits stipulated by the legislation, (1.2 g L⁻¹ HAc or 20 mEq.L⁻¹) they do not influence the sensory quality of the wines (BRASIL, 2018).

The wines were tested and quantified for novel volatile compounds belonging to various chemical families (C6 alcohols, esters and acids) (Table 2). Quantification revealed that the presence

of 2-phenylethanol was higher in concentration in the samples than were the other compounds. Wines fermented using the yeasts *S. cerevisiae* and *S. cerevisiae cerevisiae* revealed the highest concentrations of 2-phenylethanol. This superior alcohol released through yeast metabolism is able to also enable the production of derivatives like phenylethyl acetate ester, which give the wines the rose aromas (ETSCHMANN et al., 2002; WANG et al., 2016).

Ethyl octanoate was found to be the most abundant of the esters (Table 2). When the yeast of the genus *Saccharomyces* was used as a fermentation agent, the wines revealed the highest concentrations of ethyl octanoate (beyond 1,000 µg L⁻¹), while fermentation using the yeast *T. delbrueckii* produced the next highest levels. These results correspond to the results reported by SADOUDI et al. (2012), clearly showing lower concentrations of this compound in the wines produced by using the non-*Saccharomyces* yeasts as the fermenting agents. As a point of interest, all the treatments produced ethyl octanoate levels which exceeded the threshold of olfactory perception (2 µg L⁻¹), thus enhancing the aromatic quality of the wines, as their descriptors (pineapple and pear) induce a pleasing smell (JIANG & ZHANG, 2010).

In quantitative terms, the second key ester was phenylethyl acetate because it contributed the

Table 2 - Concentration of volatile compounds (µg L⁻¹) of yeast-fermented 'Riesling Italico' wines *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae cerevisiae*, *Saccharomyces bayanus*, *Torulopsis delbrueckii* and *Metschnikowia pulcherrima*.

Compound (descriptors)	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>S. bayanus</i>	<i>T. delbrueckii</i>	<i>M. Pulcherrima</i>	*LO ^{OS}
2-phenylethanol (roses ¹)	44,120 ^a ±5,812	41,031 ^{ab} ±2,872	31,905 ^c ±3,996	33,450 ^c ±2,216	36,395 ^{bc} ±3,774	10,000 ¹
Isoamyl acetate (banana ¹)	26a ± 7.5	17ab ± 5.5	11b ± 8.9	18ab ± 2.7	12b ± 9.1	30 ¹
Hexyl acetate (pear, apple ²)	62a ± 7.8	59a ± 0.8	58a ± 0.7	58a ± 1.1	58a ± 1.2	670 ²
Phenylethyl acetate (roses, honey ²)	258a ± 6.6	123b ± 26.5	135b ± 13.2	243a ± 27.8	136b ± 35.0	250 ²
Ethyl octanoate (pineapple, pear ³)	1,043a ± 59.8	1,071a ± 159.8	1,066a ± 91.8	809b ± 42.8	748b ± 291.2	2 ³
Ethyl decanoate (floral, soap ⁴)	84a ± 17.0	78a ± 1.8	78a ± 10.0	84a ± 7.2	74a ± 19.2	200 ⁴
Ethyl decanoate (floral, fruity ³)	71.2bc ± 1.0	74.0ab ± 1.7	71.2c ± 1.1	71.5c ± 1.1	76.2a ± 2.9	350 ³
Decanoic acid (rancid ¹)	1,206b ± 68.1	1,100b ± 145.8	1,685a ± 180.1	760.0c ± 26.0	926.7c ± 152.2	1,000 ¹
Dodecanoic acid (Metal ¹)	103a ± 7.1	103a ± 5.0	100a ± 0.0	100a ± 0.0	100a ± 0.0	1,000 ¹

Different letters on the same line indicate a significant difference statistically by the Tukey test, with P<0.05. *LO^{OS}: Olfactory perception threshold (µg L⁻¹). References: ¹WANG et al. (2016); ²ARCARI et al. (2017); ³JIANG & ZHANG (2010); ⁴KATARINA et al. (2014).

floral touches of rose and honey to the wine aromas, with the threshold of olfactory perception at 250 $\mu\text{g L}^{-1}$ (ARCARI et al., 2017). Only the yeast *S. cerevisiae* exceeded this threshold, followed closely by *T. delbrueckii*. Results concurring with these findings were recorded by SADOUDI et al. (2012), suggesting that *T. delbrueckii* had the capacity to produce this compound similar to *S. cerevisiae*.

As isoamyl acetate imparts a pleasant sensory aroma to the wines, it is recognized as being among the most significant of the esters. It is generated through the esterification of isoamyl alcohols and the olfactory perception it endows is very similar to a banana aroma (JIANG & ZHANG, 2010). When the *S. cerevisiae* (26 $\mu\text{g L}^{-1}$) yeasts were used in fermentation, this ester was produced in the highest concentration, while fermentation with *T. delbrueckii* and *S. cerevisiae cerevisiae* produced lower levels of it. The olfactory perception limit for this compound is 30 $\mu\text{g L}^{-1}$ (WANG et al., 2016), a concentration which none of the wines tested achieved.

In all the wines tested, the concentrations of other esters, like the hexyl acetate and ethyl decanoate esters, showed no particular differences. Further, the olfactory perception thresholds of these compounds were not exceeded in any of the samples (KATARÍNA et al., 2014; ARCARI et al., 2017). On the other hand, the decanoic acid and dodecanoic acids, which are volatile compounds that exert a negative influence upon the aromatic quality of wine, (RIBÉREAU-GAYON et al., 2006), were also assessed. Among these, the presence of decanoic acid alone was noted to be at concentrations above the prescribed olfactory perception threshold in the treatments using the *Saccharomyces* yeasts.

Table 3 lists the results of the quantitative descriptive sensory analysis (QDA) of the wines. All the sensory descriptors returned values that were average scores, without any statistical differences being noted among the treatments. The wines, however, gave evidence of the perception of the different aromatic families, indicating a high level of aromatic complexity. These results concurred with the findings of SCHÜTTLER et al. (2015) in the 'Riesling' wines, with the emphasis on the fruity notes, pear and apple in particular, apart from a floral touch. Wine fermented using the yeast *S. cerevisiae* revealed the highest aromatic profile scores, barring the floral aroma. This result is related to the presence of the volatile compounds in greater concentration in this wine, which maximized the pleasant aromas, such as 2-phenylethanol (roses), phenylethyl acetate (roses and honey) and ethyl octanoate (pineapple and pear), and minimized the perception of the defective aromas, like those imparted by decanoic acid (rancid), which crossed the olfactory perception limit in this wine (Table 2).

When *T. delbrueckii* was used as the fermenting agent, higher sensory evaluation scores were noted, than for the wines fermented with the non-*Saccharomyces* yeasts (Table 3). This finding is linked to presence of the compounds which impart the pleasant aromas in high levels: isoamyl acetate (banana), hexyl acetate (pear and apple), phenylethyl acetate (roses and honey) and ethyl decanoate (floral). In addition, it presented the highest scores for taste quality, among all evaluated treatments. In their work on red wines, RAMÍREZ et al. (2016) reported heightened taste quality imparted by *T. delbrueckii*, while the wines made with *S. cerevisiae* were noted for the most intense aromatic notes.

Table 3 - Sensory analysis of 'Riesling Italico' wines fermented by yeast *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae cerevisiae*, *Saccharomyces bayanus*, *Torulasporea delbrueckii* and *Metschnikowia pulcherrima*.

	<i>S. cerevisiae</i>	<i>S. cerevisiae cerevisiae</i>	<i>S. bayanus</i>	<i>T. delbrueckii</i>	<i>M. pulcherrima</i>	CV (%)
Intensity of aroma	4.92 ^a ± 0.5	4.60 ^a ± 0.2	4.62 ^a ± 0.5	4.82 ^a ± 0.7	4.52 ^a ± 0.3	9.93
Floral	3.77 ^a ± 0.7	3.17 ^a ± 0.3	3.37 ^a ± 0.5	3.90 ^a ± 0.3	3.80 ^a ± 0.9	16.27
Citrus fruits	3.90 ^a ± 0.7	3.52 ^a ± 0.4	3.80 ^a ± 0.2	3.72 ^a ± 0.3	3.40 ^a ± 0.3	11.33
White pulp fruits	4.15 ^a ± 0.6	4.05 ^a ± 0.6	4.02 ^a ± 0.3	4.15 ^a ± 0.7	3.87 ^a ± 0.5	13.62
Olfactory quality	5.12 ^a ± 1.1	4.97 ^a ± 0.9	4.70 ^a ± 0.4	4.60 ^a ± 0.3	4.50 ^a ± 0.4	14.61
Tasteful quality	4.77 ^a ± 1.0	4.32 ^a ± 0.9	4.77 ^a ± 0.6	4.80 ^a ± 0.1	4.37 ^a ± 0.7	15.87
Overall rating	5.22 ^a ± 0.9	4.90 ^a ± 0.6	5.07 ^a ± 0.3	5.12 ^a ± 0.4	4.67 ^a ± 0.6	11.99

Different letters on the same line indicate a significant difference statistically by the Tukey test, with $P < 0.05$.

From the findings of the present work it is evident that the *T. delbrueckii* yeast is suitable for the production of Riesling Itálico white wines as it augments the sensory quality, particularly the olfactory quality. As it possesses a lower capacity for sugar conversion into ethanol, *T. delbrueckii* can be used either together with the *Saccharomyces* yeasts or as an isolated inoculation. This is because even if the sugar to ethanol conversion is lower, the quality of the wines, according to the stipulations of Brazilian law, is not compromised.

CONCLUSION

All the yeasts evaluated in this study produced the 'Riesling Itálico' wines, compliant with the Brazilian standards of white wines in terms of identity and quality. The *M. pulcherrima* and *T. delbrueckii* yeasts produced wines well within the stipulated legislations in terms of alcohol content, volatile acidity and residual sugar. The *S. cerevisiae* yeasts produced wines rich in 2-phenylethanol, ethyl octanoate, phenylethyl acetate and isoamyl acetate, which imparted the best sensory quality among the wines tested. The *T. delbrueckii* yeast produced wines with high contents of phenylethyl acetate, isoamyl acetate and ethyl decanoate, which enhanced the olfactory quality and sensory evaluation of these wines.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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