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Integrated enzymatic–yeast biostrategy to obtain reduced-alcohol wine

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ABSTRACT

Overripening of grapes, due to global warming, can result in unbalanced wines with higher alcohol content, lower acidity and an altered sensory profile. Pre-fermentative treatment of the must with a glucose oxidase-catalase enzyme system immobilized in silica-calcium-alginate hydrogel capsules degraded up to 17.3% of the glucose in the must in 48 h to obtain wines with 1.0–1.3% vol (v/v) lower alcoholic strength. Most of the gluconic acid produced by glucose oxidation was retained in the capsules, resulting in a mild reduction in the pH, thereby avoiding a strong acidification of the must. The remainder of the gluconic acid present in the must was largely degraded during the fermentation process using a selected strain of the yeast *Schizosaccharomyces pombe (S. pombe)*. Both the enzymatic treatment of the must with the capsules and the use of *S. pombe*, either in unique inoculation or in sequential inoculation with a *Saccharomyces cerevisiae (S. cerevisiae)*, led to balanced wines with a unique chemical profile. The combination of these two strategies, pre-fermentative and fermentative, presents an innovative and promising approach, not investigated so far, to counteracting the adverse effects of rising temperatures due to global warming.

1. Introduction

An accelerated and sustained increase in temperature, generated by global warming, can significantly affect the chemical composition of grapes (Van Leeuwen et al., 2024; Venios, Korkas, Nisiotou, & Banilas, 2020). Some varieties, such as Verdejo grape, are particularly sensitive to this increase in temperature due to the agroclimatic conditions and characteristic physiological traits, such as (i) a medium-short cycle (reaching maturity earlier than other white varieties), (ii) high photosynthetic efficiency (accumulating sugars quickly), (iii) thin skin and juicy pulp (promoting dehydration and loss of acidity in extreme heat scenarios), and (iv) a heat-sensitive aromatic profile (as its aromatic compounds degrade on warm nights) (Azuara, González, Mangas, & Martín, 2023; Ramos & Yuste, 2023).

In the context of global warming, with an increase in temperature and a smaller temperature range (day-night), the process of phenolic and aromatic ripening in grapes would occur at a slower rate than that of sugar ripening. The early stage of veraison and the acceleration of the technological ripening of grapes result in two crucial adverse changes: a high concentration of fermentable sugars in the grapes and a loss of acidity with an increase in pH (Droulia & Charalampopoulos, 2022; González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015). As a consequence, the musts obtained may exhibit physico-chemical and microbiological alterations that can contribute to various problems during one or more stages of winemaking, as well as sensory imbalances and/or defects in the resulting wine. An elevated content of fermentable sugars in the must can culminate in wines with an alcoholic strength increase of 1.0–1.5% vol (v/v), which can have a detrimental impact on the quality of the wine, altering its taste and aroma (Godden, Wilkes, & Johnson, 2015; Jordão, Vilela, & Cosme, 2015). The decrease in acidity and increase in pH above 3.5 generates a loss of freshness and aromatic typicity in wines and makes them more prone to oxidation and microbiological alteration (Botezatu, Elizondo, Bajec, & Miller, 2021; Mangas, González, Martín, & Rodríguez-Nogales, 2023; Soler, Del Fresno, Bañuelos, Morata, & Loira, 2025).

To address this issue, our research group is studying, as an innovative pre-fermentative strategy, the application of the enzyme system glucose oxidase-catalase (GOX-CAT) immobilized in silica-calcium-alginate hydrogel capsules (GOX-CAT capsules) to reduce glucose levels in must and thus counteract overripening (Del-Bosque, Vila-Crespo, Ruipérez,

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Fernández-Fernández, & Rodríguez-Nogales, 2023a, 2023b) and simultaneously achieve a moderate reduction in the high pH value (3.8) of the must to optimal winemaking values (< 3.5).

GOX is a flavoprotein that catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2), thereby reducing the concentration of this yeast-fermentable sugar in the must. The H_2O_2 generated behaves as a competitive inhibitor versus O_2 for GOX (Majumdar, Khan, & Bandyopadhyaya, 2016). Excessively high levels of H_2O_2 can also lead to inhibition of subsequent fermentation of the must (Röcker, Schmitt, Pasch, Ebert, & Grossmann, 2016). Consequently, the enzyme CAT is incorporated into the hydrogel capsules, which decompose H_2O_2 into H_2O and O_2. As detailed by Del-Bosque et al. (2023a), the immobilization of the GOX-CAT enzyme system was carried out by entrapment in a silica-calcium-alginate porous hydrogel, modeled in the form of spherical capsules that maximize the contact surface with the must. The results revealed that the immobilized GOX enzyme showed a higher relative activity than the free enzyme within the pH range of 3.20–3.60 and at low temperatures (10–15 °C). Subsequently, Del-Bosque et al. (2023b) confirmed the capacity of the immobilized enzyme system to process glucose in Verdejo must, achieving a reduction in potential alcohol strength of up to 2.0% vol (v/v).

The present study aims to investigate the use of a fermentative strategy as a complement to the enzymatic pre-fermentative strategy. From this perspective, the use of a properly selected *S. pombe* strain was proposed to obtain non-significant concentrations of gluconic acid remaining in the must and simultaneously to enhance the uniqueness and to improve the attributes of the final wine, due to the distinctive metabolism of this yeast. *S. pombe* has the strain-dependent capacity to degrade gluconic acid through the transport of gluconate into the yeast via specific anion-capturing transporters located on the cell membrane and, once in the cytoplasm, by phosphorylating it to 6-phosphogluconate by the enzyme gluconate kinase. After that, it is integrated into the pentose phosphate pathway for further metabolism (Benito, 2019; Corkins, Wilson, Cocuron, Alonso, & Bird, 2017). A reduction or elimination of this acid is convenient since gluconic acid can generate microbiological instability during wine storage, due to the growth of lactic acid bacteria capable of metabolizing it. Sp3 strain of *S. pombe*, which exhibits high gluconic acid consumption, moderate malic acid consumption and high fermentative power enabling it to complete fermentation on its own (Del-Bosque et al., 2025), was chosen for the fermentation of the enzymatically treated must. The analysis included a comparison of fermentation results utilizing both unique *S. pombe* strain and in sequential inoculation with a strain of *S. cerevisiae*.

2. Materials and methods

2.1. Enzymes and chemical reagents

GOX (EC 1.1.3.4, Gluzyme® Fortis 10,000 BG from *Aspergillus niger*, 10,000 U/g) and CAT (EC 1.11.1.6, Catazyme® 25 L from *A. niger*, 25,000 U/mL) were obtained from Novozymes (Bagsvaerd, Denmark). LUDOX® HS-40 colloidal silica (420816) and sodium silicate (338443) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium alginate (A3249,0250) was acquired from Panreac Applichem (Darmstadt, Germany). The remaining chemicals were of analytical grade and were obtained from Panreac Applichem (Darmstadt, Germany).

2.2. Yeast strains and culture medium

The strain of *S. pombe*, identified as Sp3 (CECT 1378), belonging to the CECT (Colección Española de Cultivos Tipo, Spain), was chosen for its high capacity to consume gluconic acid. Additionally, a strain of *S. cerevisiae (WAM)*, commercially available as a dried active yeast (Uvaferm WAM™, Lallemand Bio, Blagnac, France), was employed as a control and in sequential inoculation with the *S. pombe* strain.

Both yeasts were grown in YPD culture medium (1% yeast extract powder, 2% peptone and 2% glucose; Labkem (Barcelona, Spain)) at 25 °C under agitation at 220 rpm (Orbital Shaker SO1, Stuart Scientific, Stone, United Kingdom) for 48–72 h. The McFarland standards and their standard curve (10 points) were used to determine cell concentration by measuring optical density at 600 nm with a UV–Vis spectrophotometer (Genesys™ 150, Thermo Fisher, Madrid, Spain).

2.3. Verdejo grape must

All trials were conducted using Verdejo grape must from a winery located in the Appellation of Origin Rueda (Spain). Must was sulfited at 60 mg/L and frozen at −20 °C in an industrial freezing chamber until its utilization. Prior to the assay, the must was thawed and homogenized at 250 rpm (Orbital Shaker SO1, Stuart Scientific, Stone, UK) for 20 min. Main analytical parameters of the must used in the study are shown in Table 1.

2.4. Enzymatic treatment of must with GOX-CAT capsules and vinification with S. Pombe and S. cerevisiae

Assay was carried out in 2 phases. The experimental design is shown in Fig. 1.

In the first phase, the initial must at pH 3.80 was divided into (i) 550 mL of control must (CT-MUST), which will not be enzymatically treated and (ii) 2400 mL of must (GOX-MUST) to be treated enzymatically with the GOX-CAT capsules. These capsules were prepared as described by Del-Bosque et al. (2023a) with the modification of using a peristaltic pump (BQ80S, Lead Fluid, Baoding, China) instead of a manual syringe (inner diameter of 1.5 mm). The pump operated with an inner tube diameter of 2.0 mm and a flow rate of 13.6 mL/min. Capsules were obtained 24 h before the must treatment and were kept refrigerated at 4 °C until use. Capsules were weighed and their diameter measured using a digital caliper (Powerfix, model Z22855, Neckarsulm, Germany) before and after the treatment. The volume of treated must was 2400 mL to which 144 g of capsules were added. This represents a ratio of 60 mg hydrogel/mL of must and assumes a dose of co-immobilized enzymes of 1.8 U/mL must for both GOX and CAT enzymes.

The choice of this enzyme dose is based on the results of previous publications (Del-Bosque et al., 2023a, 2023b), which showed that this enzyme activity would be required to achieve sufficient glucose oxidation in 48 h of treatment at 15.0 °C.

The GOX-CAT capsules were added free in the must and both bottles with CT-MUST and GOX-MUST were kept in the oven at 15 °C for 48 h under stirring at 75 rpm. Brix degree (°Brix), glucose concentration, pH, gluconic acid concentration, free and total SO_2, and CIELab coordinates, were measured in CT-MUST and GOX-MUST, initially and after 48 h following the methodology described in *sections 2.5* and *2.6*.

In the second phase, the fermentation of both CT-MUST and GOX-MUST were carried out in 500 mL Erlenmeyers flasks at 25 °C. Musts were enriched with Actimax NATURA (Agrovin, Alcázar de San Juan,

Table 1
Analytical parameters of the initial must.

Parameters	Initial Must*
Brix	21.5 ± 0.14
Glucose (g/L)	105.9 ± 2.3
Gluconic acid (g/L)	N.d.
pH	3.80 ± 0.01
Free SO_2 (mg/L)	17 ± 1
Total SO_2 (mg/L)	60 ± 6
L* (AU)	97.6 ± 0.3
C* (AU)	7.8 ± 0.2
H* (AU)	100.0 ± 0.1
a* (AU)	−1.36 ± 0.06
b* (AU)	7.7 ± 0.2

* Mean values ± standard deviation. N.d. indicates that the compound was not detected.

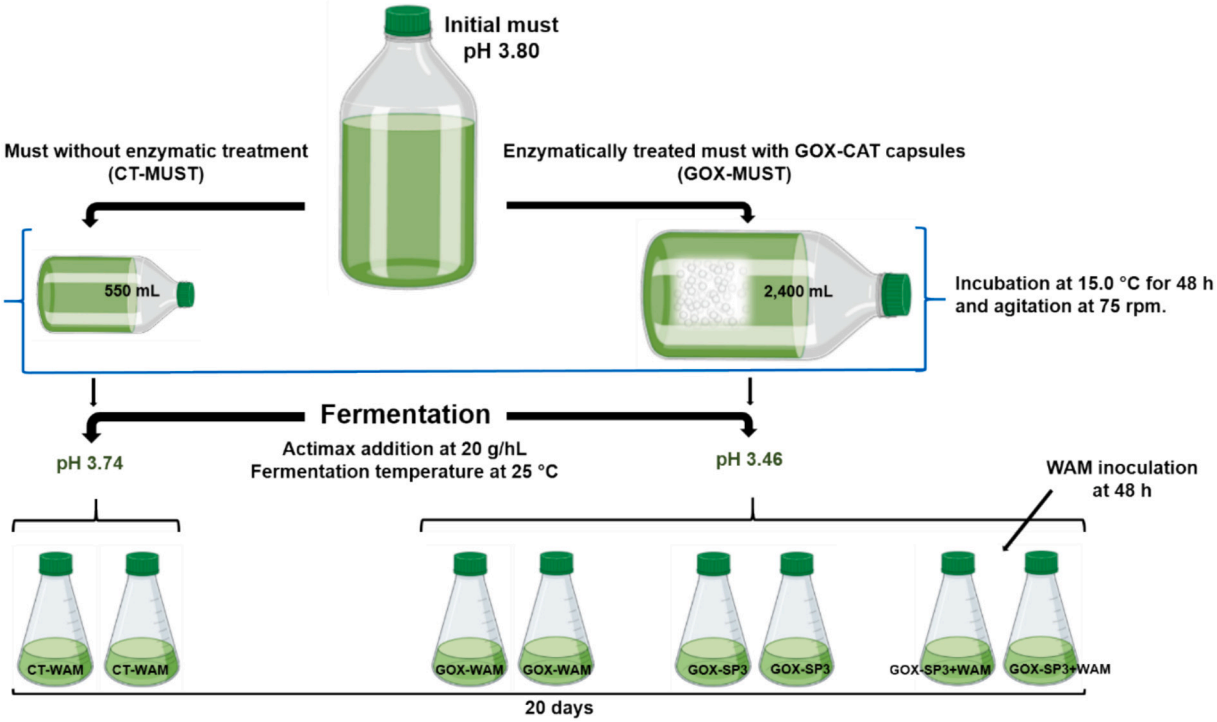

Fig. 1. Experimental design of the assay showing the enzymatic treatment phase of the must with GOX-CAT capsules and the fermentation phase of the musts.

Spain) at a concentration of 20 g/hL. CT-MUST was inoculated with the WAM strain in a 200 mL trial in duplicate (CT-WAM). The GOX-MUST volume was divided into three 200 mL trials in duplicate (GOX-WAM, GOX-SP3 and GOX-SP3 + WAM). GOX-WAM trials were also inoculated with the WAM strain. GOX-SP3 trials were inoculated with the Sp3 strain. GOX-SP3 + WAM trials were inoculated with the Sp3 strain and 48 h later with the WAM strain. This 48-h period was chosen based on the results obtained by Del-Bosque et al. (2025), where it was observed that this period allowed the development of the Sp3 strain in the must before inoculating the WAM strain. In all trials, each yeast strain (Sp3 and WAM) was inoculated at a concentration of 10^7 CFU/ml. All trials were kept in incubation at 25 °C for 20 days (T20).

Finally, glucose and glycerin concentrations, alcohol strength, gluconic, malic, tartaric, citric and succinic acid concentrations, pH, titratable and volatile acidity, potassium concentration, total polyphenol index, hydroxycinnamic acids, flavonols, catechins, CIELab coordinates and volatile organic compounds (VOCs) were measured in all trials, as indicated in *sections 2.5, 2.6* and *2.7*.

2.5. Basic composition of musts and wines

Oenological analysis of musts and wines was conducted in triplicate. Brix was calculated using a hand refractometer (ATC-1, Atago, Tokyo, Japan). The concentration of glucose, gluconic and malic acids was calculated using enzyme kits K-FRGLQR-02/17, K-GATE 04/20, and K-LMALQR 03/18, respectively (Megazyme Bray Co., Wicklow, Ireland), Gluconic acid concentration data shown in the tables were measured in the musts and wines and the percentage of gluconic acid consumption was calculated taking into account the differences between the acid concentration measured in GOX-MUST and the acid concentration measured in the different wines obtained (GOX-WAM, GOX-SP3, and GOX-SP3 + WAM). The determination of free and total SO_2 was performed with the SO_2-Matic 23 (Crison Instruments, Alella, Spain). The pH and titratable acidity were determined using a pHmeter sensION™ + (Hach Lange, Barcelona, Spain). Titratable acidity was calculated by potentiometric titration using a 0.1 N NaOH solution and expressed as tartaric acid (g/L). The volatile acidity was quantified by titrating a fraction of distilled wine with 0.01 N NaOH. The alcohol strength was measured using an ebulliometer (Gab, Barcelona, Spain). Concentrations of glycerol, catechins, potassium, and tartaric, citric and succinic acids were obtained by analysis using an infrared analyzer (FTIR) (Bacchus 3 MultiSpec, Tecnología Difusión Ibérica, Gavà, Spain).

2.6. Determination of total polyphenol index, hydroxycinnamic acids, flavonols and CIELab coordinates of musts and wines

The must and wine samples were centrifuged at 4000 rpm for 10 min. To quantify the total polyphenol index, hydroxycinnamic acids, and flavonols, the samples were diluted with deionized water by a factor 1:10 and the absorbances were measured at 280, 320, and 365 nm, respectively, using a quartz cell of 1 cm path length. CIELab coordinates (L*, C*, H*, a*, and b*) were calculated by measuring at 450, 520, 570, and 630 nm with the same cell and performing the data analysis with the MSCV® program (Universidad de la Rioja, Spain). Spectrophotometric measurements were conducted in triplicate using a UV–Vis spectrophotometer (Genesys™ 150, Thermo Fisher, Madrid, Spain). The colorimetric difference (ΔE*) between two wines was calculated according to the OIV-MA-AS2–11 method. Equivalent colors of the CIELab coordinates of the musts and wines obtained in the RGB model were calculated using a CIELAB to RGB converter (https://colordesigner.io/convert/labtorgb; URL available in July 2025).

2.7. Quantification of volatile organic compounds of wine by headspace-solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS)

A CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) was utilized in conjunction with a 7890 A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a 5977 mass selective detector (Agilent Technologies, Santa Clara, CA, USA) for the determination of wine VOCs. The HS-SPME method was employed for the extraction of wine volatile compounds, following the procedure established by Massera et al. (2012), with minor modifications. 20-mL vials were filled with 5 mL of wine from each trial with 3 g of NaCl

and 50 μL of methyl nonanoate (0.051 mg/L) as internal standard. The vials were sealed with a magnetic screw cap provided with a PTFE/silicone septum and incubated at 40 °C for 15 min with agitation (250 rpm). Extraction of VOCs was performed in the headspace vial at 40 °C for 30 min with agitation (250 rpm) using the SPME Fiber Assembly 50/30 μm DVB/CAR/PDMS Stableflex (2 cm) (Supelco, Inc., Belle- fonte, USA), previously preconditioned at 270 °C for 15 min. After extraction, the fiber was introduced into the injector of gas chromatograph (250 °C) to desorb the volatiles for 15 min. The injector temperature was 250 °C, working in splitless mode (1 min). The chromatographic separation of the volatiles was performed on a HP-Innowax column (60 m, 0.250 mm, 0.5 μm) (J &W Scientific, Folsom, CA, USA). The oven temperature program was as follows: 40 °C held for 5 min, then ramped up to 230 °C at 2.5 °C/min and finally held for 20 min. Helium gas at a flow of 1.2 mL/min (pressure of 22.413 psi) was used as carrier gas. MS was operated under the SCAN mode and the mass range studied was from 30 to 500 *m/z*. The identification was performed by comparing GC mass spectra with pure standards and with spectra from the NIST08 y Wiley7 libraries and using only the spectral data libraries in the absence of available standards (Sánchez et al., 2022). Quantification was performed using the internal standard quantification method as equivalents (Ayestarán et al., 2019; Sieiro, Villa, Da Silva, García-Fraga, & Vilanova, 2014) of 2-octanol (0.251 mg/L). Samples were analyzed in triplicated.

2.8. Microscopy images

Images of the yeasts present in the fermenting musts were taken 7 days (T7) after the initial inoculation (T0) using a Nikon ECLIPSE 90i microscope (Nikon Corporation, Tokyo, Japan) equipped with a Nikon DS-Fi3 camera, a Plan Fluor 60× DIC M objective (with numerical aperture 0.85) and NIS-Elements AR v4.60.00 LO, 64 Bit image analysis software (Nikon Corporation, Tokyo, Japan).

2.9. Statistical analysis

All results were expressed as mean values ± standard deviation. Data management and graphs were carried out with Microsoft Excel (2019 MSO 64 bits) and Statgraphics Centurion 19 (version 19.2.01, The Plains, VA, USA). Analysis of variance (ANOVA) and principal component analysis (PCA) of the data were carried out using Statgraphics Centurion 19 with a significance level of $\alpha = 0.05$. In PCA, in the equations for each principal component, the values of the variables were standardized by subtracting their mean and dividing them by their standard deviations.

3. Results and discussion

3.1. Enzymatic treatment of the must with GOX-CAT capsules

To reduce glucose levels in the Verdejo must, enzymatic treatment with GOX-CAT capsules was carried out. As shown in Table 2, the enzymatic treatment significantly reduced the glucose concentration in GOX-MUST to 87.1 ± 2.0 g/L, which represents a reduction of 17.3 ± 0.5% with respect to CT-MUST. These results align with those previously obtained in Del-Bosque et al. (2023a, 2023b). The reduction in glucose concentration was also reflected in the °Brix value, with values for CT-MUST and GOX-MUST of 21.4 ± 0.1 and 20.0 ± 0.1, respectively (Table 2). The 18.2 g/L of glucose that were consumed in GOX-MUST during enzymatic treatment would theoretically represent a 1.07% vol. (v/v) reduction in the alcoholic strength of the final wine (estimating that 1.0% vol. (v/v) corresponds to a consumption of ~17 g/L of glucose by yeasts during alcoholic fermentation) (Gonzalez, Guindal, Tronchoni, & Morales, 2021).

The concentration of gluconic acid measured in GOX-MUST after enzymatic treatment was 2.18 ± 0.2 g/L (Table 2). Since 18.2 g/L of glucose was consumed during the 48 h of treatment, the estimated concentration of gluconic acid produced would be 21.8 g/L (estimating that 1 mol of glucose corresponds to 1 mol of gluconic acid). Botezatu et al. (2021) also reported stoichiometric concentrations between glucose oxidation and gluconic acid generation. This difference between the gluconic acid concentration measured in GOX-MUST and the estimated concentration implies that the GOX-CAT capsules managed to retain 90.01% (19.65 g/L) of the theoretical gluconic acid produced in glucose oxidation.

Table 2
Physicochemical parameters of enzymatically untreated and treated musts.

Parameters	MUSTS*	
	CT-MUST	GOX-MUST
°Brix	21.4 ± 0.1[a]	20.0 ± 0.1[b]
Glucose (g/L)	105.3 ± 2.0[a]	87.1 ± 2.0[b]
% glucose consumption	–	17.3 ± 0.5
Gluconic acid (g/L)	N.d.	2.18 ± 0.2
pH	3.74 ± 0.01[a]	3.46 ± 0.01[b]
Free SO_2 (mg/L)	16 ± 1[a]	7 ± 1[b]
Total SO_2 (mg/L)	55 ± 7[a]	26 ± 2[b]
L* (AU)	97.8 ± 0.2[a]	92.6 ± 0.1[b]
C* (AU)	7.0 ± 0.1[a]	21.6 ± 0.1[b]
H* (AU)	97.6 ± 0.1[a]	90.8 ± 0.2[b]
a* (AU)	−0.92 ± 0.03[a]	−0.31 ± 0.06[b]
b* (AU)	6.9 ± 0.1[a]	21.6 ± 0.1[b]
$\Delta E^*_{CT\text{-}MUST—GOX\text{-}MUST}$	15.6	

* Mean values ± standard deviation. N.d. indicates that the compound was not detected (below the detection limit of the method). For each parameter, different letters in each row indicate significant differences between musts at the 95.0% confidence level according to Fisher's Least Significant Difference (LSD) procedure.

The initial pH of the must before enzymatic treatment were 3.80 ± 0.01 (Table 1). After the 48 h treatment, CT-MUST and GOX-MUST showed pH values of 3.74 ± 0.01 and 3.46 ± 0.01, respectively (Table 2). Evidently, the production and release of gluconic acid in GOX-MUST generated a decrease in pH with respect to the initial values and to the CT-MUST values where there was no treatment with GOX-CAT capsules. Unlike the enzymatic treatment of the must with the free enzymes (not immobilized), which release all the gluconic acid into the must (Del-Bosque et al., 2023b; Mangas et al., 2023), the pH drop in GOX-MUST was moderate, thanks to the retention of most of the gluconic acid inside the GOX-CAT capsules, allowing a pH more suitable for the yeast activity and an optimal alcoholic fermentation.

The oxidation of glucose by GOX generates gluconic acid and H_2O_2. It has been reported that SO_2 can interact with both γ- and δ-gluconolactones derived from gluconic acid forming esters (which may reduce its positive effects against oxidation and unwanted microbial growth) (Barbe, De Revel, & Bertrand, 2002; Müller & Rauhut, 2018) and with H_2O_2 which would oxidize SO_2 to sulfate (SO_4^{2-}) (Wang & Kumar, 2024). Even, SO_2 may bind reversibly to the prosthetic group of GOX (a flavin adenine dinucleotide) forming a flavin-sulfite adduct (Pickering, Heatherbell, & Barnes, 1999). These possible interactions were reflected in the SO_2 measurements taken after the 48 h treatment, which showed free/total SO_2 values of 16 ± 1 / 55 ± 7 mg/L and 7 ± 1 / 26 ± 2 mg/L, in CT-MUST and GOX-MUST, respectively (Table 2), with a significant decrease for GOX-MUST in both values of ~50%.

CT-MUST exhibited very small differences in the CIELab coordinates values with the initial must (Tables 1 and 2). However, GOX-MUST clearly exhibited, at the end of the enzymatic treatment, a browning of its color (Fig. 2) and CIELab coordinates values showed significant differences between CT-MUST and GOX-MUST (Table 2).

The minimal color difference (ΔE*) between two samples detectable by the human eye through a wine tasting glass has been fixed as $\Delta E^* = 2.7$ (Aleixandre-Tudo, Buica, Nieuwoudt, Aleixandre, & Du Toit, 2017; Pérez-Gil, Pérez-Lamela, & Falqué-López, 2022). CT-MUST and GOX-MUST showed a $\Delta E^*_{CT\text{-}MUST—GOX\text{-}MUST} = 15.6$ (Table 2). Most likely, this browning is due to the action of H_2O_2 generated as a by-product of

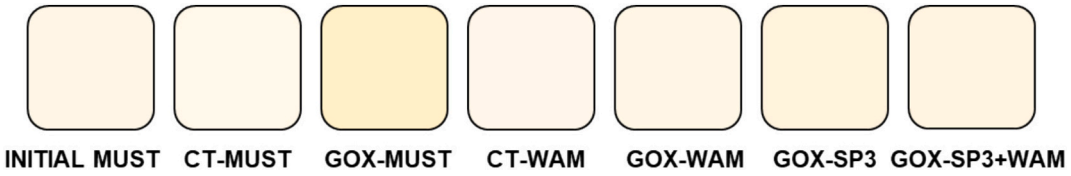

Fig. 2. Colors of initial MUST before treatment, CT-MUST and GOX-MUST after 48 h of treatment and of the resulting wines at T20 are illustrated. Colors shown above, obtained in the RGB model, were calculated from the corresponding CIELAB coordinates of each must and wine.

glucose oxidation and which the CAT enzyme activity was not able to fully compensate. This browning of the must by the release of H_2O_2 was also observed by Valencia, Espinoza, Ramirez, Franco, and Urtubia (2017) and Botezatu et al. (2021). Even so, as will be explained in *section 3.2.*, after alcoholic fermentation and stabilization up to T20, the CIELab coordinates values of the wines obtained from GOX-MUST will return to values more similar to those of CT-MUST, thereby recovering much of the original color.

Fig. 3. shows the GOX-CAT capsules (a) before and (b) after enzymatic treatment. To separate the GOX-CAT capsules from the must, a metal sieve with a pore size of 1 mm was used. No deformation of the geometry or breakage of the capsule structure was observed at the end of the treatment.

It was obtained (i) a change in the shade of the capsules, probably due to the absorption of the dyes from the must and (ii) an increase in their weight, due to the filling of the internal porous structure of the hydrogel, with the must and the retention of gluconic acid. The weight increased from 27 ± 10 mg/capsule to 31 ± 10 mg/capsule, i.e., a weight increase of 12.9%. The average diameter of the capsules was 3.79 ± 0.06 mm and 3.69 ± 0.04 mm before and after enzymatic treatment, respectively.

3.2. Alcoholic fermentation of musts with strains of S. cerevisiae and S. Pombe and physicochemical analysis of wines

After the enzymatic treatment, both CT-MUST and GOX-MUST were inoculated (T0) with their respective yeasts (as indicated in the experimental design, Fig. 1) and kept in incubation for 20 days. All trials experienced fermentation without incident (stuck or sluggish) or apparent contamination. Biyela, du Toit, Divol, Malherbe, and van Rensburg (2009), Valencia et al. (2017) and Botezatu et al. (2021) also reported no fermentation difficulties after enzymatic treatment.

At 7 days (T7) after the initial inoculation, microscopy images of all trials were taken to check for yeast presence (Fig. 4).

The rectangular morphology of *S. pombe* and its binary fission system are easily identified and microscopically differentiated from the oval morphology of *S. cerevisiae,* which reproduces by budding. As observed in Fig. 4, there is a unique population of *S. cerevisiae* in CT-WAM and GOX-WAM trials. In GOX-SP3 trial, there is a very predominant population of the Sp3 strain. In the trial with sequential inoculation, GOX-SP3 + WAM, mixed populations of Sp3 and WAM strains can be observed, with a strong presence of the Sp3 strain. From these observations, it can be accepted that the Sp3 strain was capable of growing and developing in a non-sterile must and, therefore, has the potential to consume gluconic acid and to produce compounds that provide sensory uniqueness to the final wine. (Taillandier, Gilis, & Strehaiano, 1995) reported that *S. pombe* species (G2 strain) is able to grow in the presence of *S. cerevisiae* and can even partially inhibit its growth in sequential mixed inoculations as a function of time to *S. cerevisiae* inoculation and temperature.

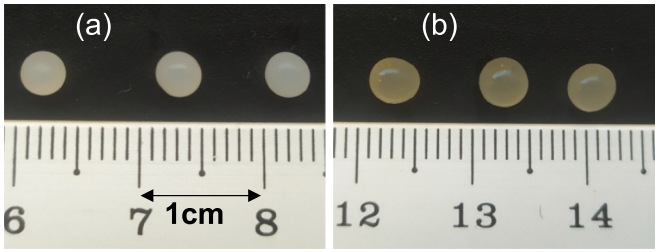

Fig. 3. GOX-CAT capsules, (a) before and (b) after enzymatic must treatment.

Measurement of glucose concentration at T20 showed that in all trials 100% of the glucose had been consumed (Table 3) in accordance with the results described in Del-Bosque et al. (2025). Although *S. pombe* strain showed a lower glucose consumption rate than WAM strain, Sp3 has a high fermentative power, being able to complete the fermentation by itself.

The measurement of alcoholic strength showed significant differences between the wine obtained from CT-MUST (11.88 ± 0.18% vol. (v/v)) and the wines obtained from GOX-MUST (Table 3). The lower glucose concentration in GOX-MUST resulted in wines with a drop in alcoholic strength of between 1.08 and 1.38% vol. (v/v) compared to the wine obtained from CT-MUST. These results show a very close range with the estimated reduction in alcoholic strength (1.07% vol. (v/v)) calculated from the glucose consumption and confirm the effectiveness of the enzymatic treatment with GOX-CAT capsules in compensating for the overripening of the grapes due to the increase in temperature. Ethanol acts as part of the liquid matrix of wine and decreases the effective vapor pressure activity of volatile compounds through three main mechanisms (i) solubility effect (ethanol increases the solubility of VOCs in the liquid phase, reducing their partition to the gas phase) (ii) surface activity effect (ethanol modifies the intermolecular forces of the medium, reducing the partial pressure of volatile compounds), and (iii) competition for the liquid-gas interface (ethanol has a high affinity for the air/liquid interface, partially displacing volatile compounds and limiting their transfer to the gas) (De-La-fuente-blanco, Arias-Pérez, Escudero, Sáenz-Navajas, & Ferreira, 2024; Muñoz-González, Pérez-Jiménez, Criado, & Pozo-Bayón, 2019). Therefore, the reduction achieved in the alcohol concentration in wines made from enzymatically treated must may improve the aromatic composition.

It is noteworthy that there was a significant increase in glycerin concentration in wines with the presence of Sp3 strain, with 7.06 ± 0.11 g/L and 6.67 ± 0.08 g/L for GOX-SP3 and GOX-SP3 + WAM, respectively (Table 3). Very similar glycerol concentrations were found by other researchers (Benito, Calderón, & Benito, 2019; Mylona et al., 2016; Scansani, Rauhut, Brezina, Semmler, & Benito, 2020) using selected strains of *S. pombe*. This suggests that Sp3 strain also possesses an active glycerol-pyruvic pathway, as no significant differences were found between CT-WAM and GOX-WAM and a higher concentration was obtained in GOX-SP3 (single inoculation) than in GOX-SP3 + WAM (sequential inoculation). It has been reported that *S. pombe* is a strain-dependent glycerol producer, reaching glycerol concentrations higher than 9.0–11.4 g/L, slightly higher than those of *S. cerevisiae* (Domizio, Liu, Bisson, & Barile, 2017). Increased glycerin concentration would improve mouthfeel in wine (Mulyono, Tania, Heryson, Felix, & Ratnasari, 2022; Mylona et al., 2016).

There was no significant difference between the concentration of gluconic acid present in GOX-MUST and the concentration of gluconic acid found after fermentation at T20 in the trial inoculated only with WAM, (GOX-WAM) (Tables 2 and 3). As WAM strain does not consume gluconic acid (Del-Bosque et al., 2025), thus the results are consistent

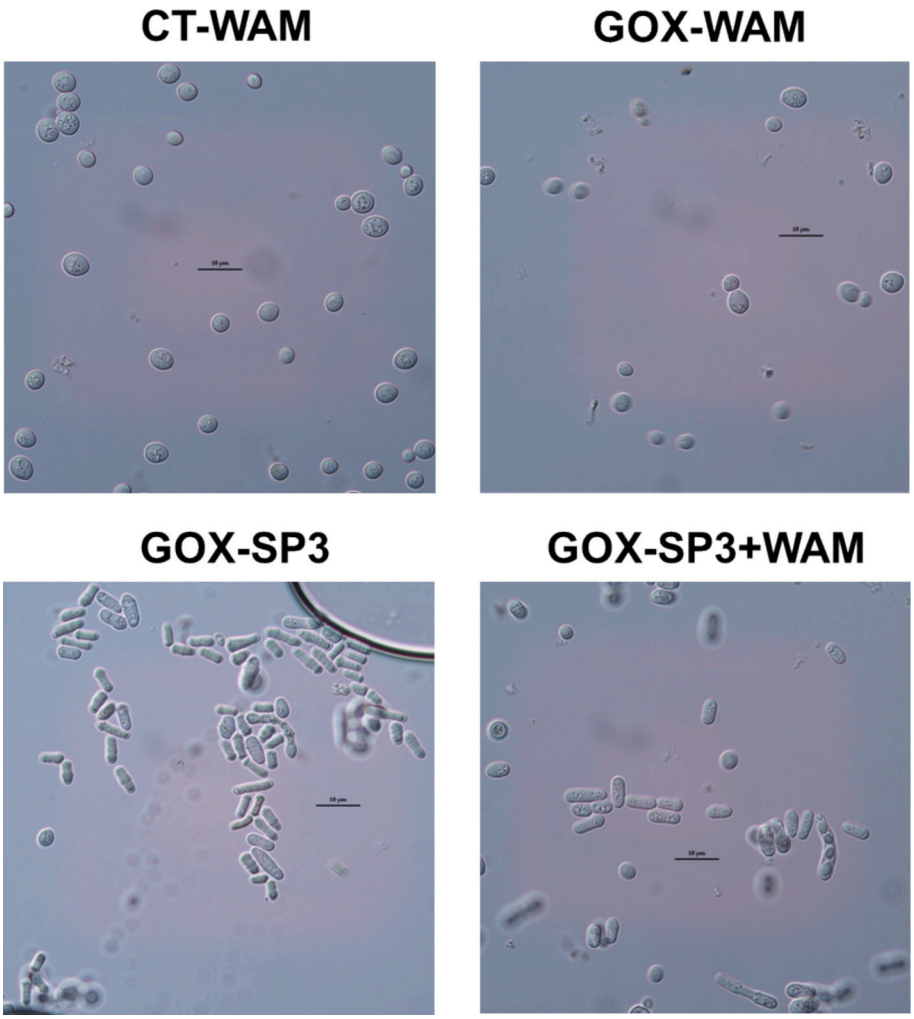

Fig. 4. Microscopy images of the presence of WAM and Sp3 strains in CT-WAM, GOX-WAM, GOX-SP3 and GOX-SP3 + WAM trials, at T7.

with expectations. On the contrary, the trials containing the Sp3 strain, both GOX-SP3 and GOX-SP3 + WAM, showed a high consumption of gluconic acid at T20, with values of 80.8 ± 4.3% and 85.8 ± 4.3%, respectively (Table 3). These percentages correspond significantly with the values found for the same Sp3 strain in Del-Bosque et al. (2025), both for the trials containing the single strain (90.5 ± 3.7%) and in sequential inoculation with WAM (88.7 ± 3.7%). This reduction allows obtaining residual levels of gluconic acid in the must below 0.5 g/L which avoids the undesirable effects of this acid, such as those occurring in musts from grapes with grey rot (Cornelissen, Kidd, Aleixandre-Tudo, & Nieuwoudt, 2022; Peinado, Maestre, Mauricio, & Moreno, 2009).

With regard to the consumption of malic acid (Table 3), a consumption of this acid was observed in the wines containing only the Sp3 strain. The percentage of consumption was 25.5 ± 2.6% and 41.6 ± 2.6% for GOX-SP3 and GOX-SP3 + WAM, respectively, with a significantly higher consumption in the sequential inoculation trial. The presence and competition of both strains in this wine, where WAM strain has a higher glucose consumption rate than *S. pombe* strain (Del-Bosque et al., 2025; Mylona et al., 2016; Taillandier et al., 1995), could stimulate the consumption of malic acid by Sp3 to obtain an additional energy resource or precursors for biomass synthesis (Loira, Morata, Palomero, Gonzalez, & Suarez-Lepe, 2018; Luo et al., 2023). Unlike other *S. pombe* strains that consume a high percentage of the malic acid present in the must (Del Fresno et al., 2017; Del-Bosque et al., 2025; Loira et al., 2015; Mulyono et al., 2022; Scansani et al., 2020; Silva et al., 2003; Taillandier et al., 1995), the use of a strain such as Sp3 that only consumes around 25–40% of the malic acid may also be of interest to maintain acidity and freshness in a white wine. The consumption of malic acid generates pyruvic acid, which in turn is metabolized to ethanol and carbon dioxide under fermentative conditions through the so-called maloethanolic pathway (Loira et al., 2018).

Differences in tartaric and succinic acid concentration were not found among the four wines (Table 3). The presence of citric acid was detected only in wines from GOX-MUST.

With regard to the final pH value measured at T20 (Table 3), a large significant difference was found between CT-WAM wine from CT-MUST, with a value of 3.71 ± 0.01, and the remaining wines from GOX-MUST, with values of 3.43 ± 0.01, 3.40 ± 0.01 and 3.38 ± 0.01 for GOX-WAM, GOX-SP3 and GOX-SP3 + WAM, respectively.

Differences in titratable acidity values among the four wines were also found. CT-WAM, with the highest pH value (3.71 ± 0.01), showed, as expected, the lowest titratable acidity value (4.46 ± 0.05 g/L). However, GOX-WAM, which presented a pH value (3.43 ± 0.01) slightly higher than GOX-SP3 and GOX-SP3 + WAM, showed the highest titratable acidity value (5.44 ± 0.05 g/L). There were no significant differences between GOX-SP3 and GOX-SP3 + WAM, neither in pH value nor in titratable acidity value (Table 3). The values obtained for volatile acidity (Table 3) were within the usual range for a white wine, highlighting the lowest value of 0.24 ± 0.01 g/L obtained by the GOX-SP3 wine. Wines containing the WAM strain (CT-WAM, GOX-WAM and GOX-SP3 + WAM) showed no differences between their volatile acidity values. This suggests that the Sp3 strain possesses another metabolic

Table 3
Physicochemical parameters of wines.

Code	Parameters	WINES*			
		CT-WAM	GOX-WAM	GOX-SP3	GOX-SP3 + WAM
Gc	Glucose (g/L)	N.d.	N.d.	N.d.	N.d.
%G	% glucose consumption	100.0 ± 2.3[a]	100.0 ± 2.3[a]	100.0 ± 2.3[a]	100.0 ± 2.3[a]
Ad	Alcohol strength (%, v/v)	11.88 ± 0.18[a]	10.80 ± 0.10[b]	10.73 ± 0.04[b]	10.50 ± 0.71[b]
Gy	Glycerin (g/L)	5.53 ± 0.04[c]	5.67 ± 0.16[c]	7.06 ± 0.11[a]	6.67 ± 0.08[b]
Ga	Gluconic acid (g/L)	N.d.	2.11 ± 0.2[a]	0.42 ± 0.2[b]	0.31 ± 0.2[b]
%Ga	% gluconic acid consumption	N.c	3.0 ± 2.8[b]	80.8 ± 4.3[a]	85.8 ± 4.3[a]
Ma	Malic acid (g/L)	3.5 ± 0.2[a]	3.2 ± 0.2[a]	2.7 ± 0.2[b]	2.0 ± 0.2[c]
%M	% malic acid consumption	1.2 ± 2.5[c]	6.4 ± 2.6[c]	25.5 ± 2.6[b]	41.6 ± 2.6[a]
Ta	Tartaric acid (g/L)	**1.17 ± 0.14**	**1.34 ± 0.02**	**1.33 ± 0.14**	**1.34 ± 0.11**
Sa	Succinic acid (g/L)	**0.58 ± 0.09**	**0.55 ± 0.12**	**0.64 ± 0.05**	**0.64 ± 0.01**
Ca	Citric acid (g/L)	N.d.	0.13 ± 0.01[a]	0.14 ± 0.01[a]	0.12 ± 0.01[a]
pH	pH	3.71 ± 0.01[a]	3.43 ± 0.01[b]	3.40 ± 0.01[c]	3.38 ± 0.01[c]
Ty	Titratable acidity (g/L)	4.46 ± 0.05[c]	5.44 ± 0.05[a]	4.82 ± 0.08[b]	4.74 ± 0.03[b]
Vy	Volatile acidity (g/L)	0.47 ± 0.02[a]	0.47 ± 0.04[a]	0.24 ± 0.01[b]	0.50 ± 0.01[b]
Pt	Potassium (mg/L)	1003.5 ± 51.6[a]	808.5 ± 24.7[b]	820.0 ± 82.0[b]	825.5 ± 14.8[b]
Tp	Total polyphenol index (A_{280} nm)	9.28 ± 0.12[a]	6.40 ± 0.06[b]	5.97 ± 0.01[c]	5.71 ± 0.15[c]
Ha	Hydroxycinnamic acids (A_{320} nm)	4.29 ± 0.01[a]	1.92 ± 0.01[d]	2.02 ± 0.01[c]	2.10 ± 0.04[b]
Fl	Flavonols (A_{365} nm)	0.85 ± 0.06[a]	0.52 ± 0.01[c]	0.62 ± 0.01[b]	0.61 ± 0.02[b]
Ct	Catechins (mg/L)	182.0 ± 12.7[a]	149.0 ± 2.8[b]	181.5 ± 7.8[a]	181.5 ± 14.8[a]
L*	L* (AU)	97.7 ± 0.2[a]	97.8 ± 0.1[a]	96.1 ± 0.4[c]	96.8 ± 0.2[b]
C*	C* (AU)	6.7 ± 0.3[c]	8.4 ± 0.1[b]	10.7 ± 0.3[a]	10.3 ± 0.3[a]
H*	H* (AU)	95.7 ± 0.2[c]	97.9 ± 0.1[a]	96.0 ± 0.2[c]	96.8 ± 0.3[b]
a*	a* (AU)	−0.67 ± 0.05[a]	−1.16 ± 0.04[b]	−1.12 ± 0.01[b]	−1.21 ± 0.01[b]
b*	b* (AU)	6.7 ± 0.3[c]	8.3 ± 0.1[b]	10.7 ± 0.3[a]	10.2 ± 0.3[a]
	$\Delta E^*_{CT\text{-}WAM—GOX\text{-}WAM}$		1.7	–	–
	$\Delta E^*_{CT\text{-}WAM—GOX\text{-}SP3}$		–	4.3	–
	$\Delta E^*_{CTWAM—GOX\text{-}SP3+WAM}$		–	–	3.7
	$\Delta E^*_{GOX\text{-}WAM—GOX\text{-}SP3}$	–		2.8	–
	$\Delta E^*_{GOX\text{-}WAM—GOX\text{-}SP3+WAM}$	–		–	2.1
	$\Delta E^*_{GOX\text{-}SP3—GOX\text{-}SP3+WAM}$	–	–		0.8

* Mean values ± standard deviation. N.d. indicates that the compound was not detected (below the detection limit of the method). N.c. indicates that the data cannot be calculated. For each parameter, different letters in each row indicate significant differences between wines at the 95.0% confidence level according to Fisher's Least Significant Difference (LSD) procedure. Data in bold indicates that no significant differences were found between the wines.

trait of great enological interest, generating a lower volatile acidity than the *S. cerevisiae* strain, contrary to that obtained with the *S. pombe* strains used by other researchers (Benito et al., 2016; Del Fresno et al., 2017; Scansani et al., 2020; Vicente et al., 2023). In contrast, very similar volatile acidity values (0.23 and 0.21 g/L) were reported respectively by Gardoni et al. (2021) in Riesling must and Silva et al. (2003) using dry immobilized strains of *S. pombe* in must blended from several varieties, including 12% Muscat grapes.

The wines obtained from GOX-MUST showed a lower potassium concentration (Table 3). This decrease is probably associated with the precipitation of potassium bitartrate (Coulter et al., 2015) induced by pH drop in the GOX-MUST (Table 2) generated by the presence of gluconic acid from the enzymatic treatment. Potassium salts from citric, succinic, malic and gluconic acids are quite soluble so it is unlikely that the drop in potassium is due to their formation (Payan, Gancel, Jourdes, Christmann, & Teissedre, 2023).

It should be noted that in the GOX-SP3 and GOX-SP3 + WAM wines, the consumption of gluconic and malic acids by Sp3 strain did not increase the initial pH value of GOX-MUST (3.46 ± 0.01) (Table 2), but even a slight decrease in pH was obtained. This may be due to the above-mentioned potassium decrease by precipitation in the form of potassium bitartrate (KTa), which would favor the lowering of pH by leaving more free tartaric acid (Ta), with the Ta/KTa buffer system being modified.

This reduction in potassium would have positive effects as it would improve the tartaric stability of the wine (Coulter et al., 2015) and favor lowering the pH, which could help increase microbiological stability, decrease the need for SO_2 (Lisanti, Blaiotta, Nioi, & Moio, 2019), enhance color stability (Kanavouras, Coutelieris, Karanika, Kotseridis, & Kallithraka, 2020) and augment the sensation of acidity in the wine.

From the values of total polyphenol index, hydroxycinnamic acids and flavonols shown in Table 3, it can be observed that in all cases there are significant differences between CT-WAM and the rest of the trials using GOX-MUST regardless of the strain used. Therefore, enzymatic treatment of the must with GOX-CAT capsules generate differences with a reduction in these parameters. Harlen, Prakash, Yuliani, and Bhandari (2024), using alginate matrices, reveal interactions between the carboxyl group of alginate and the hydroxyl groups of polyphenols. These interactions may contribute to the partial retention of these compounds in GOX-CAT capsules, which would explain their reduction in the must. A slight decrease in the total polyphenol index was also found by Fernández-Fernández et al. (2019) using silica-calcium-alginate capsules. Mangas et al. (2023) also reported a decrease of hydroxycinnamic acids and flavonols in wines enzymatically treated with GOX. Among the different trials using GOX-MUST, there is very little difference between the wines using the Sp3 strain (GOX-SP3 and GOX-SP3 + WAM) and a greater significant difference is observed with the GOX-WAM wine. Therefore, the presence of the Sp3 strain may have a significant influence on the values obtained for these parameters.

After alcoholic fermentation and stabilization up to T20 during which part of the dyes present decanted, the colors obtained in all wines showed more proximate CIELab coordinates, although still with significant differences (Table 3). The wines obtained with WAM strain were slightly lighter (higher L* value), while those inoculated with Sp3 strain were slightly more golden (higher b* value) and intense o saturated (higher C* value). These results are in line with the data reported by other researchers (Botezatu et al., 2021; Scansani et al., 2020). This increase, with higher values of b* (yellow component) and C* (saturation) corresponding to wines fermented with *S. pombe*, is particularly relevant because these wines have the lowest final pH values of the set (3.40 in GOX-SP3 and 3.38 in GOX-SP3 + WAM), which rules out the possibility that the increases in the yellow component are due to chemical oxidation associated with high pH. Therefore, the cause would lie in the specific fermentation biochemistry of *S. pombe* and its interaction with the phenolic matrix (hydroxycinnamic acids and flavonols) of the must to form stable yellow pigments. In addition, the redox activity of *S. pombe* generates mild oxidation that intensifies color saturation without drastically reducing brightness (L*). CT-WAM and GOX-WAM showed a value of $\Delta E^*_{CT\text{-}WAM—GOX\text{-}WAM} = 1.7$ (Table 3), below the established value of 2.7. There would also be no discernible difference between GOX-SP3 and GOX-SP3 + WAM with a value of $\Delta E^*_{GOX\text{-}SP3—GOX\text{-}SP3+WAM} = 0.8$ (Table 3).

3.3. Principal component analysis of the physicochemical parameters and VOCs of the wines

A PCA (Fig. 5(a)) was applied using the statistically significant parameters from Table 3 to identify the parameters that differentiate the four wines.

As shown in Fig. 5(a), the first component (PC1), explaining 64.01% of the variability of the data, significantly distinguishes the CT-WAM wine (on the positive side of PC1) coming from the must without enzymatic treatment (CT-MUST), from the wines coming from the must treated with GOX-CAT capsules, GOX-WAM, GOX-SP3 and GOX-SP3 + WAM (on the negative side of PC1). PC1 correlated positively with 11 of the 18 parameters, mainly pH, total polyphenol index (Tp), hydroxycinnamic acids (Ha), potassium concentration (Pt), and alcohol strength (Ad). On the other hand, PC1 correlated negatively with 7 parameters, the most contributing being citric acid concentration (Ca), CIELab coordinates C* and b*, and glycerin concentration (Gy).

The second component (PC2), containing 29.28% of the variability, significantly differentiates the wines from GOX-MUST that were inoculated with the Sp3 strain, GOX-SP3 and GOX-SP3 + WAM (on the negative side of PC2) from those inoculated only with the WAM strain (on the positive side of PC2). PC2 correlated positively with 10 parameters. Those with the highest weights were gluconic acid concentration (Ga), CIELab coordinates H* and L*, and titratable acidity concentration. On the negative side, PC2 correlated with catechin concentration (Ct), glycerin concentration (Gy), and CIELab coordinates b* and C*.

The impact of both inoculation regimens and the enzymatic

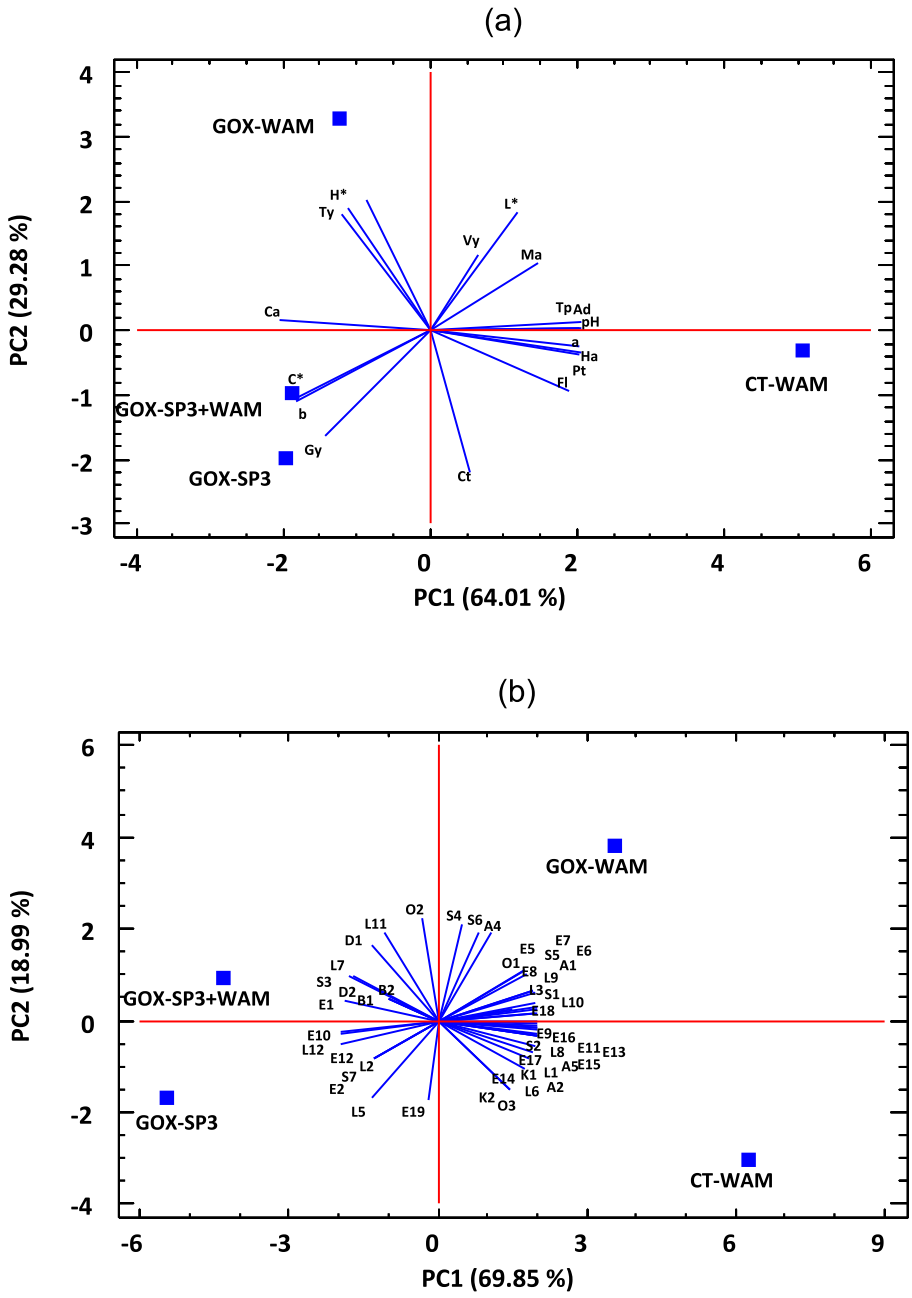

Fig. 5. Principal component analysis biplot of (a) physicochemical parameters and wines and (b) VOCs and wines. Codification of wines: blue filled square. Codifications of parameters: see Table 3. Codifications of VOCs: see Table 4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment on the final aromatic profile of the wines was addressed by analyzing VOCs. Table 4 shows the VOCs in all wines. Fifty-two compounds were identified and grouped into families (ethyl esters, esters, acids, alcohols, aldehydes, benzenes, ketones and others). Significant differences in the presence-absence and concentration of VOCs between the four wines were detected.

Of the 52 VOCs analyzed, only ethyl butanoate (E3), ethyl hexanoate (E4) and hexan-1-ol (L4) showed no significant differences among the four wines. These compounds present an odor descriptor of fruity, sweet, tutti-frutti, and apple and fresh (E3), green apple, fruity, sweet and pineapple (E4) and floral, green and grass (L4) (Cuevas, 2020; Luo et al., 2023; Marais, 2017; Vararu, Moreno-García, Zamfir, Cotea, & Moreno, 2016).

PCA, with the statistically significant VOCs in Table 4, was applied to identify the main variables that characterize the aromatic profile among the four wines. As shown in Fig. 5(b), the first component (PC1), explaining 69.85% of the variability of the data, and the second component (PC2), with (18.99%), significantly distinguish the four wines.

Again, the same pattern of wine differentiation as in Fig. 5(a) can be observed in Fig. 5(b). PC1 reflects a gradient influenced by the yeast species, but also by their relative proportion and possible interactions between strains: on the right, wines with only *S. cerevisiae* (CT-WAM, GOX-WAM); on the left, wines with *S. pombe* (GOX-SP3 and GOX-SP3 + WAM). The latter, in a more intermediate position, containing both species but closer to the *S. pombe* profile. PC1 was positively correlated with 31 VOCs. Those with the highest weights were ethyl dec-9-enoate (E11), 2-phenylethanol (L10), ethyl (*E*)-dec-4-enoate (E9), decan-1-ol (L8), ethyl hexadecanoate (E16), 3-methylbutyl octanoate (S2), ethyl tetradecanoate (E15), 3-methylbutan-1-ol (L3) y 2-phenylethyl acetate (S1). On the other hand, PC1 was negatively correlated with 16 VOCs. Those that contributed the most weights were 3,7-dimethylocta-1,6-dien-3-ol (L12), ethyl 2-phenylacetate (E12), ethyl benzoate (E10), ethyl acetate (E1) y diethyl butanedioate (S3). PC2 reflects an interaction between enzymatic treatment and yeast used: positive, GOX-WAM and GOX-SP3 + WAM (enzymatic treatment with at least *S. cerevisiae*); negative, CT-WAM and GOX-SP3 (*S. cerevisiae* without enzymatic treatment and *S. pombe* with enzymatic treatment, respectively). PC2 correlated positively with 24 VOCs, most notably 2,4,5-trimethyl-1,3-dioxolane (O2), [2,2,4-trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-Methylpropanoate (S4), 2-ethylhexan-1-ol (L11), decanoic acid (A4), propyl (*Z*)-tetradec-9-enoate (S6), and 3-methylbutanoic acid (A1). In its negative zone, PC2 correlated with the other 24 VOCs, with ethyl (Z)-octadec-9-enoate (E19), nonan-2-ol (L5), undecan-2-one (K2), 6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptane (O3), and ethyl tridecanoate (E14) being the most influential.

Therefore, the effect of enzymatic treatment on the volatile profile of wine depends not only on the use of GOX-CAT capsules, but also on the yeast used and their interaction.

It is reasonable to deduce that the modification of the glucose and gluconic acid concentration of the treated must and its pH (shown in Table 2), together with the different metabolic capacity of each yeast strain (Del-Bosque et al., 2025), will lead to the generation of differences in certain VOCs. Similarly, modifying the SO_2 concentration in enzymatically treated must could influence the survival rate of the yeasts present in the must and, therefore, the effects of their metabolism. It should be noted that both yeast strains used, WAM and Sp3, show positive catalase activity (Gröger et al., 2023; Martins, Nguyen, & English, 2019), which means that they possess enzymatic mechanisms to protect themselves against oxidative stress caused by the possible presence of a low amount of H_2O_2 produced by the oxidation of glucose and which would not have been degraded by catalase activity.

The absence of certain non-detected compounds (N.d.) reinforces the differences between wines with different strains and treatments and contributes to define the unique volatile profile of the wines.

Nonan-1-ol (L7), 2-ethylhexan-1-ol (L11), nonanal (D1) and 2,4,5-

Table 4
Concentration (mg/L) of each of the VOCs found in the wines.*

VOCs		WINES*			
Code	IUPAC name	CT-WAM	GOX-WAM	GOX-SP3	GOX-SP3 + WAM
	Ethyl esters				
E1	Ethyl acetate	1.25 ± 0.70^{b}	1.40 ± 0.16^{ab}	1.66 ± 0.23^{ab}	1.83 ± 0.77^{a}
E2	Ethyl 2-methylpropanoate	N.d.	N.d.	0.04 ± 0.02	N.d.
E3	Ethyl butanoate	**0.25 ± 0.14**	**0.23 ± 0.03**	**0.26 ± 0.04**	**0.23 ± 0.12**
E4	Ethyl hexanoate	**4.03 ± 2.15**	**3.91 ± 0.85**	**4.53 ± 0.50**	**4.82 ± 1.77**
E5	Ethyl heptanoate	0.15 ± 0.04^{a}	0.18 ± 0.03^{a}	0.09 ± 0.02^{b}	0.11 ± 0.03^{b}
E6	Ethyl octanoate	23.04 ± 1.88^{a}	23.51 ± 0.96^{a}	10.98 ± 1.25^{c}	14.82 ± 1.20^{b}
E7	Ethyl nonanoate	1.20 ± 0.06^{b}	1.37 ± 0.18^{a}	0.38 ± 0.14^{c}	0.45 ± 0.05^{c}
E8	Ethyl decanoate	13.21 ± 1.68^{b}	17.05 ± 1.05^{a}	4.43 ± 0.63^{d}	6.53 ± 0.36^{c}
E9	Ethyl (*E*)-dec-4-enoate	0.10 ± 0.02^{a}	0.08 ± 0.02^{a}	N.d.	N.d.
E10	Ethyl benzoate	N.d.	N.d.	0.06 ± 0.01^{b}	0.08 ± 0.01^{a}
E11	Ethyl dec-9-enoate	22.87 ± 0.13^{a}	16.54 ± 0.60^{b}	0.71 ± 0.12^{d}	3.04 ± 0.49^{c}
E12	Ethyl 2-phenylacetate	N.d.	N.d.	0.14 ± 0.01^{a}	0.09 ± 0.01^{b}
E13	Ethyl dodecanoate	4.36 ± 0.43^{a}	3.75 ± 0.26^{b}	2.47 ± 0.49^{d}	2.86 ± 0.11^{c}
E14	Ethyl tridecanoate	0.21 ± 0.01^{a}	0.17 ± 0.02^{b}	0.16 ± 0.01^{bc}	0.15 ± 0.02^{c}
E15	Ethyl tetradecanoate	0.53 ± 0.11^{a}	0.47 ± 0.04^{a}	0.36 ± 0.08^{b}	0.36 ± 0.02^{b}
E16	Ethyl hexadecanoate	2.03 ± 0.18^{a}	1.79 ± 0.15^{b}	1.17 ± 0.38^{c}	1.33 ± 0.09^{c}
E17	Ethyl (*E*)-hexadec-9-enoate	0.63 ± 0.14^{a}	0.43 ± 0.03^{b}	0.09 ± 0.08^{c}	0.06 ± 0.04^{c}
E18	Ethyl octadecanoate	0.21 ± 0.04^{a}	0.19 ± 0.02^{a}	0.12 ± 0.03^{c}	0.15 ± 0.02^{b}
E19	Ethyl (*Z*)-octadec-9-enoate	0.23 ± 0.01^{a}	0.18 ± 0.02^{b}	0.20 ± 0.07^{ab}	0.22 ± 0.03^{a}
	Esters				
S1	2-phenylethyl acetate	2.20 ± 0.05^{a}	2.05 ± 0.04^{b}	0.22 ± 0.05^{d}	0.31 ± 0.01^{c}
S2	3-methylbutyl octanoate	0.52 ± 0.08^{a}	0.36 ± 0.03^{b}	N.d.	N.d.
S3	Diethyl butanedioate	0.13 ± 0.03^{d}	0.32 ± 0.04^{c}	0.40 ± 0.08^{b}	0.47 ± 0.05^{a}
S4	[2,2,4-trimethyl-3-(2-methylpropanoyloxy) pentyl] 2-Methylpropanoate	0.09 ± 0.04^{b}	0.13 ± 0.03^{a}	0.08 ± 0.01^{bc}	0.11 ± 0.02^{ab}
S5	3-methylbutyl decanoate	0.20 ± 0.03^{a}	0.24 ± 0.06^{a}	N.d.	N.d.
S6	Propyl (*Z*)-tetradec-9-enoate	N.d.	0.06 ± 0.01	N.d.	N.d.
S7	Diethyl benzene-1,2-dicarboxylate	N.d.	N.d.	0.04 ± 0.01	N.d.
	Acids				
A1	3-methylbutanoic acid	0.40 ± 0.04^{a}	0.41 ± 0.07^{a}	N.d.	N.d.
A2	(*Z*)-dodec-5-enoic acid	0.15 ± 0.01^{a}	0.07 ± 0.02^{b}	N.d.	N.d.
A3	Nonanoic acid	**0.11 ± 0.01**	**0.11 ± 0.03**	**0.12 ± 0.02**	**0.13 ± 0.06**
A4	Decanoic acid	1.16 ± 0.31^{b}	1.64 ± 0.18^{a}	0.97 ± 0.31^{b}	1.18 ± 0.32^{b}
A5	Dec-9-enoic acid	0.90 ± 0.06^{a}	0.55 ± 0.11^{b}	N.d.	0.14 ± 0.06^{c}

(*continued on next page*)

Table 4 (*continued*)

VOCs		WINES*			
Code	IUPAC name	CT-WAM	GOX-WAM	GOX-SP3	GOX-SP3 + WAM
Alcohols					
L1	2-methylpropan-1-ol	4.11 ± 0.16[a]	2.55 ± 0.50[b]	N.d.	N.d.
L2	Butan-1-ol	N.d.	N.d.	0.09 ± 0.01	N.d.
L3	3-methylbutan-1-ol	18.83 ± 1.66[a]	17.91 ± 0.64[a]	5.70 ± 1.12[b]	6.89 ± 1.65[b]
L4	Hexan-1-ol	**1.37 ± 0.19**	**1.27 ± 0.12**	**1.25 ± 0.27**	**1.24 ± 0.25**
L5	Nonan-2-ol	0.18 ± 0.02[b]	0.12 ± 0.01[c]	0.21 ± 0.01[a]	0.19 ± 0.03[b]
L6	Octan-1-ol	0.11 ± 0.01[a]	0.08 ± 0.01[b]	0.06 ± 0.01[c]	0.06 ± 0.01[c]
L7	Nonan-1-ol	N.d.	0.04 ± 0.01[b]	0.06 ± 0.01[a]	0.05 ± 0.01[ab]
L8	Decan-1-ol	0.11 ± 0.01[a]	0.08 ± 0.01[b]	N.d.	N.d.
L9	3,7-dimethyloct-6-en-1-ol	0.12 ± 0.01[a]	0.10 ± 0.01[b]	0.04 ± 0.01[c]	0.10 ± 0.04[ab]
L10	2-phenylethanol	9.37 ± 0.14[a]	8.54 ± 0.12[b]	3.55 ± 0.95[d]	4.24 ± 1.03[c]
L11	2-ethylhexan-1-ol	N.d.	0.08 ± 0.01[a]	0.05 ± 0.02[b]	0.07 ± 0.02[a]
L12	3,7-dimethylocta-1,6-dien-3-ol	N.d.	N.d.	0.09 ± 0.01[a]	0.09 ± 0.01[a]
Aldehydes					
D1	Nonanal	N.d.	0.07 ± 0.01[a]	0.06 ± 0.01[a]	0.07 ± 0.01[a]
D2	3-methylbenzaldehyde	N.d.	N.d.	N.d.	0.15 ± 0.13
Benzenes					
B1	1,2-xylene	N.d.	N.d.	N.d.	0.06 ± 0.02
B2	(4*R*)-1-methyl-4-prop-1-en-2-ylcyclohexene	N.d.	N.d.	N.d.	0.07 ± 0.01
Ketones					
K1	Nonan-2-one	0.29 ± 0.01[a]	0.13 ± 0.02[b]	N.d.	N.d.
K2	Undecan-2-one	0.08 ± 0.02	N.d.	N.d.	N.d.
Others					
O1	Ethenoxyethane	0.12 ± 0.01[b]	0.15 ± 0.03[a]	0.08 ± 0.04[c]	0.07 ± 0.02[c]
O2	2,4,5-trimethyl-1,3-dioxolane	N.d.	1.90 ± 0.35[a]	0.67 ± 0.30[c]	1.23 ± 0.36[b]
O3	6,6-dimethyl-2-methylidenebicyclo[3.1.1] heptane	0.11 ± 0.04	N.d.	N.d.	N.d.

* Mean values and their standard deviation are shown. N.d. indicates that the compound was not detected (below the detection limit of the method). For each VOCs, different letters in each row indicate significant differences between wines at the 95.0% confidence level according to Fisher's Least Significant Difference (LSD) procedure. Data in bold indicate that no significant differences were found between the wines.

trimethyl-1,3-dioxolane (O2) were detected only in wines from GOX-MUST. Among them, nonanal stands out with an odor descriptor of citrus, fresh, rose and waxy (Tang, Xi, Ma, Zhang, & Xu, 2019; Vararu et al., 2016). In contrast, undecan-2-one (K2) with fruity notes (Xia, Liu, Wang, & Shuang, 2020) and 6,6-dimethyl-2-methylidenebicyclo[3.1.1] heptane (O3) (Delač Salopek et al., 2022) appeared only in wine from CT-MUST.

CT-WAM was characterized by a higher concentration of ethyl dec-9-enoate (E11) (fruity, rose and green notes) (Shi et al., 2022), together with those of higher molecular weight, such as ethyl dodecanoate (E13) (with fruity, flowery, green, earthy, herbal and waxy notes) (Radeka et al., 2023; Shi et al., 2022), ethyl tridecanoate (E14), ethyl tetradecanoate (E15), ethyl hexadecanoate (E16) and ethyl (E)-hexadec-9-enoate (E17). The formation of these medium- and long-chain ethyl esters occurs intracellularly in *S. cerevisiae* through acyltransferase enzymes (ester synthases) that catalyze the condensation of an alcohol (usually ethanol) with an acyl-CoA (acetyl-CoA or fatty acid acyl-CoA) (Scott, Henriques, Smid, Notebaart, & Balsa-Canto, 2023). This wine also has a higher concentration of 3-methylbutyl octanoate (S2), (fruity, cheese, cream and sweet notes) (Shi et al., 2022), dec-9-enoic acid (A5) (wax, honey, ripe fruit notes) (López-Enríquez, Vila-Crespo, Rodríguez-Nogales, Fernández-Fernández, & Ruipérez, 2023) and alcohols 2-methylpropan-1-ol (L1) (bitter and green notes) (Cuevas, 2020), octan-1-ol (L6) (alcoholic and fatty notes) (Han et al., 2022) and decan-1-ol (L8) (floral, fruity and sweet notes) (Scutarasu et al., 2022). It also has a higher concentration of ketones, nonan-2-one (K1) and undecan-2-one (K2) (fruity notes) (Xia et al., 2020) which derive from the incomplete β-oxidation of alcohols and the partial oxidation of alcohols or long-chain fatty acids, respectively.

GOX-WAM stood out for a higher concentration of lower molecular weight ethyl esters, ethyl heptanoate (E5), ethyl octanoate (E6), ethyl nonanoate (E7) and ethyl decanoate (E8), which have odor descriptors of floral, fruity, apple, apricot, banana, pear and brandy (Cuevas, 2020; Iobbi, Tomasino, & Di, 2025; Vararu et al., 2016). Higher concentrations of esters, generated mainly via the Ehrlich pathway from its corresponding amino acid, such as 2-phenylethyl acetate (S1), [2,2,4-trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-Methylpropanoate (S4), 3-methylbutyl decanoate (S5) and propyl (*Z*)-tetradec-9-enoate (S6) were also detected, with floral, apple, cherry, pear, banana, fruity and sweet notes (Cuevas, 2020; Naranjo et al., 2021; Shi et al., 2022). Higher presence of acids, 3-methylbutanoic acid (A1) (sweet, rancid and acid notes) (Cuevas, 2020) and decanoic acid (A4) (fatty and rancid notes) (Cuevas, 2020) and alcohols, 3-methylbutan-1-ol (L3) (fusel, bitter, banana notes) (Cuevas, 2020; Shi et al., 2022), 3,7-dimethyloct-6-en-1-ol (L9) (citrus and lime notes) (Baron, Prusova, Tomaskova, Kumsta, & Sochor, 2017; Marais, 2017) and 2-phenylethanol (L10) (floral and roses notes) (Cuevas, 2020; Waterhouse, Sacks, & Jeffery, 2017) were found.

The GOX–SP3 wine exhibited the most distinctive volatile profile, notably producing several ethyl esters, including ethyl 2-methylpropanoate (E2), detected exclusively in this wine and associated with fruity notes (Chen et al., 2025). Although this compound can originate from early valine catabolism via the Ehrlich pathway (Gonzalez & Morales, 2017; Zhu et al., 2024), *S. pombe* typically leaves higher residual valine than *S. cerevisiae* (Benito, 2019), making enhanced valine degradation an unlikely source. Instead, its presence may reflect the esterification of small precursor amounts formed during the initial steps of this pathway, facilitated by the metabolic environment of *S. pombe* in GOX-treated must. Other ethyl esters contributing to the distinctive profile of GOX-SP3 wines included ethyl benzoate (E10), associated with fruity notes (Guo et al., 2025); ethyl 2-phenylacetate (E12), responsible for rose-like aromas (Wang et al., 2023); and ethyl (Z)-octadec-9-enoate (E19), linked to fatty nuances (J. Wang, Chen, Wu, & Zhao, 2022).

Also alcohols, nonan-2-ol (L5) (fruity note) (Antolak, Jeleń, Otlewska, & Kręgiel, 2019) and 3,7-dimethylocta-1,6-dien-3-ol (L12) (flower and lavender notes) (Luo et al., 2023; Marais, 2017), were characteristic. On the other hand, VOCs as diethyl benzene-1,2-dicarboxylate (S7) and butan-1-ol (L2) appeared only in GOX-SP3 wine, with odorless for S7 (Vararu et al., 2016) and fusel for L2 (López-Enríquez et al., 2023).

Finally, GOX-SP3 + WAM presented the highest concentration of the lowest molecular weight ethyl ester, ethyl acetate (E1) (fruity note) (Cuevas, 2020; Iobbi et al., 2025) and ester, diethyl butanedioate (S3) (fruity note) (Scutarasu et al., 2022). Also noteworthy is the unique presence of benzenes, 1,2-xylene (B1) (pungent note) (Voce et al., 2019) and (4*R*)-1-methyl-4-prop-1-en-2-ylcyclohexene (B2) (limon note) (Hu et al., 2020). B2 or D-limonene belongs to the family of monoterpenes derived from isoprenoid metabolism (mevalonate pathway) in grape

tissues, not in yeast (Chigo-Hernandez & Tomasino, 2023). In white grape wines (e.g., Muscat, Riesling, Verdejo), these terpenes are glycosylated precursors that are released by enzymatic hydrolysis during fermentation. The content of aldehydes, nonanal (D1) (fresh, rose and waxy notes) (Vararu et al., 2016) and 3-methylbenzaldehyde (D2) (Wang et al., 2024), derived from the partial oxidation of medium-chain fatty acids (C9–C10) or the incomplete reduction of nonanoil-CoA during β-oxidation, and from the oxidative catabolism of aromatic compounds derived from the amino acids phenylalanine or tyrosine, respectively (Álvarez-Fernández et al., 2019; Sipiczki, 2022; Zilelidou & Nisiotou, 2021), with a unique presence in GOX-SP3 + WAM wine, stood out.

Mangas et al. (2023) indicated that the enzymatic treatment of Verdejo must reduced the values of some compounds with floral notes such as 2-phenylethanol (L10) and ketones. Our results showed the same trend in these compounds. It was also reported that there was a decrease in long chain ethyl esters (with soapy notes). As can be observed in Table 4, the same trend is shown (esters from E13 to E19) with greater effect in the wines inoculated with Sp3 strain. On the other hand, an increase in ethyl hexanoate (E4) was detected in the same publication while our results found no significant differences in this ester with fruity aroma.

The sensory analysis carried out by Botezatu et al. (2021) showed that the wines obtained from a Riesling must treated with GOX were perceived as brighter in color, tasted (in the mouth) of "acid, citrus, floral, grape, pear and apple" and with aromas of "floral, citrus, fruity, sweet and caramel".

Our results showed that the compounds most associated with the presence of *S. cerevisiae*, regardless of must treatment, include ethyl (*E*)-dec-4-enoate (E9) (fruity and pineapple notes) (Kliks, Kawa-Rygielska, Gasiński, Rębas, & Szumny, 2021), 3-methylbutyl decanoate (S5), 3-methylbutanoic acid (A1) and 3-methylbutan-1-ol (L3).

VOCs ethyl benzoate (E10), ethyl 2-phenylacetate (E12) and (L12) were only detected in wines from musts inoculated with Sp3 strain, so in theory, they could be linked to the metabolism of this strain. These compounds have been described associated with fruity notes (E10) (Guo et al., 2025), roses notes (E12) (Wang et al., 2023) and flower and lavender notes (L12) (Luo et al., 2023; Marais, 2017).

Mylona et al. (2016) and Scansani et al. (2020) also reported lower 2-phenylethyl acetate (S1) concentrations in wines fermented with *S. pombe*.

The analysis of volatile compounds carried out by Delač Salopek et al. (2022) indicated that must of the Malvazija Istarska variety fermented with *S. pombe* showed (with respect to *S. cerevisiae*) a significant increase in terpenes (linalool, menthol, camphene, geranyl acetate), C13-norisoprenoids (β-damascenone, β-ionone), acetate esters (isoamyl acetate, hexyl acetate) and ethyl esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate), which would provide greater floral and varietal character, increased aroma of cooked apple and violets, greater fruity contribution (banana, pineapple) and improvement of fruity/floral aromas, respectively. On the other hand, less concentration of higher alcohols (2-phenylethanol, isoamyl), less volatile acids (butyric acid) would also be obtained by Delač Salopek et al. (2022). The same trend showed our results for 3,7-dimethylocta-1,6-dien-3-ol (L12) and decanoic acid (A4). However, disparate results were found for 2-methylpropan-1-ol (L1), 3-methylbutan-1-ol (L3), 2-phenylethanol (L10), ethyl hexanoate (E4), ethyl octanoate (E6) and ethyl decanoate (E8). Also in Gardoni et al. (2021), Scansani et al. (2020) and Mylona et al. (2016), lower amounts of higher alcohols and volatile fatty acids were shown in the results with *S. pombe*, as for example, for 2-phenylethanol (L10) and ethyl decanoate (E8), in agreement with our results.

4. Conclusion

Our results showed that a Verdejo must subjected to a 48 h prefermentative treatment with our GOX-CAT capsules, reduced its glucose concentration by up to 17.3 ± 0.5% and thus decreased its potential alcoholic strength by up to 1.38% vol (v/v). This would compensate in many wineries for the effects of the increase in average temperature due to global warming. Up to 90.01% of the gluconic acid generated in the oxidation of glucose can be retained in the capsules allowing a moderate lowering of the pH. In addition to a lower alcoholic strength, the decrease in potassium content obtained could help improve tartaric stability and microbiological stability due to the decrease in pH with lower doses of SO_2.

GOX-MUST fermentation can be carried out with a selected *S. pombe* strain either in unique inoculation or in sequential inoculation at 48 h with a *S. cerevisiae* strain. The wines obtained possess their own characteristic physicochemical parameters (acidity, color, glycerin and phenolic) and volatile profile highlighting the presence of lower concentrations of long-chain ethyl esters (which usually contribute soapy notes). Wines obtained with inoculation of the Sp3 strain, both single and sequential, increase their glycerin concentration which would improve the mouthfeel of the wine. Must fermentation by Sp3 would also increase wine color intensity and showed inferior amounts of higher alcohols resulting in less masking of the varietal aroma.

CRediT authorship contribution statement

David Del-Bosque: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Josefina Vila-Crespo:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Violeta Ruipérez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Encarnación Fernández-Fernández:** Writing – review & editing, Methodology, Investigation, Conceptualization. **José Manuel Rodríguez-Nogales:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Informed consent statement

Informed consent was obtained from all authors involved in the study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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