

1479-OE-CHARACTERISATION OF *Schizosaccharomyces pombe* STRAINS METABOLISING GLUCONIC ACID USING GOX/CAT IN OENOLOGY



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Abstract

This study evaluated the use of an immobilized glucose oxidase/catalase (GOX/CAT) system combined with selected *Schizosaccharomyces pombe* strains to reduce alcohol content and metabolize the gluconic acid generated during enzymatic treatment of Verdejo must. Four *S. pombe* strains (Sp1, Sp3, Sp4, and Sp7) were evaluated under microvinification conditions. GOX/CAT treatment reduced the final alcohol content but increased gluconic acid concentration and acidity. Significant strain-dependent differences were observed. Among the strains tested, Sp3 showed the most balanced oenological performance, combining lower residual gluconic acid with suitable acidity and pH values. Phenolic composition and color were only slightly affected by the treatments. These results identify *S. pombe* Sp3 as a promising strain for integration into GOX/CAT-based alcohol reduction strategies in winemaking.

Introduction

Climate change is altering grape composition during ripening, leading to wines with higher alcohol content and lower acidity. One promising strategy to mitigate these effects is the oxidation of glucose using the GOX/CAT enzymatic system. However, this process generates gluconic acid, which can affect wine composition and quality (Del Bosque et al., 2026). *S. pombe* has the ability to metabolize gluconic acid and may therefore complement GOX/CAT treatments in winemaking (Del Bosque et al., 2025). The aim of this study was to characterize selected *S. pombe* strains under oenological conditions and identify the most suitable strain for use in Verdejo microvinifications treated with immobilized GOX/CAT.

Materials and methods

The enzymes used for co-immobilization were GOX (Gluzyme®Fortis, Novozymes®) and CAT (Catazyme® Novozymes®). The biocomposite capsules consist of an interpenetrating polymeric network of silica-calcium-alginate (del-Bosque et al., 2023) that entraps the enzymes.

Three fermentation treatments were evaluated: (i) control fermentation without enzyme treatment, inoculated with *Saccharomyces cerevisiae* WAM; (ii) prefermentative treatment with immobilized GOX/CAT for 48 h followed by WAM inoculation (GOX); and (iii) simultaneous application of immobilized GOX/CAT and selected *S. pombe* strains (Sp1, Sp3, Sp4, and Sp7) during the first 48 h, followed by capsule removal and WAM inoculation. All fermentations were carried out at 21°C for 21 days (Figure 1).

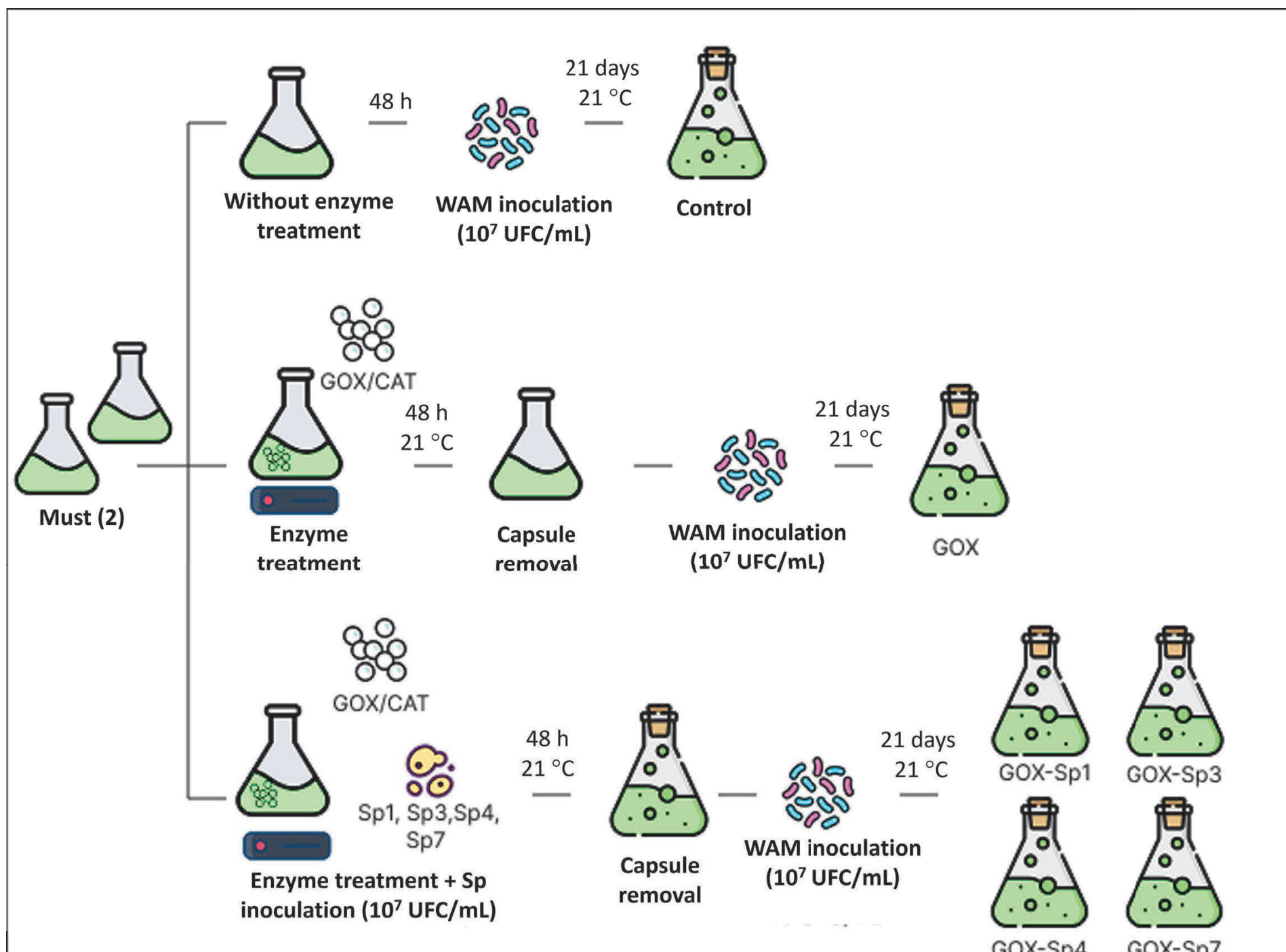


Figure 1. Experimental design of Verdejo microvinifications

Results

In must after 48 h of treatment, GOX/CAT significantly increased gluconic acid concentration compared to the control. Although *S. pombe* strains did not significantly affect gluconic acid levels, they reduced residual H₂O₂ concentrations, with Sp4 showing the greatest reduction (Table 1).

Table 1. Gluconic acid and H ₂ O ₂ concentrations in must after 48 h of treatment		
Must	Gluconic acid (g/L)	H ₂ O ₂ (mg/L)
Control	0.00±0.00 ^a	-
GOX	23.24±4.43 ^b	100
GOX-Sp1	19.35±2.51 ^b	50
GOX-Sp3	20.18±1.04 ^b	51
GOX-Sp4	21.10±1.80 ^b	20
GOX-Sp7	21.46±3.50 ^b	60

The analysis of the final wines showed that GOX treatment alone achieved the greatest reduction in alcohol content (8.96% v/v), although it resulted in high gluconic acid levels, increased total acidity, and a very low pH. Among the *S. pombe* strains evaluated, Sp3 exhibited the most balanced oenological profile, with lower residual gluconic acid and suitable acidity and pH values (Table 2).

Table 2. Basic composition of wines							
Wine	Alcohol degree (% vol)	pH	Total acidity (g/L)	Volatile acidity (g/L)	Gluconic acid (g/L)	Malic acid (g/L)	H ₂ O ₂ (mg/L)
Control	10.79±0.26 ^c	3.59±0.00 ^d	4.87±0.00 ^b	0.28±0.01 ^b	0.00±0.00 ^a	1.68±0.01 ^c	-
GOX	8.96±0.13 ^a	2.12±0.18 ^a	11.23±0.86 ^d	0.10±0.00 ^a	20.86±2.10 ^b	2.46±0.22 ^d	0
GOX-Sp1	10.04±0.43 ^b	3.57±0.01 ^{cd}	2.89±0.04 ^a	0.40±0.01 ^{cd}	1.40±1.14 ^a	0.03±0.04 ^a	0
GOX-Sp3	9.75±0.09 ^b	3.18±0.33 ^c	5.54±1.45 ^b	0.31±0.07 ^{bc}	4.70±4.10 ^a	1.50±0.08 ^c	0
GOX-Sp4	9.53±0.16 ^{ab}	3.54±0.05 ^{cd}	3.79±0.01 ^{ab}	0.41±0.04 ^d	3.25±0.91 ^a	0.00±0.00 ^a	0
GOX-Sp7	9.47±0.14 ^{ab}	2.68±0.17 ^b	7.69±0.83 ^c	0.04±0.05 ^a	14.53±3.18 ^b	0.62±0.37 ^b	0

Phenolic composition of wines was largely unaffected by the treatments. Minor differences were observed in hydroxycinnamic acids, flavonoids, and color parameters, with Sp7 showing the highest lightness (L*) and Sp4-Sp7 the lowest flavonoid levels (Table 3).

Table 3. Phenolic composition and color characteristics of wines						
Wine	Total Polyphenol Index	Hydroxycinnamic acids	Flavonoids	L*	a*	b*
Control	5.17±0.16 ^a	1.85±0.08 ^c	0.59±0.04 ^b	97.38±0.72 ^{ab}	-0.89±0.01 ^{abc}	7.76±0.04 ^{ab}
GOX	4.80±0.20 ^a	1.72±0.12 ^{bc}	0.63±0.08 ^b	95.78±0.55 ^a	-0.74±0.04 ^{bc}	8.58±0.57 ^{bc}
GOX-Sp1	4.62±0.30 ^a	1.80±0.09 ^c	0.61±0.05 ^b	96.30±0.69 ^a	-0.71±0.04 ^c	9.17±0.22 ^c
GOX-Sp3	4.72±0.36 ^a	1.85±0.14 ^c	0.62±0.04 ^b	96.85±0.64 ^{ab}	-1.00±0.28 ^a	9.11±0.37 ^c
GOX-Sp4	4.85±0.5 ^a	1.55±0.05 ^{ab}	0.49±0.02 ^a	96.98±0.10 ^{ab}	-0.87±0.09 ^{abc}	7.62±0.07 ^a
GOX-Sp7	4.73±0.29 ^a	1.49±0.02 ^a	0.50±0.02 ^a	98.40±0.12 ^b	-0.96±0.09 ^{ab}	7.21±0.11 ^a

Conclusions

- The immobilized GOX/CAT system effectively reduced the final alcohol content of Verdejo wines, although it promoted gluconic acid accumulation during the prefermentative stage.
- The evaluated *S. pombe* strains showed different metabolic behaviors, leading to significant differences in gluconic acid degradation and acid composition.
- Strains Sp1 and Sp4 completely consumed malic acid, while Sp7 maintained higher gluconic acid and total acidity levels.
- Among the strains tested, Sp3 exhibited the most balanced oenological profile, combining alcohol reduction with lower residual gluconic acid and acceptable pH and acidity values.

References

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