# EFFECT BENZENE COMPOUNDS ON GROWTH AND FERMENTATION OF WINE YEASTS

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## INTRODUCTION

In industrial winemaking processes, *Saccharomyces cerevisiae* strains are commonly used to ensure proper fermentation development. In recent years, there has been a notable shift towards the production of wines with greater differentiation. The selection of non-*Saccharomyces* yeast strains can provide different organoleptic characteristics to the wine. *Hanseniaspora vineae* is a uruguayan native yeast strain that produces positive aromas, enhancing the quality of the final wine. This species is typically employed in mixed cultures with *S. cerevisiae* to guarantee complete sugar consumption and optimal alcoholic fermentation. The timing of inoculation of *S. cerevisiae* is of great importance, as it can influence the performance of *H. vineae*, which in turn can result in changes to the wine's aromatic profile. The interaction between these yeast species can be regulated by the release of molecules into the extracellular medium. Some cell communication activators derived from aromatic amino acids, such as 2-phenylethanol, tryptophol and tyrosol, have been previously described in *S.cerevisiae*. It is noteworthy that *H. vineae* is capable of rapidly and efficiently acetylate these alcohols, thereby reducing the toxic effects of the alcohols in the extracellular medium (Carrau et al., 2023). The discovery that aromatic compounds produced by the wine yeast *H. vineae* during fermentation may play a signalling role during fermentation has sparked interest in the identification of compounds involved in cell communication. This could potentially lead to the development of more efficient fermentations with an impact on the sensory quality of the final product.

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## METHODOLOGY

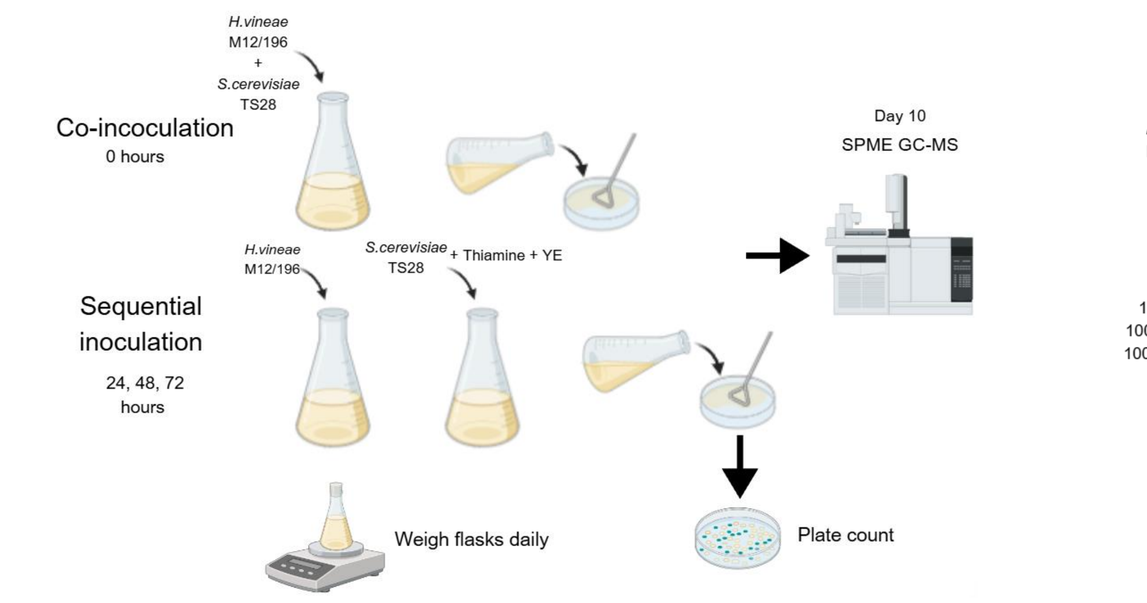



**Fig 1**. Microfermentations, co-inoculation *H.vineae* and *S.cerevisiae* at time 0 and sequential inoculation starting with *H.v ineae* followed by *S.cerevisiae* at either 24, 48, or 72 hours. Plated the day after inoculation and halfway through it. Daily weighing of flasks. SPME GC-MS analysis of samples taken from day 10 of fermentation.

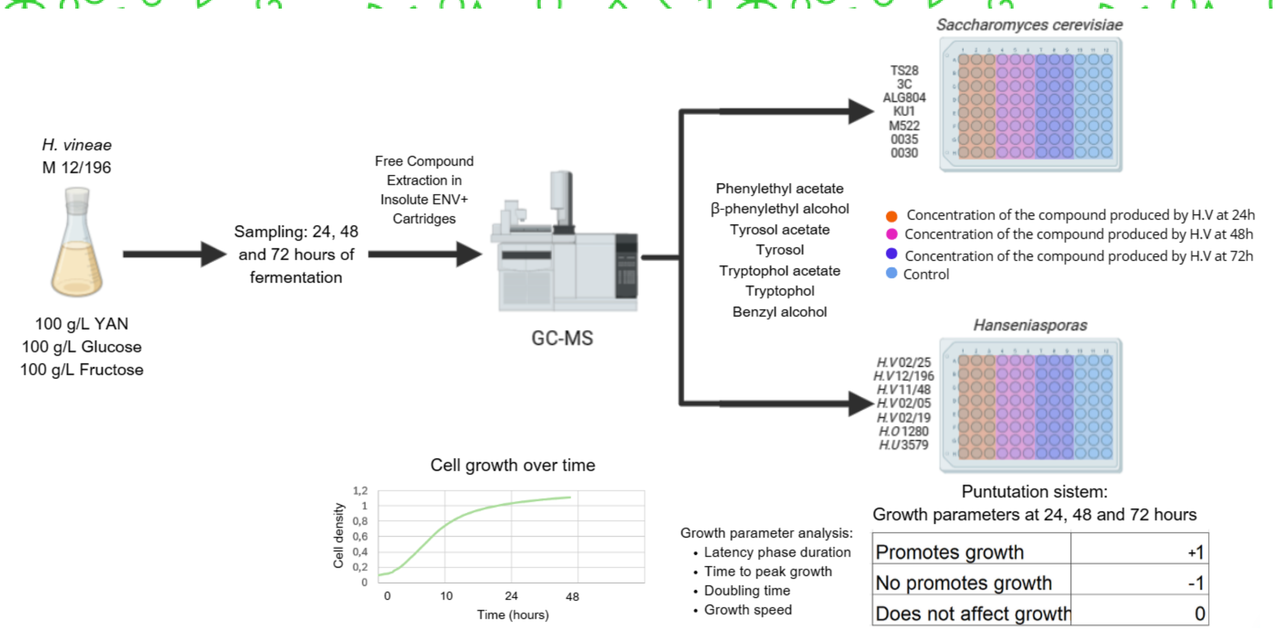
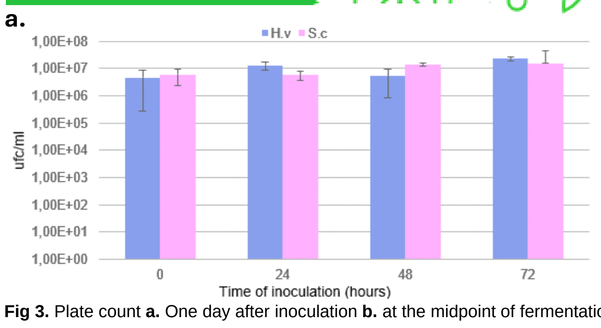



**Fig 2**. Fermentation of *H.vineae* , extraction of free volatile compounds with Insolute ENV+ Cartidges of samples taken at 24, 48 and 72 hours of fermentation. Identification by GC-MS, quantification of compounds and addition of these concentrations in 96-well plates with seven strains of *S.cerevisiae* and seven strains of *H.vineae* to evaluate their growth for 48 hours. Statistic analysis of growth parameters.

## RESULTS



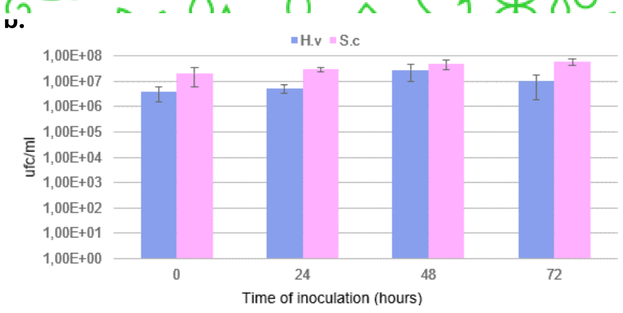
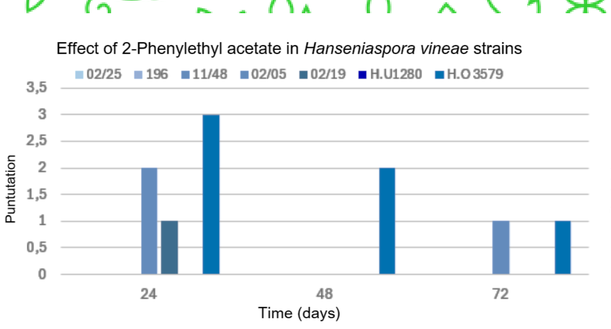
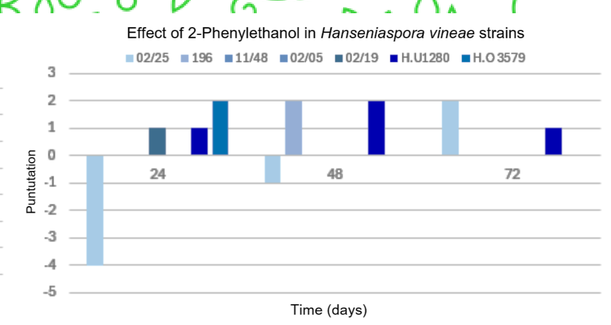




**Fig 3.** Plate count **a.** One day after inoculation **b.** at the midpoint of fermentation.



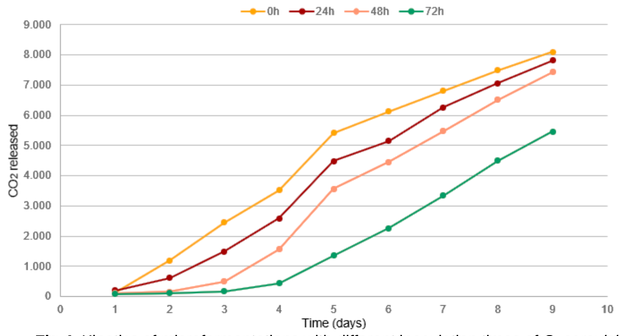




**Fig 4**. Kinetics of microfermentations with different inoculation times of *S. cerevisiae*.

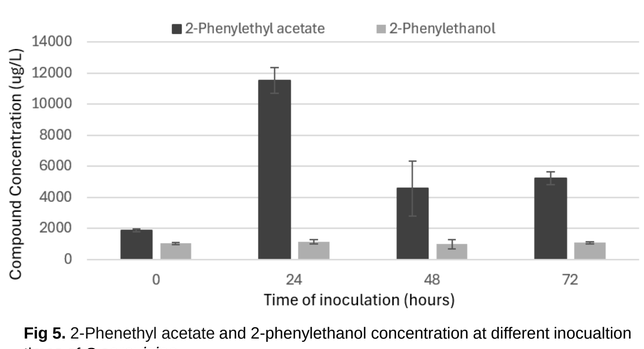
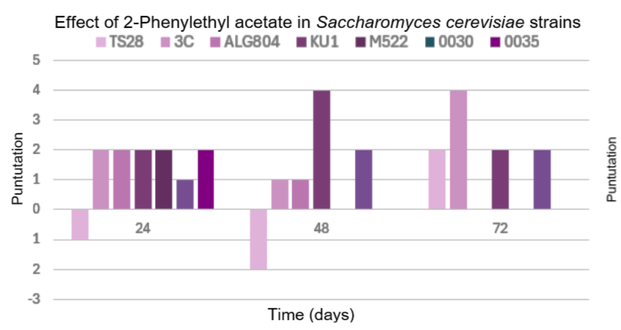



**Fig 5.** 2-Phenethyl acetate and 2-phenylethanol concentration at different inocualtion times of *S.cerevisiae*.



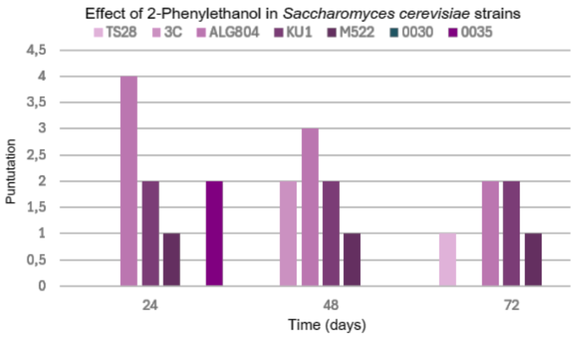




**Fig 6**.Effect of compounds in *S.cerevisiae* and *H.vineae* growth study by 96-well plates with puntuation system designed.

| Sample (hours of inoculation) | Ethanol (%v/v) | Volatile acidity (g $H_2SO_4$/L) |
|---|---|---|
| 0 | 12,6 $^{c}$ ± 0,7 | 0,5 ± 0,02 |
| 24 | 11,4 $^{b, c}$ ± 0,1 | 0,5 ± 0,02 |
| 48 | 11,1 $^{a, b}$ ± 0,3 | 0,5 ± 0,02 |
| 72 | 9,9 $^{a}$ ± 0,4 | 0,5 ± 0,01 |

Table 1. Measurement of ethanol, sugar, and volatile acidity.

## CONCLUSIONS

- Sequential inoculation of *S. cerevisiae* 24 hours after starting *H. vineae* fermentation results in higher levels of 2-phenylethyl acetate due to *H. vineae*'s ability to acetylate and more efficient fermentation.
- The physiological concentration of 2-phenylethyl acetate produced by *H. vineae* after 24 hours of fermentation has been observed to stimulate the growth of *S. cerevisiae* and to facilitate a more efficient fermentation process. This evidence points towards the existence of a synergistic interaction between the two species, with *S. cerevisiae* generating the alcohol precursor of 2-phenylethyl acetate, which is subsequently acetylated by *H. vineae*.

## ACKNOWLEDGEMENTS

PROYECTO ANII
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