

Optimizing Fermentation Time and pH in Primary Souring of Beer through Co-Fermentation with *Saccharomyces cerevisiae* and *Lachancea thermotolerans*

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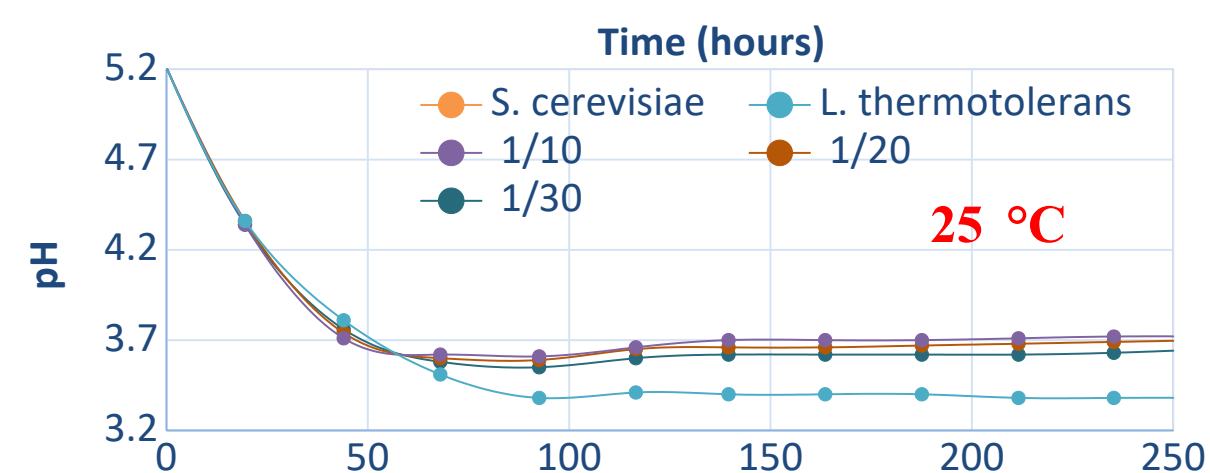
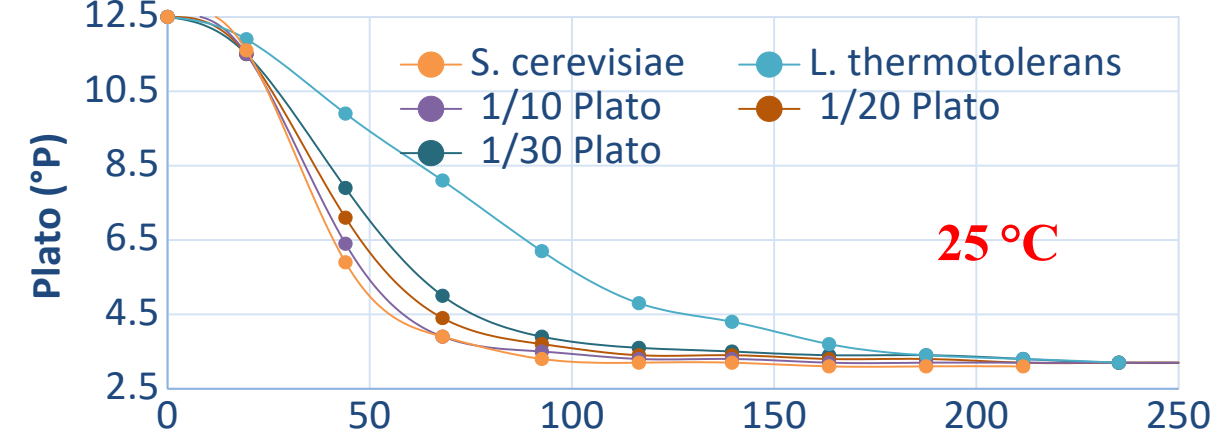
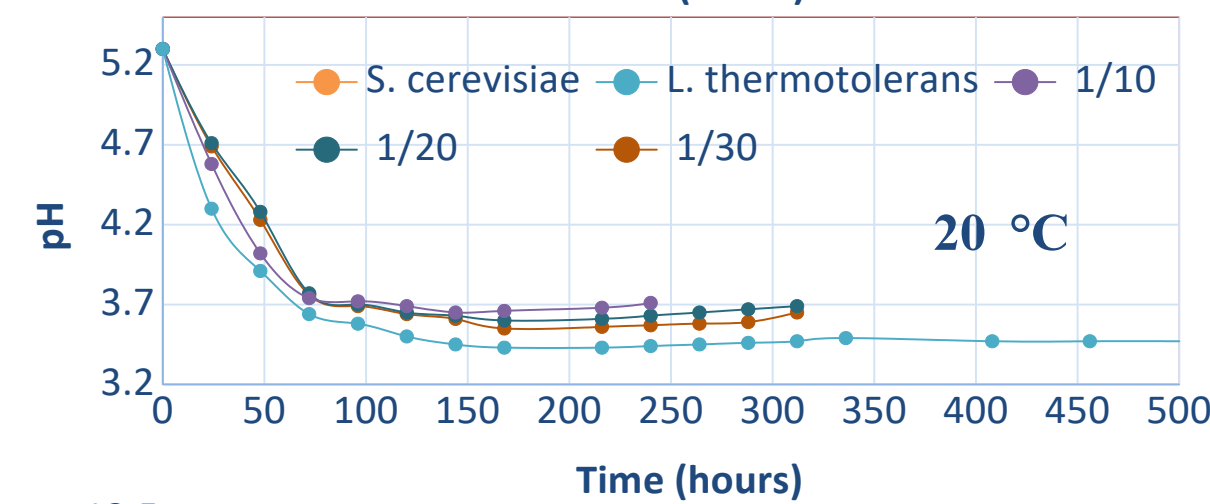
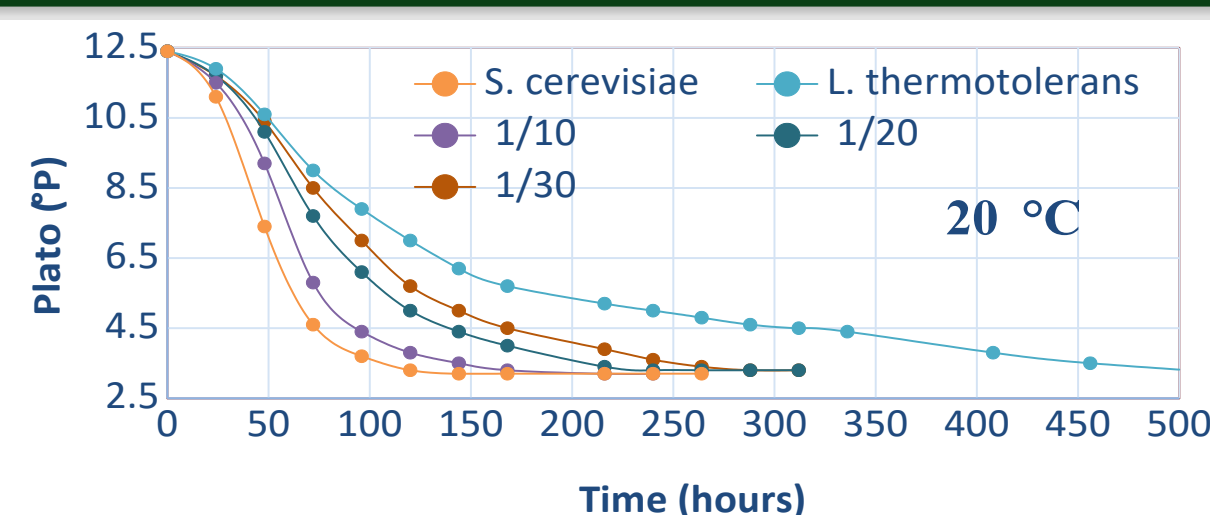
INTRODUCTION & AIM

This study investigates mixed fermentations of *Lachancea thermotolerans* and *Saccharomyces cerevisiae* as a strategy to reduce fermentation time in sour beer production while preserving the acidification benefits of *L. thermotolerans*, emphasizing the optimization of fermentation duration, pH, acidity and sensory characteristics. The primary aim was to improve the fermentation performance of *L. thermotolerans* (*L. t.*) through co-inoculation with *S. cerevisiae* (*S. c.*) and examine how different inoculation ratios affect the speed of fermentation, the final pH, and the overall quality of the beer.

METHOD

For this series of experiments, an all-grain wort in three batches was prepared using a step mashing method (60 min at 64 °C, 15 min at 72 °C, and 5 min at 78 °C), followed by a 1-hour boil. The resulting wort had an extract of 12.5 °P, a pH of approximately 5.2 ± 0.05 , and a bitterness level of 18 IBU. Two identical fermentation trials were then carried out at 25 °C and 20 °C using different fermentation containers: (1) pure *S. cerevisiae*, (2) pure *L. thermotolerans*, and mixed cultures of *S. cerevisiae* to *L. thermotolerans* at ratios of (3) 1:10, (4) 1:20 and (5) 1:30. Subsequently, an additional set of fermentations was performed at 25 °C and 20 °C with pure *L. thermotolerans* and mixed cultures at ratios of (a) 1:30 and (b) 1:40. Density and pH were measured daily to monitor fermentation progress. Final analyses included alcohol content, pH, total acidity and FAN according to the ASBC methods of analysis, followed by a sensory evaluation.

RESULTS & DISCUSSION



Mixed fermentations with *Lachancea thermotolerans* and *Saccharomyces cerevisiae*

➤ Alcohol concentration:

Alcohol levels were slightly higher at 25 °C compared to 20 °C in both pure and mixed-culture fermentations, irrespective of the inoculation ratio between *L. thermotolerans* and *S. cerevisiae*.

➤ pH values:

Fermentations carried out at 25 °C exhibited the same or slightly lower final pH values than those conducted at 20 °C. Except for pure *S. cerevisiae* fermentations (>3.95), none of the other samples exceeded a final pH of ~ 3.7 . In mixed-culture fermentations, an increased proportion of *L. thermotolerans* resulted in slightly lower final pH values.

➤ Total acidity:

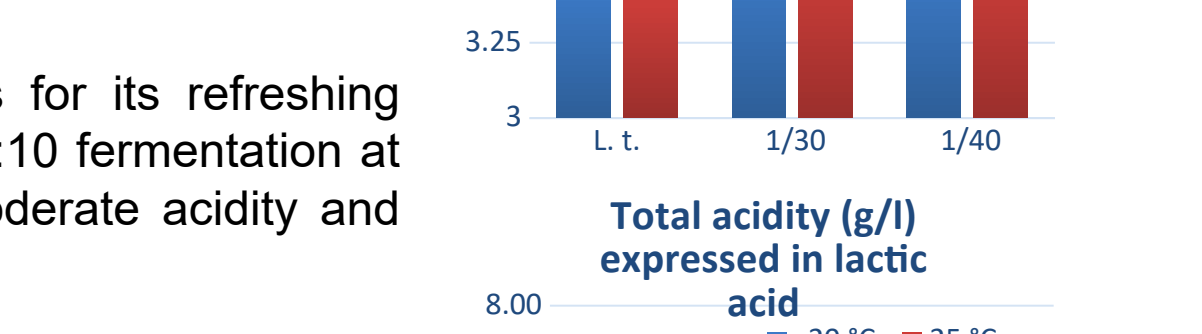
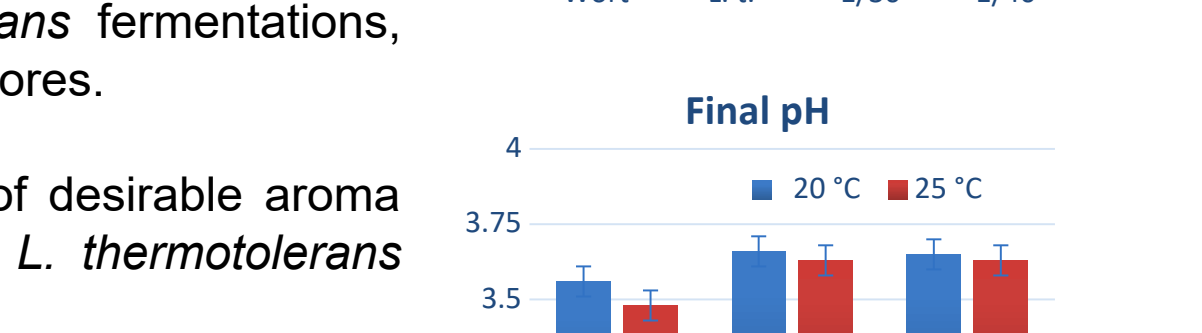
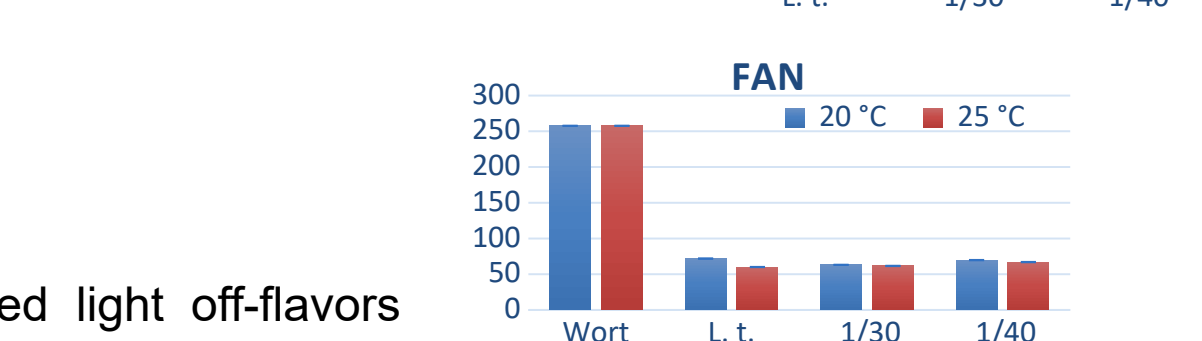
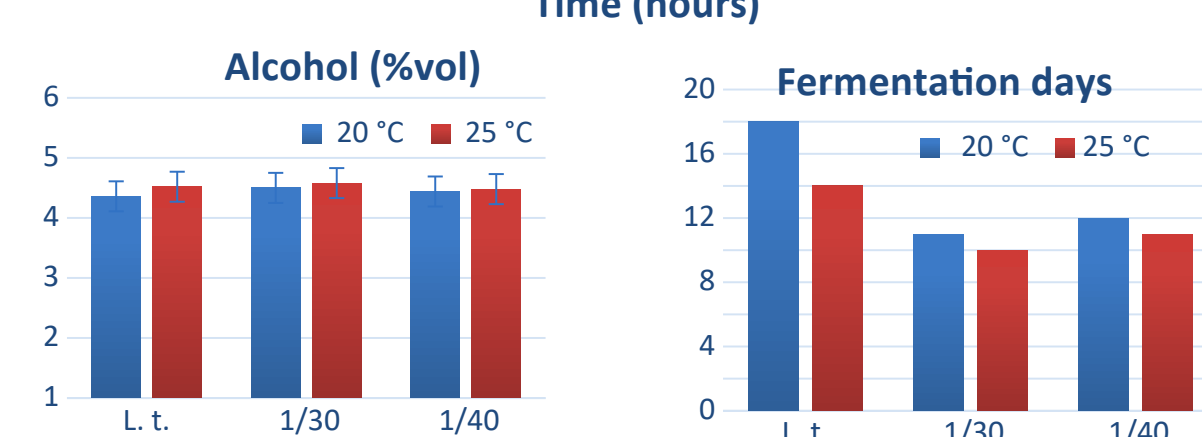
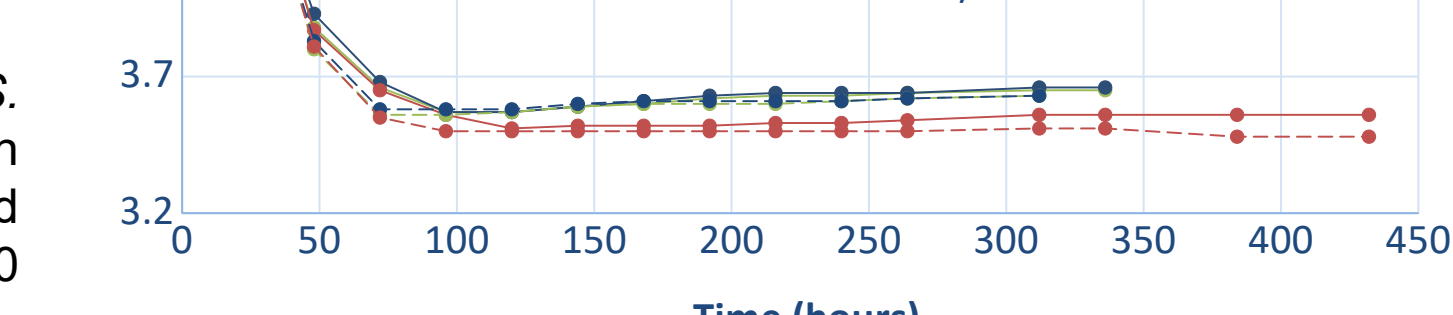
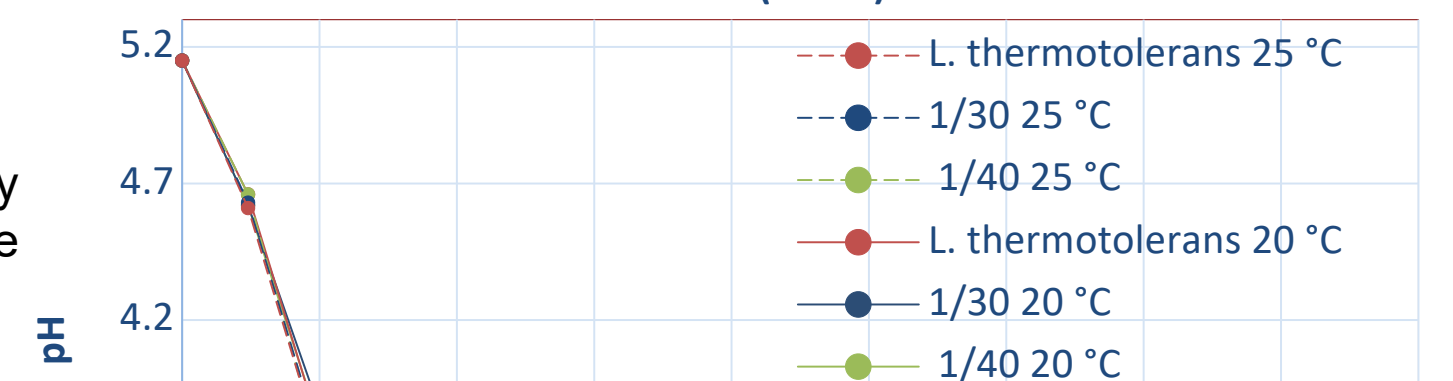
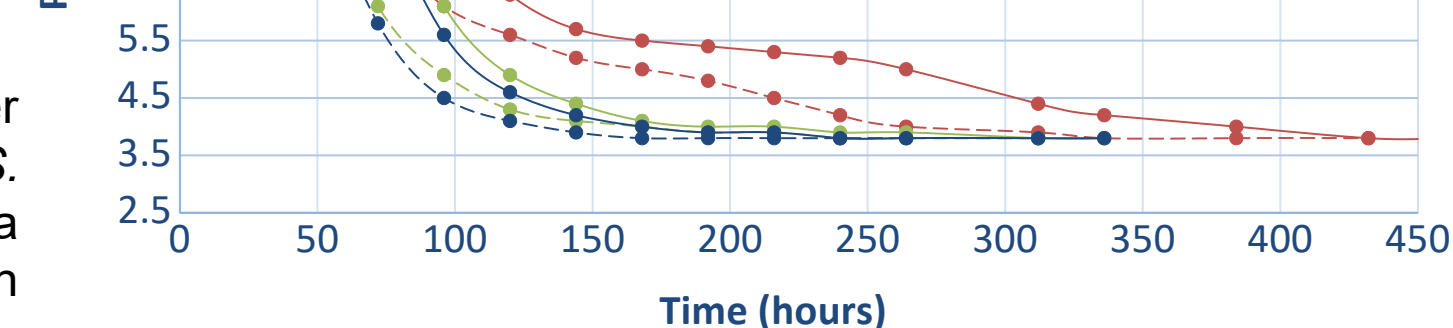
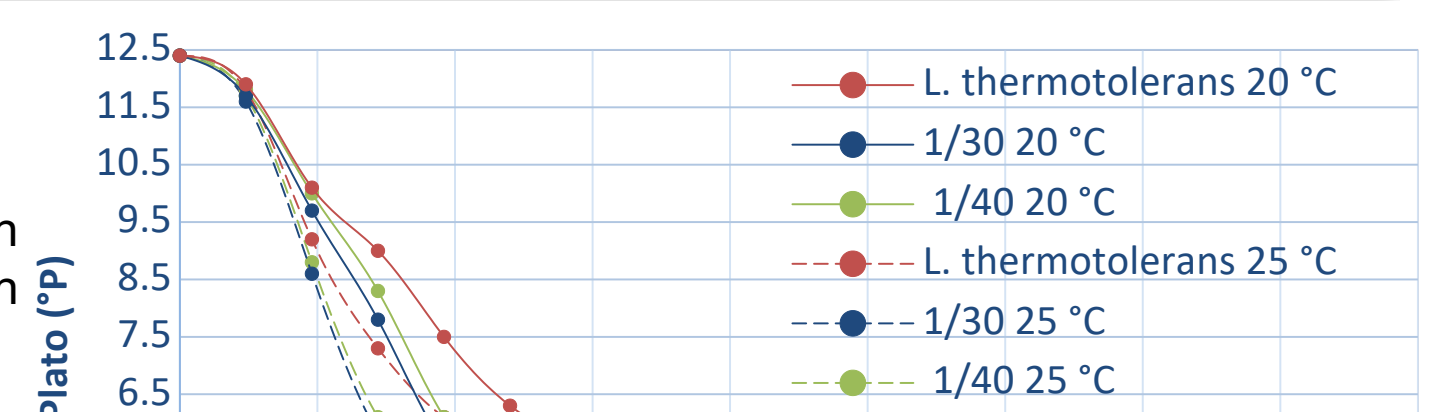
Mixed-culture fermentations showed a 30–50% reduction in total acidity compared to pure *L. thermotolerans* fermentations, depending on the inoculation ratio (from 1:40 to 1:10, respectively).

➤ Fermentation duration:

At 25 °C, the fermentation duration decreased as the proportion of *S. cerevisiae* increased, with the 1:10 ratio showing a similar fermentation time to the pure *S. cerevisiae* control. At 20 °C, the fermentation period was shortened by approximately 60% for the 1:10 and 20% for the 1:40 ratios, respectively, relative to pure *L. thermotolerans*.

➤ Free Amino Nitrogen (FAN):

The FAN concentration profiles were comparable across all the different fermentations. The wort initially contained approximately 250–270 mg/L FAN, which decreased to 60–70 mg/L. This demonstrates that FAN utilization was similar in both pure and mixed-culture fermentations.



Sensory evaluation and characteristics:

- ✓ At 25 °C, *S. cerevisiae* fermentations—both pure and mixed—developed light off-flavors described as metallic and animal-like. In contrast, pure *L. thermotolerans* fermentations, particularly at 20 °C, were free of off-flavors and received higher sensory scores.
- ✓ Mixed-culture fermentations at 20 °C generated greater concentrations of desirable aroma compounds compared to those at 25 °C. In fermentations with higher *L. thermotolerans* ratios, the citrus-like acidity was more prominent.
- ✓ The 1:10 ratio was preferred by consumers unfamiliar with sour beers for its refreshing character, despite its lower pH (~ 3.7) compared to “classic” beers. The 1:10 fermentation at 20 °C was the most favored overall, offering a balanced profile with moderate acidity and pleasant citrus–honey notes.
- ✓ The 1:40 ratio fermentations, at both 20 °C and 25 °C, produced flavor profiles more closely resembling the sour character of typical pure *L. thermotolerans* fermentations, which were preferred by experienced sour beer aficionados.

CONCLUSION

Mixed fermentations of *Lachancea thermotolerans* with *Saccharomyces cerevisiae* significantly shortened the fermentation period of sour beers while maintaining low final pH values (3.6–3.7). Fermentations with higher *L. thermotolerans* ratios at 20 °C yielded the most desirable sensory profiles, featuring balanced acidity and pleasant citrus–floral notes. Overall, these findings confirm that controlled mixed fermentations improve fermentation performance, boosting fermentation speed. Furthermore, by adjusting different yeast ratios between the two yeasts allows for a variety of sour beer profiles and lower cooling energy needs during fermentation.