The 1st International Online Conference on Fermentation



12-13 November 2025 | Online

Effects of Sequential Inoculation of Non-Saccharomyces and S. cerevisiae Strains on the Fermentation Process of High-Sugar Chardonnay

Ipek Aktuna^{1,2}, Jonathan D. Brumley¹, G. Candan Gurakan³, Charles G. Edwards¹

¹ School of Food Science, Washington State University, Pullman, WA, USA ² Department of Biotechnology, Middle East Technical University, Ankara, Turkey

³ Department of Food Engineering, Middle East Technical University, Ankara, Turkey

ipek_aktuna@hotmail.com

INTRODUCTION & AIM

- > Increasing grape sugar levels, driven by climate change, impacts the alcohol content of wine. One of the possible solutions to reduce alcohol content is the use of non-Saccharomyces yeasts.
- > In this study, chaptalized Chardonnay must (28°Brix) was sequentially inoculated with one of non-Saccharomyces
 - M. pulcherrima P01A016,
 - M. quillermondii P40D002,
 - W. anomalus O37,
 - L. thermotolerans O9, or
 - M. pulcherrima D11 and commercial S. cerevisiae Lalvin NBC™ with YAN supplementation (Fermaid K™) to track
 - fermentation kinetics,
 - YAN consumption,
 - ethanol and by-products concentrations.

METHOD Day 5 & 8 Day 0 Day 0 Day 4 M. guillermondi S. cerevisiae YAN Add. P40D002 M. pulcherrima S. cerevisiae YAN Add. P01A016 Filtered W. anomalus Chardonnay YAN Add. S. cerevisiae 037 Juice ¹ S. cerevisiae YAN Add. thermotolerans 09 M. pulcherrima YAN Add. S. cerevisiae D11

*For the control treatment, the Chardonnay fermentation inoculated only S. cerevisiae was started on day 4 and YAN was added on the day the other treatments were added.

ACKNOWLEDGEMENT: This research was made possible by the financial support of the Washington

State Grape & Wine Research Program, the Northwest Center for Small Fruits Research, and the PhD

scholarship of the Turkish Higher Education Council (YÖK 100/2000). This work was also supported in

part by the USDA National Institute of Food and Agriculture Hatch project 1016366.





Yeast colonies on WL & Lysine Agars to measure the yeasts' cultivability

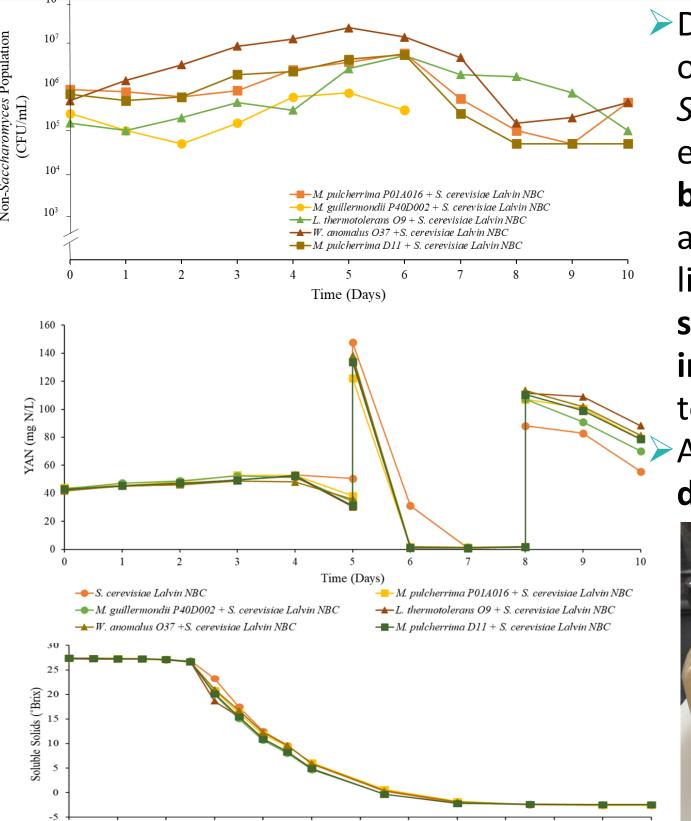




Chardonnay must in Wheaton fermenter

HPLC for wine analysis

RESULTS & DISCUSSION



−●− M. guillermondii P40D002 + S. cerevisiae Lalvin NBC - k-- L. thermotolerans O9 + S. cerevisiae Lalvin NBC

→S. cerevisiae Lalvin NBC

→ W. anomalus O37 +S. cerevisiae Lalvin NBC

- During the early stages of fermentation, non-Saccharomyces strains exhibited lower growth, biomass production, and YAN consumption, likely due to **osmotic** stress and their inadequate adaptation to high-sugar must.
- All treatments reached dryness.



Chardonnay wines at the end of the fermentation

Treatments	Ethanol (% v/v)	Glycerol (g/L)	Glucose (g/L)	Fructose (g/L)	Acetic Acid (g/L)	Succinic Acid (g/L)
S. cerevisiae Lalvin NBC	16.3 ^{a,b}	9.11 ^a	1.60 ^c	0.511 ^{a,b}	0.378 ^c	0.417 ^{c,d}
<i>M. pulcherrima</i> P01A016*	16.0 ^b	8.93 ^a	1.75 ^{b,c}	0.359 ^{a,b}	0.580 ^a	0.366e
<i>M. guilliermondii</i> P40D002*	16.6ª	9.18 ^a	1.73 ^{b,c}	0.349 ^{a,b}	0.405 ^{b,c}	0.478 ^{a,b}
<i>L. thermotolerans</i> O9*	16.7 ^a	8.91 ^a	1.95 ^{a,b}	0.656ª	0.469 ^b	0.433 ^{b,c}
<i>W. anomalus</i> O37*	16.7 ^a	9.18 ^a	2.00 ^a	0.492 ^{a,b}	0.611 ^a	0.376 ^{d,e}
<i>M. pulcherrima</i> D11*	16.5 ^{a,b}	9.44 ^a	1.85 ^{a,b}	0.209 ^b	0.432 ^{b,c}	0.488 ^a

--- M. pulcherrima P01A016 + S. cerevisiae Lalvin NBC

-■- M. pulcherrima D11 + S. cerevisiae Lalvin NBC

Means within a column with different superscript letters indicate significantly different at $p \le 0.05$. represents sequential inoculation with S. cerevisiae Lalvin NBC.

CONCLUSION

- >M. pulcherrima P01A016 exhibited a 0.6-0.7%(v/v) alcohol reduction.
- > Although *M. pulcherrima* strains from various regions of the world produced similar alcohol levels, their profiles of acetic and succinic acids differed, highlighting how strain selection affects wine composition.

FUTURE WORK / REFERENCES

- To decrease osmotic stress and enhance adaptation to high-sugar environments, future research should concentrate on optimizing fermentation conditions for non-Saccharomyces yeasts.
- Aplin, J. J., & Edwards, C. G. (2021). Impacts of non-Saccharomyces species and aeration on sequential inoculation with Saccharomyces cerevisiae to produce lower alcohol Merlot wines from Washington state. Journal of the Science of Food and Agriculture, 101(4), 1715–1719. https://doi.org/10.1002/JSFA.10769
- Rodrigues, A. J., Raimbourg, T., Gonzalez, R., & Morales, P. (2016). Environmental factors influencing the efficacy of different yeast strains for alcohol level reduction in wine by respiration, LWT, 65, 1038-1043, https://doi.org/10.1016/i.lwt,2015.09.046