## Comparative Analysis of Glutathione Production by Commercial Non-Saccharomyces Yeast Strains in Wine Fermentation

Ivonne C. Dresser<sup>1</sup>, David L. Spector<sup>2</sup>, Lars C. Schrübbers<sup>3</sup>, Katja Sander Jensen<sup>3</sup>, Amalie Stavnstrup Rasmussen<sup>3</sup>, Helle Simonsen<sup>3</sup> and <u>Duncan J. Hamm<sup>3</sup></u>



1. Gusmer Enterprises, Santa Rosa CA, USA

2. Novonesis, Inc., Milwaukee WI, USA

3. Novonesis A/S, Hørsholm, Denmark

Presenting author: dunha@novonesis.com

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Introduction

L-y-glutamyl-L-cystinyl-glycine, commonly known as Glutathione (GSH), plays a crucial role as an antioxidant in wine, helping to mitigate the loss of volatile flavor compounds susceptible to oxidation, as well as helping to prevent oxidative browning and aroma changes. In recent years particular interest in utilizing GSH in winemaking has developed, and as a result yeast derivatives rich in this compound have been marketed along the lines of maximizing and preserving flavor expression. Non-saccharomyces yeasts (NSY) have been associated with producing higher concentrations of GSH compared to *Saccharomyces cerevisiae*, yet data on commercial strains has been limited<sup>1</sup>. The potential of four commercial NSY strains from three species - *Lachancea thermotolerans, Pichia kluyveri*, and *Torulaspora delbrueckii* - to increase GSH concentration in the final wine was investigated. Conducted in two different musts, designed to emulate conditions for red and white or rosé wine fermentations respectively, the initial yeast inoculation with NSY was followed after 48 hours with *S. cerevisiae*. The selected NSY strains exhibit varied capabilities in enhancing final GSH levels, with *Torulaspora delbrueckii* CHCC5755 giving the highest concentrations in both wines. This potentially offers winemakers an additional tool for improving wine quality and stability through natural antioxidant mechanisms. Further research and trials will illuminate the practical implications of these findings for winemaking practices, particularly in flavor preservation and oxidative stability.

Methodology	Results		
Two musts were selected for the experiment to represent red and white/rosé conditions:	GSH conc. before, at day two and at end of fermentation Chardonnay (high pH / high temperature)	<b>GSH conc. before, at day two and at end of fermentation</b> Pinot Noir (low pH / low temperature)	
<ul> <li>Chardonnay, adjusted with NaOH to pH 3.60,</li> </ul>	20.0	20.0	
to be fermented at 25 °C	18.0	18.0	
• Pinot Noir adjusted with tartaric acid to nH	16.0	16.0	

### 3.10, to be fermented at 15°C

#### Each must was fermented with NSY or controls:

Two *S. cerevisiae* starters were used. A second control with one of the *S. cerevisiae* yeasts plus 30g/hL of a commercially available GSH-rich yeast derivative (YD) was included. Run in duplicate, all fermentations were 200ml in size.





#### Figure 1. Concentrations of GSH in must before yeast inoculation, at day two and at end of alcoholic fermentation All samples were centrifuged upon sampling then frozen, to prevent oxidation, and no $SO_2$ was added to these in order to prevent GSH forming glutathione-S-sulfonate (GSSO<sub>3</sub>H)<sup>2</sup>. Samples were thawed immediately before analysis.

Conclusions

Ferment to	Ferment to	Ferment to	G/F
dryness	dryness	dryness	<2g/L
			)

3. The resultant wines were sampled prior to inoculation, at 48 hours and at sugar dryness for GSH (without SO<sub>2</sub>). Enzymatic kit MAK440 was used for the analysis. (Merck, Germany).

- Torulaspora delbrueckii CHCC5755, a commercial NSY (trade name Viniflora<sup>™</sup> PRELUDE<sup>™</sup>) was the most effective at increasing the GSH concentration across both wines, performing better than the GSH-rich yeast derivative.
- This work could easily be scaled-up to commercial scale and extended to measure markers for oxidation and/or sensory analysis.
- Measuring the related compounds of glutathione disulfide (GSSG) and GSSO<sub>3</sub>H, in addition to GSH itself, could also be beneficial.

#### References:

- 1. Binati, R. L., W. J. F. Lemos Junior, and S. Torriani. "Contribution of non-Saccharomyces yeasts to increase glutathione concentration in wine." *Australian Journal of Grape and Wine Research* 27.3 (2021): 290-294.
- 2. Dienes-Nagy, Ágnes, et al. "Simultaneous quantification of glutathione, glutathione disulfide and glutathione-S-sulfonate in grape and wine using LC-MS/MS." *Food Chemistry* 386 (2022): 132756.