Innovative Yeast Solutions

Leveraging Non-*Saccharomyces* Strains for Better Wine

Gusmer Enterprises, Inc.®

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CONTENTS

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Selecting the proper **Viniflora™** yeast strain for your wine

Viniflora® yeast range for 2024

	Wine Style	Alcohol Tolerance	Species	Optimal Temperature	Inoculation Rate	Fermentation Speed	SO_2 Tolerance	Key Characteristics
Non Saccharomyces Yeasts								
FROOTZEN™	Sauvignon Blanc, Pinot Gris, Chardonnay, Riesling & Pinot Noir	6% v/v	*Pichia kluyveri*	15-25°C	100ppm	✓	45ppm	High level of volatile thiols Direct inoculation Frozen Product Oxygen scavenging
PRELUDE™	Pinot Noir, Bordeaux varieties, Grenache, Rosé, Barrel matured whites	9% v/v	*Torulaspora delbreuckii*	10-25°C	200ppm	✓	30ppm	Produces polysaccharides for texture Caramel / pastries flavour note Perfect for pre-fermentation maceration
CONCERTO™	Light to medium weight reds including Mediterranean styles & Pinot Noir	10% v/v	*Lachancea thermotolerans*	15-25°C	200ppm	✓	30ppm	Lower pH naturally (lactic acid production) Produces polysaccharides for mouthfeel Fruit lift from ethyl lactate Inhibition of Kloeckera & acetic acid bacteria
OCTAVE™	Rosé, Pinot Gris, Chardonnay, Riesling	11% v/v	Lachancea thermotolerans	13-25oC	200ppm	✓	30ppm	Inhibition of spontaneous MLF Colour vibrancy in Rosé Lower pH naturally (lactic acid production) Lifted stone fruit character
Saccharomyces Yeasts								
MERIT	Traditional red varieties	17% v/v	*Saccharomyces cerevisiae*	15-30°C	200ppm	✓✓	90ppm	Resistance to high alcohol Red & black fruit flavour Spicy notes
JAZZ™	Traditional red varieties	17% v/v	*Saccharomyces cerevisiae*	10-30°C	200ppm	✓✓✓	90ppm	Fruit lift without being confected Elegant structure Velvety complex tannins
Blend of Saccharomyces and Non Saccharomyces Yeasts								
MELODY™	Chardonnay Pinot Noir Grenache	17% v/v	*Saccharomyces cerevisiae* (60%) *Lachancea thermotolerans* (20%) *Torulaspora delbreuckii* (20%)	15-28°C	200ppm	✓✓	30ppm	Increases aromatic complexity Rounded mouthfeel

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Viniflora® yeast range for 2024

Addition Point / Purpose: Cold Soak or Onset of Primary Fermentation/ Bio-Protect Wines - Flavor, Aroma, Tactile, Development	Addition Point / Purpose: Primary Fermentation/ Alcoholic Fermentation	Product	Features	Benefits
Chr. Hansen Non-*Saccharomyces* yeasts				
✓		FrootZen 	• *Pichia kluyveri* • High levels of volatile thiols, esters, terpenes • Increases mouthfeel / palate weight • Direct inoculation yeast • Low SO_2 and H_2S production • Used with your favorite *Saccharomyces* yeast	• Grapefruit, passion fruit and citrus • Fuller wines • No rehydration or acclimatization required • Easy on malolactic bacteria
		Recommended Wines	Fruit forward white and rosé wines and light red wines | Sauvignon Blanc | Chardonnay | Riesling | Pinot Gris	
		Temperature Range: 50 to 82 °F (10 to 28 °C)	Sugar / Alcohol Yield: 16.8 g/L sugar for 1% alcohol; Fermentation Speed: Slow	Alcohol Tolerance: 6.0%; Relative Nitrogen Needs: Medium; Lag Phase: Short
✓		Concerto 	• *Lachancea thermotolerans* • Increase total acidity (lactic acid formation) • Low SO_2 and H_2S production • Used with your favorite *Saccharomyces* yeast	• Integrated red and black fruit, spice • Softer palate • Easy on malolactic bacteria
		Recommended Wines	Fruit forward red and rosé wines | Medium body wines | Merlot | Zinfandel | Grenache | Tempranillo | Sangiovese	
		Temperature Range: 50 to 82 °F (10 to 28 °C)	Sugar / Alcohol Yield: 16.8 g/L sugar for 1% alcohol; Fermentation Speed: Slow	Alcohol Tolerance: 10.0%; Relative Nitrogen Needs: Medium; Lag Phase: Moderate
✓		Prelude 	• *Torulaspora delbrueckii* • Heavy producer of mannoproteins / polysaccharides • Low SO_2 and H_2S production • Used with your favorite *Saccharomyces* yeast	• Silky, round wines, increases palate weight • For wines matured in oak and increases perceived fullness • Easy on malolactic bacteria
		Recommended Wines	Perfect with red & white wines fermented or aged in oak | Chardonnay | Cabernet Sauvignon | Syrah | Pinot Noir	
		Temperature Range: 50 to 82 °F (10 to 28 °C)	Sugar / Alcohol Yield: 16.8 g/L sugar for 1% alcohol; Fermentation Speed: Slow	Alcohol Tolerance: 9.0%; Relative Nitrogen Needs: Medium; Lag Phase: Moderate to Long
✓		Octave 	• *Lachancea thermotolerans* • Enhance fruit flavors (Esters) • Low SO_2 and H_2S production • Used with your favorite *Saccharomyces* yeast	• Very low volatile phenols • Pre-fermentation product used on harvested grapes, on crushed grapes, or in the must • Easy on malolactic bacteria
		Recommended Wines	Ideal for white/rosé wines from warm climates	
		Temperature Range: 50 to 82 °F (10 to 28 °C)	Sugar / Alcohol Yield: 17.0 g/L sugar for 1% alcohol; Fermentation Speed: Slow	Alcohol Tolerance: 10 - 11.0%; Relative Nitrogen Needs: Medium; Lag Phase: Moderate to Long
Chr. Hansen Blend of *Saccharomyces* and non-*Saccharomyces* yeasts				
✓	✓	Melody 	• Mixed Cultures: *S. cerevisiae, L. thermotolerans, T. delbrueckii* • Three yeast strains - 60:20:20% blend • Can tolerate high alcohol • Low SO_2 and H_2S production	• More intense complexity and fruit • Enhanced mouthfeel • Easy on malolactic bacteria • One of the most popular strains
		Recommended Wines	Dry white and red wines | Chardonnay | Pinot Gris | Cabernet Sauvignon | Pinot Blanc	
		Temperature Range: 50 to 90 °F (10 to 32 °C)	Sugar / Alcohol Yield: 17.7 g/L sugar for 1% alcohol; Fermentation Speed: Moderate	Alcohol Tolerance: 17.0%; Relative Nitrogen Needs: Medium; Lag Phase: Moderate

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MICROBIAL ECOLOGY, POPULATION DYNAMICS
'WILD-FERMENT' CHARDONNAY, KUMEU RIVER WINERY - NZ

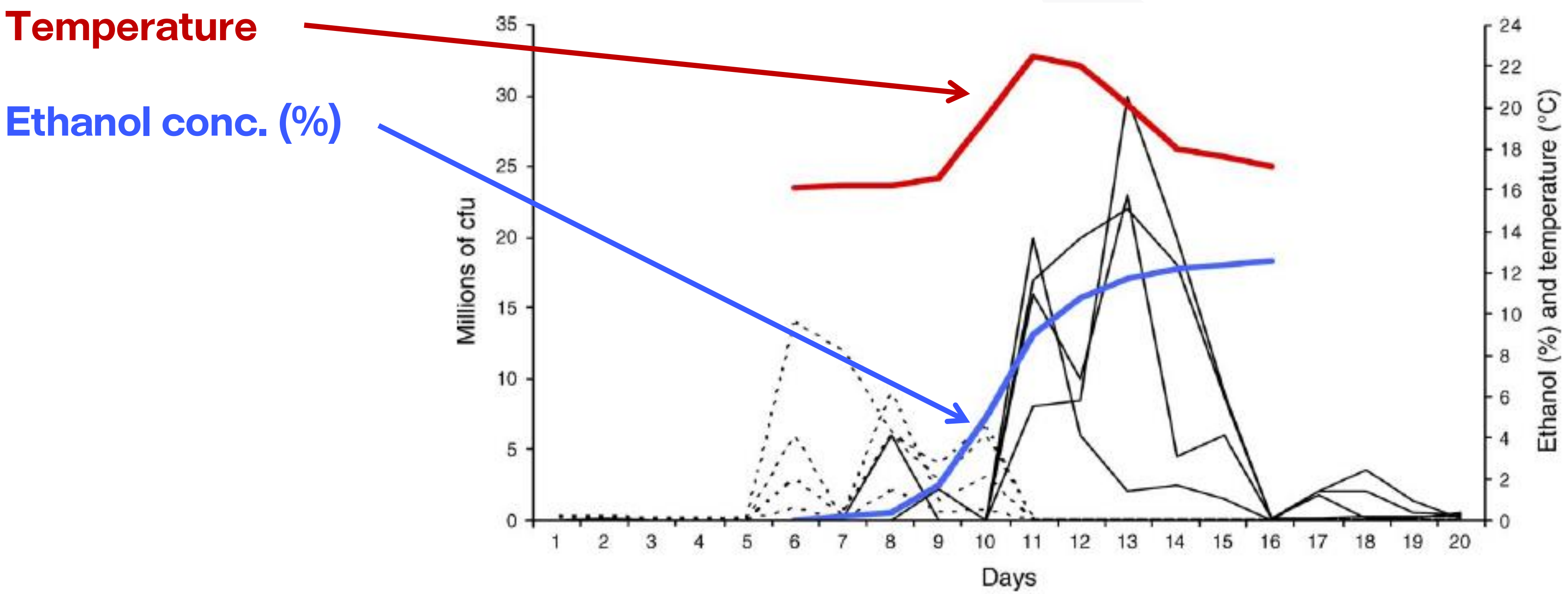


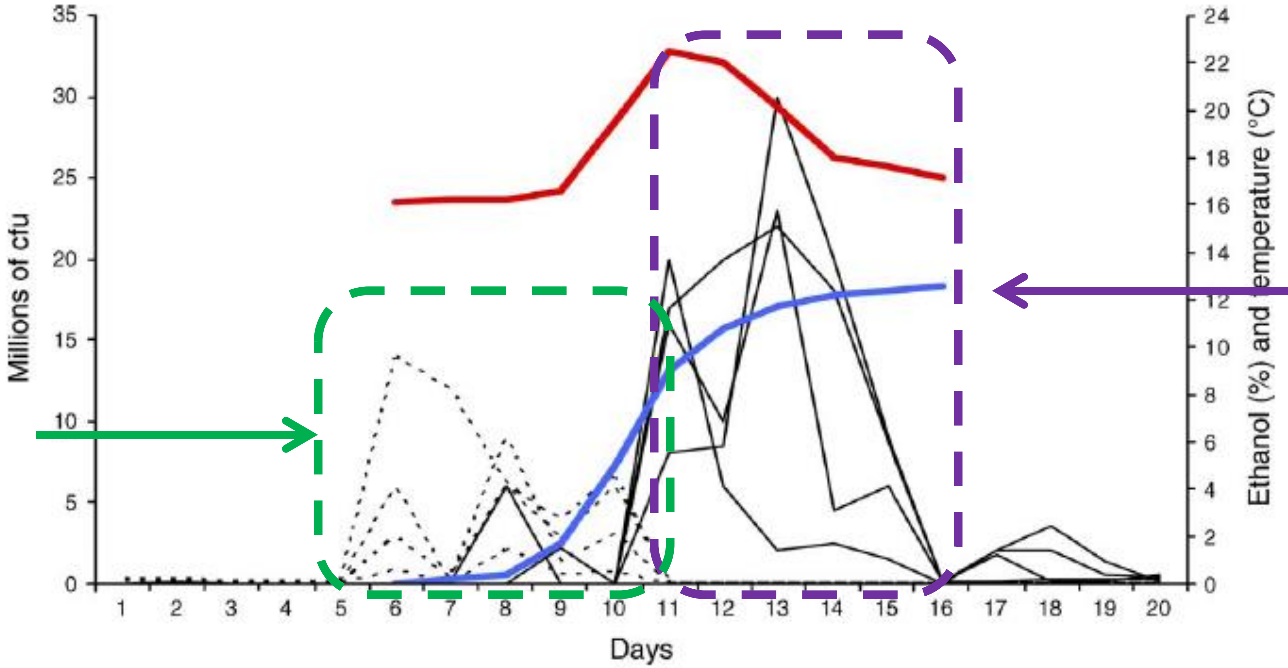
FIG. 1. The change in yeast community composition, temperature, and ethanol concentration during a traditional wine ferment. Shown is the change in population size (colony forming units, cfu) of the non-*Saccharomyces* yeasts (thin black dashed lines) and *S. cerevisiae* (thin black solid lines) in four separate barrels over 20 days of ferment. Also shown is the average change in temperature (heavy red line) and ethanol levels estimated from the change in specific gravity (heavy blue line) for these four barrels over days 6–16 of the ferment.

Source: Goddard MR. 2008. Ecology 89: 2077-2082

MICROBIAL ECOLOGY, POPULATION DYNAMICS

'WILD-FERMENT' CHARDONNAY, KUMEU RIVER WINERY - NZ

NSY proliferate at early stages



Saccharomyces takes over, after starting from a low conc.

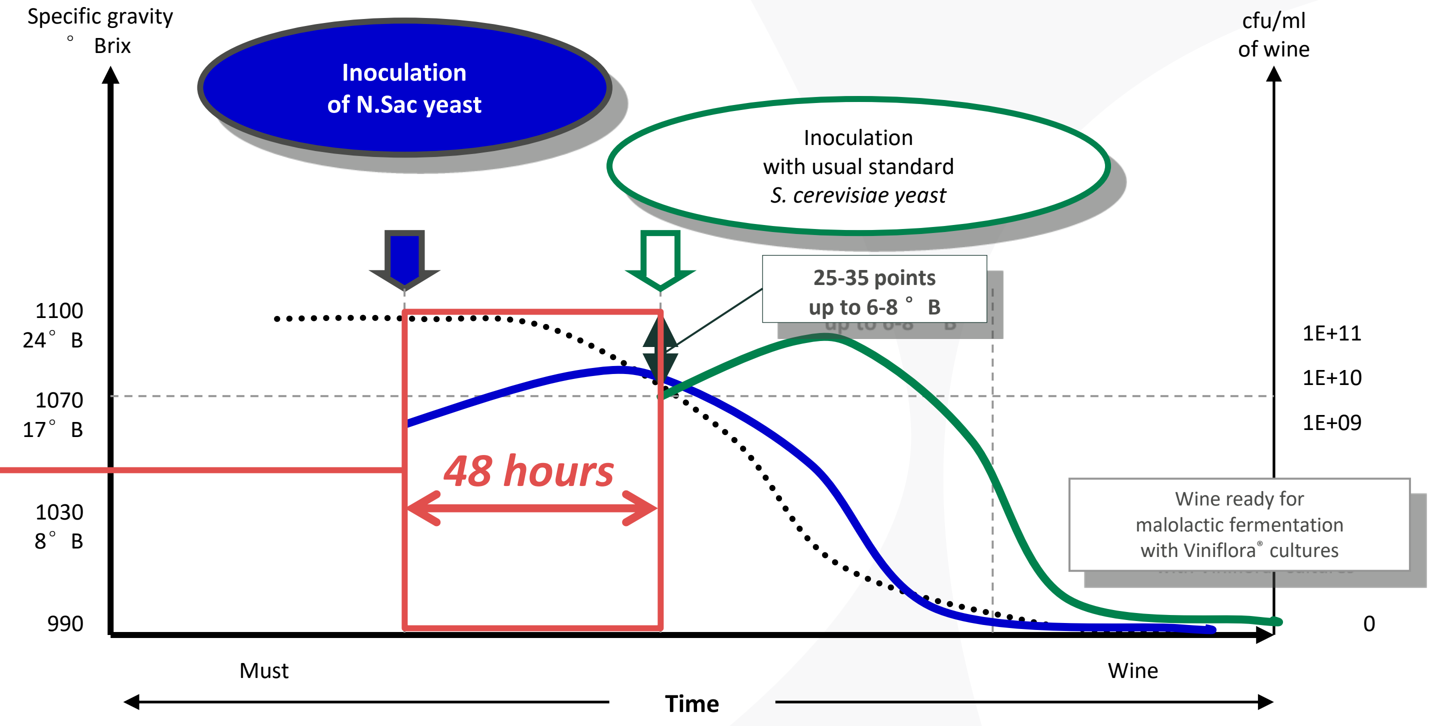
FIG. 1. The change in yeast community composition, temperature, and ethanol concentration during a traditional wine ferment. Shown is the change in population size (colony forming units, cfu) of the non-*Saccharomyces* yeasts (thin black dashed lines) and *S. cerevisiae* (thin black solid lines) in four separate barrels over 20 days of ferment. Also shown is the average change in temperature (heavy red line) and ethanol levels estimated from the change in specific gravity (heavy blue line) for these four barrels over days 6–16 of the ferment.

Source: Goddard MR. 2008. Ecology 89: 2077-2082

HOW TO USE NON-SACCHAROMYCES YEASTS - TIMING

- Sequential inoculation is recommended to get the most from a Non-Saccharomyces yeat
- Standard dosage for NSY is one 500g pack per 530-660 gal (or 200-250ppm)
- Inoculate the Non-Sacc when you would normally add yeast, followed by Saccharomyces after 48 hours:

This 48hr window allows the N.Sac to dominate before Saccharomyces takes control



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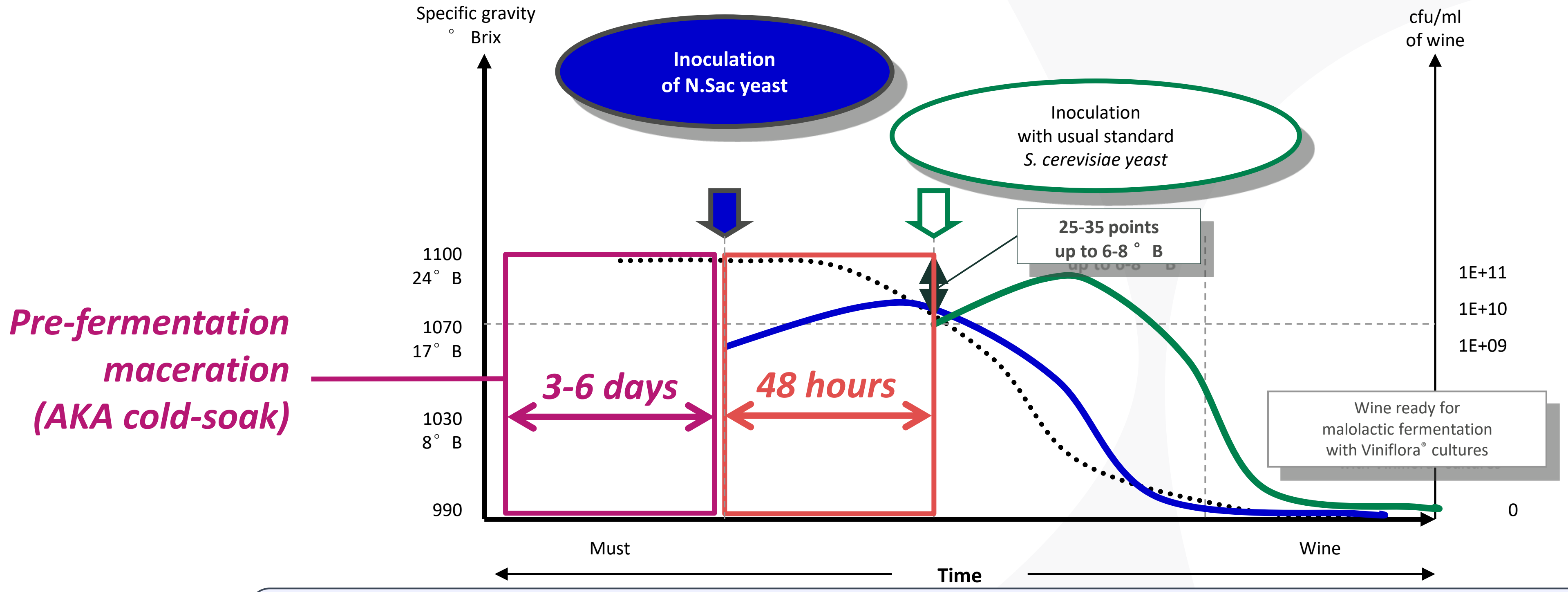


For red wines undergoing pre-fermentation maceration (cold-soak), NSY can be added at the start of this process, with Saccharomyces added once must is warmed

HOW TO USE NON-SACCHAROMYCES YEASTS - REHYDRATION

Attention to chlorine!

Attention to the T-SO_2!

Attention to the temperature!

Check out this clip on rehydration of Non-*Saccharomyces* yeasts: youtube.com/watch?v=wefPAyKbflE

HOW TO USE NON-SACCHAROMYCES YEASTS – N. MANAGEMENT

- The N demand across different NSY species and strains does vary considerably
- FrootZen can release Amino N into must relatively quickly, while others have similar demand to *S. cerevisiae* and will not release back into must[1]
- It is therefore important to make sure the *S. cerevisiae is well*

01

Measure the YAN in must before inoculation
Get a picture of the needs for each parcel before any yeast starts to deplete it

02

Inoculate with NSY
The NSY can use the native Nitrogen already present in the must

03

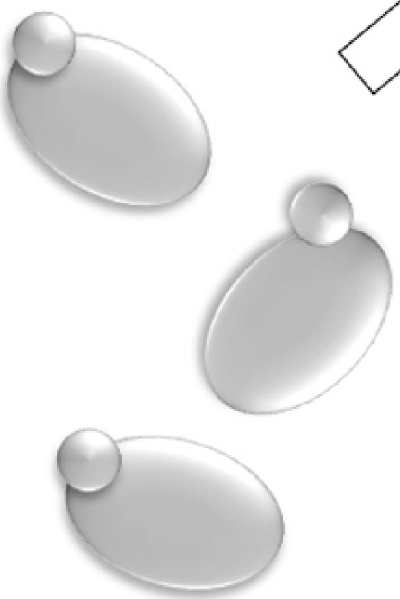
Once Saccharomyces is added 2-3 days later, start N addition
The Saccharomyces needs to go the distance to it is crucial that is well supplied with the Nitrogen it needs

1. Kelly J. Prior, Florian F. Bauer, Benoit Divol, The utilisation of nitrogenous compounds by commercial non-Saccharomyces yeasts associated with wine, Food Microbiology, Volume 79, 2019, Pages 75-84

Advantages of *Non-Saccharomyces* yeasts: biological-protection, flavor & texture, acidity

How does biological-protection work?

Yeasts



Passive strategies: competition for

- Nutrient
  - Carbohydrates
  - Nitrogen
  - Sterols
  - Vitamins
  - Micronutrients
- Oxygen

Space Multicellular aggregation

Lactic Acid bacteria

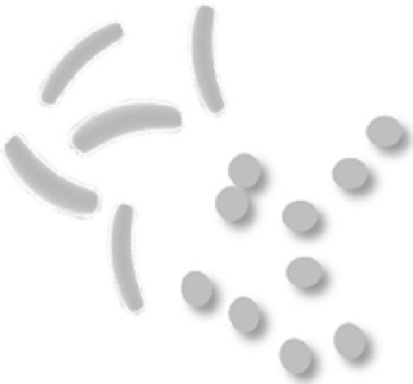


Table taken from: Di Gianvito *et al.* Bioprotection strategies in winemaking. International Journal of Food Microbiology, Volume 364, 2022

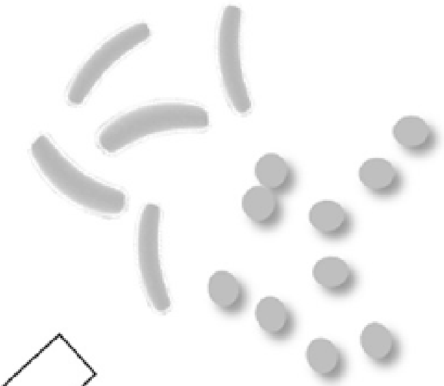
How does biological-protection work?

Yeasts

Passive strategies: competition for

- Nutrient
  - Carbohydrates
  - Nitrogen
  - Sterols
  - Vitamins
  - Micronutrients
- Oxygen
- Space Multicellular aggregation

Lactic Acid bacteria



Active competition strategies

- Production of antimicrobial compounds
- Cell-to-cell contact

For more on this topic check out:
Hu *et al.* The effects of cell-cell contact between *Pichia kluyveri* and *Saccharomyces cerevisiae* on amino acids and volatiles in mixed culture alcoholic fermentations.
Food Microbiology Vol 103, 2022

Table taken from: Di Gianvito *et al.* Bioprotection strategies in winemaking. International Journal of Food Microbiology, Volume 364, 2022

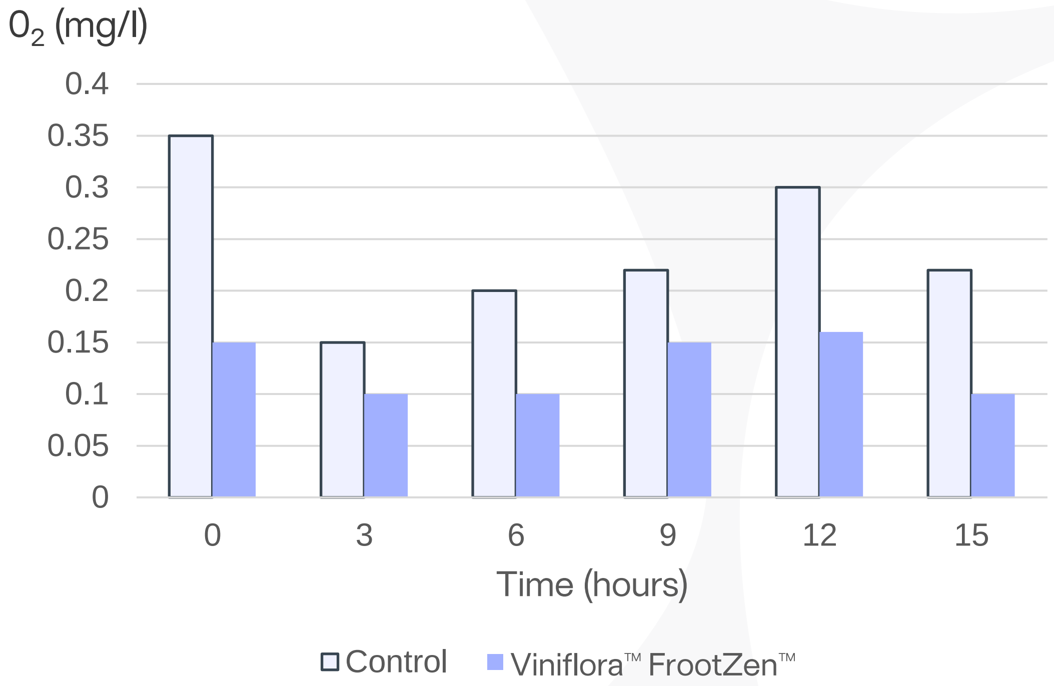
Biological-protection example – protection against oxidation

COLOR AT END OF ALCOHOLIC FERMENTATION

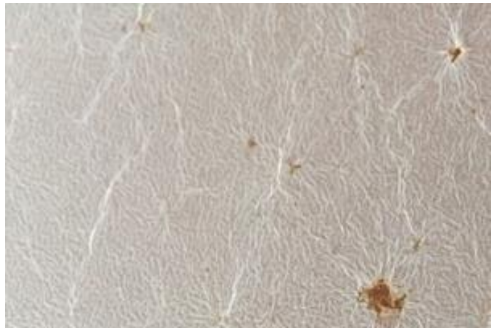
Control[1]

Viniflora™ FrootZen™

DISOLVED OXYGEN IN SAUVIGNON MUST AT COLD-SETTLING[2]



Viniflora™ FrootZen™ generates a biofilm at the surface of the tank that works as a barrier to oxygen. The layer will dissolve later during the fermentation process.



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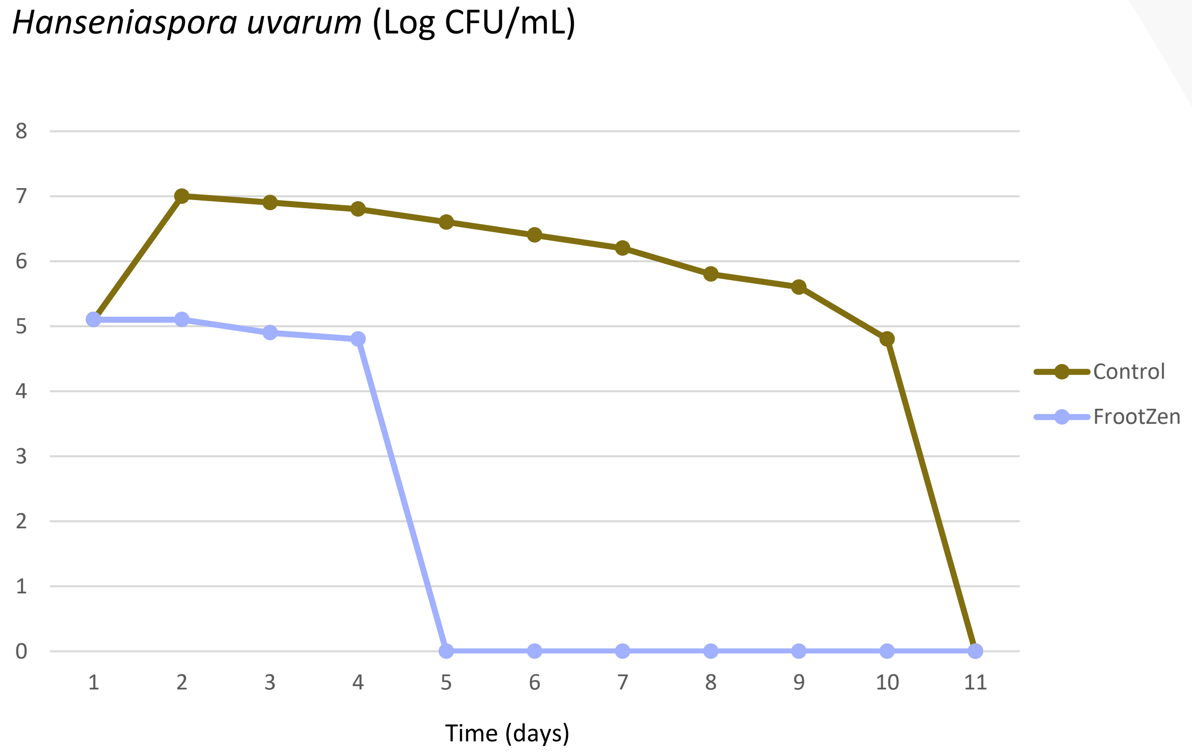
1. Another bioprotective yeast was used 2 . Inoculation at crusher

Biological-protection example – protection against *Hanseniaspora uvarum*

REDUCTION OF APICULATED YEAST IN CABERNET SAUVIGNON 25°C



No growth of *H.uvarum* in presence of Viniflora™ FrootZen™ and fast drop down of the contaminant after 4 days.

With FrootZen™ at day 4

The white colonies are FrootZen™ yeast and *S. cerevisiae*. There is no growth of unwanted *H. uvarum.*

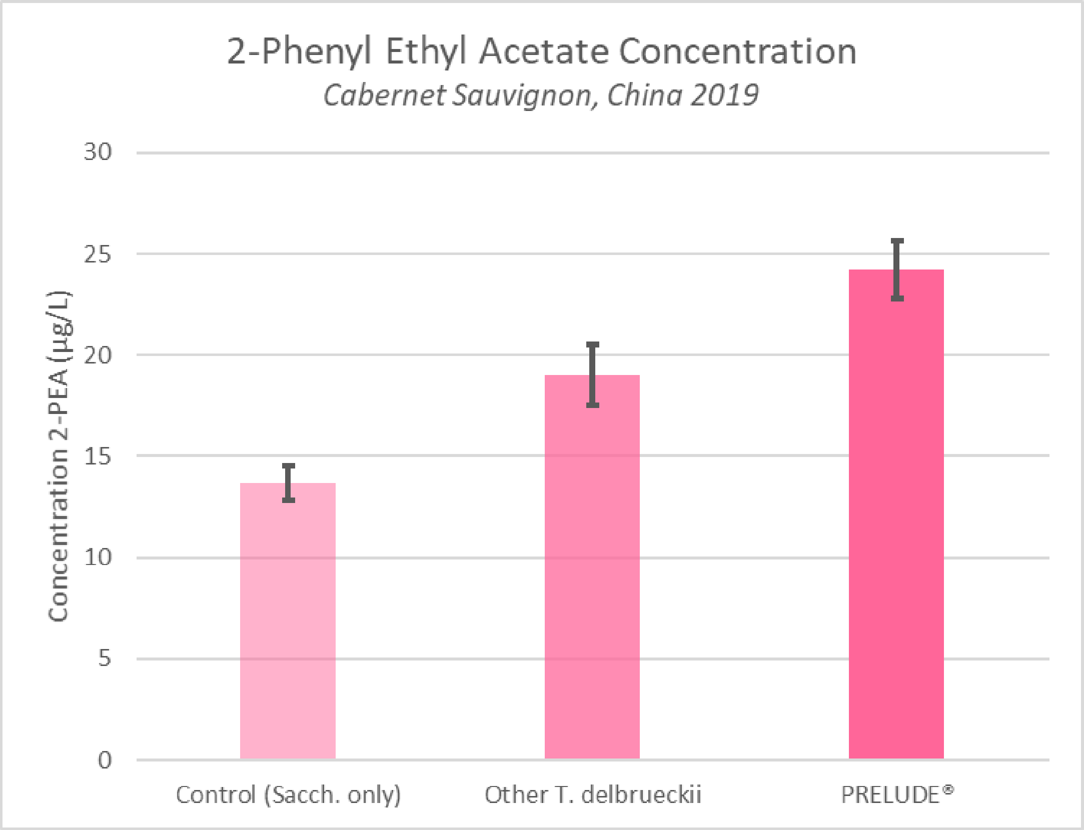
Control at day 4

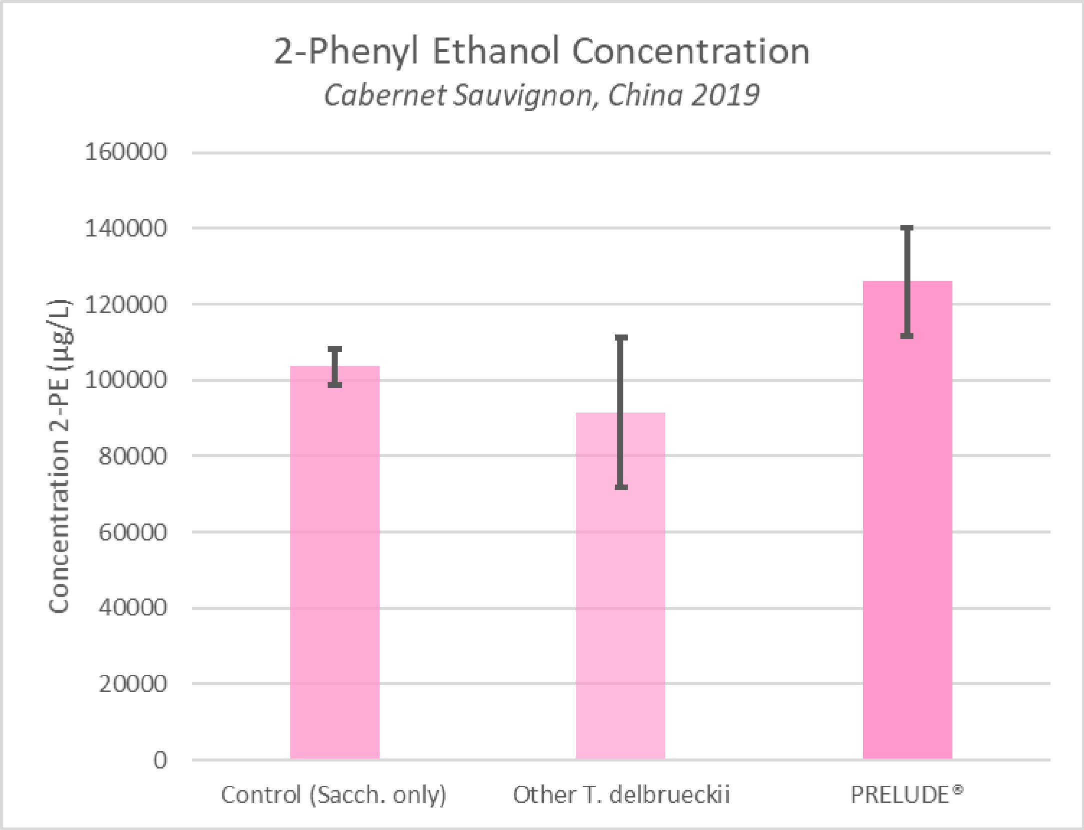
The dark colonies are unwanted *H. uvarum.*

The white colonies are *S. cerevisiae.*

Impact of flavor and texture – example of 2-PE and 2-PEA from PRELUDE

CABERNET SAUVIGNON, XINJIANG PROVINCE, CHINA 2019





Taken from : Boqin Zhang, Violeta Ivanova-Petropulos, Changqing Duan, Guoliang Yan. Distinctive chemical and aromatic composition of red wines produced by Saccharomyces cerevisiae co-fermentation with indigenous and commercial non-Saccharomyces strains. Food Bioscience, Volume 41, 2021.

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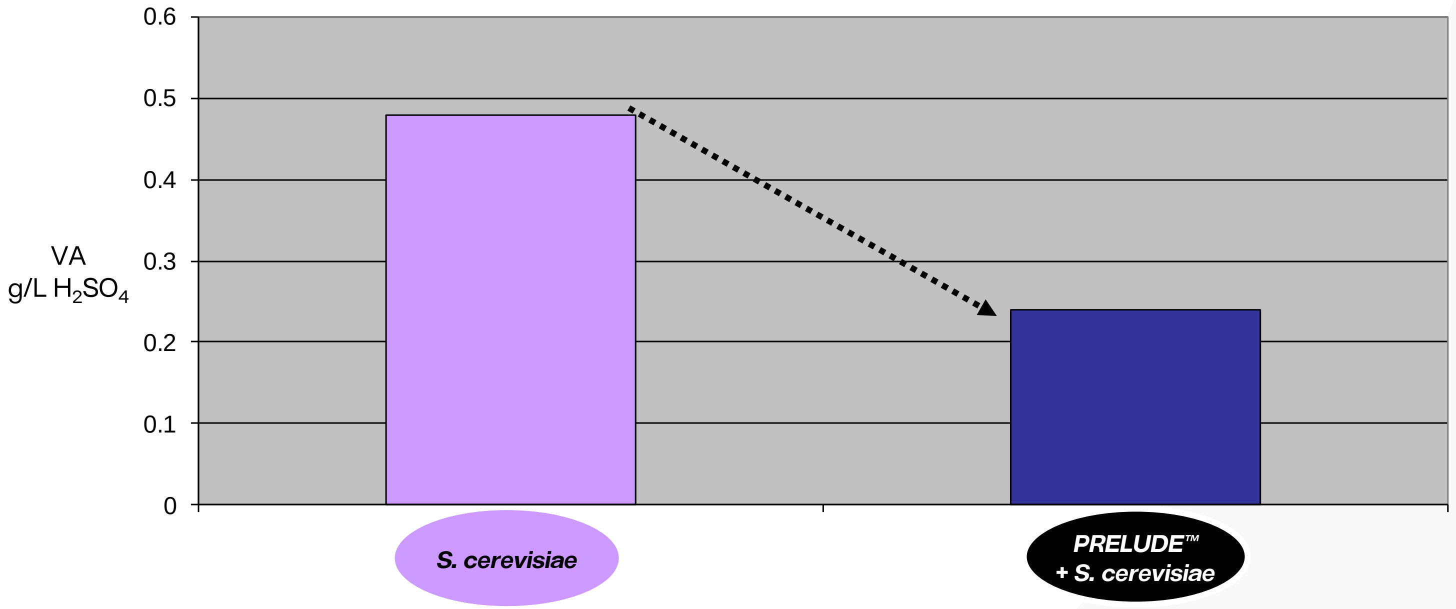
Impact of flavor and texture – example of less acetic acid with PRELUDE™

WINE MADE FROM HIGH-SUGAR SEMILLON MUST, FRANCE 2009



Torulaspora delbrueckii has been shown to produce less acetic acid under high osmotic stress when compared to *Saccharomyces cerevisiae*[1]

Bely, Marina, et al. "Impact of mixed Torulaspora delbrueckii–Saccharomyces cerevisiae culture on high-sugar fermentation." *International journal of food microbiology* 122.3 (2008): 312-320.

Impact of flavor and texture – example polysaccharide production

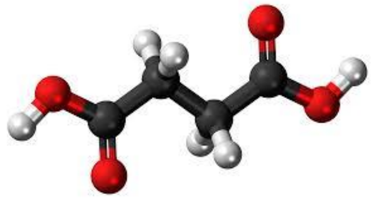
Torulaspora delbrueckii **shows highest concentration of polysaccharide**

Inoculum, (cells ml^{-1})	Ethanol (% v/v)	pH	Total acidity ($g\ l^{-1}$)	Volatile acidity ($g\ l^{-1}$)	Glycerol ($g\ l^{-1}$)	Δ Polysaccharides ($mg\ l^{-1}$)
S. cerevisiae 10^7	13.93 ± 0.06[a]	3.20 ± 0.04[a]	7.05 ± 0.04[a]	0.46 ± 0.01[a]	6.23 ± 0.54[a]	97 ± 10[a]
S. cerevisiae 10^5	13.87 ± 0.00[a]	3.16 ± 0.05[a]	7.12 ± 0.11[a]	0.47 ± 0.01[a]	6.65 ± 0.05[a]	98 ± 11[a]
S. cerevisiae 10^3	13.88 ± 0.03[a]	3.17 ± 0.21[a]	7.02 ± 0.05[a]	0.50 ± 0.06[a]	6.46 ± 0.52[a]	68 ± 3.0[a]
C. zemplinina + *S. cerevisiae* 10^7	13.83 ± 0.04[a]	3.21 ± 0.01[a]	6.88 ± 0.27[a]	0.43 ± 0.04[a]	6.25 ± 0.30[a]	123 ± 42[a]
C. zemplinina + *S. cerevisiae* 10^5	13.78 ± 0.05[a]	3.15 ± 0.05[a]	6.84 ± 0.02[a]	0.44 ± 0.06[a]	7.18 ± 1.30[b]	140 ± 42[a]
C. zemplinina + *S. cerevisiae* 10^3	13.64 ± 0.04[b]	3.08 ± 0.18[b]	6.88 ± 0.04[a]	0.52 ± 0.01[a]	7.95 ± 1.28[b]	181 ± 48[a]
L. thermotolerans + *S. cerevisiae* 10^7	13.80 ± 0.02[a]	3.16 ± 0.01[a]	7.30 ± 0.07[a]	0.38 ± 0.01[b]	6.95 ± 0.20[b]	133 ± 1.0[a]
L. thermotolerans + *S. cerevisiae* 10^5	13.80 ± 0.01[a]	2.97 ± 0.03[b]	9.00 ± 1.96[b]	0.40 ± 0.00[a,b]	7.29 ± 0.96[b]	139 ± 10[a]
L. thermotolerans + *S. cerevisiae* 10^3	13.70 ± 0.18[a]	2.90 ± 0.01[b]	9.20 ± 1.93[b]	0.40 ± 0.00[a,b]	7.58 ± 0.46[b]	158 ± 3.0[a]
T. delbrueckii + *S. cerevisiae* 10^7	13.90 ± 0.04[a]	3.19 ± 0.01[a]	7.12 ± 0.02[a]	0.38 ± 0.01[b]	5.88 ± 0.04[a]	157 ± 16[a]
T. delbrueckii + *S. cerevisiae* 10^5	13.85 ± 0.08[a]	3.10 ± 0.08[a,b]	7.36 ± 0.51[a]	0.40 ± 0.04[a,b]	6.14 ± 0.22[a]	269 ± 44[b]
T. delbrueckii + *S. cerevisiae* 10^3	13.76 ± 0.04	3.08 ± 0.11[a,b]	7.34 ± 0.49[a]	0.41 ± 0.01[a,b]	6.29 ± 0.61[a]	308 ± 42[b]
M. pulcherrima + *S. cerevisiae* 10^7	13.87 ± 0.01[a]	3.40 ± 0.08[c]	6.33 ± 0.27[a]	0.30 ± 0.04[b]	6.53 ± 0.27[a]	120 ± 10[a]
M. pulcherrima + *S. cerevisiae* 10^5	13.79 ± 0.13[a]	3.39 ± 0.14[c]	6.50 ± 0.16[a]	0.34 ± 0.07[b]	6.98 ± 0.00[b]	126 ± 10[a]
M. pulcherrima + *S. cerevisiae* 10^3	13.65 ± 0.19[b]	3.40 ± 0.00[c]	6.64 ± 0.37[a]	0.33 ± 0.01[b]	7.25 ± 0.25[b]	154 ± 17[a]

Data are means ± standard deviations of two independent experiments. Values displaying different superscript letters ([a, b, c]) within each column are significantly different according to the Duncan test (0.05%).

Taken from: Francesca Comitini, Mirko Gobbi, Paola Domizio, Cristina Romani, Livio Lencioni, Ilaria Mannazzu, Maurizio Ciani, Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with Saccharomyces cerevisiae, Food Microbiology, Volume 28, Issue 5, 2011, Pages 873-882

Increase acidity with natural lactic acid



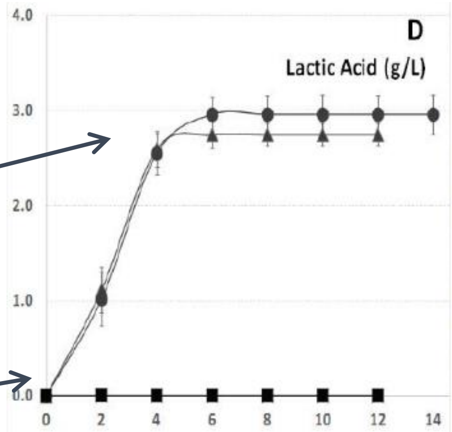
While *S. cerevisiae* predominantly converts sugar to ethanol, *Lachancea thermotolerans* can divert some sugar into lactic acid

Relevant for hot climates / low-acid musts/juices

Also relevant for EtOH reduction

Compounds	SC	SC + MLF	KT···SC	KT···SC + MLF
L-Lactic Acid (g/L)	0.01 ± 0.01a	0.54 ± 0.08b	2.75 ± 0.12c	3.27 ± 0.19d
L-Malic Acid (g/L)	0.92 ± 0.02b	0.01 ± 0.01a	0.89 ± 0.04b	0.01 ± 0.01a
Acetic Acid (g/L)	0.36 ± 0.01b	0.44 ± 0.05c	0.32 ± 0.02a	0.39 ± 0.04bc
Residual Sugar (g/L)	2.08 ± 0.30b	0.12 ± 0.04a	2.22 ± 052b	0.16 ± 0.04a
Glycerol (g/L)	5.96 ± 0.02a	5.89 ± 0.05a	6.48 ± 0.05b	6.36 ± 0.06b
Free SO_2 (mg/L)		26.12 ± 2.38a		25.25 ± 3.43ab
Total SO_2 (mg/L)		56.52 ± 2.43b		44.13 ± 3.16a
Alcohol (% v/v)	14.56 ± 0.01c	14.54 ± 0.02c	14.20 ± 0.04b	14.18 ± 0.06b
pH	3.94 ± 0.01c	3.99 ± 0.02d	3.74 ± 0.02a	3.79 ± 0.02b
Urea	1.43 ± 0.01b		1.45 ± 0.02b	
Color Intensity	6.16 ± 0.03b	5.38 ± 0.06a	6.29 ± 0.06c	5.51 ± 0.07a
Citric Acid (g/L)	0.22 ± 0.01a	0.03 ± 0.02b	0.24 ± 0.03a	0.04 ± 0.03b

Lachancea thermotolerans followed by *Saccharomyces cerevisiae* (no MLF)



Saccharomyces cerevisiae only (no MLF)

Taken from: Benito, A. et al. Combine Use of Selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* Yeast Strains....in Red Wine Production. Molecules (20) 2015

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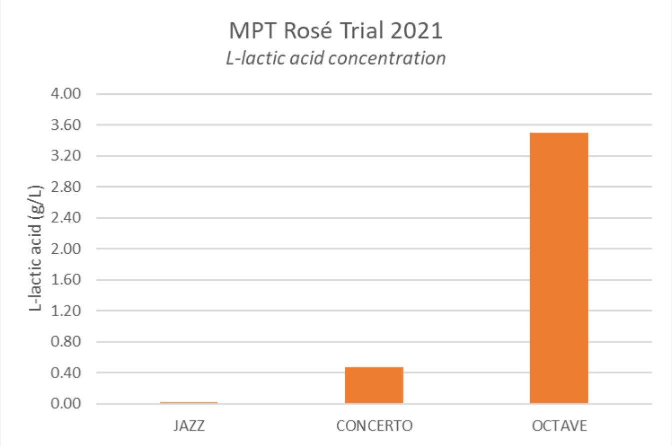
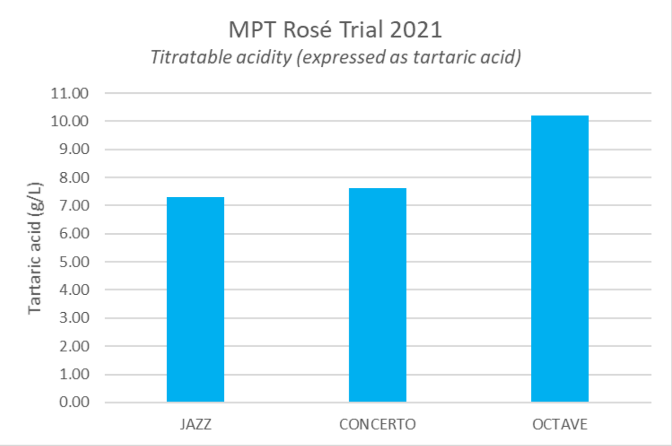
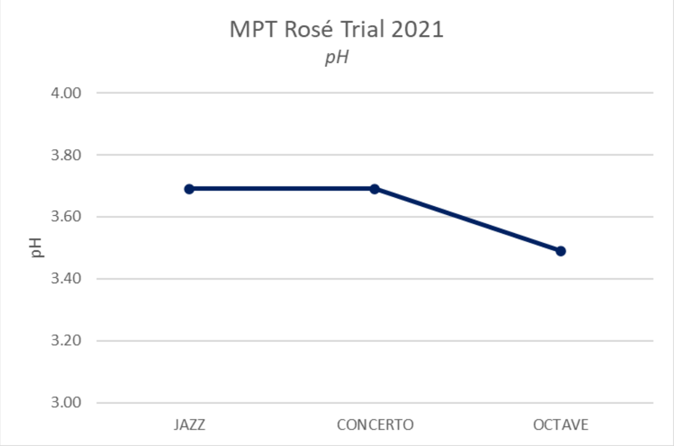
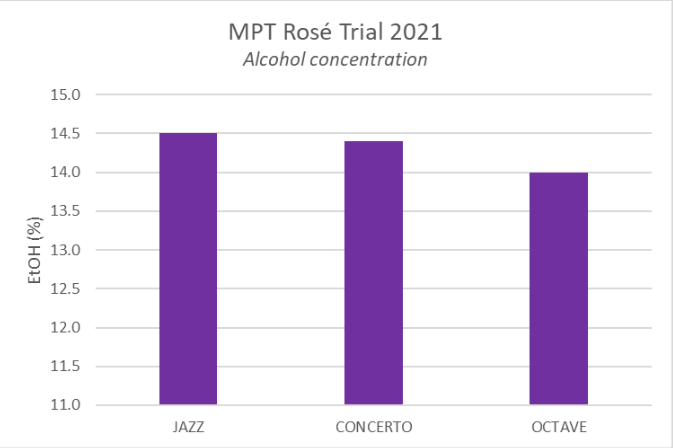
Lachancea thermotolerans comparison

CONCERTO AND OCTAVE IN ROSÉ FROM SYRAH, AUSTRALIA 2021









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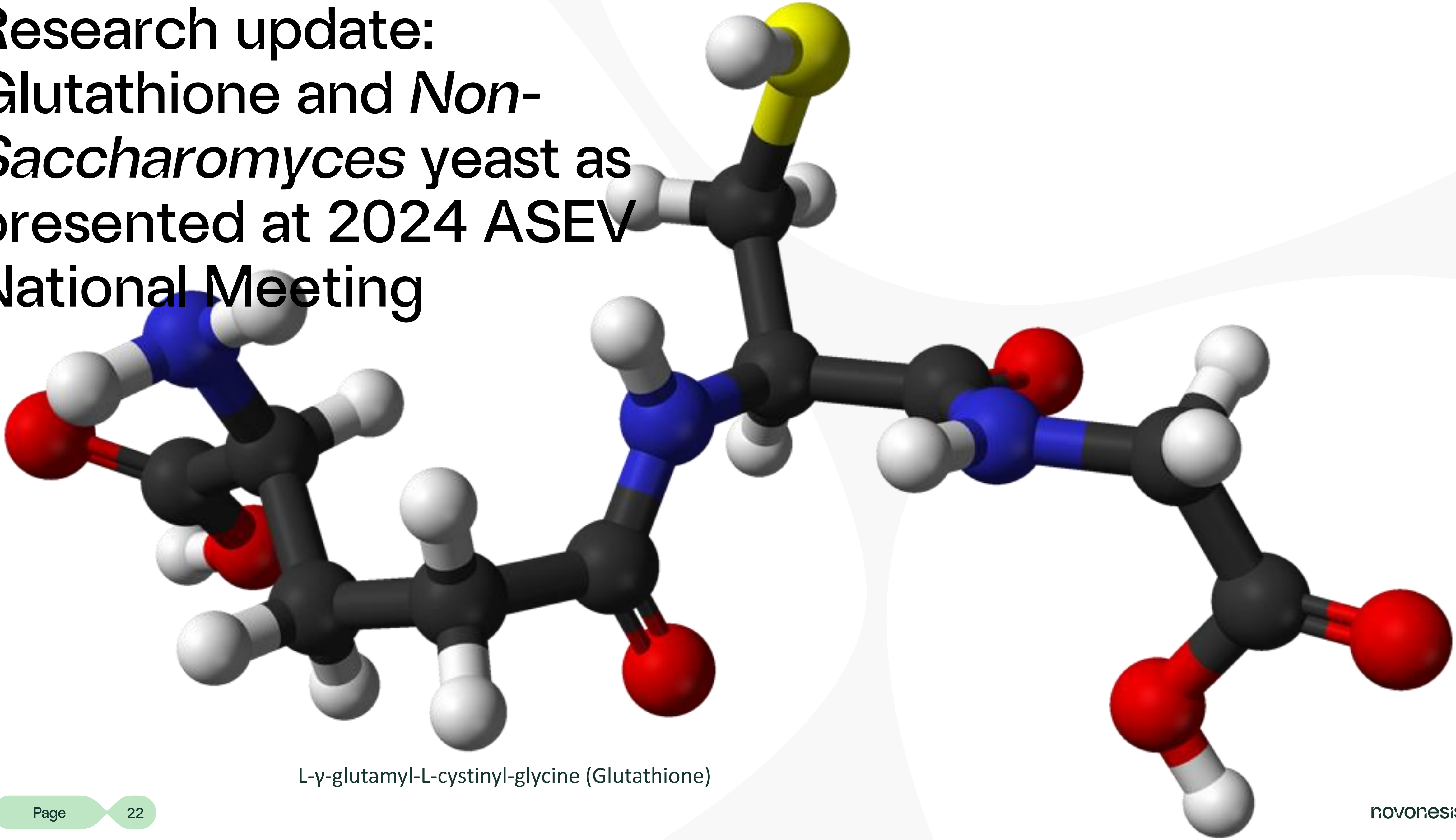
Research update: Glutathione and *Non-Saccharomyces* yeast as presented at 2024 ASEV National Meeting



L-γ-glutamyl-L-cystinyl-glycine (Glutathione)

Background

– L-γ-glutamyl-L-cystinyl-glycine (aka Glutathione or GSH for short) has become recognised as an important anti-oxidant in wine

– NSY have often been seen as producers of GSH, but this has never been quantified in literature for commercial culture[1]

– We therefore set out to investigate if commercial strains could be useful as GSH-producers

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.

Experimental design

01. Must preparation

Two musts were selected for the experiment to represent red and white/rosé conditions:

Chardonnay, adjusted with NaOH to pH 3.60, to be fermented at 25 °C. (YAN = 400mg/L)

Pinot Noir, adjusted with tartaric acid to pH 3.10, to be fermented at 15°C.
(YAN = 169mg/L)

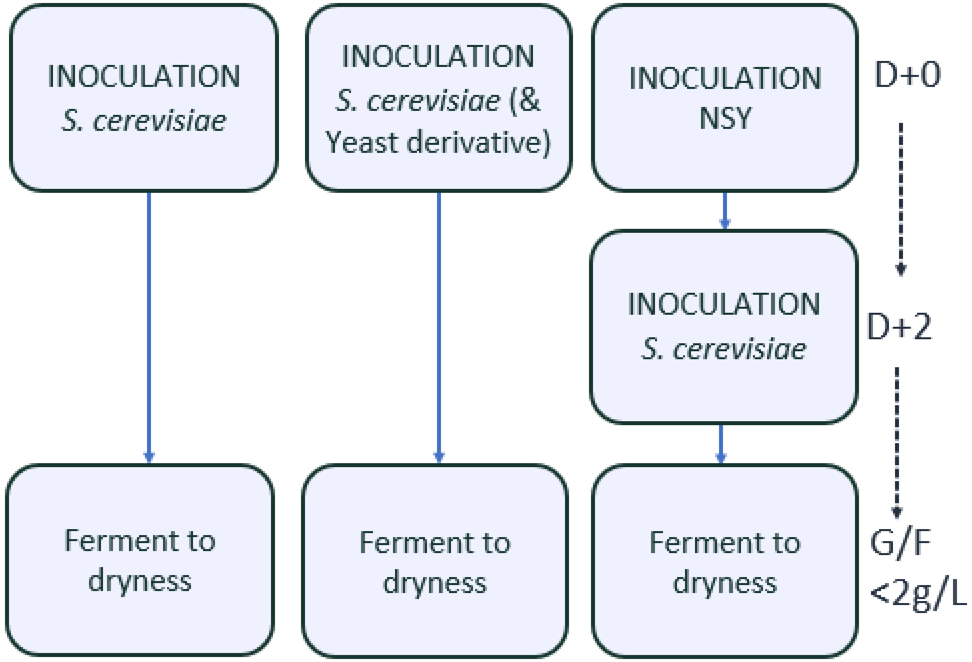
02. Fermentation

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.



The resultant wines were sampled prior to inoculation, at 48 hours and at sugar dryness for GSH (without SO_2). Enzymatic kit MAK440 was used for the analysis. (Merck, Germany).

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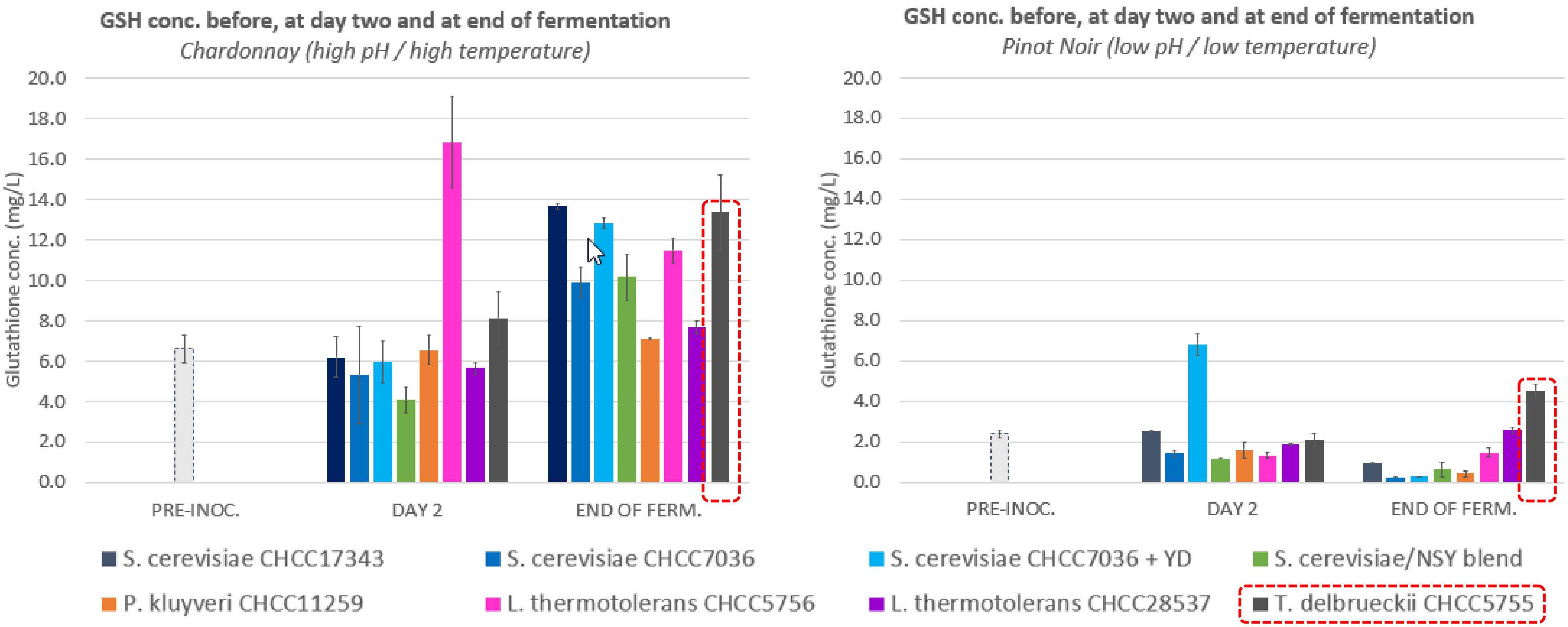
Results



GSH conc. before, at day two and at end of fermentation
Chardonnay (high pH / high temperature)
Glutathione conc. (mg/L)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
PRE-INOC.
DAY 2
END OF FERM.
GSH conc. before, at day two and at end of fermentation
Pinot Noir (low pH / low temperature)
Glutathione conc. (mg/L)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
PRE-INOC.
DAY 2
END OF FERM.
S. cerevisiae CHCC17343
S. cerevisiae CHCC7036
S. cerevisiae CHCC7036 + YD
S. cerevisiae/NSY blend
P. kluyveri CHCC11259
L. thermotolerans CHCC5756
L. thermotolerans CHCC28537
T. delbrueckii CHCC5755

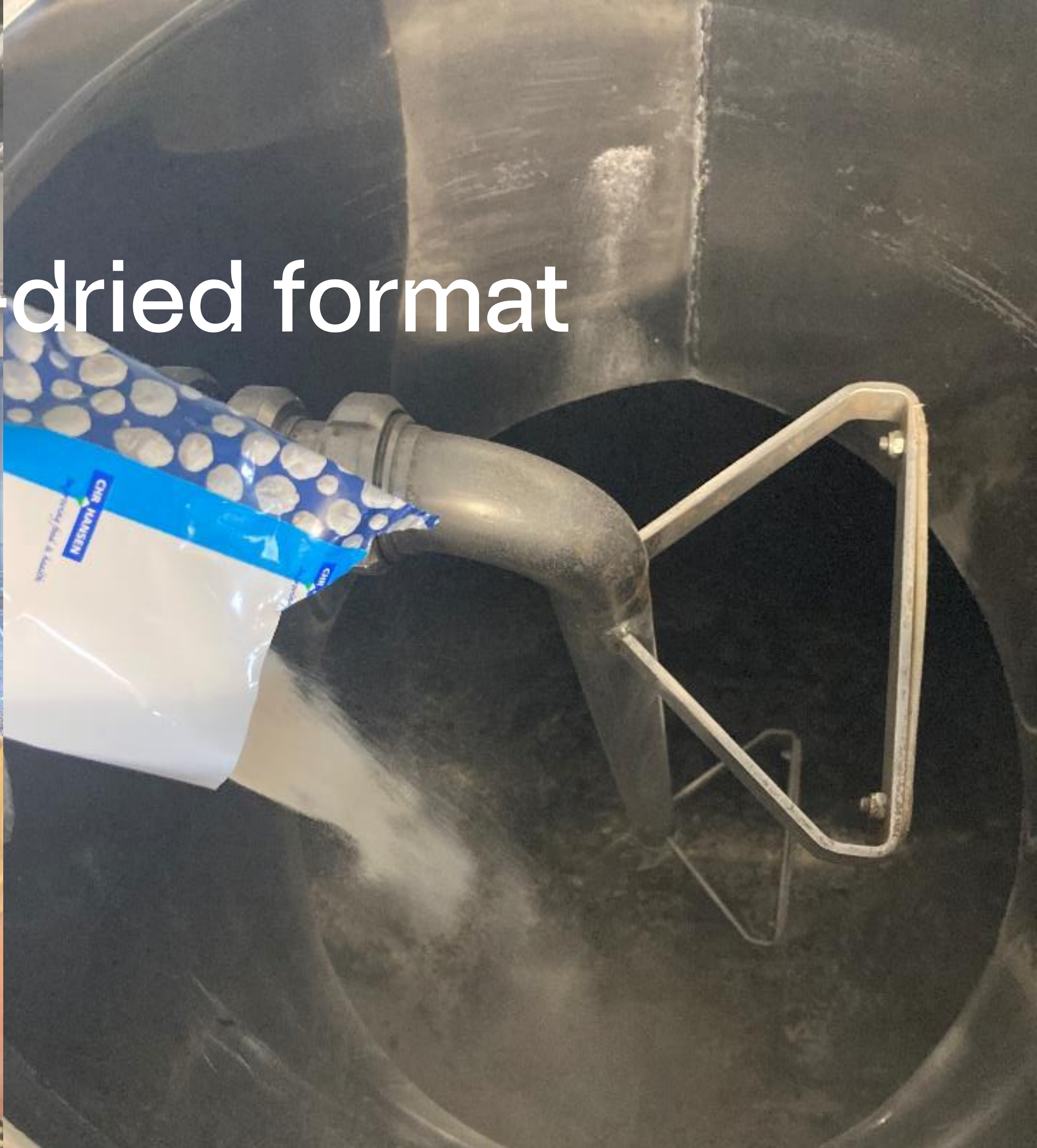
Conclusion

- Viniflora™ PRELUDE™ (*Torulaspora delbrueckii* CHCC5755) was the most effective at increasing the GSH concentration across both wines, out-performing 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 $GSSO_3H$, in addition to GSH itself, could also be beneficial.

Product Update:
FrootZen™ in freeze-dried format



Frozen vs freeze-dried

FROZEN LIQUID YEAST

A unique format in the market since 12 years

Yeast cream is deep-frozen just after production.

- Can be used as frozen block or thawed in fridge or water-bath.
- To ship with dry-ice
- To store at -45°C
- Customer can store it at -18°C (<2 month at -18°C)
- Shelf life : 2 years

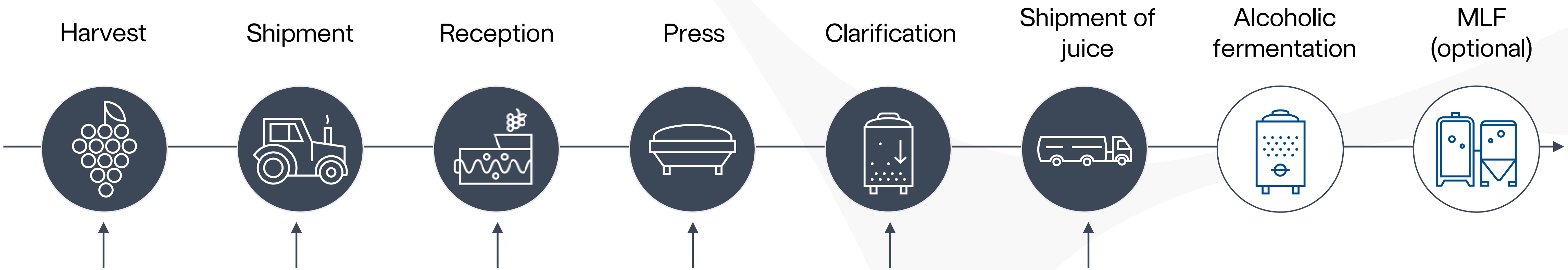
FREEZE-DRIED YEAST

A new development by Novonesis scientists

Yeast cells are gently dried under vacuum.

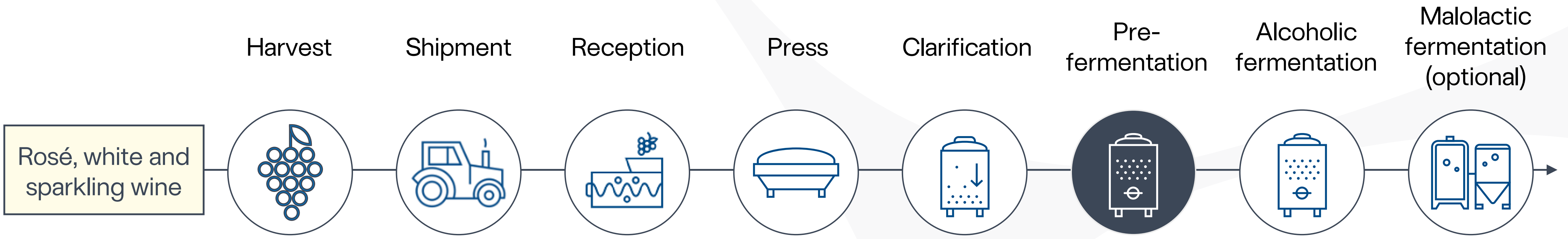
- Can easily be rehydrated in water or must.
- Can be shipped at ambient temperature (except in hot season if shipment exceed 2 days).
- Store at -18°C
- Shelf life: 1 year

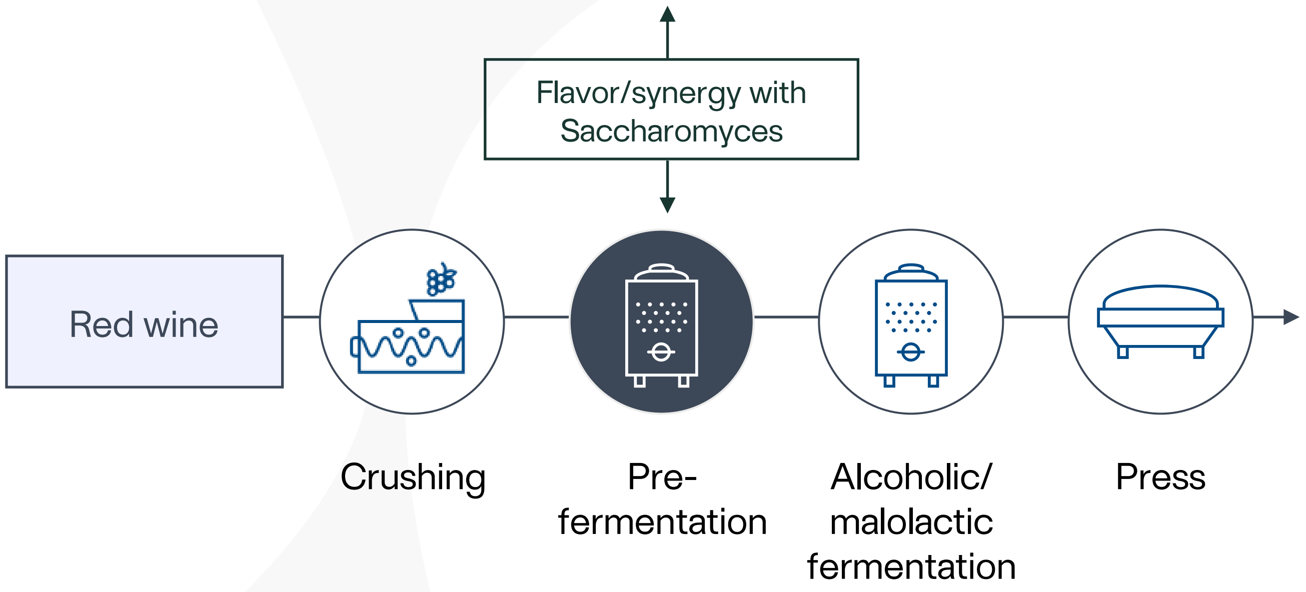
How to use FrootZen™ for bio-protection



GRAPE BIO-PROTECTION	MUST BIO-PROTECTION
• 1 pouch of 375g/ 3-5 tons • Sprinkle the content of the pouches on top of the grapes at the start of truck filling/harvesting machine or at the crusher • Reduce sulfites: From 4 - 6 g/hl to 0 – 2.5 g/hl • Wait for 2-3 days before an inoculation of Saccharomyces	• 1 pouch of 375g/ 2500 L • Sprinkle the content of the pouches in the settling tank or dilute in must. Mix thoroughly. • Reduce sulfites: From 4 - 6 g/hl to 0 – 2.5 g/hl • Inoculate 1-2 days before an inoculation of Saccharomyces • For an optimal effect, prefer a clarification by means of cold settling versus flotation

How to use Viniflora™ FrootZen™ to increase the flavor or to boost Saccharomyces





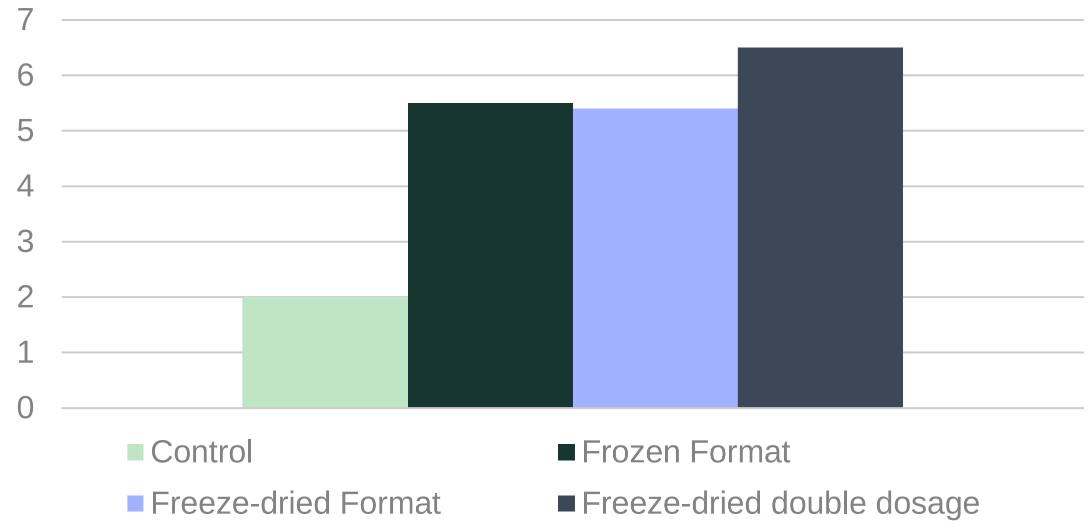
HOW TO USE

- Use 1 bag (375g) for 2500 L/ 660 gal
- Red wines: sprinkle the content of the pouches in the fermentation tank at the filling and achieve some pump over to homogenize or dilute in must for 20 minutes and pour in the tank.
- Rosés and white wines: inoculate in the clarified must.
- Wait for 1-2 days before an inoculation of Saccharomyces

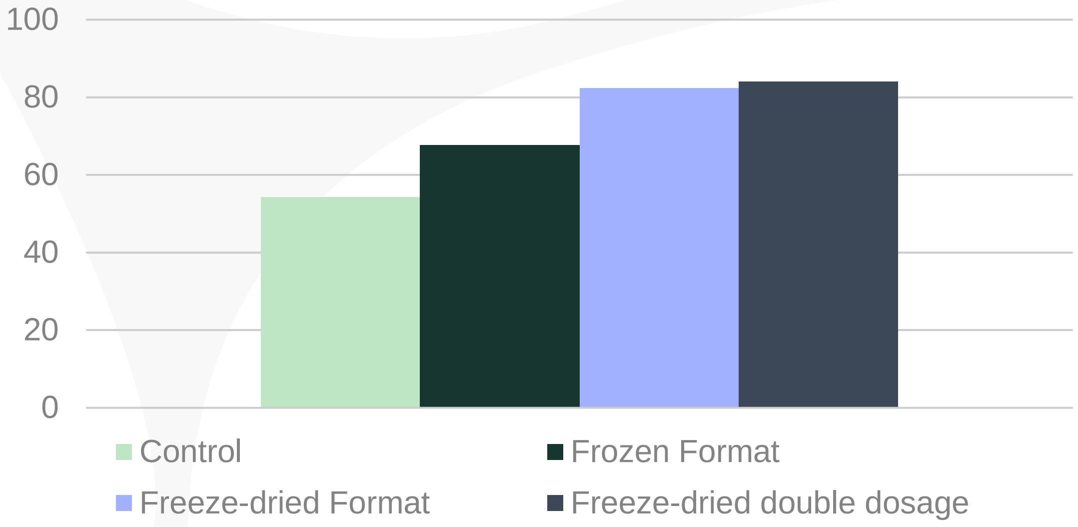
- For non-interventionists process, control the temperature and nutrition and leave the wine ferment to dryness with indigenous yeast

Frozen and freeze-dried formats of FrootZen™ show similar aroma performance

ISO-AMYL ACETATE
mg/l



3-MERCAPTO-HEXYL-ACETATE (3MHA)
µg/l



A sequential inoculation of FrootZen™ and Saccharomyces JAZZ (added after 3 days) in Chardonnay shows similar production of acetate esters and thiols with the two formats. The level of inoculation recommended is optimized. A double dosage does not significantly change the benefits of the strain.

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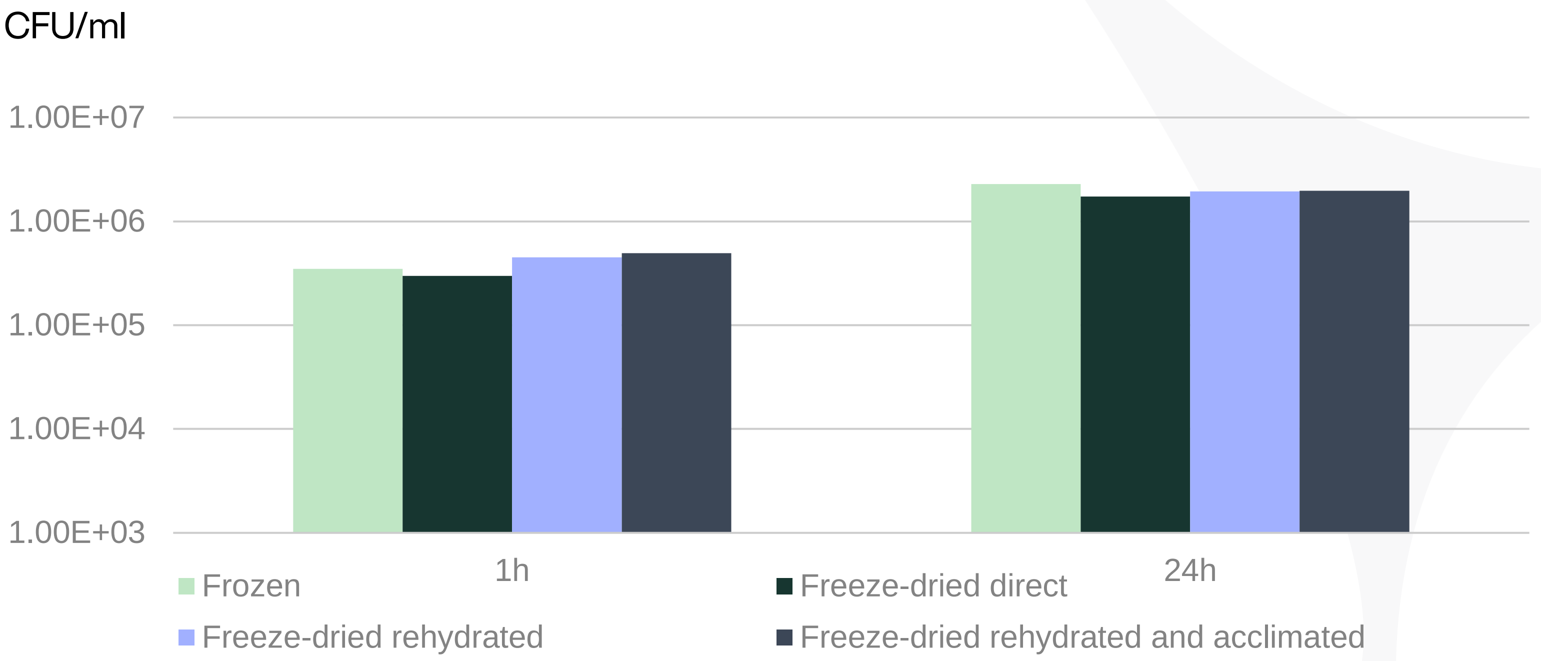
Viniflora™ FrootZen™ can be used by direct pitching or after rehydration

GROWTH ACCORDING TO VARIOUS MODUS OF PREPARATION



FrootZen™ showed similar growth within 24 hours in Chardonnay, whatever inoculation method was used, in anaerobic conditions.

Comment: The growth rate is higher when oxygen is added (can reach 1.0E+08 CFU/ml)

METHODS OF PREPARATION

- Frozen block thawed overnight at 4°C
- Freeze-dried direct: Powder sprinkled
- Freeze-dried rehydrated: 10 min in 25°C unchlorinated water
- 10 min in 25°C unchlorinated water + activated for 20 min by adding Chardonnay 3:1

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Pichia kluyveri vs. *Metschnikowia pulcherrima*

	***Pichia kluyveri* (FrootZen™)**	***Metschnikowia pulcherrima***
Format	Freeze-dried or Frozen	Active-dried yeast
Rehydration required	No	Yes
Pellicle formation	Yes	No
O_2 uptake	Rapid	Medium
Fruit impact	High	Medium
T-SO_2 tolerance	45mg/l	40mg/l
Nitrogen demand	Low	Low
Symbiosis with S. cerevisiae and O. oeni	High	Medium
BioP capability (oxidation)	High	Medium
BioP capability (microbial spoilage)	High	High
Cold tolerant?	Yes	Yes
Suitability for ‘Go Natural’	High	Medium

Any questions?

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Thank you!