The use of Non-Saccharomyces yeasts in winemaking

5 April 2022

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AGENDA

1. What is the yeast landscape beyond Saccharomyces cerevisiae?
2. How Non-Saccharomyces have a positive organoleptic impact
3. How Non-Saccharomyces can be a crucial tool against spoilage
4. How when and why to apply Non-Saccharomyces yeast

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VINIFLORA® YEAST RANGE FOR 2022

	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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What is the yeast landscape beyond Saccharomyces?

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YEAST ECOLOGY IN WINE

› The ecology of micro-organisms during winemaking is very complex. The following genera of yeast can be found:

- *Brettanomyces / Dekkera*
- *Candida*
- *Cryptococcus*
- *Debaromyces*
- *Hanseniaspora / Kloeckera*
- *Hansenula*
- *Lachancea*
- *Torulaspora*
- *Saccharomycodes*
- *Schizosaccharomyces*
- *Zygosaccharomyces*
- *Metschnikowia*
- *Pichia*
- *Rhodotorula*
- ***Saccharomyces***

Alcoholic fermentation is always completed by Saccharomyces (generally *Saccharomyces cerevisiae*)

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MICROBIAL ECOLOGY, POPULATION DYNAMICS

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

Temperature

Ethanol conc. (%)

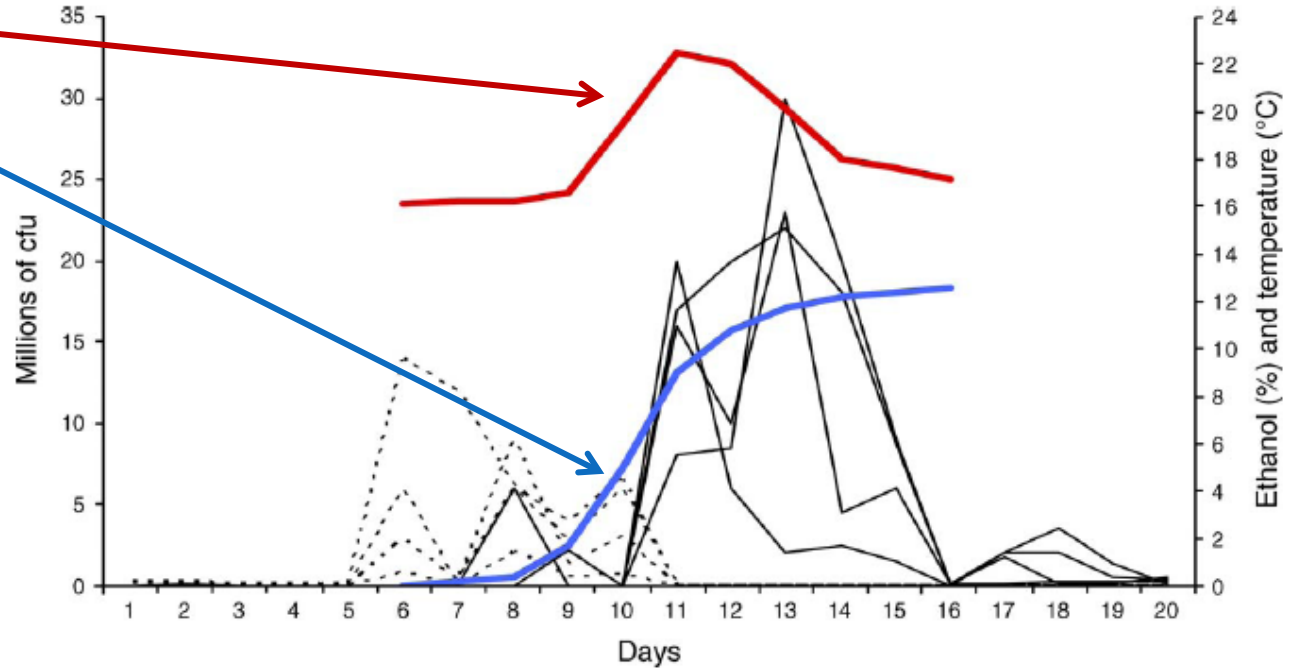


FIG. 1. The change in yeast community composition, temperature, and ethanol concentration during a traditional wine ferment. Shown is the change in population size (colony forming units, cfu) of the non-*Saccharomyces* yeasts (thin black dashed lines) and *S. cerevisiae* (thin black solid lines) in four 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.

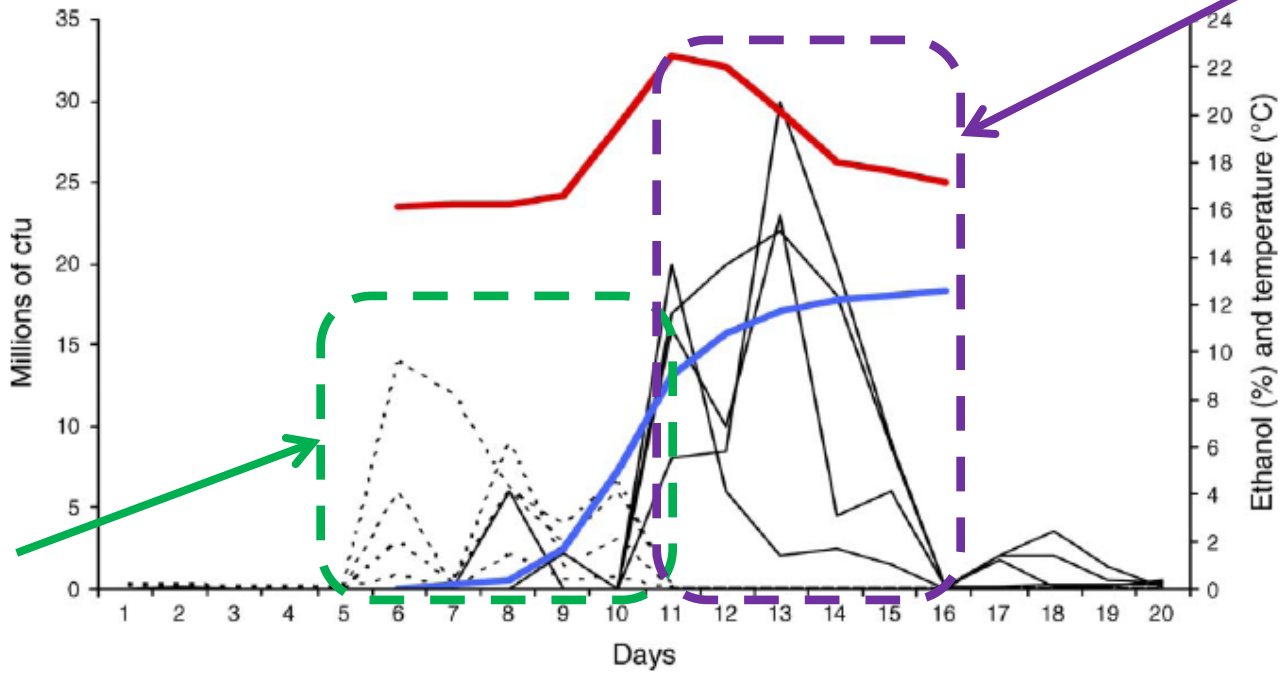
Source: Goddard MR. 2008. Ecology 89: 2077-2082

MICROBIAL ECOLOGY, POPULATION DYNAMICS

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

Saccharomyces takes over, after starting from a low conc.

Non-Sacc's proliferate at early stages



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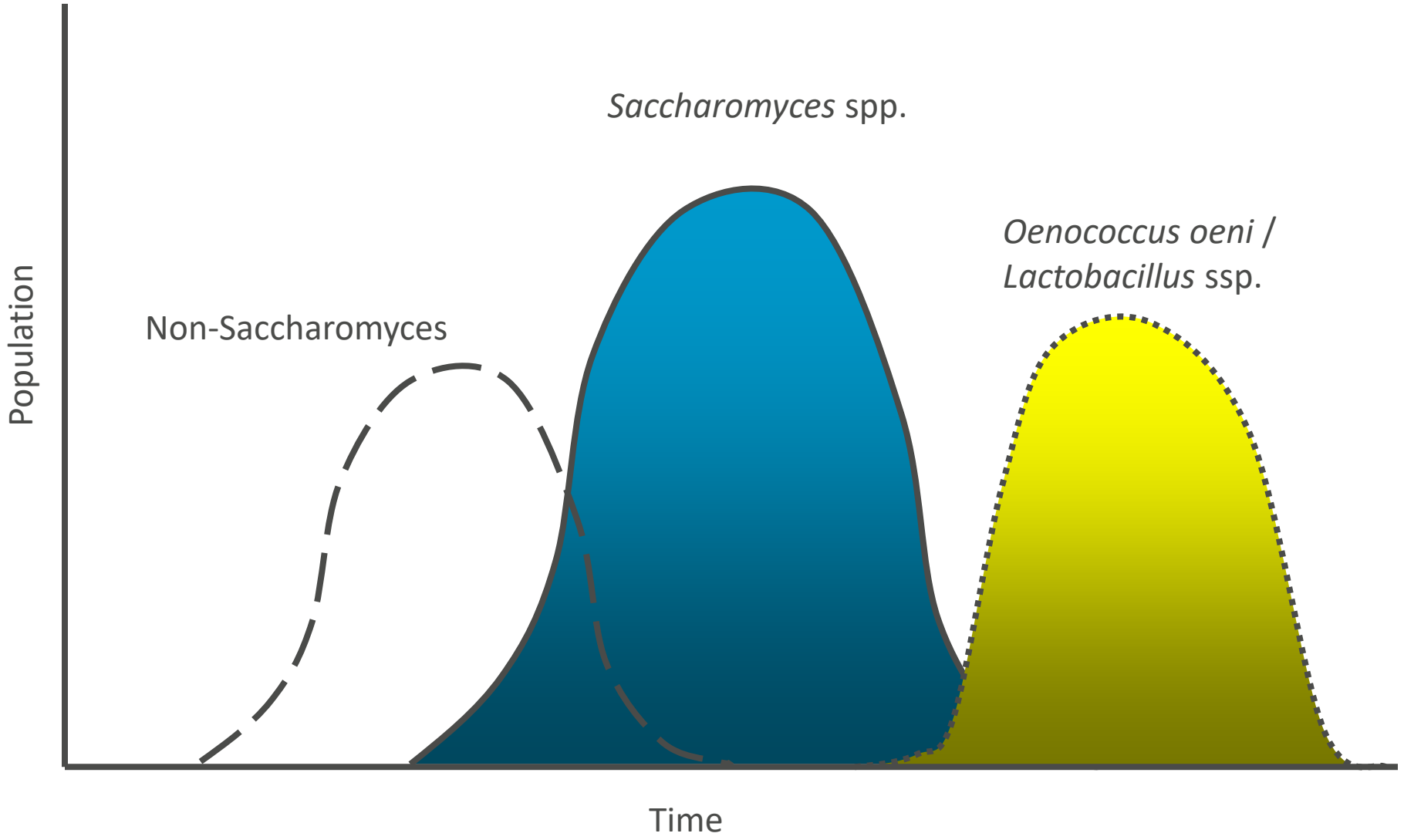
MICROBIAL ECOLOGY, POPULATION DYNAMICS

SIMPLIFIED GRAPHICAL REPRESENTATION OF NATURAL PROGRESSION



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How Non-Saccharomyces have a positive organoleptic impact

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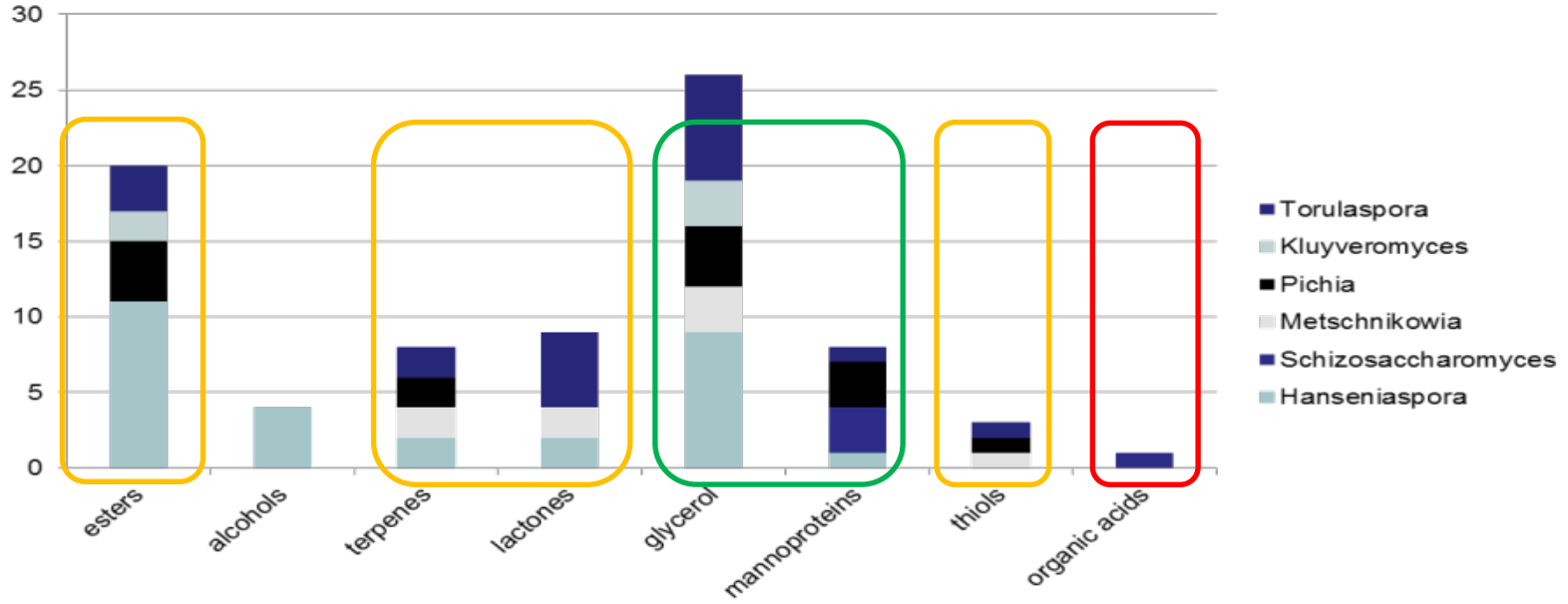
POSSIBLE IMPACTS ON A WINE FROM NON-SACCHAROMYCES YEAST SPECIES

LITERATURE SEARCH FOCUSED ON THE FOLLOWING TOPICS



Flavors

Precursor conversion
Metabolism differences

Acid balance

Organic acids
production/reduction

Mouth-feel

Polysaccharides
Mannoproteins

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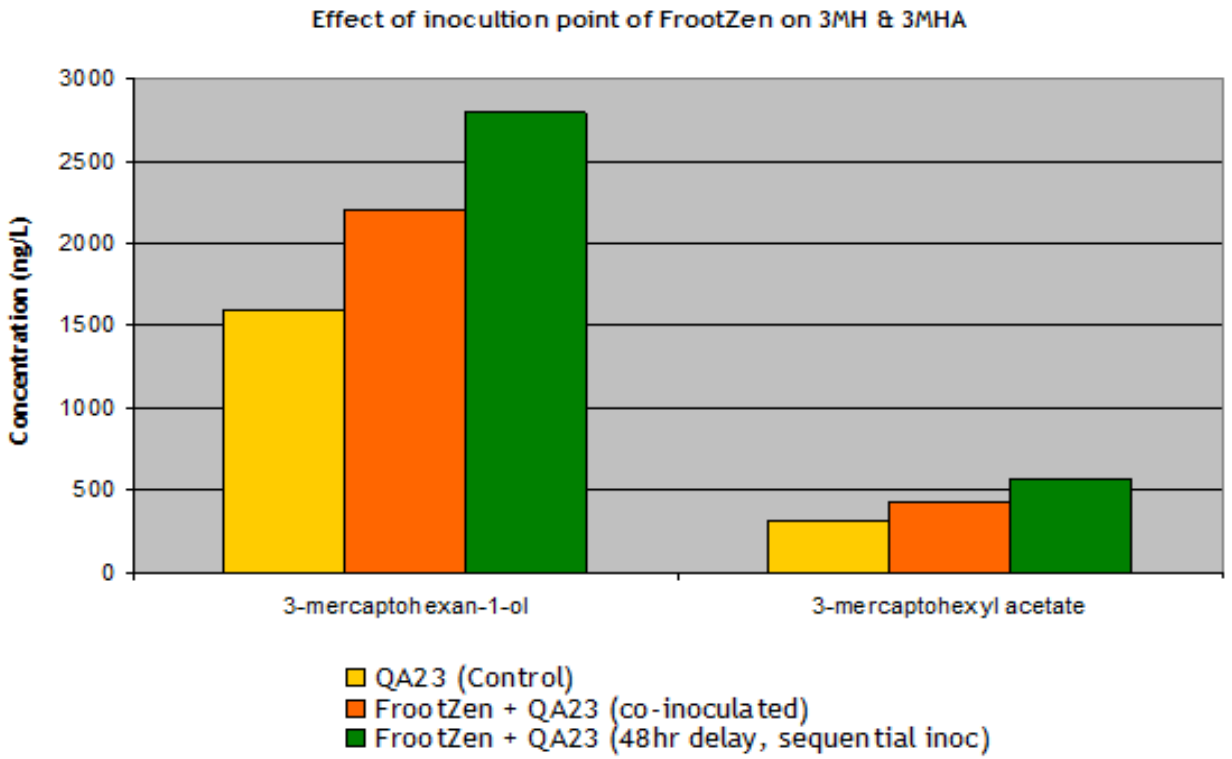
VINIFLORA® FROOTZEN™

VOLATILE THIOLS



3-mercaptohexan-1-ol: *passionfruit, citrus, grapefruit*

3-mercaptohexyl acetate: *Broom/boxtree, passionfruit*

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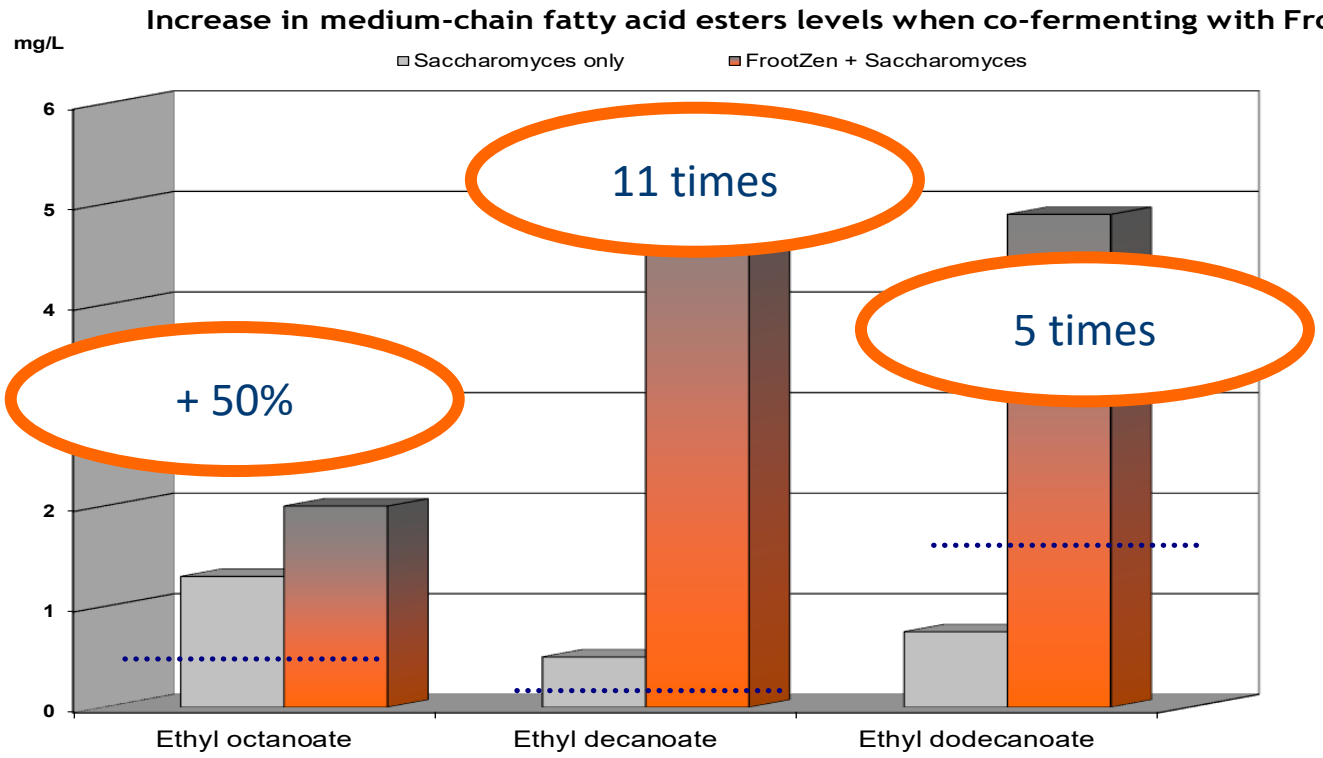
VINIFLORA® FROOTZEN™

ETHYL ESTERS



Ethyl octanote: pineapple, brandy, pear

Ethyl decanoate: apricot, sweet, brandy

Ethyl dodecanoate: waxy, floral

..... *Aroma threshold in wine*

Long chain esters are known to give a 'fruity' character, wine-like with a longer life span. They also provide part of the 'complexity' found in 'wild ferment' wines*

**Varela et. al. Australian Journal of Grape and Wine Research 15, 3, 2009, pp. 238-248*

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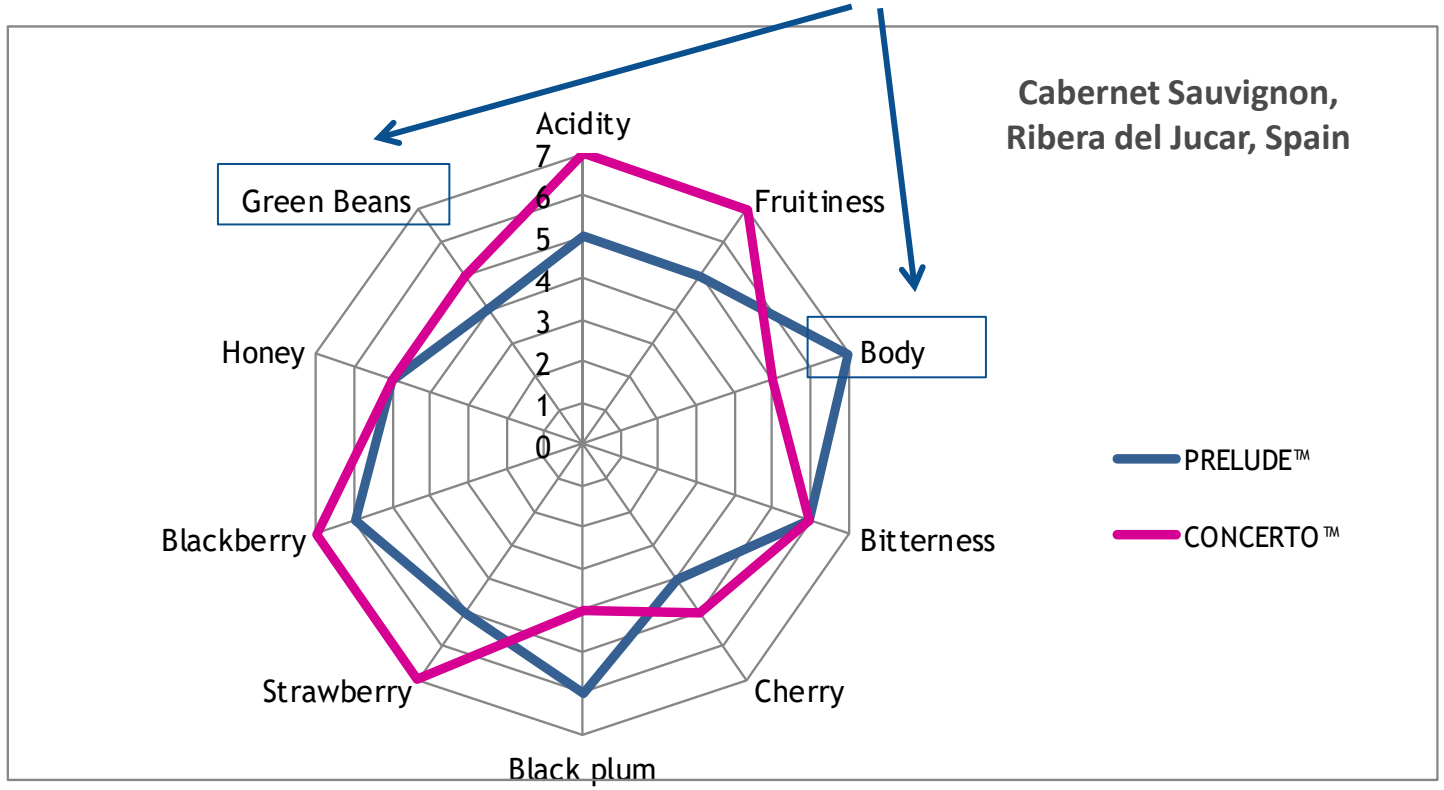
VINIFLORA® PRELUDE™

EFFECT ON PALATE WEIGHT AND GREEN FLAVORS

PRELUDE™ is effective for increasing body/palate-weight, as well as masking 'green' characters



The production of polysaccharides by T. delbreuckii has been extensively published. For an example see Comitini, F., et al., Selected non-Saccharomyces wine yeasts in controlled multistarter f..., Food Microbiology (2010)

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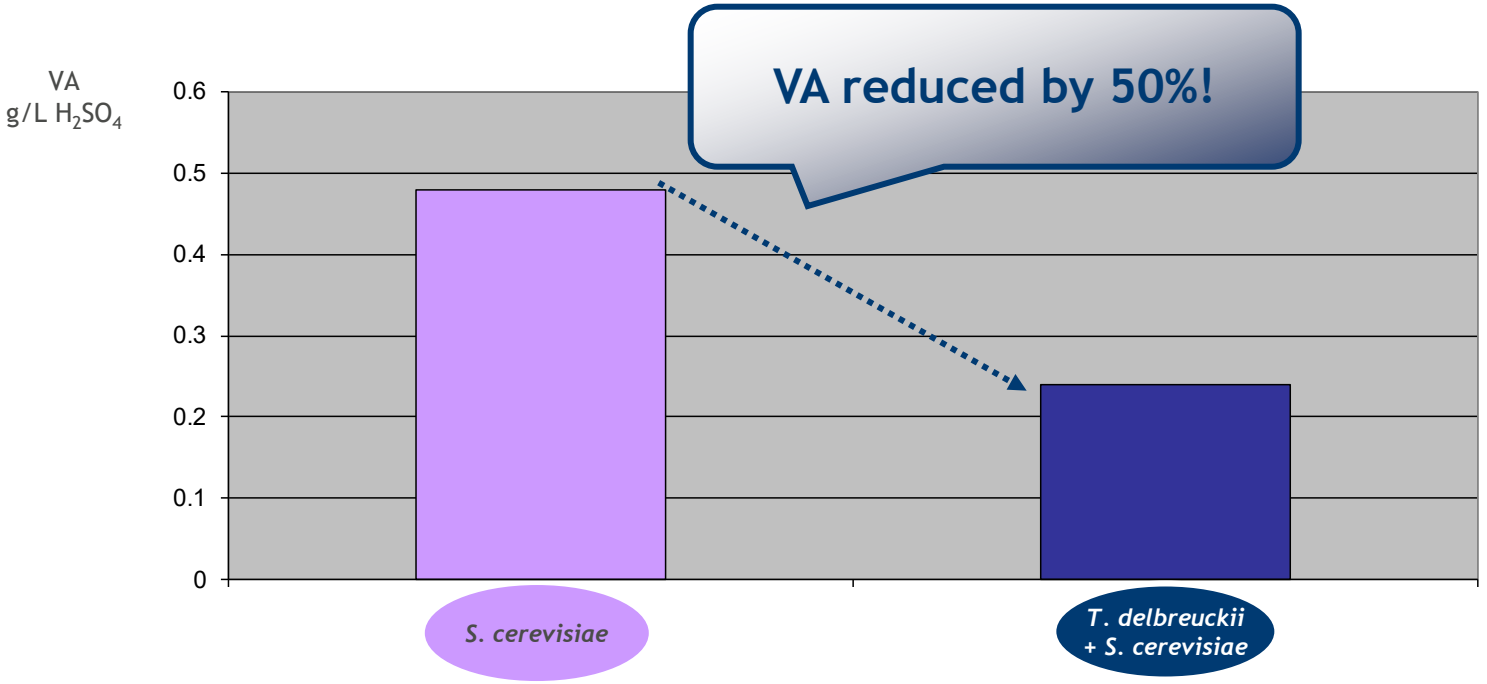
VINIFLORA® PRELUDE™

ACETIC ACID REDUCTION



Wine made from high sugar Semillon must, Bordeaux, France 2009
ISVV Bordeaux

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Two *Lachancea thermotolerans* for two different applications

Viniflora® OCTAVE for whites and rosé

Viniflora® CONCERTO™ for reds

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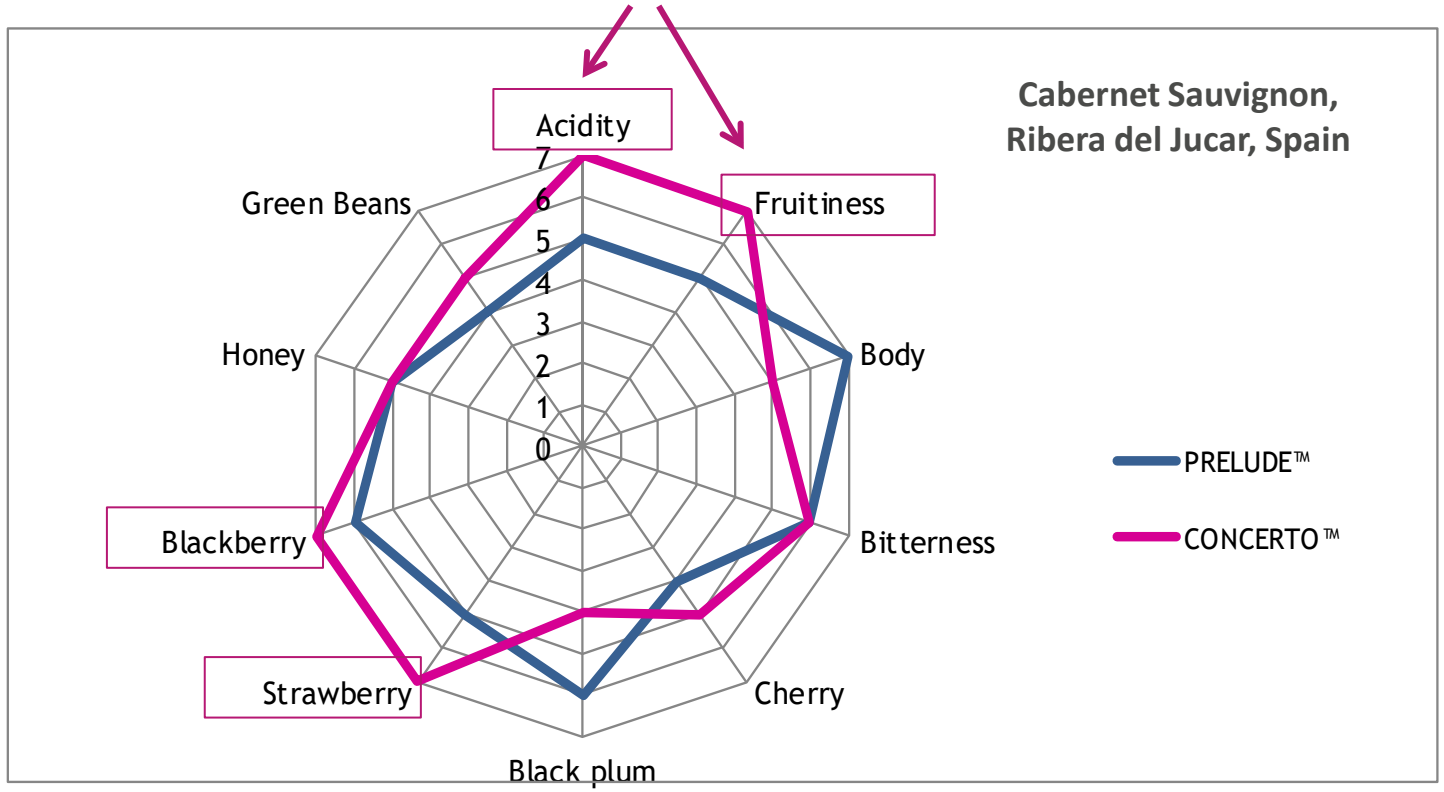
VINIFLORA® CONCERTO™

EFFECT ON FRUIT WEIGHT AND ACIDITY

CONCERTO™ is effective for lifting berry-fruit characters and bringing freshness/acidity



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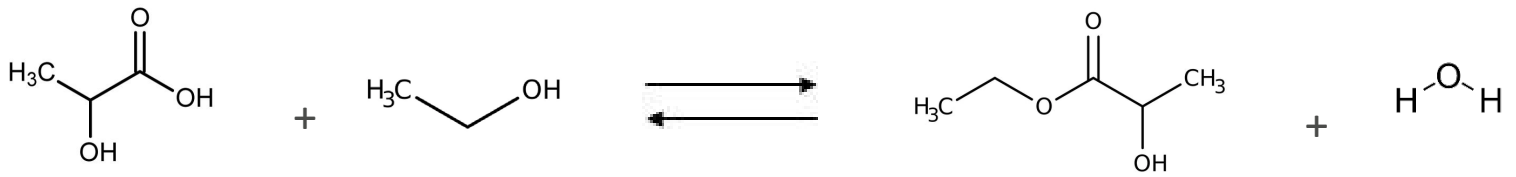
NON-SACCHAROMYCES AND EFFECTS ON FLAVOR

ETHYL ESTERS

Study carried out at Centre du Rosé (Provence - France)

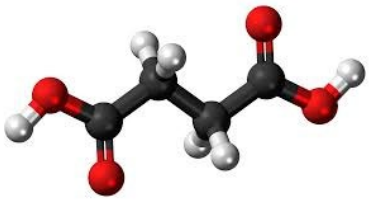
	Descriptor	Sacch. control	L. thermotolerans A 3d + Sacch.	L. thermotolerans B 3d + Sacch.
Total acetates	Flowery and fruity	52,8	57,9	46,1
Total acids	Cheesy	190,35	189,8	171,05
Total linear ethyl esters	Fruity	1,04	0,7	1,02
Total alcohols	Fusel and green	315,54	354,6	314,02
Ethyl lactate[1]	Cream, fruity (strawberry, coconut)	5,87	**9,31**	**78,89**
γ-Butyrolactone	Peach	1,35	3,62	3,98

[1] Odour threshold: 154 mg/L

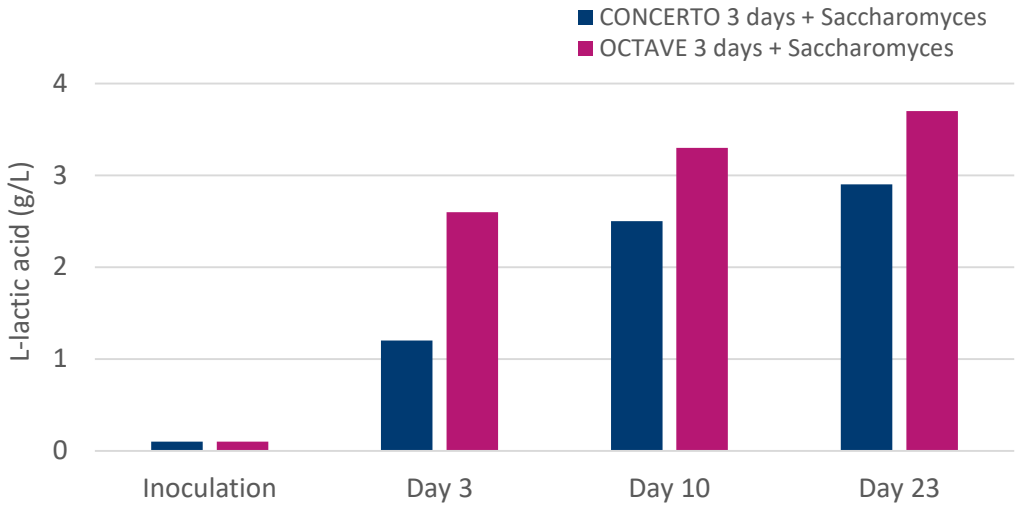


Lactic acid + **Ethanol** ⇌ **Ethyl lactate** + **Water**

INCREASE ACIDITY WITH NATURAL LACTIC ACID



LACTIC ACID IN NON-SULFITED ROSÉ WINE



Compared to Viniflora® CONCERTO, OCTAVE is more efficient in releasing L-lactic acid

VINIFLORA® OCTAVE ENABLES A SULFITE REDUCTION

Values from vinification of rosé wine, Languedoc, 2019

Microorganisms	Final wine pH	% sulfites as molecular
Control *Saccharomyces*	3.52	1.9
CONCERTO 3 days + *Saccharomyces*	3.45	2.2
OCTAVE 3 days + *Saccharomyces*	3.35	2.8

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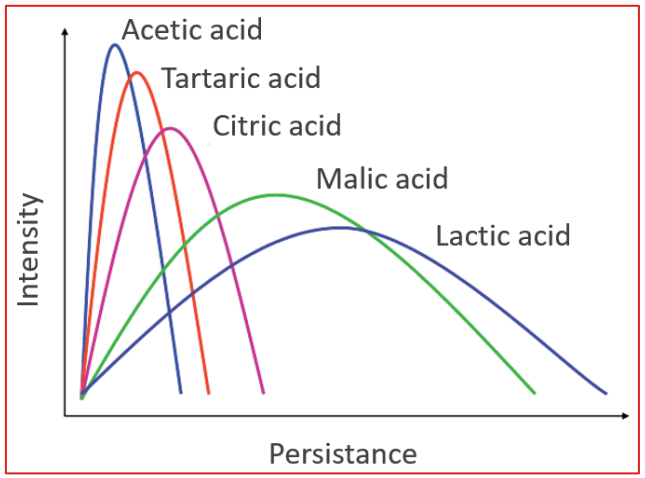
TARTARIC ACID IS COMMONLY USED TO INCREASE ACIDITY, BUT WITH NATURAL LACTIC ACID, LESS TARTARIC ACID IS NEEDED

TARTARIC ACID

- Added in high quantity (up to 400 g/hL)
- Price is unstable and financial unpredictable, e.g. in 2018, the price was € 8/kg equal to € 1.2-3.0/hL
- Leads to dryness on the palate
- Can be lost by (late) precipitation in the bottle



LACTIC ACID BY VINIFLORA® OCTAVE

- Produced naturally during pre-fermentation
- Constant cost in use
- Pleasant taste with mild and round acidity
- Very stable acid

VINIFLORA® OCTAVE ENABLES TO REPLACE 150 G/HL OF TARTARIC ACID

Tartaric acid	H_2SO_4	Equivalent lactic acid
150 g/hL (20 meq)	+1.0 g/l	180 g/hl
250 g/hL (33 meq)	+1.6 g/l	300 g/hl

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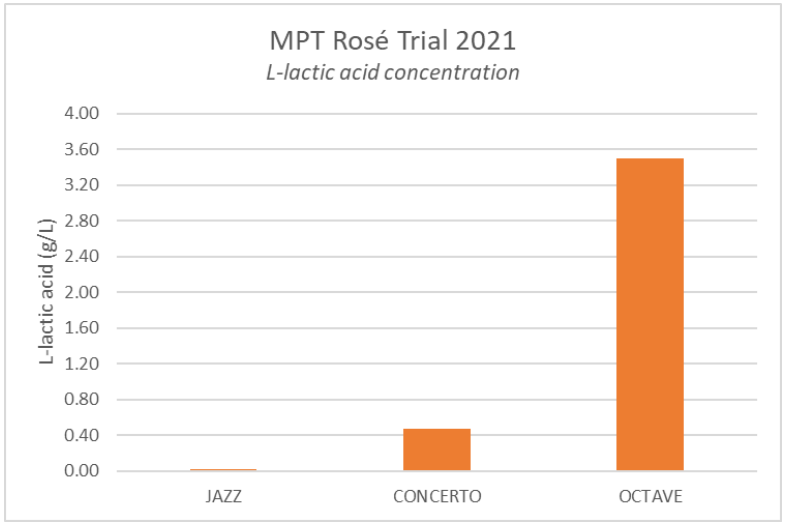
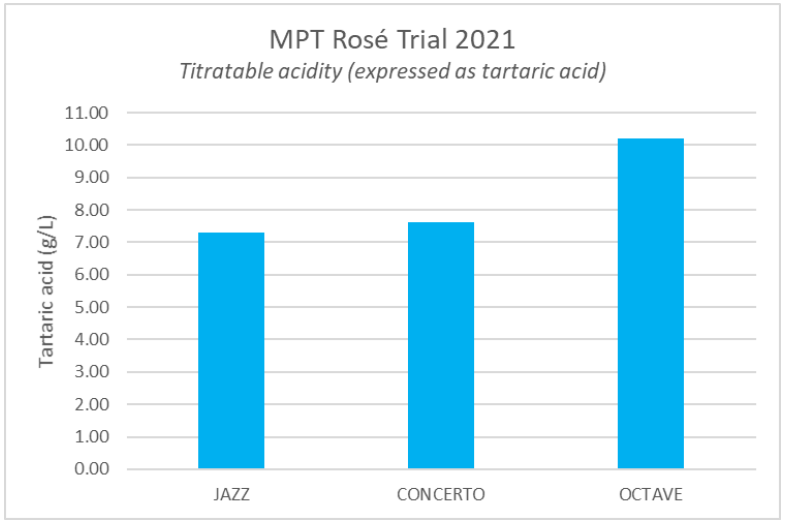
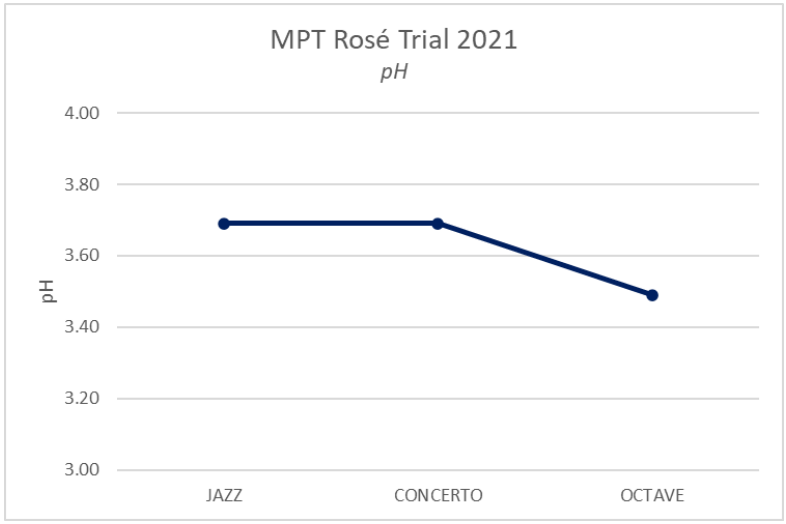
LACHANCEA THERMOTOLERANS COMPARISON

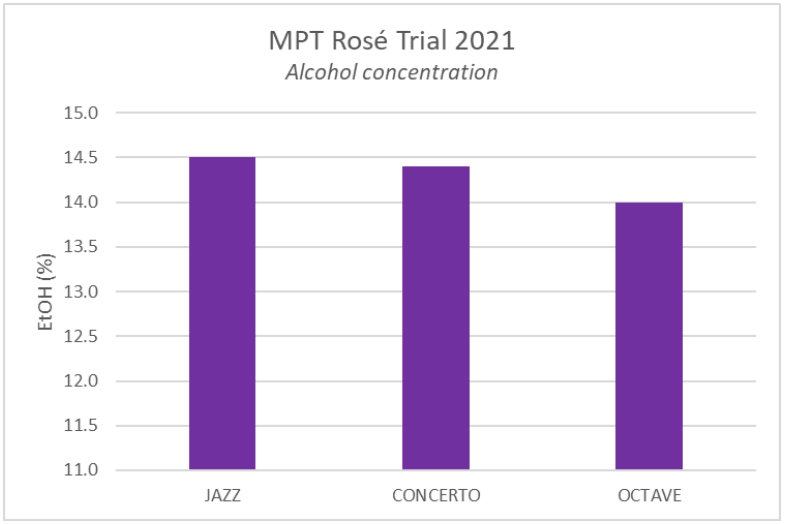
CONCERTO AND OCTAVE IN ROSÉ FROM SYRAH

Field trial from Australia, Grampians Region 2021. Shiraz (saignée)









Two *Lachancea thermotolerans* for different applications

Rosé wine

OCTAVE

White wine

OCTAVE

Red wine

CONCERTO

OCTAVE

in special cases

Mind the risk of difficult malo

Blending recommended

Sparking wine

CONCERTO

Traditional method

OCTAVE

Charmat method

Blending recommended

Fortified wine/Late harvest

CONCERTO

OCTAVE

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How Non-Saccharomyces can be a crucial tool against spoilage

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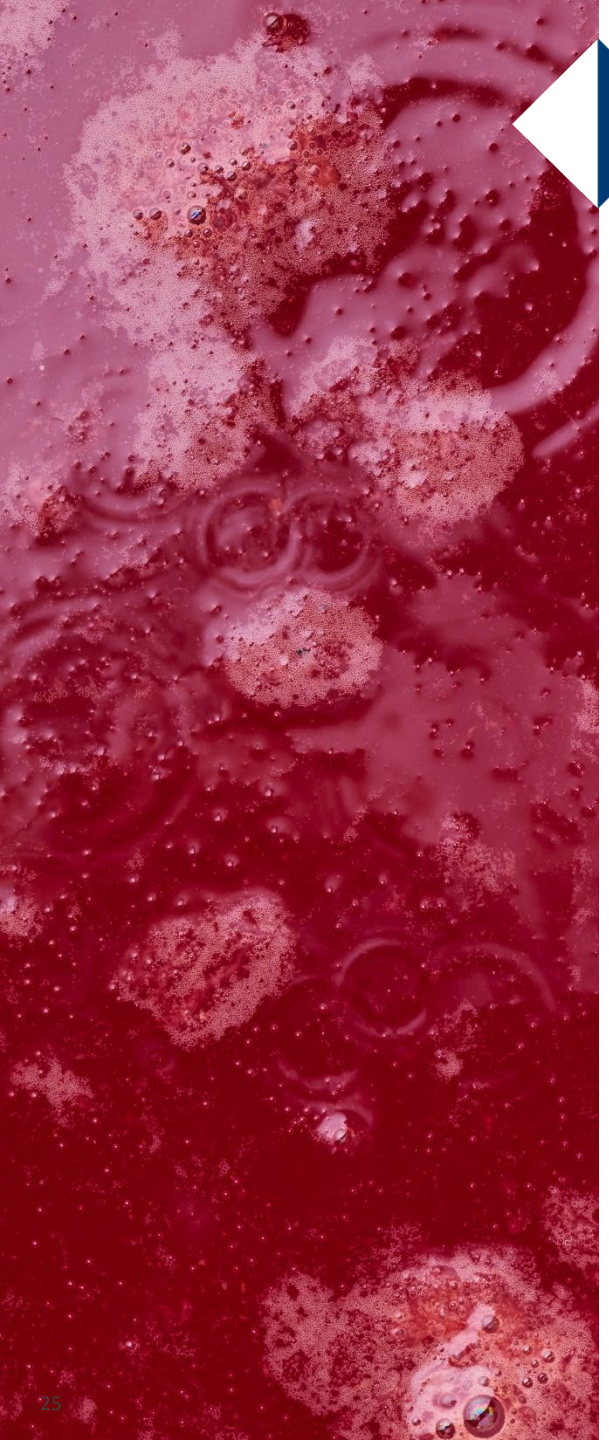

WHAT IS BIOLOGICAL PROTECTION?

"Bioprotection is a current concept, so its definition is still under discussion. However, it can be considered the active or passive use of some microorganisms to preserve foods and beverages and to exclude other spoilage microorganisms, thus avoiding the production of off-flavors, sensory alterations, or even the formation of toxic molecules"

Non-*Saccharomyces* as Biotools to Control the Production of Off-Flavors in Wines, Morata et al, *Molecules* 2021, *26*(15), 4571

The vast majority of biological protection applications incorporate the use of Non-Saccharomyces yeast species

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ACTIVE VS. PASSIVE….

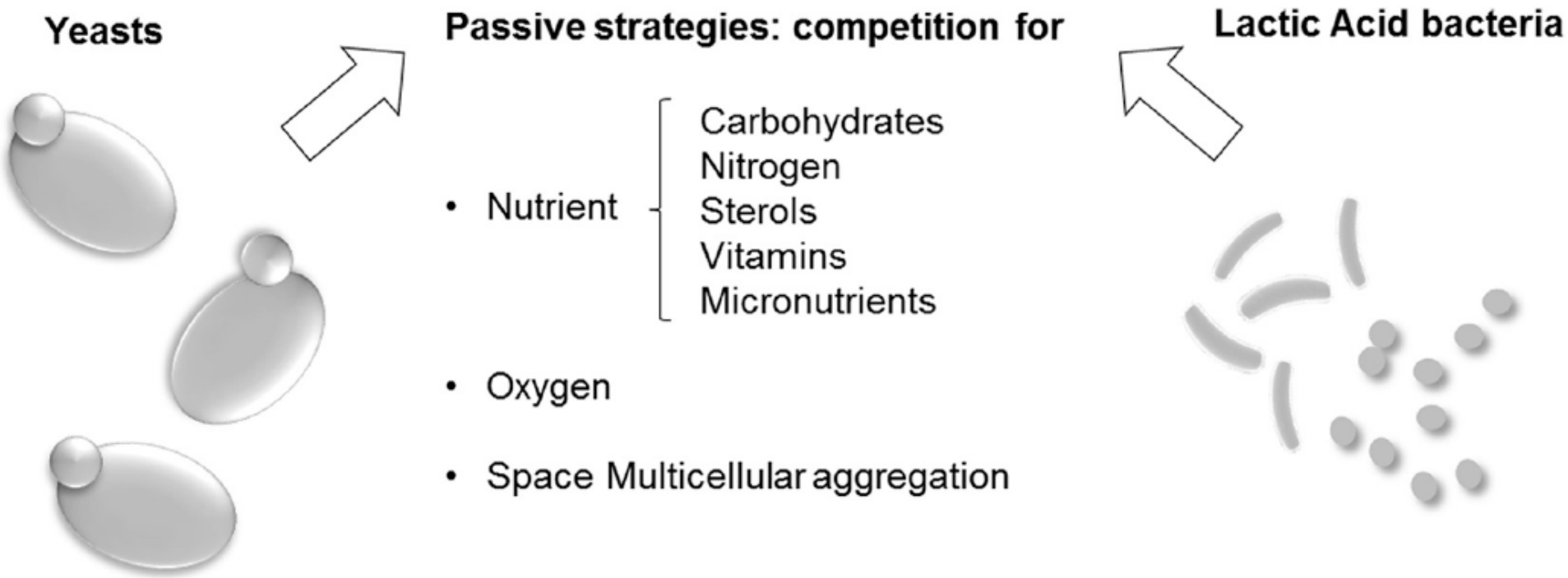
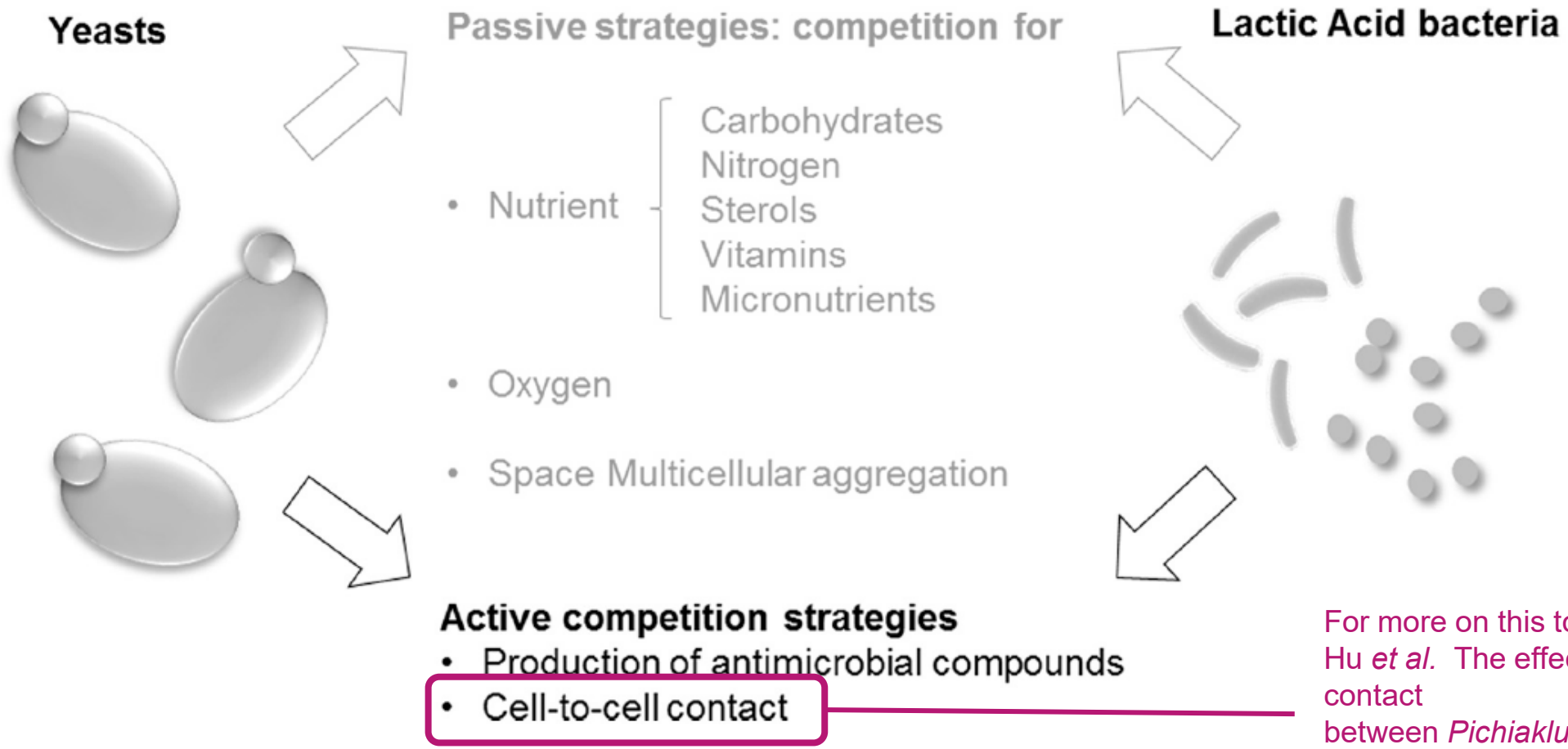


Table taken from: Di Gianvito *et al*. Bioprotection strategies in winemaking. International Journal of Food Microbiology, Volume 364, 2022

ACTIVE VS. PASSIVE….



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

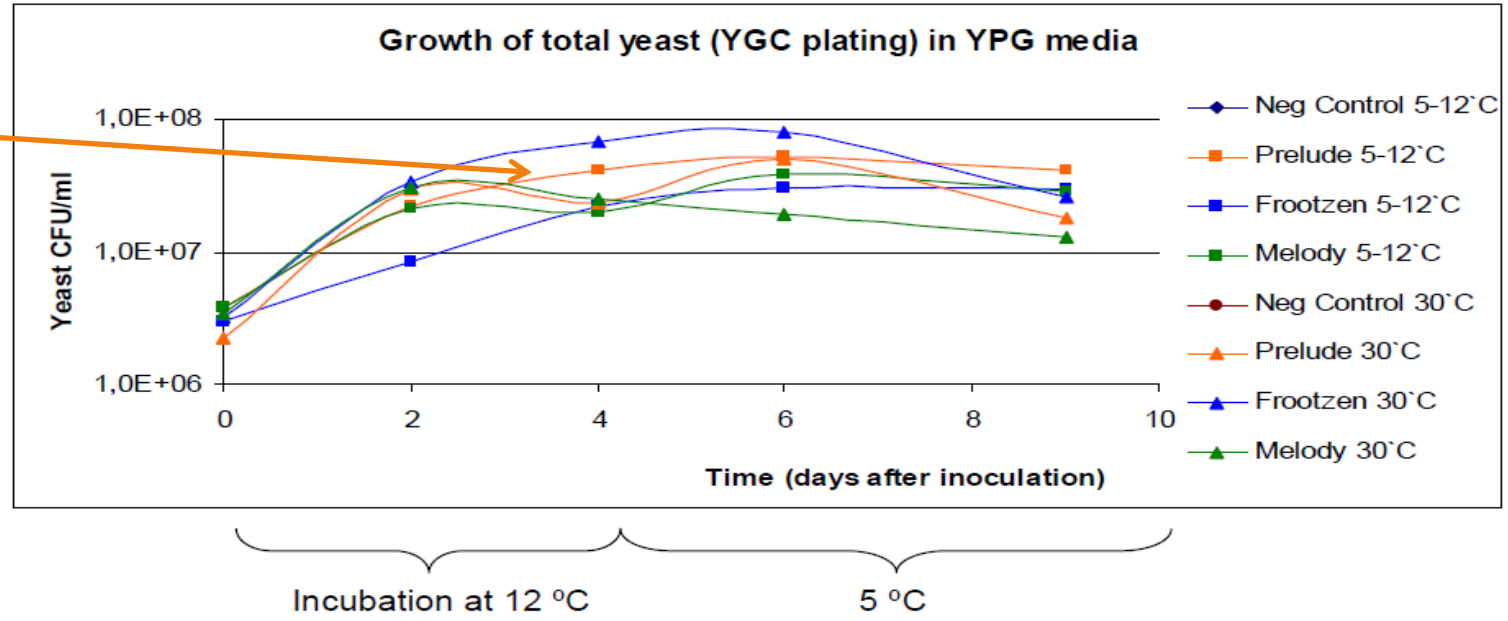
Table taken from: Di Gianvito *et al*. Bioprotection strategies in winemaking. International Journal of Food Microbiology, Volume 364, 2022

PASSIVE BIOLOGICAL PROTECTION

EXAMPLE – TORULASPORA DELBREUCKII AT LOW TEMPERATURES

› PRELUDE™ (*Torulaspora delbreuckii*) is very well suited to being used during pre-fermentation maceration due to its ability to dominate must, even at low temperatures

Implantation and development of different N.Sac yeast at 12°C then 5°C vs. 30°C



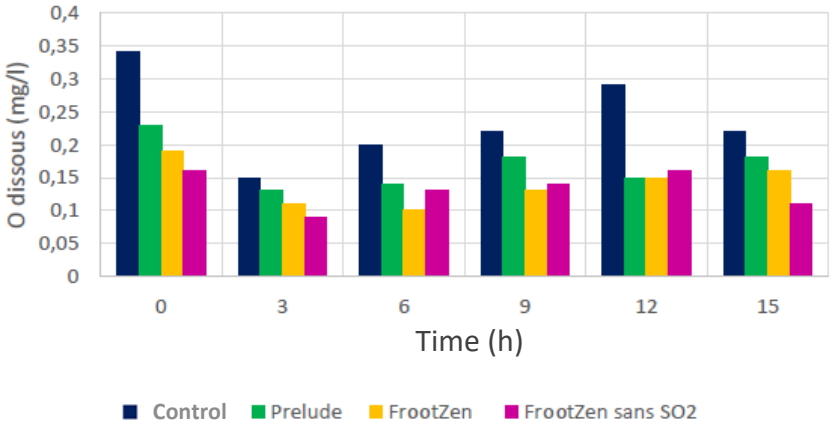
Population (CFU/ml) for Prelude™ which is represented in orange, has the best growth among the different strains and blends assessed.

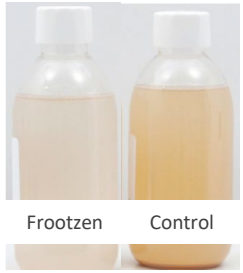
PASSIVE BIOLOGICAL PROTECTION

EXAMPLE – PICHIA KLUYVERI AND OXYGEN CONSUMPTION

› FROOTZEN™ (*Pichia kluyveri*) has been shown to rapidly take up oxygen in grape must

Dissolved oxygen (mg/L) vs. Time (hrs) in Sauvignon blanc juice
Sancerre, France - 2012

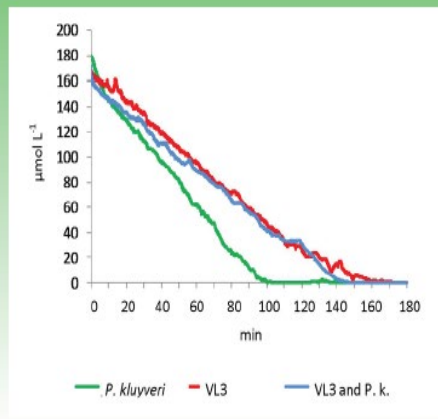




End of fermentation

› Helps to avoid oxidation of juice thereby reducing browning.

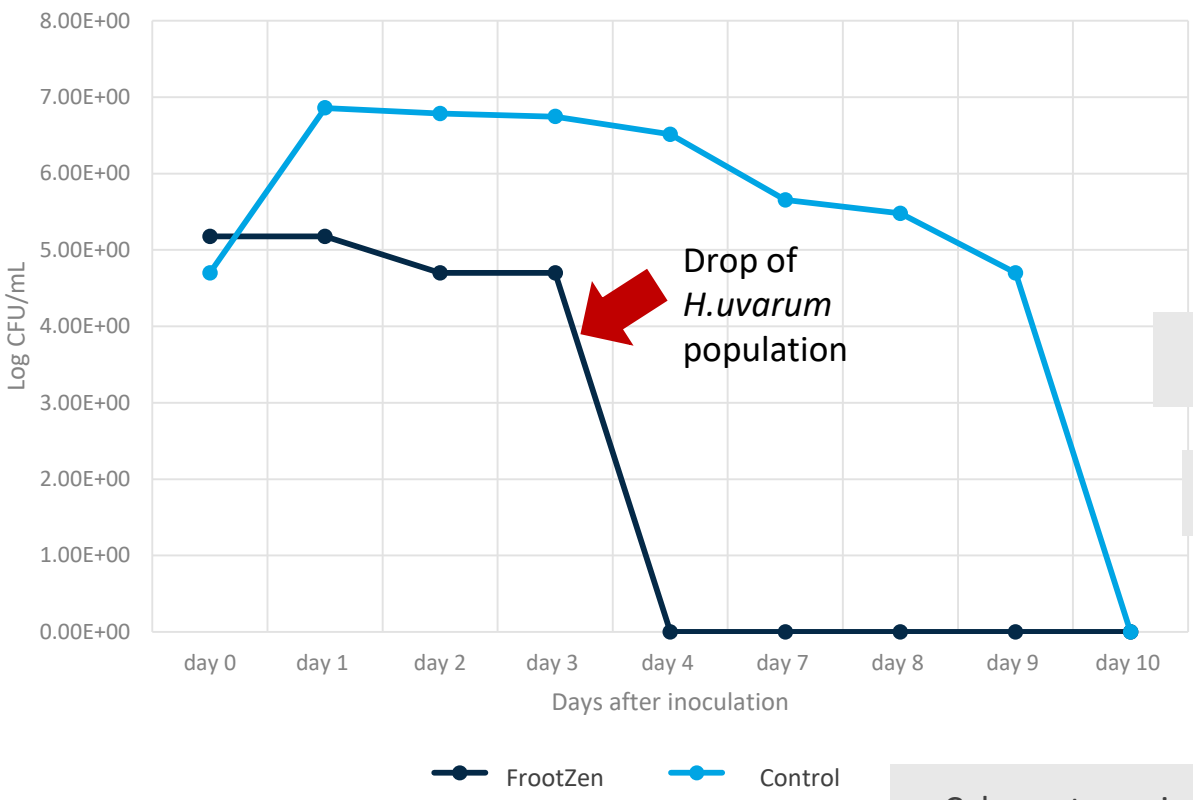
Ferments are anaerobic within 2-4 hours of inoculation, no dissolved oxygen can be detected afterwards



*P. Kluyveri:*Frootzen™
VL3:*S.cerevisiae*

PASSIVE BIOLOGICAL PROTECTION

EXAMPLE – PICHIA KLUYVERI AND OXYGEN CONSUMPTION



Cabernet sauvignon 25°C

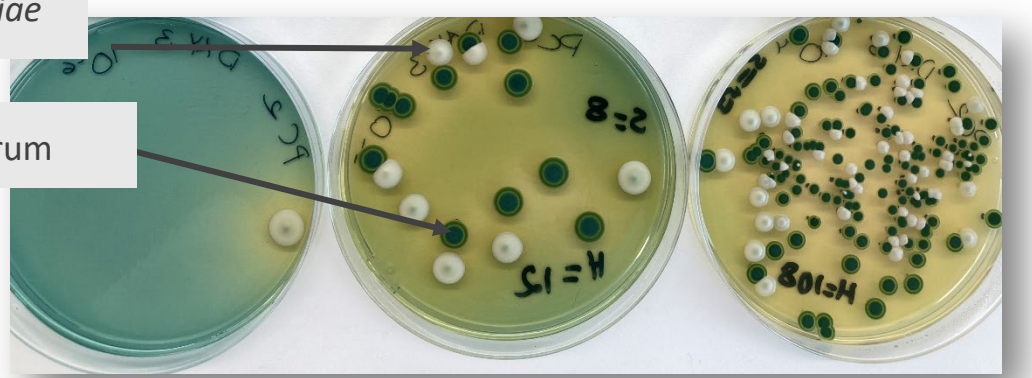
Trial (FrootZen + *H. uvarum* + *S. cerevisiae*) Day 4

FrootZen

S.cerevisiae

Control (*H. uvarum* + *S. cerevisiae*) Day 4

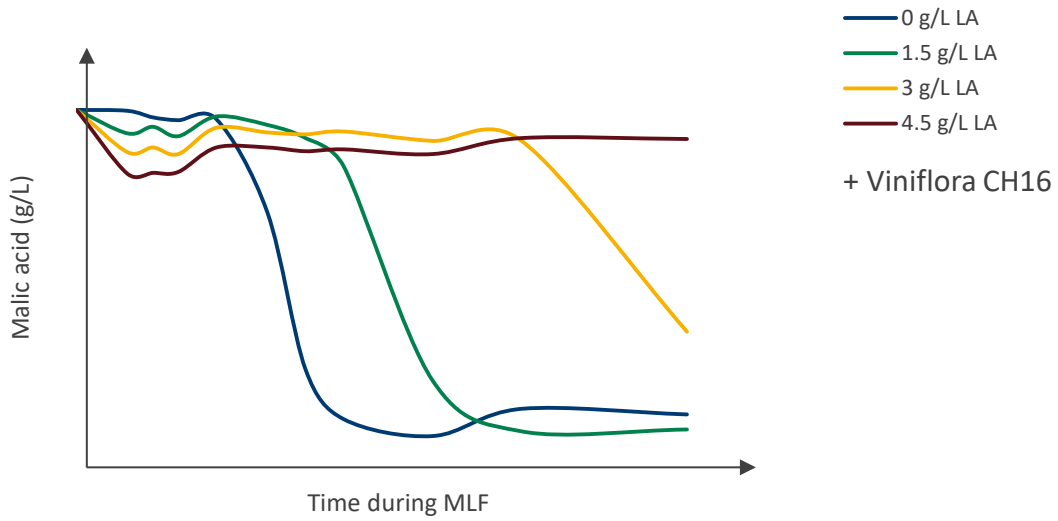
H. uvarum



ACTIVE BIOLOGICAL PROTECTION

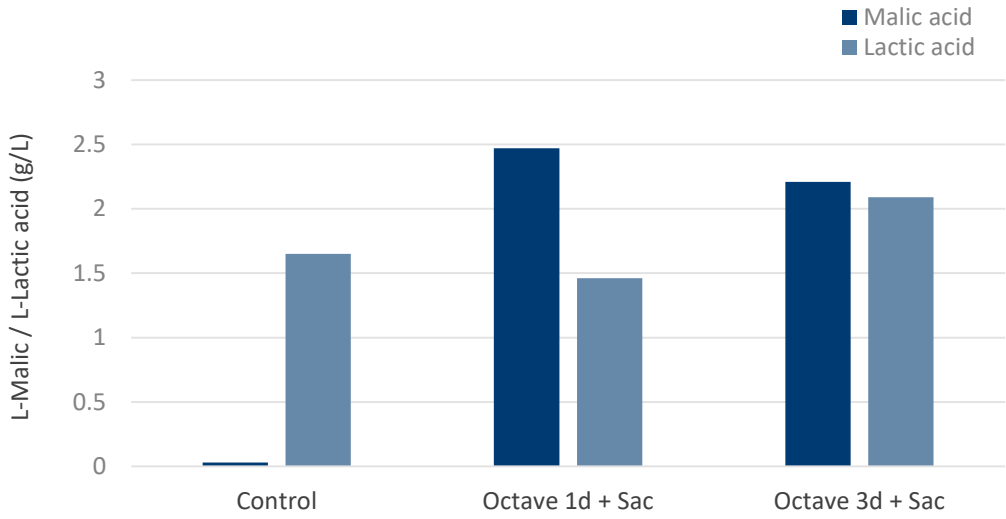
EXAMPLE – LACHANCEA THERMOTOLERANS AND L-LACTIC ACID

ADDITION OF L-LACTIC ACID



Lactic acid at levels >1.5 g/L are already inhibitory to indigenous malolactic bacteria, with significant delay from 3 g/L onwards

AFTER ALCOHOLIC FERMENTATION



Viniflora® OCTAVE used in pre-fermentation can inhibit the development of spontaneous MLF, keeping most of the malic acid and adding with lactic acid

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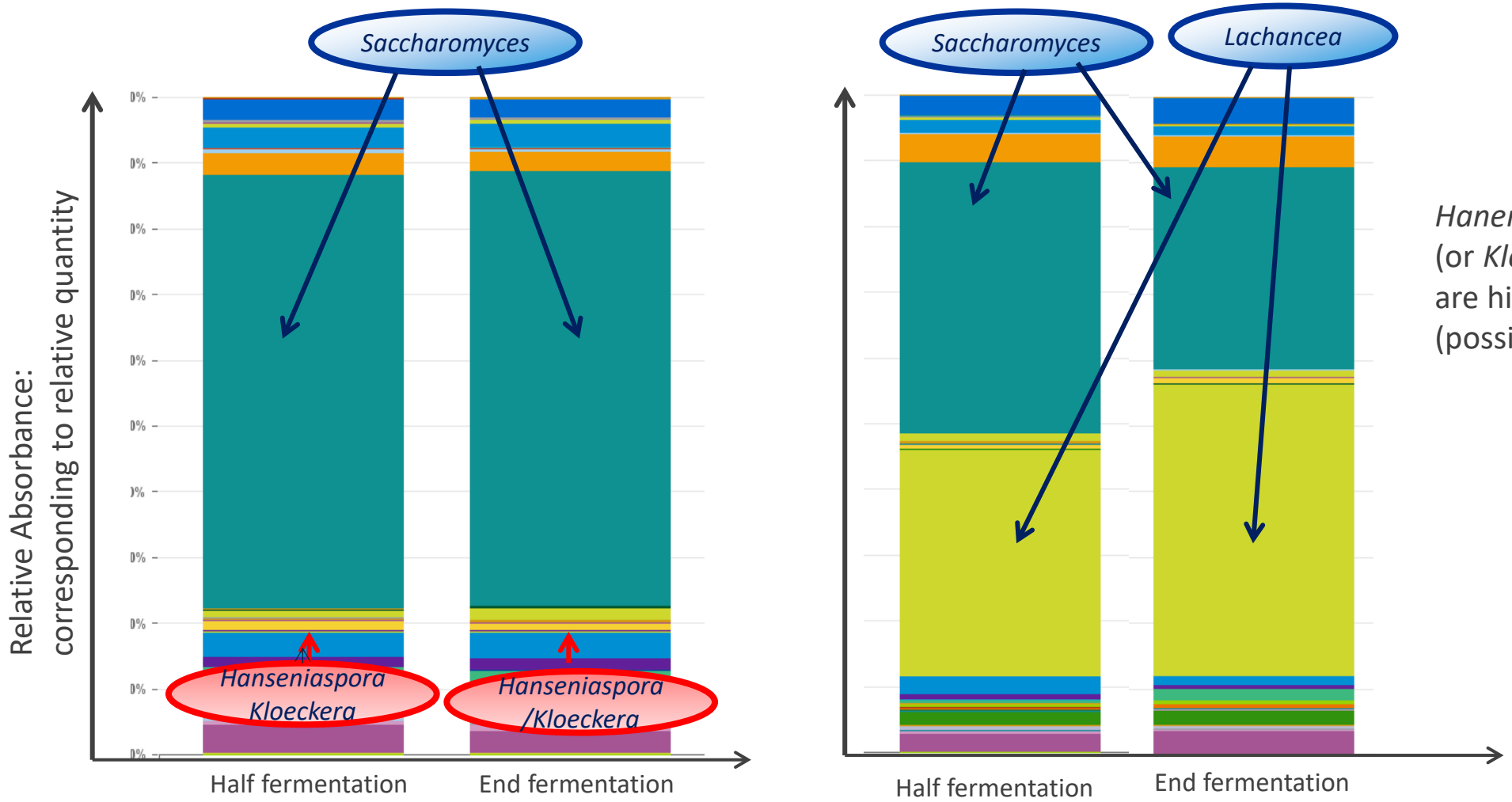
ACTIVE BIOLOGICAL PROTECTION

EXAMPLE – LACHANCEA THERMOTOLERANS AND L-LACTIC ACID

History of fungal microflora using metagenomics (DNA fragments….so not ‘live’ cells)



Haneniaspora uvarum
(or *Kloeckera apiculata*)
are higher in the control wine
(possible ethyl acetate production)

How when and why to apply Non-Saccharomyces yeast

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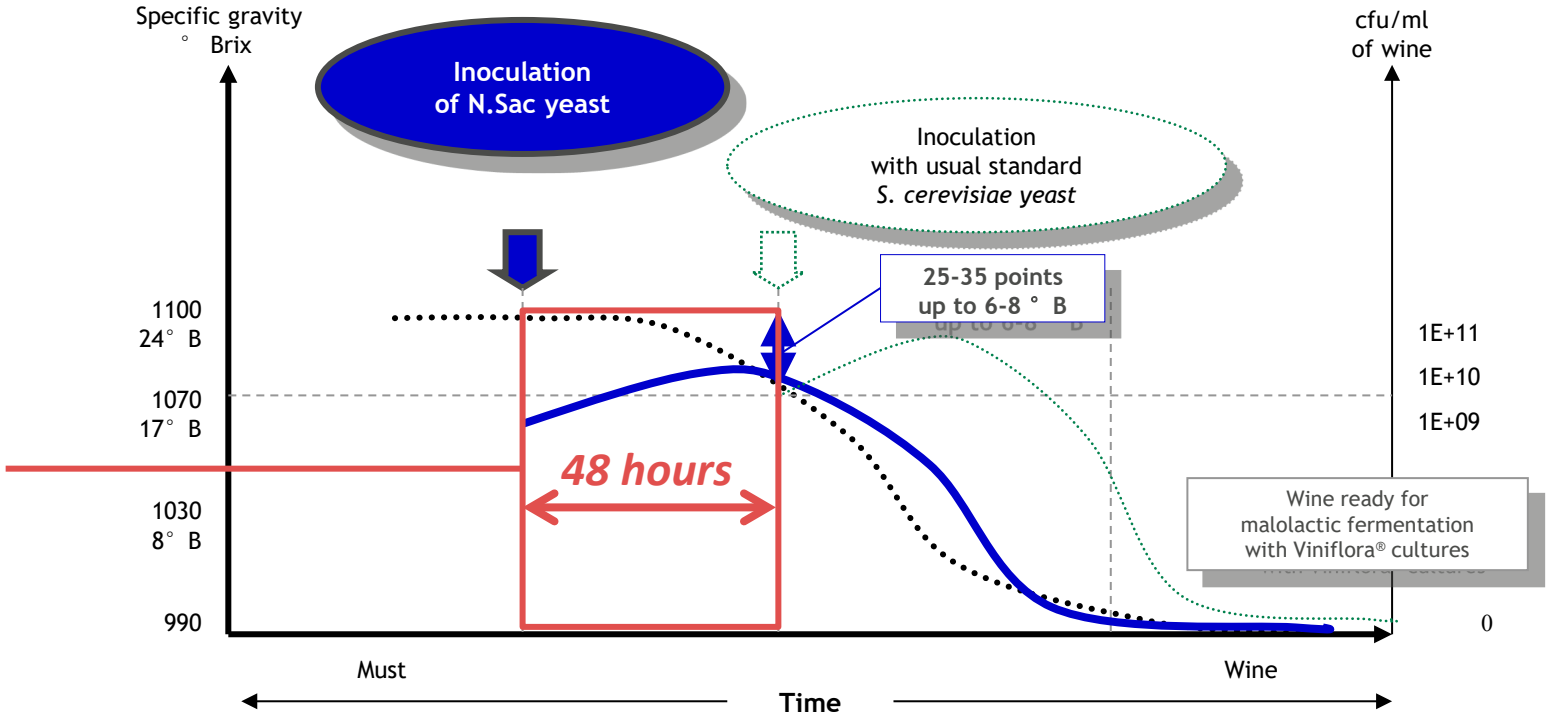
HOW TO USE NON-SACCHAROMYCES YEAST
INOCULATION TIMING

- Sequential inoculation is recommended to get the most from a Non-Saccharomyces yeat
- Standard dosage for Non-Sacc yeast is 20-25g/hL (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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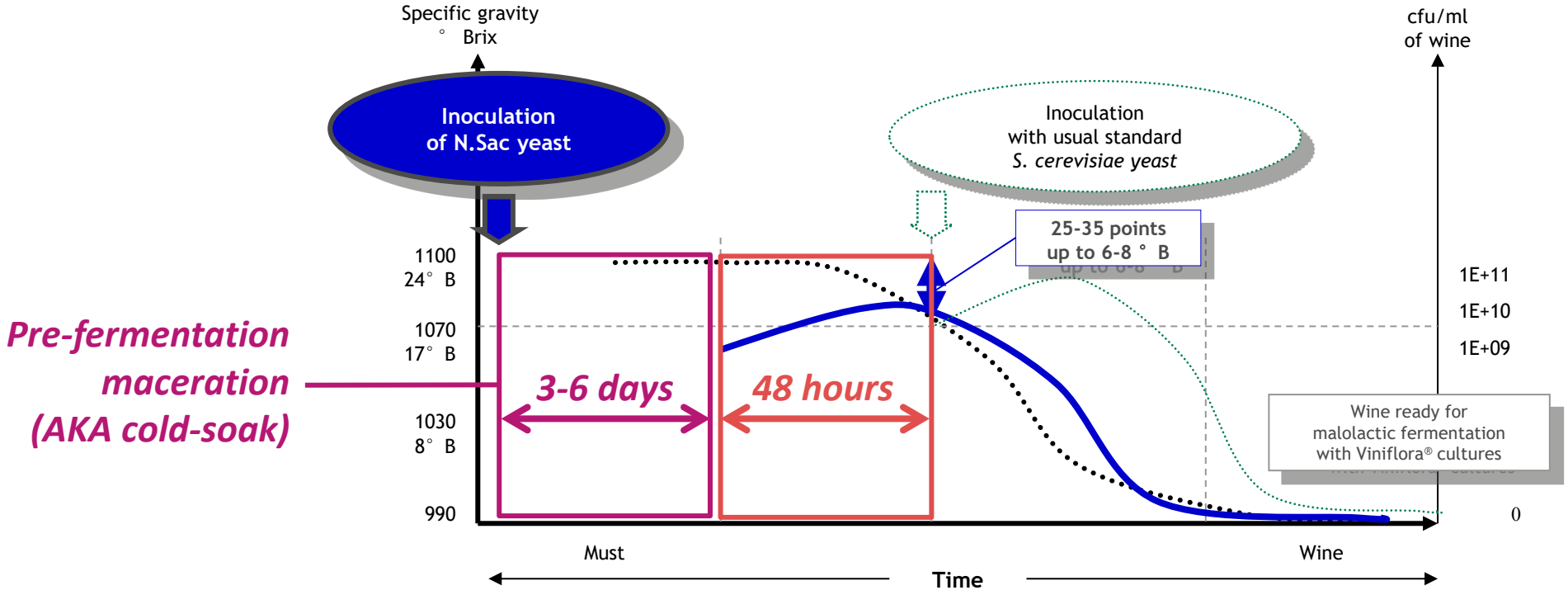

For red wines undergoing pre-fermentation maceration (cold-soak), N.Sac can be added at the start of this process, with Saccharomyces added once must is warmed

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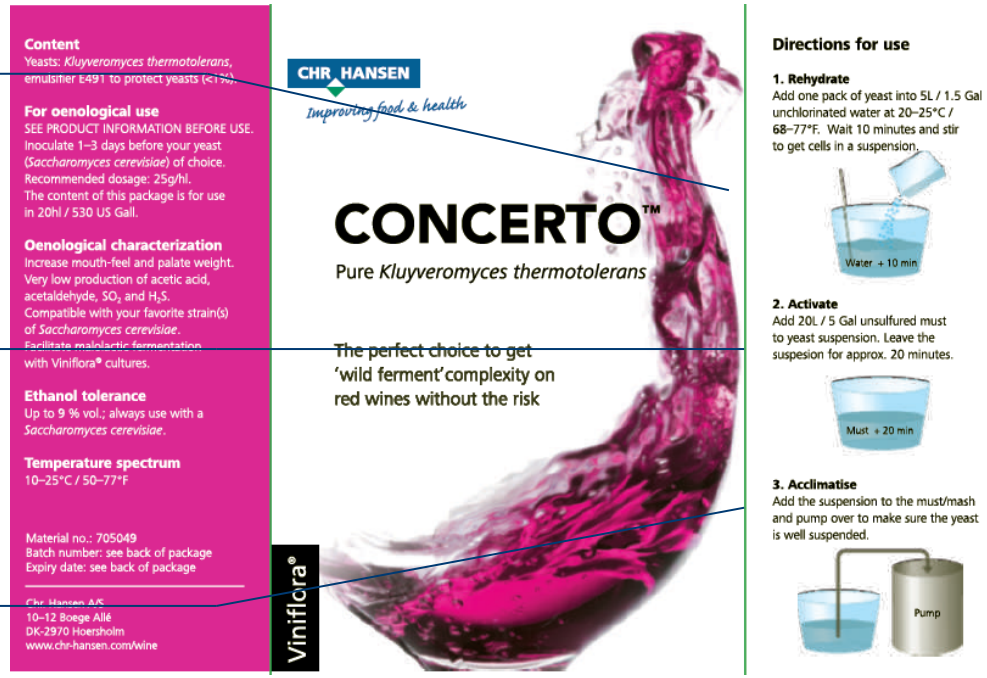
HOW TO USE NON-SACCHAROMYCES YEAST

REHYDRATION PROCESS SIMILAR BUT NOT THE SAME TO OTHER WINE YEASTS

Attention to chlorine!

Attention to the T-SO_2!

Attention to the temperature!



How about Nitrogen?

- As with any wine yeast, Chr. Hansen strongly suggest measuring YAN before any yeast inoculation
- If Nitrogen supplementation is required, then this should be added once the Saccharomyces is inoculated
- An ideal YAN target when using Non-Sacc yeast during a fermentation is 250mg/L

Thank you

Questions ?

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866.213.1131

Contact Your Gusmer Enterprises Technical Sales Representative

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