Using non-*Saccharomyces* yeasts during alcoholic fermentation: taking Advantage of Yeast Biodiversity

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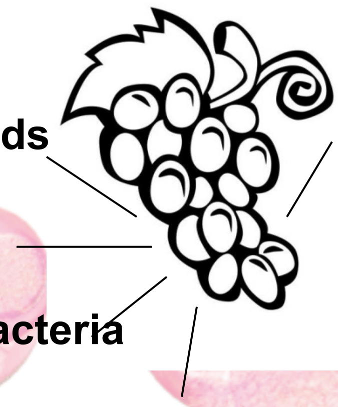
Wineries Unlimited, 2012. Richmond, VA

LAFFORT

Non-*Saccharomyces* in Winemaking

Native Microflora



non – *Saccharomyces*

- *Pichia*
- *Metchnikowia*
- *Kloeckera*
- *Kluyveromyces*
- *Candida*
- *Zygosaccharomyces*
- *Torulaspora*
- *Cryptococcus*
- *Brettanomyces*
- *Hanseniaspora*

Non-*Saccharomyces* on Grapes

Flowering → Nouaison → Harvest

Total yeasts/grape

Dominant species

- *Cryptococcus*
- *Candida*
- *Pichia*

And others

- *Torulaspora delbrueckii*

Beginning of AF → End of AF

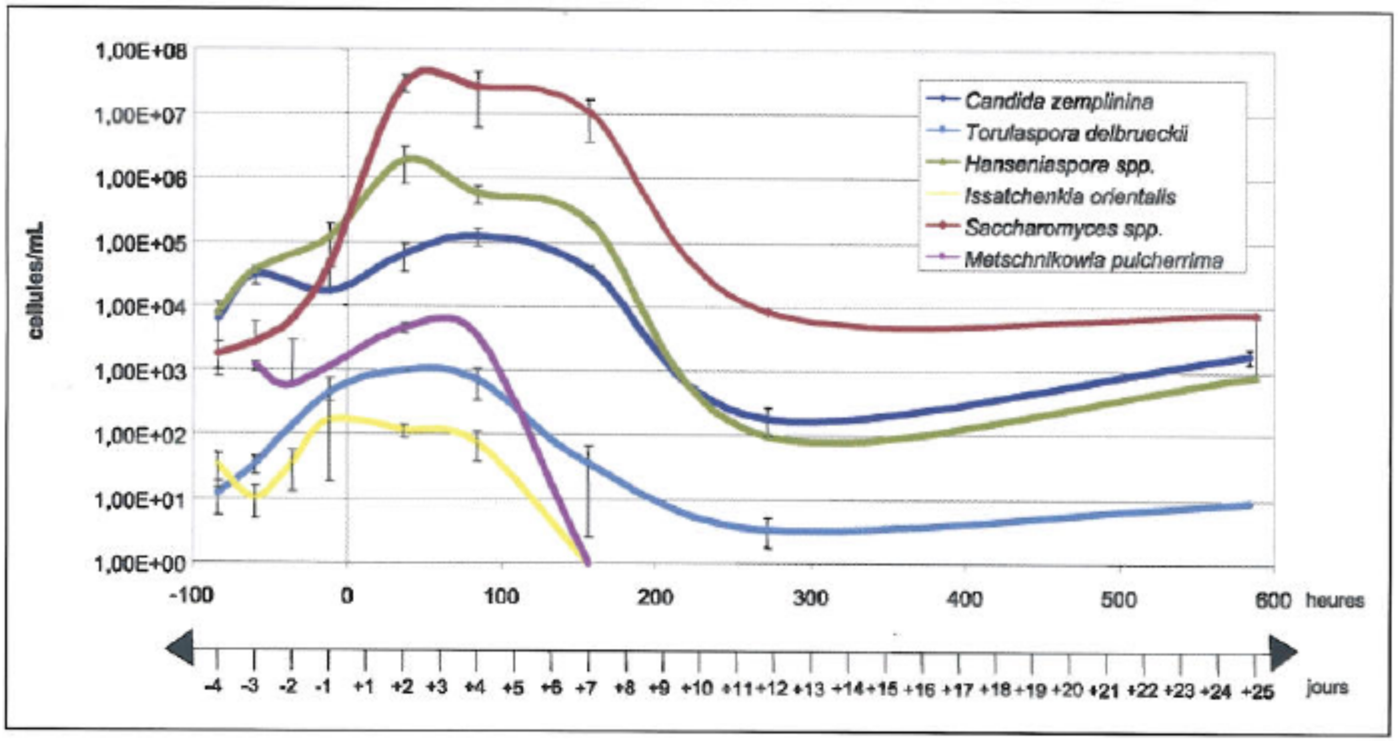
Decrease in the non-*Saccharomyces* population

50% non-*Saccharomyces*
50% *S. cerevisiae*

> 99% *S. cerevisiae*

Dynamics and Diversity

Dynamics of yeast populations established by real time PCR, from cold soaking until barrel settling for FML (Zott, 2009)



0 hours = addition of a commercial yeast.

Taking Advantage of Biodiversity

Espèce utilisée en association avec *S. cerevisiae*	But	Références
Candida cantarellii	Augmentation teneur en glycérol	Toro et Vazquez (2002)
Candida pulcherrima	Modulation aromatique	Jolly *et al.* (2003); Zohre et Erten (2002)
Candida stellata	Augmentation teneur en glycérol	Ciani et Ferraro (1995, 1998); Ferraro *et al.* (2000)
	Modulation aromatique	Soden *et al.* (2000)
Debaryomyces vanriji	Augmentation teneur en géraniol	Garcia *et al.* (2002)
Hanseniaspora guilliermondii	Modulation aromatique	Zironi *et al.* (1993)
Hanseniaspora uvarum (Kloeckera apiculata)	Modulation aromatique	Ciani *et al.* (2006); Herraiz *et al.* (1990); Mendoza *et al.* (2007); Moreira (2005); Moreira *et al.* (2008); Zironi *et al.* (1993); Zohre et Erten (2002)
Issatchenkia orientalis	Réduction teneur en acide malique	Kim *et al.* (2008)
Kluyveromyces thermotolerans	Réduction production acide acétique	Ciani *et al.* (2006); Mora *et al.* (1990)
	Augmentation acidité totale	Kapsopoulou *et al.* (2007)
Pichia fermentans	Modulation aromatique	Clemente-Jimenez *et al.* (2005)
Pichia kluyveri	Augmentation teneur en thiols volatils	Anfang *et al.* (2009)
Pichia anomala	Modulation aromatique	Kurita *et al.* (2008)
Schizosaccharomyces pombe	Dégradation acide malique	Ciani (1995); Magyar et Panic (1989); Snow et Gallender (1979); Yokotsuka et al.(1993)
Torulaspora delbrueckii	Réduction production acide acétique	Bely *et al.* (2008); Ciani *et al.* (2006); Lafon-Lafourcade (1981); Salmon *et al.* (2007)
	Modulation aromatique	Herraiz *et al.* (1990)

Source : P. Renault 2010

Many ecological studies bring light to the role of non-*Saccharomyces* yeasts

Specific metabolisms: different technological interests

***Torulaspora delbrueckii*: A non-*Saccharomyces* <u>with no organoleptic defects and</u> compatible with *S. cerevisiae* species**

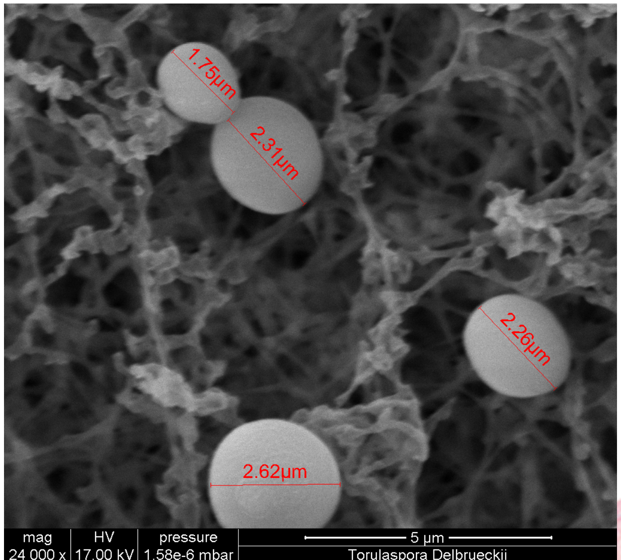
Torulaspora delbrueckii:

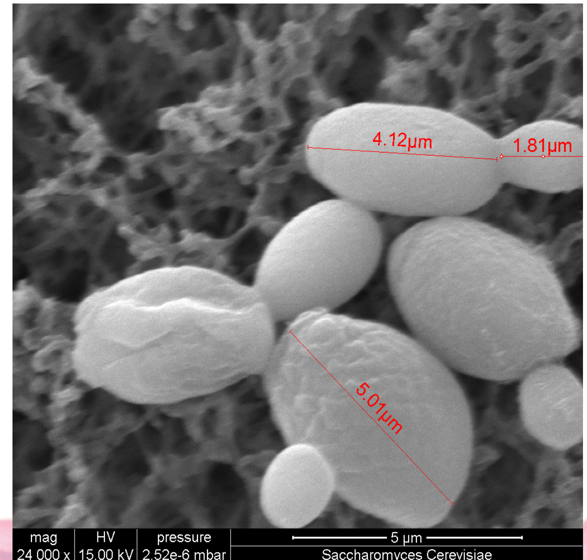
Natural Positive Traits

- ✓ Low VA production
- ✓ No volatile phenols production (POF negative)
- ✓ Low ethanal and ethyl acetate production

Biodiversity

Torulaspora delbrueckii *Saccharomyces cerevisiae*



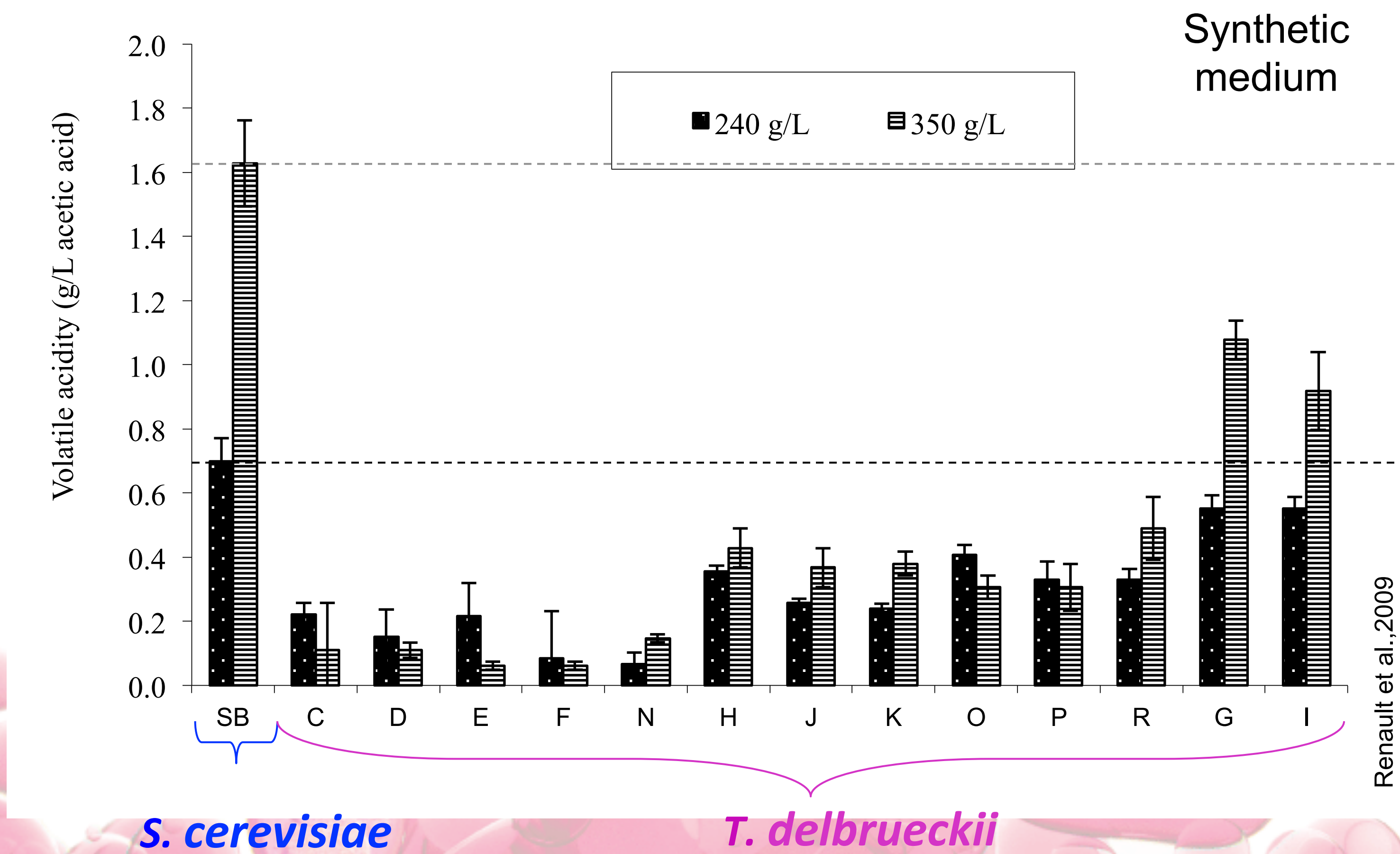


Microscopie électronique à Balayage, grossissement X 24000
Bordeaux Imaging Center - Pôle d'Imagerie Electronique - Université Bordeaux 2.

6 chromosomes **16 chromosomes**

- ✓ **Complex aromatic profile**
- ✓ **Positive and significant impact on mouthfeel**

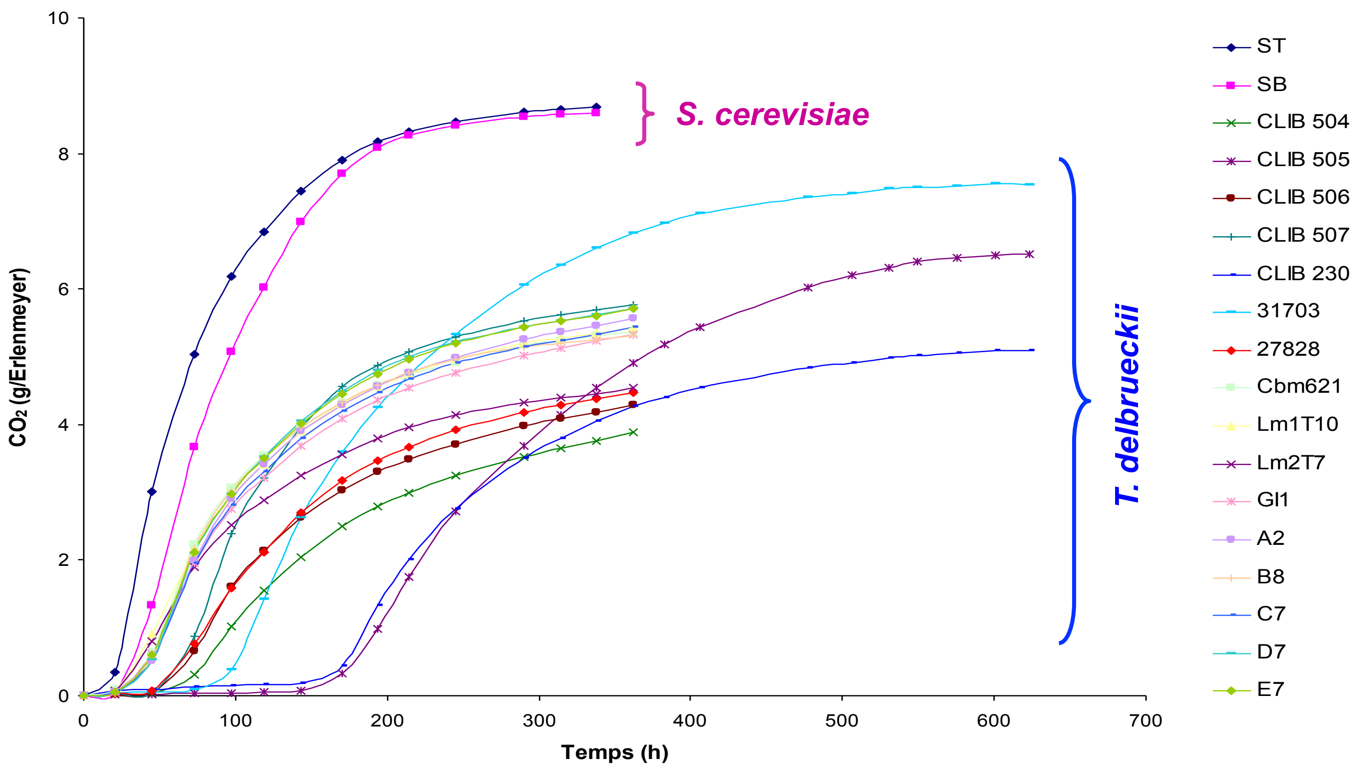
Natural Advantages: Low VA Production



→ Important phenotypic variability: Essential for the selection of a top performing strain

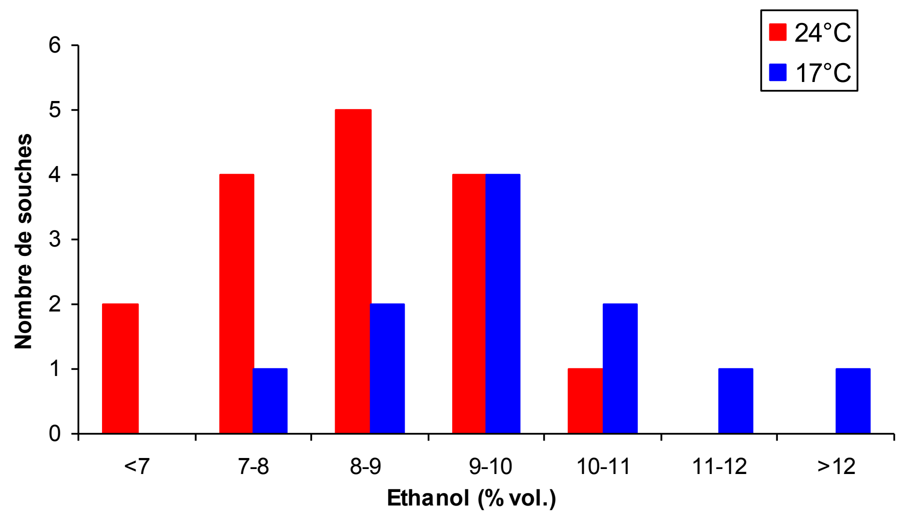
Fermentative Properties

Collection of 30 strains of Td.
Only few with excellent fermentative capacity for a non-*Saccharomyces* but a slower growth rate and fermentation kinetics than *S. cerevisiae*



Fermentative Properties

Td collection of strains: low ethanol production (7- 9 % v/v) hence not a complete fermentation solution



→ **Mixed yeasting necessary:**

- **Ensures complete fermentation**
- **Increases wine complexity and uniqueness**

Mixed Yeasting

Torulaspora delbrueckii and *Saccharomyces cerevisiae*

→ **Sequential inoculation:** ***S. cerevisiae*** **24 – 72 hrs after *Torulapora* introduction:**

Rehydration temperature:
25-30 °C / 77-86 ° F

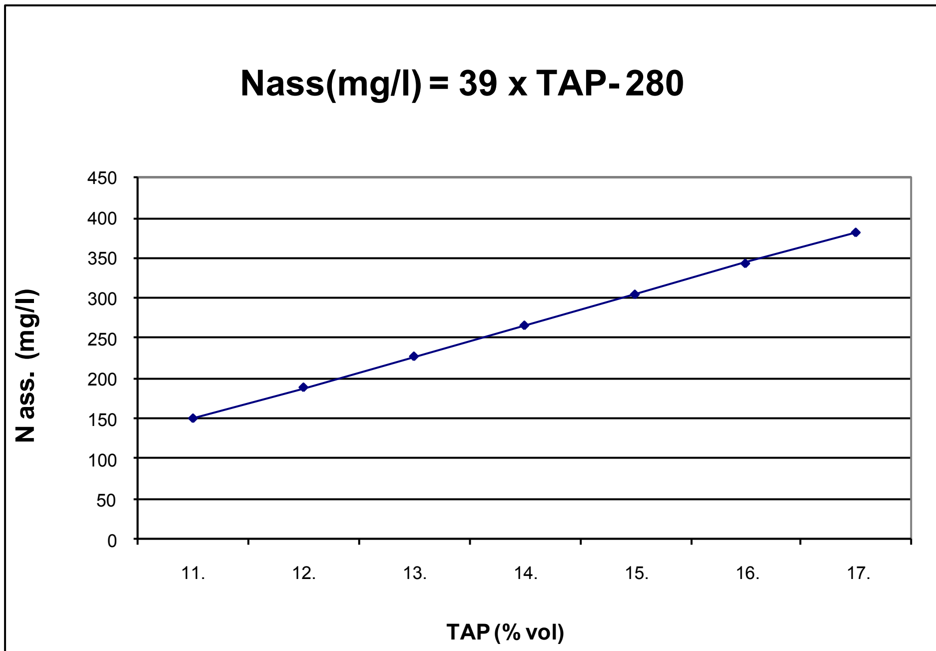
I.300 ppm TD n.sacch.
Important: rehydration in 77-86F water, <u>without nutrient</u>.

II.24 - 72hrs* after TD n.sacch. addition:
S. cerevisiae **addition at 200ppm; rehydration nutrient recommended.**

Complex nutrient additions recommended

*** In the case of sweet wines, our trials show that the best reults are achieved when *S. cerevisiae* is inoculated 5-10 hrs after TD (at 400 ppm).**

Yeast Nutrition Management



Bisson et Butzke, 2000

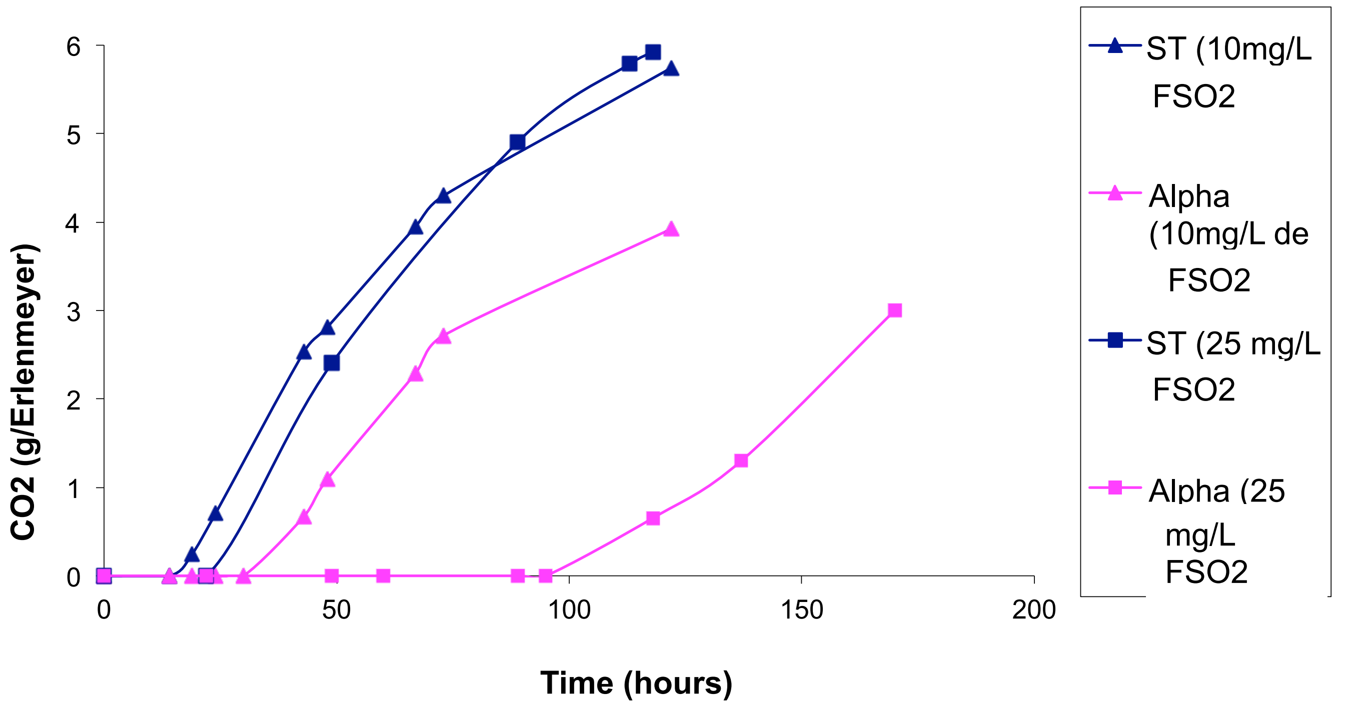
Yeast Nutrition Recommendations

1.Torulaspora
(+ ½ dose of ammonium source of low YAN)

2. **24hrs** (in red wines) **– 72hrs** (in white and rose wines) **after**:
S. cerevisiae. **Rehyration nutrient** recommended

3.24hrs after: ½ dose or entire dose of **complex nutrient**
(A thiamine addition is important to ensure the implantation and the activity of *S. cerevisiae*)

SO2 Effect



SO2 effect at 10 and 25 ppm SO2 in a synthetic medium at 75F

→ The higher the SO2 concentration the longer the lag phase.

→ Alpha is resistant (good viability) and can start fermenting in musts with high [SO2]. There is a variability in *Torulaspora* strains!

Sauvignon Blanc
Pessac Leognan 2010

Must Analyses:

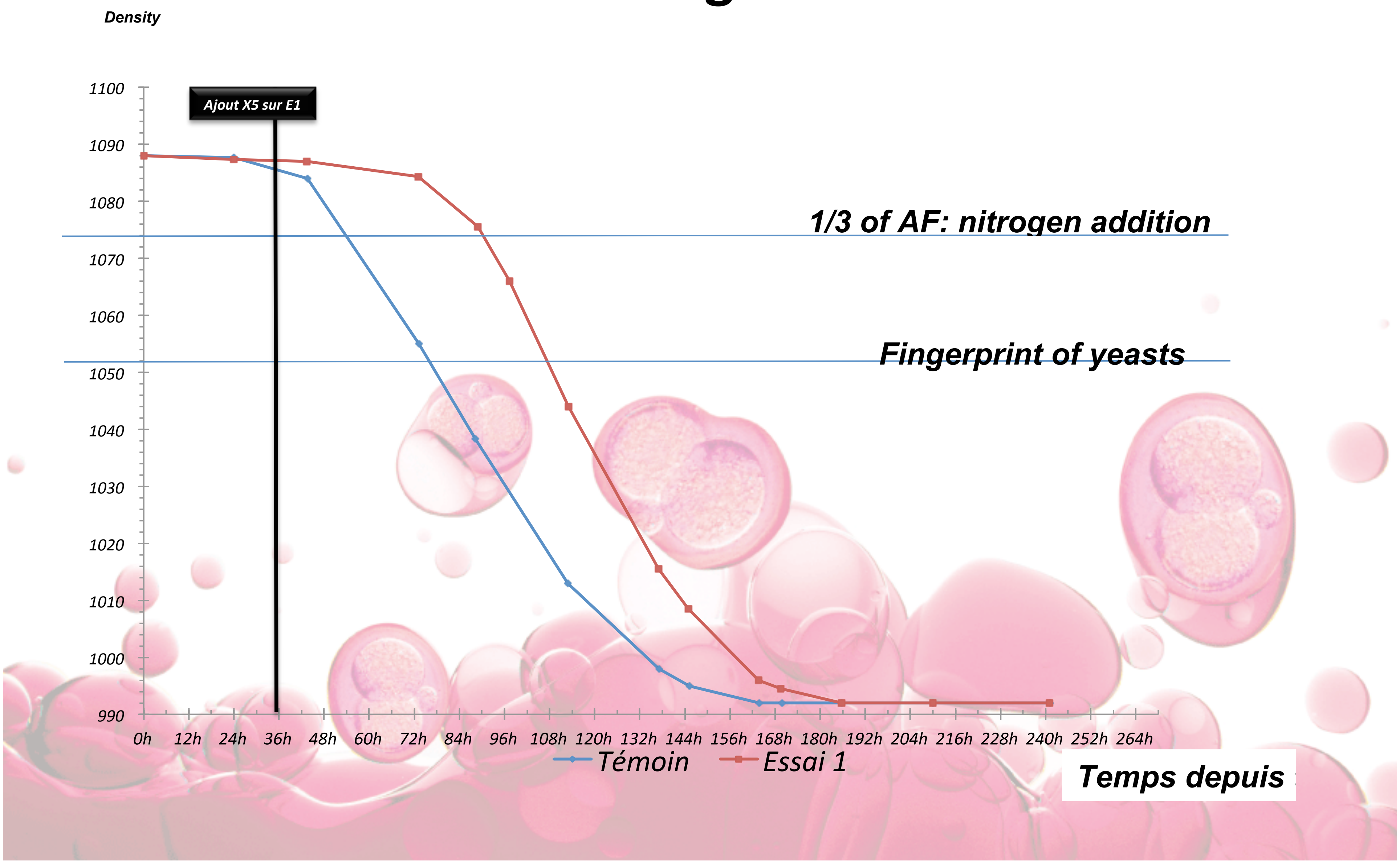
Sugar (g/l): 216
Pot. Alcohol (% v/v): 12.77
TA (g/l): 6,04
MA (g/l): 3,5
pH: 3,25
Free SO2 (mg/l): 12
Active SO2 (mg/l): 0,62
Total SO2 (mg/l): 51
YAN (mg/l): 179 (adjusted to 218 mg/L 1/3 through fermentation)
Turbidity (NTU): 190

Trial tanks:

T : Control Zymaflore X5
E1 : Zymaflore Alpha + X5

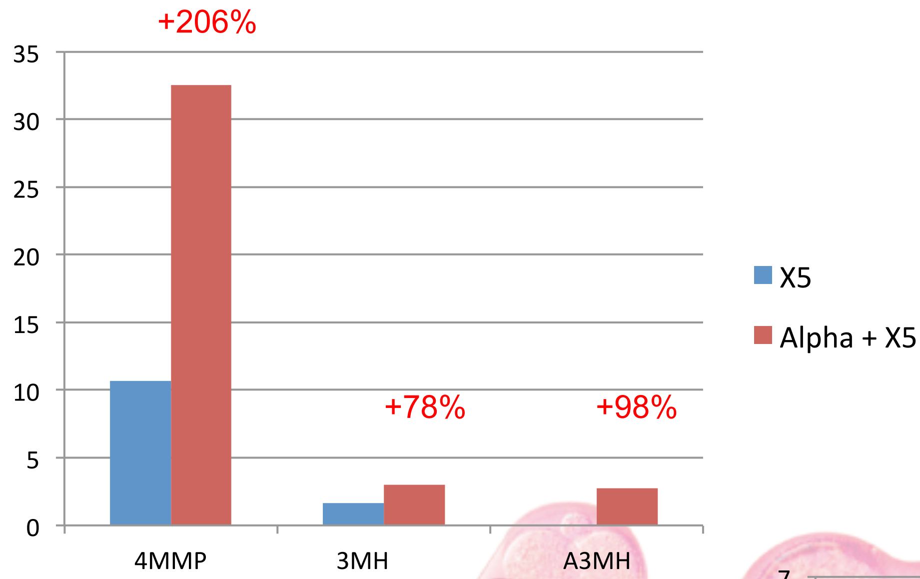
Sauvignon Blanc

Pessac Leognan 2010

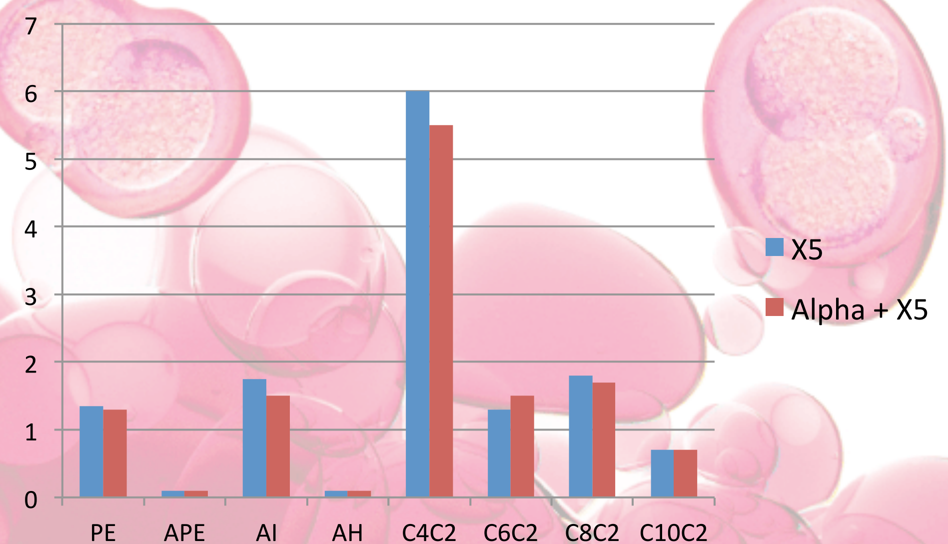


Sauvignon Blanc

Pessac Leognan 2010

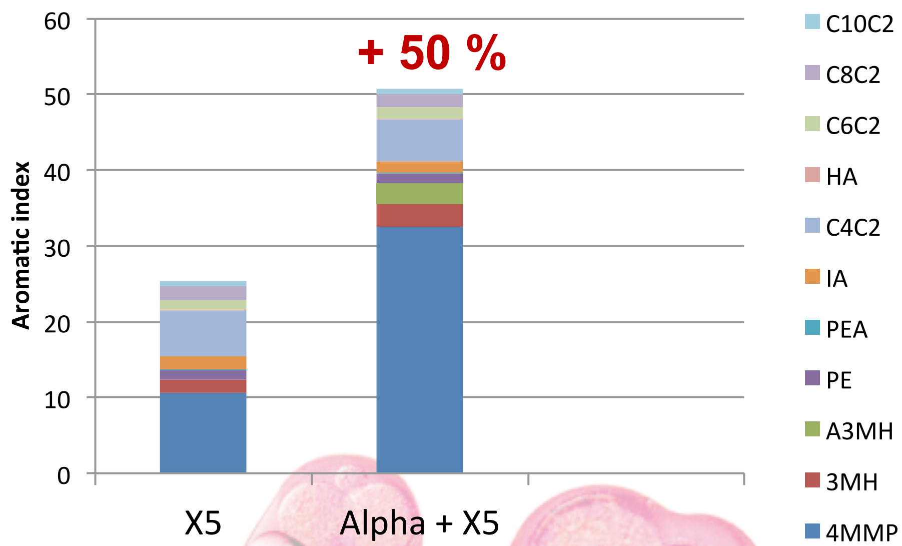


More *complex* aromatic perception (synergistic effects between flavors) and more aromatic *volume*.



Sauvignon Blanc

Pessac Leognan 2010



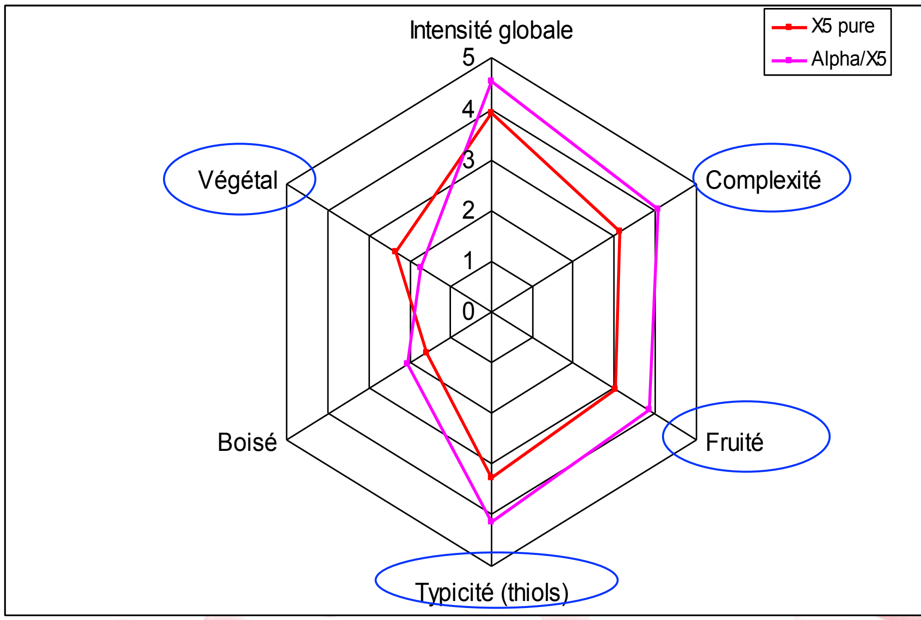
Increased aromatic intensity due to higher expression of thiols

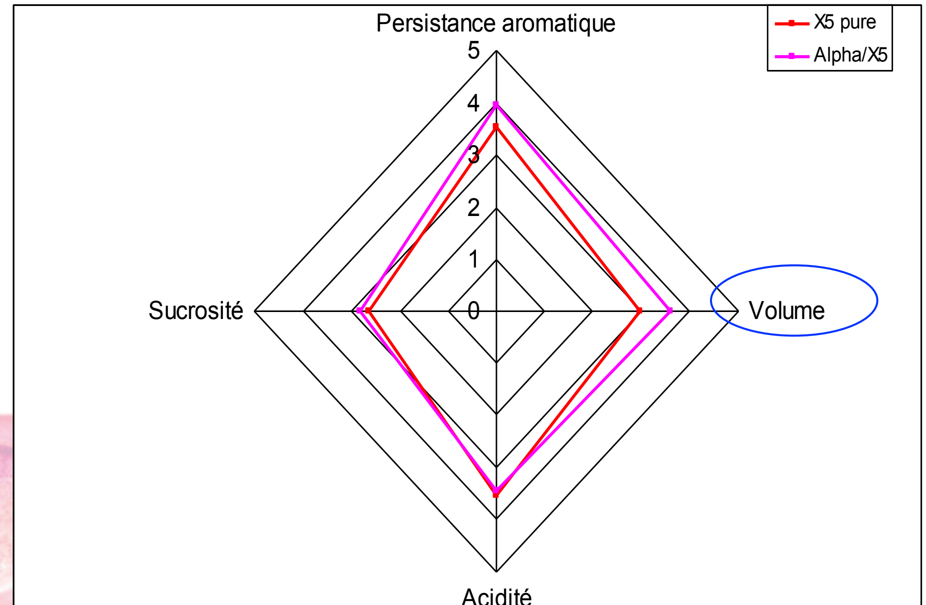
	X5	Alpha + X5
RS (g/l)	**0.1**	**0.1**
Alc (% v/v)	**13**	**13.05**
TA (g/l H2SO4)	**4.45**	**4.5**
Malic acid (g/l)	**2.9**	**2.9**
pH	**3.23**	**3.22**
Free SO2 (mg/l)	**1**	**1**
Active SO2 (mg/l)	**0.05**	**0.06**
VA (g/L H2SO4)	**0.18**	**0.16**

Sauvignon Blanc

Pessac Leognan 2010

- Descriptive analysis (22 member tasting panel) (ISVV, Bordeaux)





Wine Alpha/X5:

Significant difference:
+ More Complexity
+ More Fruitiness
+ More Thiols
- Less Vegetal

Wine Alpha/X5:

Significant difference:
+ More Volume

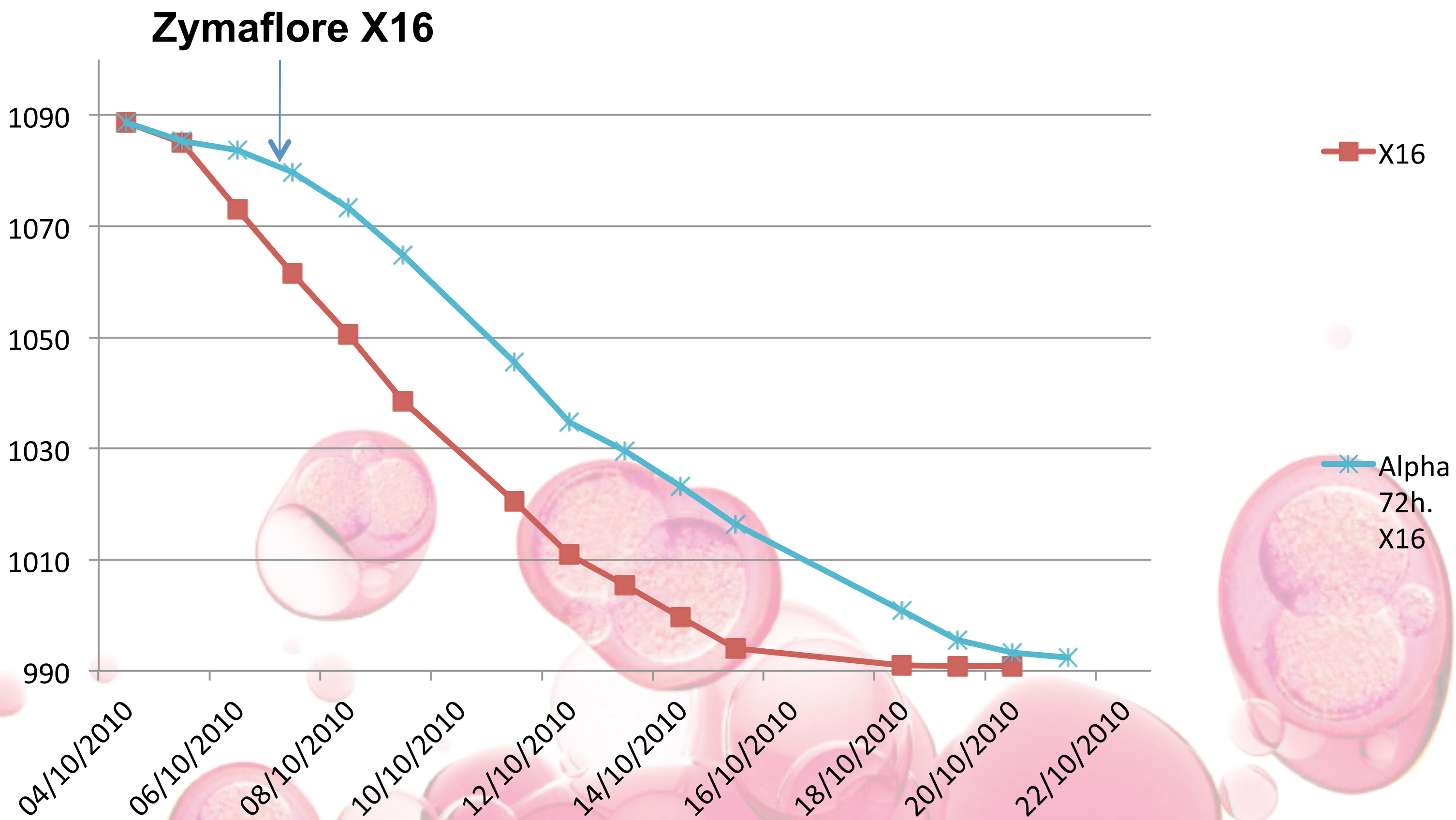
Rosé Merlot 2010

- Lafazym CL 30 ppm
- Turbidity: 80 NTU
- AF Temperature: 61-68 °F
- Initial YAN: 112 ppm
- Nitrogen correction: 200 ppm Thiazote after X16 addition + 200 ppm Nutristart after 1/3 Fermentation

	X16 (200 ppm)	Alpha (300 ppm) + X16 (200ppm) (72hrs after Alpha)
Alcohol % vol.	12,5	12,5
TA g/L	5,2	5,1
VA g/L H2SO4	0,13	0,17
AF duration (days)	15	18

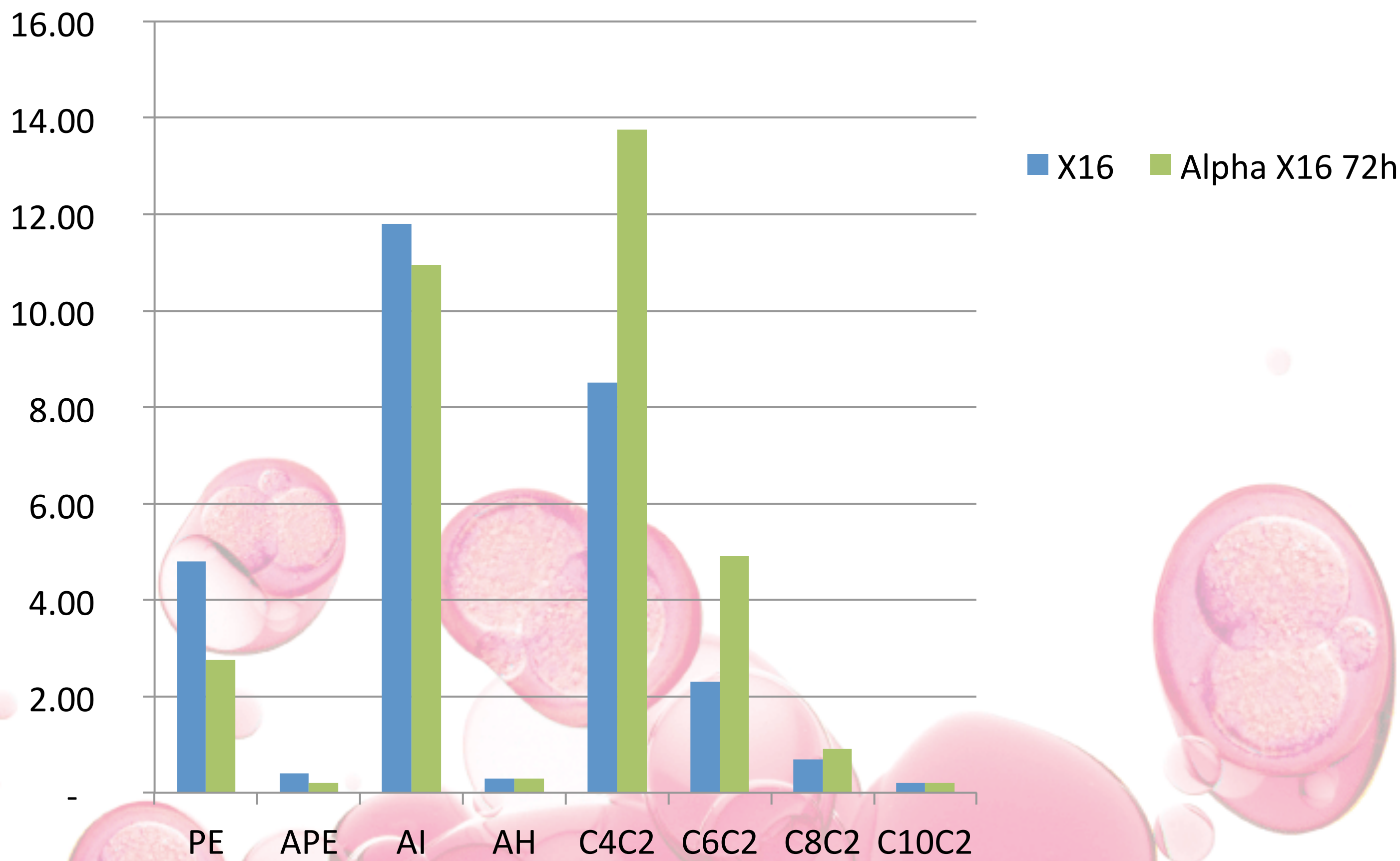
Rosé Merlot 2010

Entre deux mers



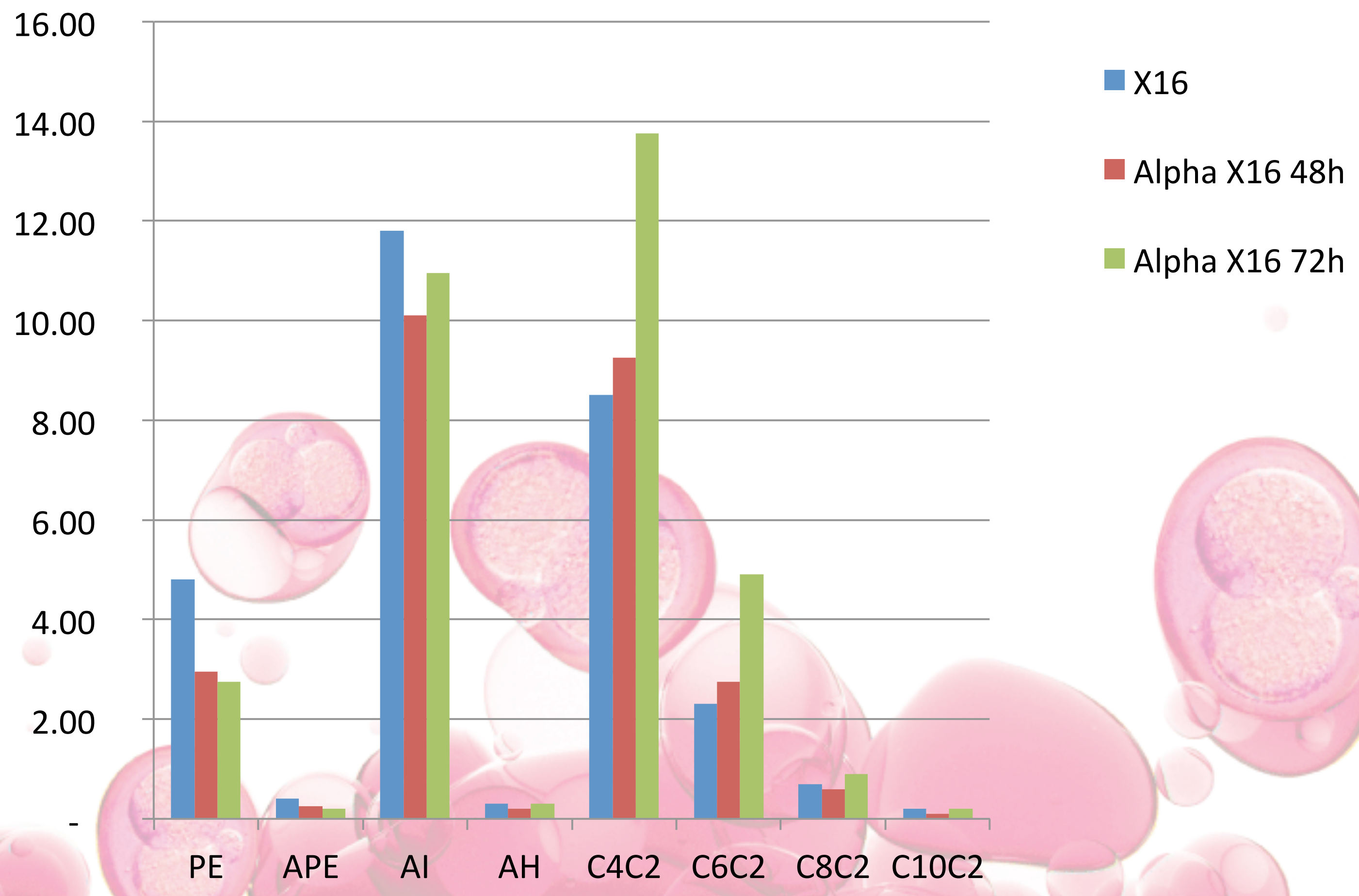
Rosé Merlot 2010

Entre deux mers



Rosé Merlot 2010

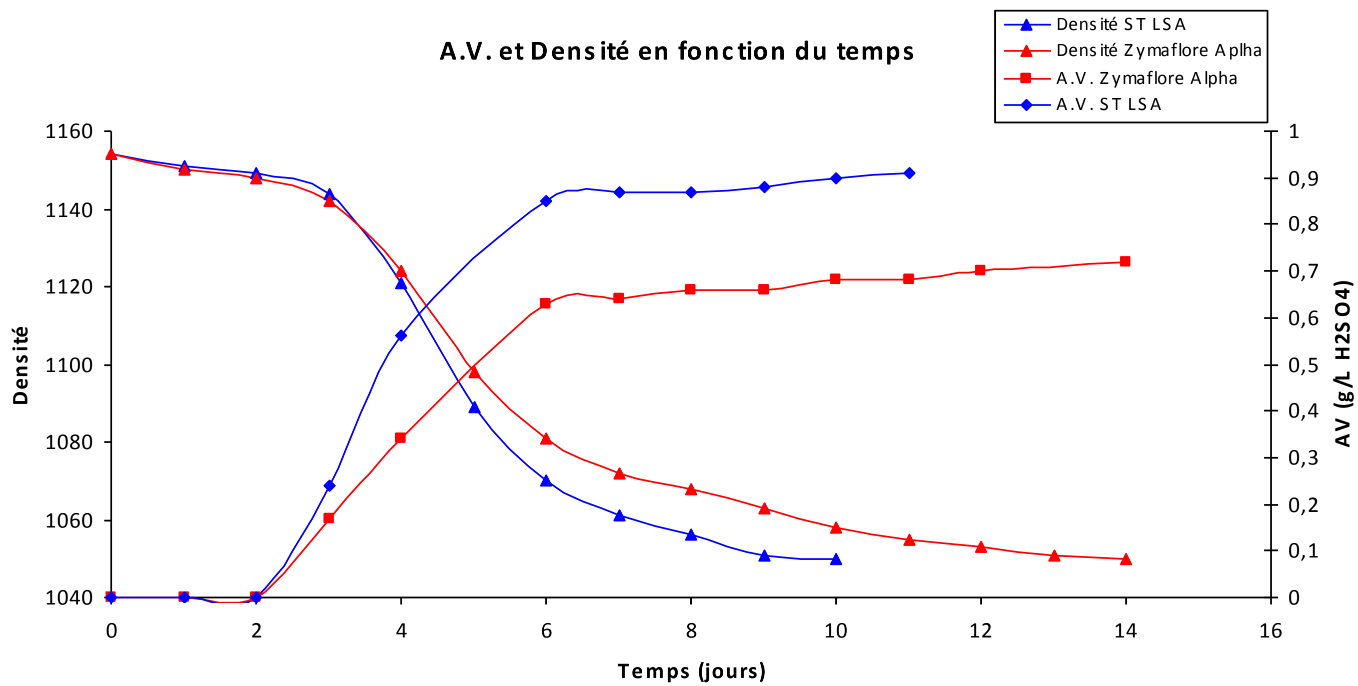
Entre deux mers



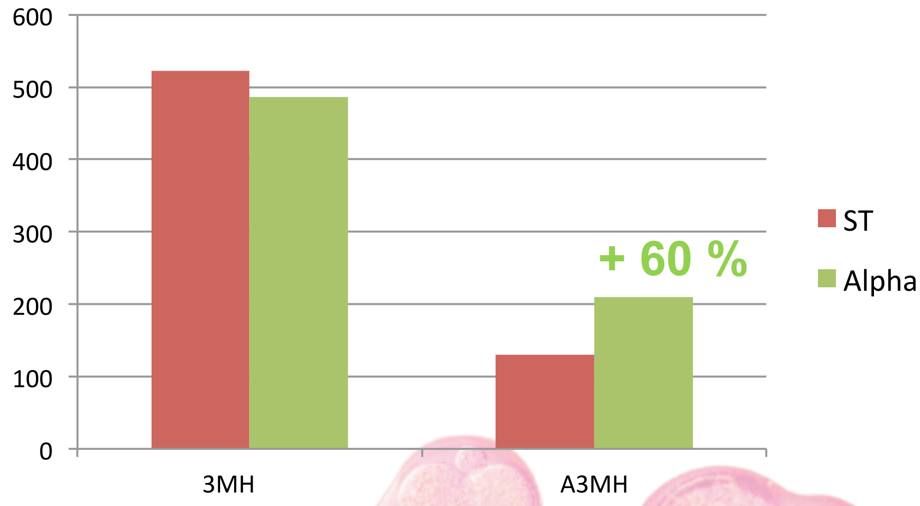
Sauternes 2010

		Zymaflore ST	Zymaflore Alpha
Alcohol	% Vol.	14,2	13,93
Sugars	g/L	145	148
TA	g/L H2SO4	3,88	3,81
VA	**g/L H2SO4**	**1**	**0,73**
pH		3,85	3,83
Free SO2	mg/L	33	35
Total SO2	mg/L	183	179

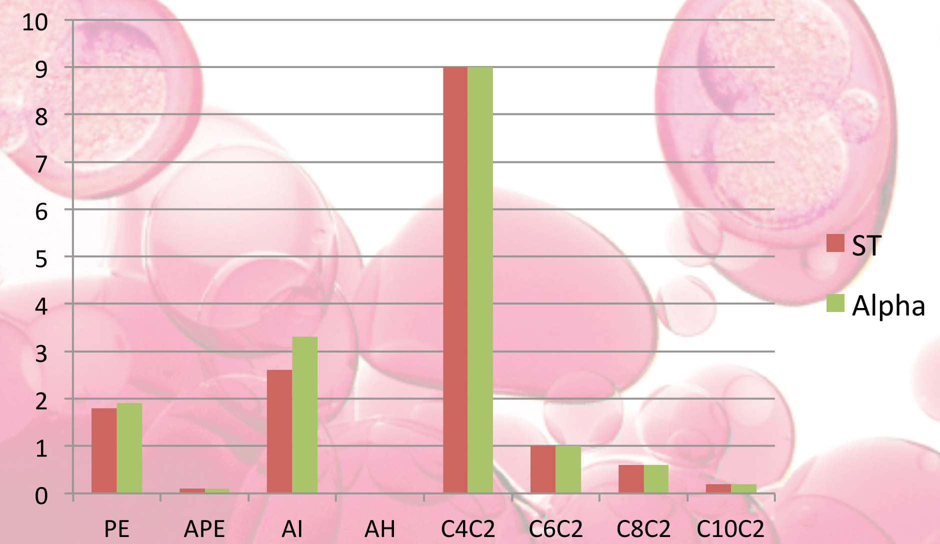
27 % VA reduction



Sauternes 2010

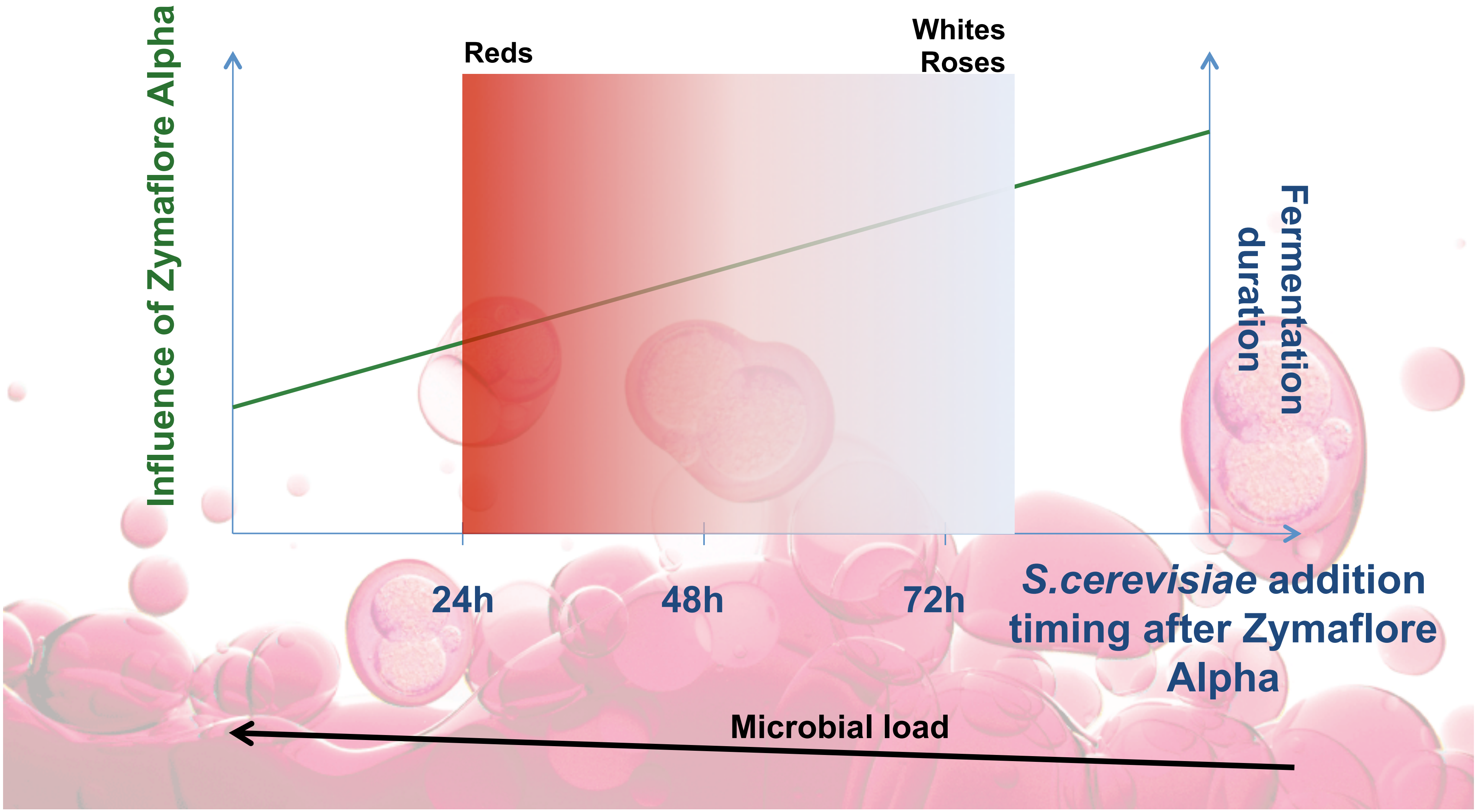


Zymaflore Alpha wine perceived as more complex, fresher and fruitier compared to control wine



Sequential Inoculation Protocol

Schematic Representation



Winemaker Testimonial

We are making the best Bonarda in all Argentina! We have added 300ppm Alpha to our high end Bonarda on the Thursday. After 3 days of cold soak at theoretically 46F, we have added RX60 after having raised the temperature.
We have a control with the exact same grapes and same protocol (same Optizym enzyme,etc.), inoculated with RX60 only.

The tasting is impressive:
-Nose: very floral (rose, peony), fresh strawberries (impressive), blackberry but still very fresh.
-Mouth: an incredible volume, much more than in the control.

The control is at 11.5 Beaume and the Alpha tank is at 7.5 Beaume. It is much rounder. Hard to believe that both tanks come from the same grapes!

Torulaspora delbrueckii: summary

- **Mouthfeel and volume** increase.
- Higher aromatic **complexity** on all varieties – differentiated organoleptic profile.
- **VA decrease**, especially in high Brix grapes and sweet wines.
- Recreates conditions of a **native/ecological fermentation**, in a more controlled way and without negative aromatic impact by indigenous microflora.

Thank You for your Attention!

Let's taste some Virginia wines made with TD

For more information: charlotte.gourraud@laffort.com
and booth #925!