# Yeast Nutrition Management

**Belgrade 2019**

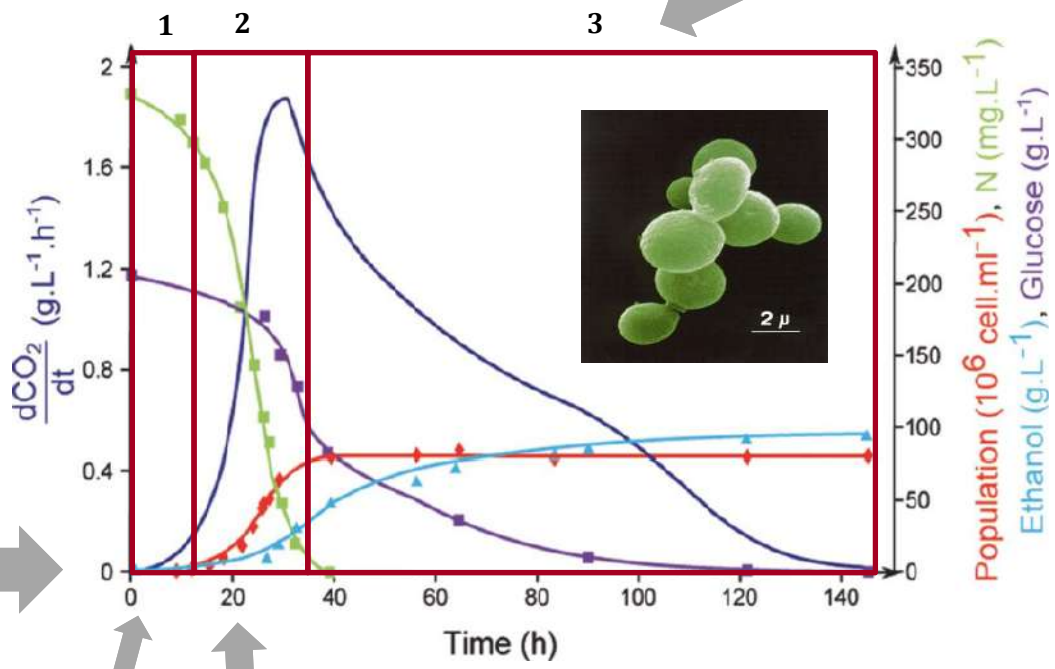
*Edouard LORDAT*

# Yeast Nutrition management to reveal aromas : New research

## Key points for a successful fermentation & an optimum wine quality

# Enological conditions: hostiles for yeast

**Ethanol stress (up to 15 % vol)**



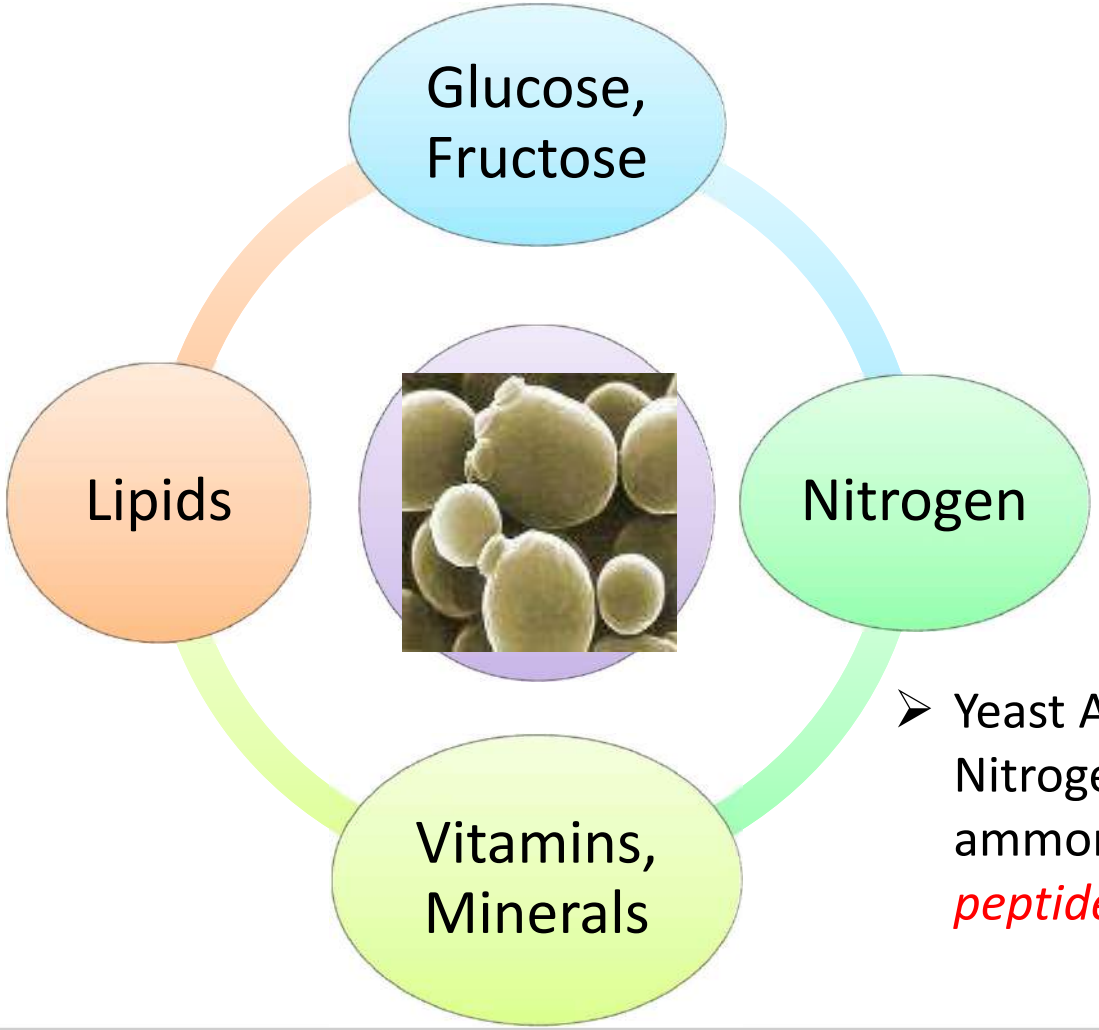

**Sulfites**
**Anaerobiosis**

**Acid stress**
pH (3.0 - 3.6)

**Osmotic stress**
glucose - fructose
180 - 260 g/L

**Starvation stress :**
(nutritional deficiencies:
N, lipids, vitamins)

# *Saccharomyces cerevisiae* : Nutritional needs to perform the alcoholic fermentation




- Yeast Assimilable Nitrogen : YAN = ammonium + aa + *peptides*

# Yeast nutrition management in alcoholic fermentation :

## Evolution over the years

→ **First objectives in the 90's:**
**limit the risk of stuck or sluggish fermentation**

- **Nitrogen (YAN) is a key compound:**
  **- defines biomass (Xmax)**
  **- direct impact on the fermentation rate (Vmax)**
  **- on the yeast fermentative activity**

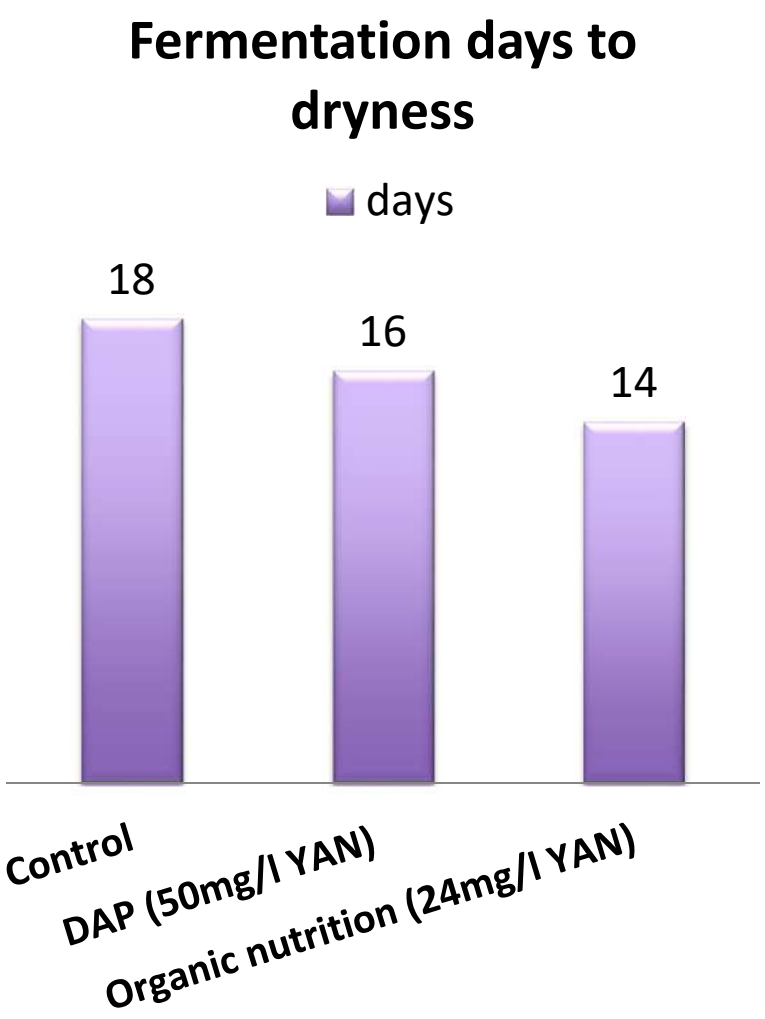
# Yeast nutrition management in alcoholic fermentation :
## Evolution over the years

→ **10 years ago : Nitrogen is nitrogen? …NO:**
**YAN « quality » as new focus:**
**importance of nitrogen source**

Organic Nitrogen (aa) better than Mineral N (NH4+) :

- More efficient on fermentation for same YAN added
- Positive impact on **fermentative & varietal aromas**

# Nitrogen source efficiency on fermentation




The Australian Wine Research Institute

Adelaide Hills, 2010
*Chardonnay*

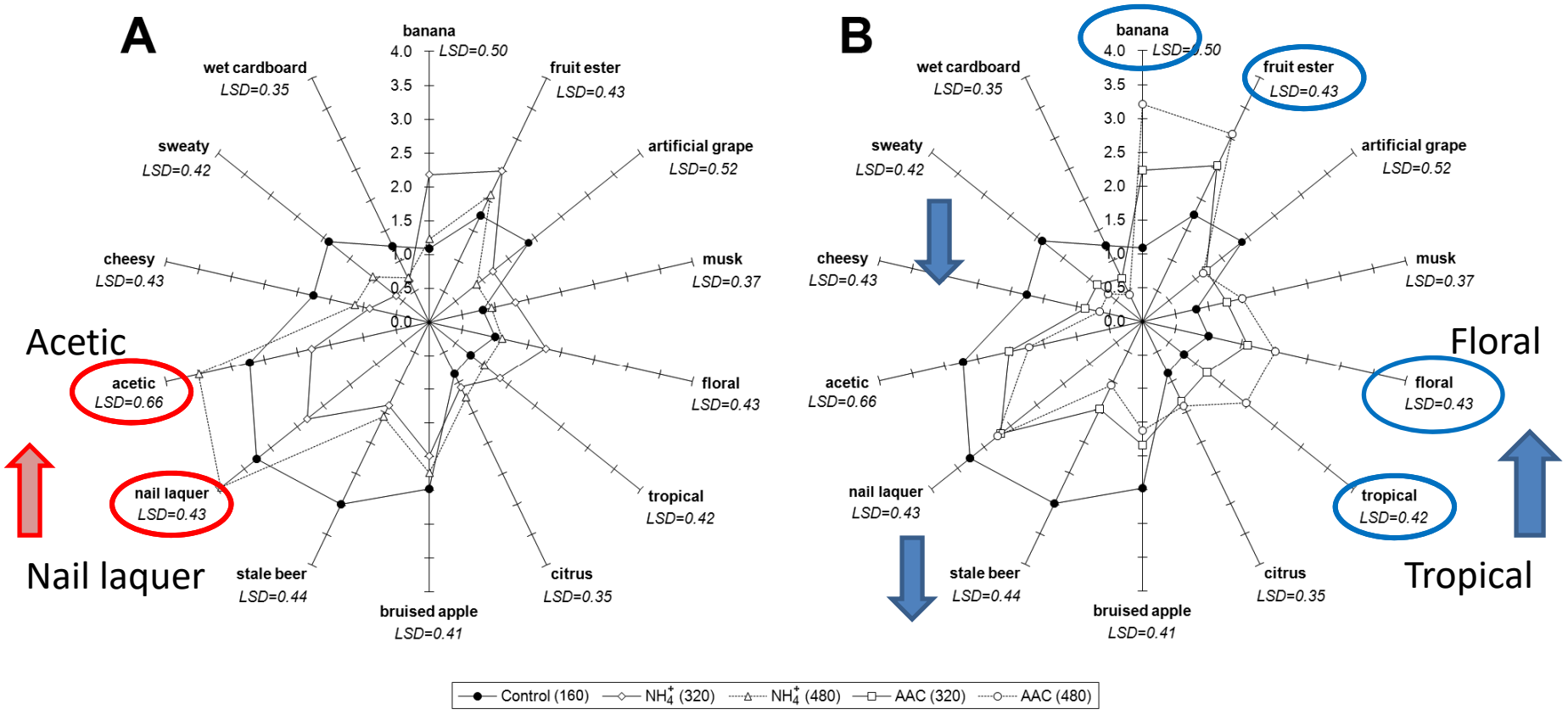
**⇨ Organic nutrition is 2 times more efficient that inorganic nitrogen to complete fermentation.**

# Nitrogen source impacts on wine sensory profile

The Australian Wine Research Institute

+ Ammonium

+ Amino acids




*Henschke, 2010*

# Yeast nutrition management in alcoholic fermentation :
## Evolution over the years

**→ These last 4 years : new knowledge, new understanding**

**Need for a well balanced nutrition:**

1. to assure yeast viability & vitality
2. to optimize yeast aroma biosynthesis and release

Nitrogen source management: YES, but not only:

✓ vitamins, minerals, sterols

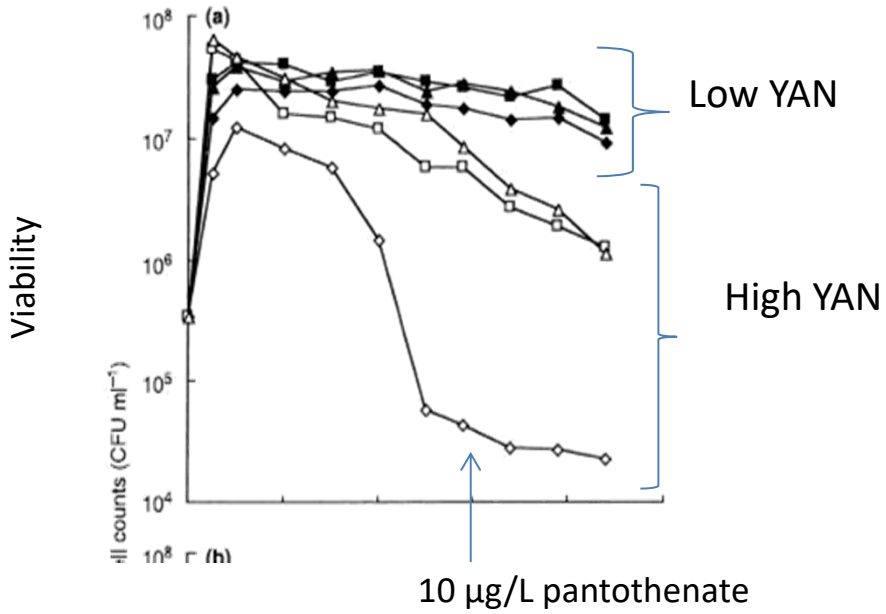
✓ limit the risk of **nutritional imbalances** responsible for :

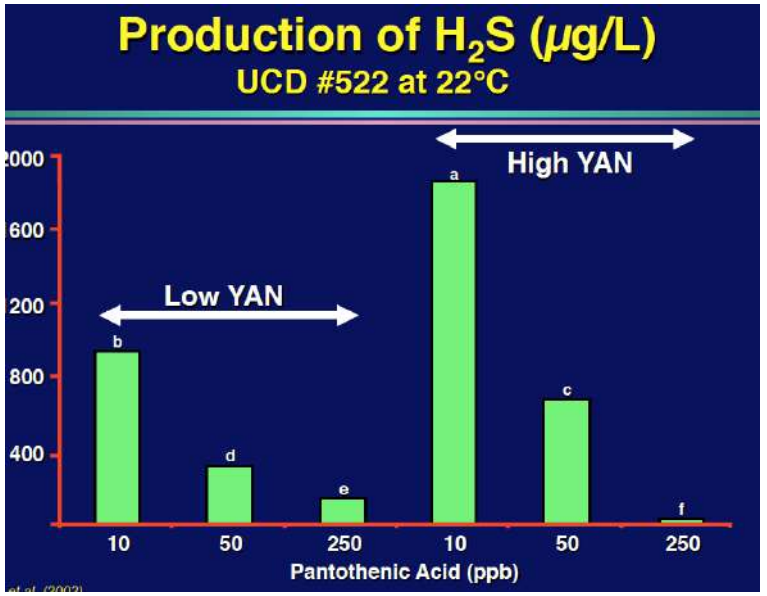
⇨loss of viability, activity

⇨ loss of aroma release

⇨ H2S overproduction

# **Nutritional imbalances :** Impact of Pantothenic acid level on yeast cell viability and H2S production



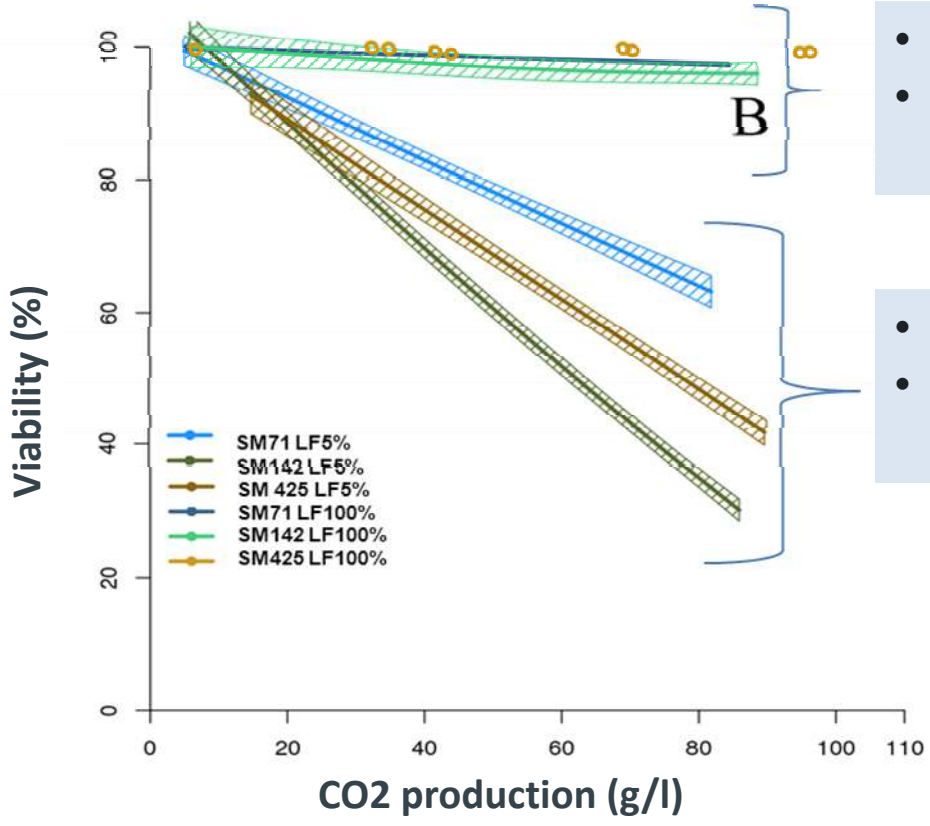




*Wang et al., 2003*

**Pantothenate deficiency**

- ⇨ **Decrease of viability with high YAN content**
- ⇨ **leads to high H2S production, even more with high YAN**

# **Nutritional imbalances :** Impact of lipids level on yeast cell viability




- High lipids content
- 3 levels of nitrogen : from 70 to 425 mg/l YAN)

- Lipids deficiency
- 3 levels of nitrogen : from 70 to 425 mg/l YAN)

*Blondin et Tesnières., 2013, PlosOne : 8, e1645*

**Lipids deficiency** :

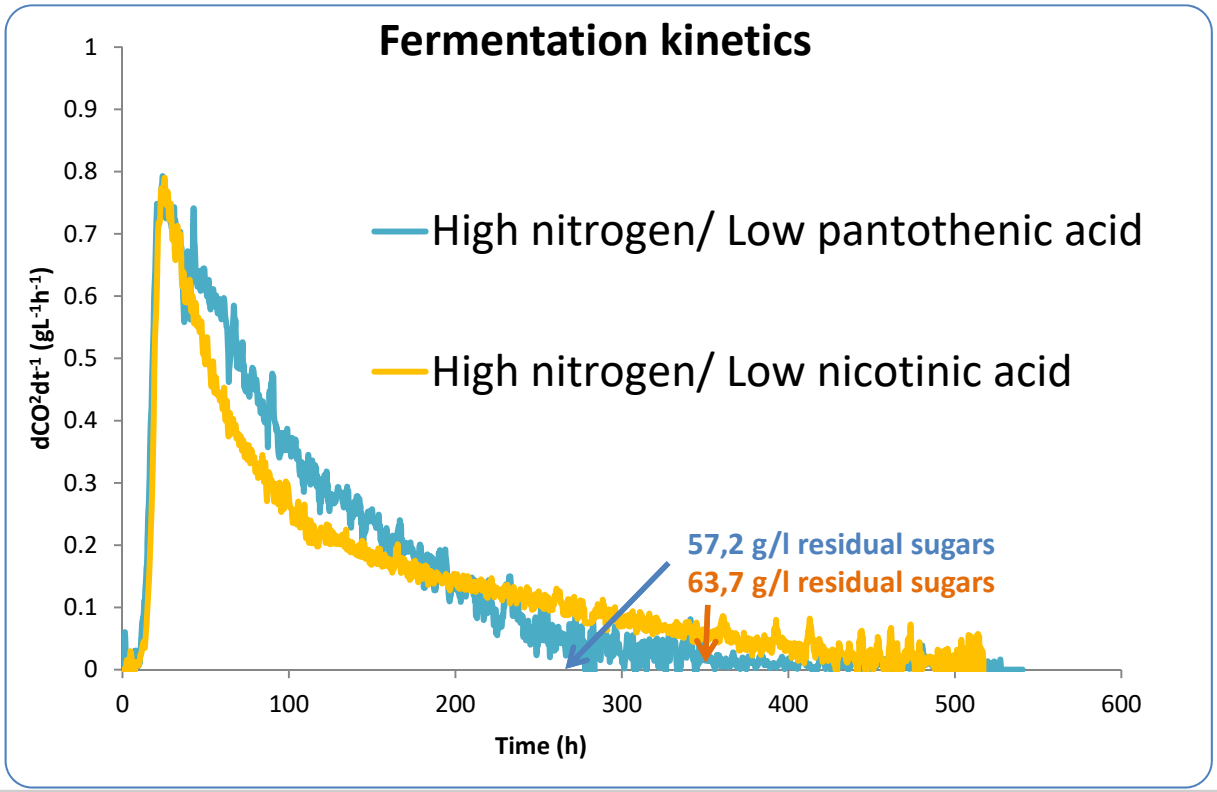
**⇨Mortality rate modulated by nitrogen concentration:**

**⇨ the highest YAN with low lipids, the highest mortality.**

# **Nutritional imbalances :** impact on fermentation kinetics

## High nitrogen with low vitamins content

(pantothenate & nicotinic acid)




***Duc C., 2017***
***(PhD Lallemand – INRA)***



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# Nutrient interactions are key
# Which impact on the aromas?

Maximize & reveal the aromatic potential of the grapes

**Yeast Nutrition & micronutrition = Stimulation of it's metabolism and enzymatic activities**

**Yeast**


**Yeast protection : to take care of yeast vitality and membrane health (transport system..)**

**Grapes : aromatic potential**

# Thiols and Sauvignon blanc wine

- Positive thiols :

  – Beneficial thiols

3-mercaptohexanol 

3-mercaptohexylacetate 

4-mercapto-4-methyl-pentan-2-one 

.....

*Handbook of Enology, vol. 2, 2006*
*Coetzee and du Toit, 2015*

# Where do they originate?
# Release of thiols from grape precursors

Grape Must

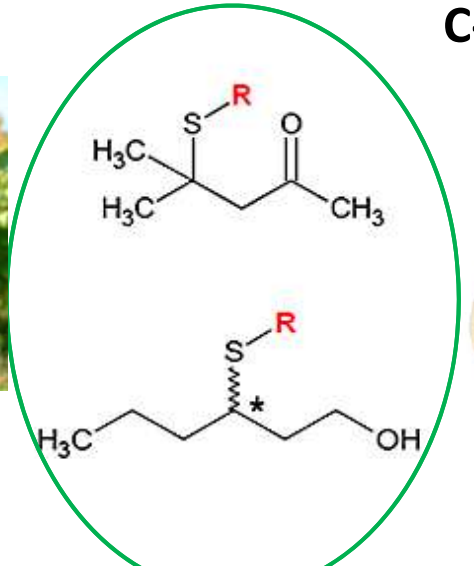
Wine

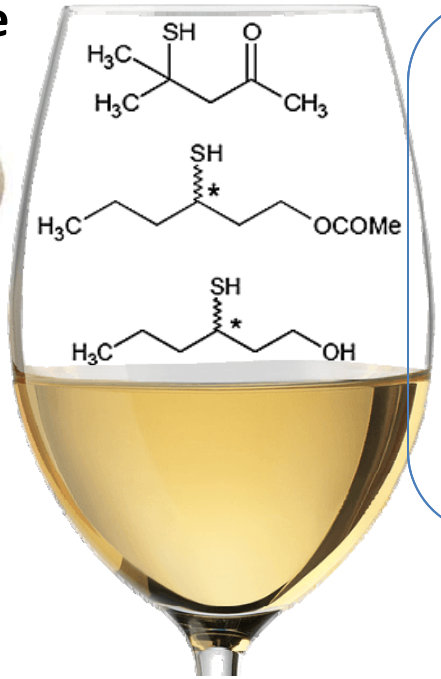
**PRECURSORS**

**Yeast**

**C-S- lyase**

**VARIETAL THIOLS**



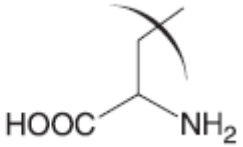




4-mercapto-4-methyl-pentan-2-one **(4MMP)**

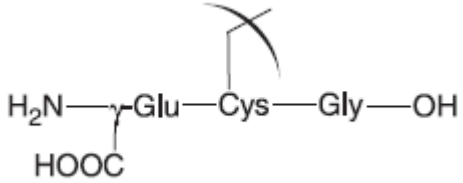
3-mercaptohexyl acetate **(A3-MH)**

3-mercaptohexan-1-ol **(3-MH)**

**R=**




**R=**

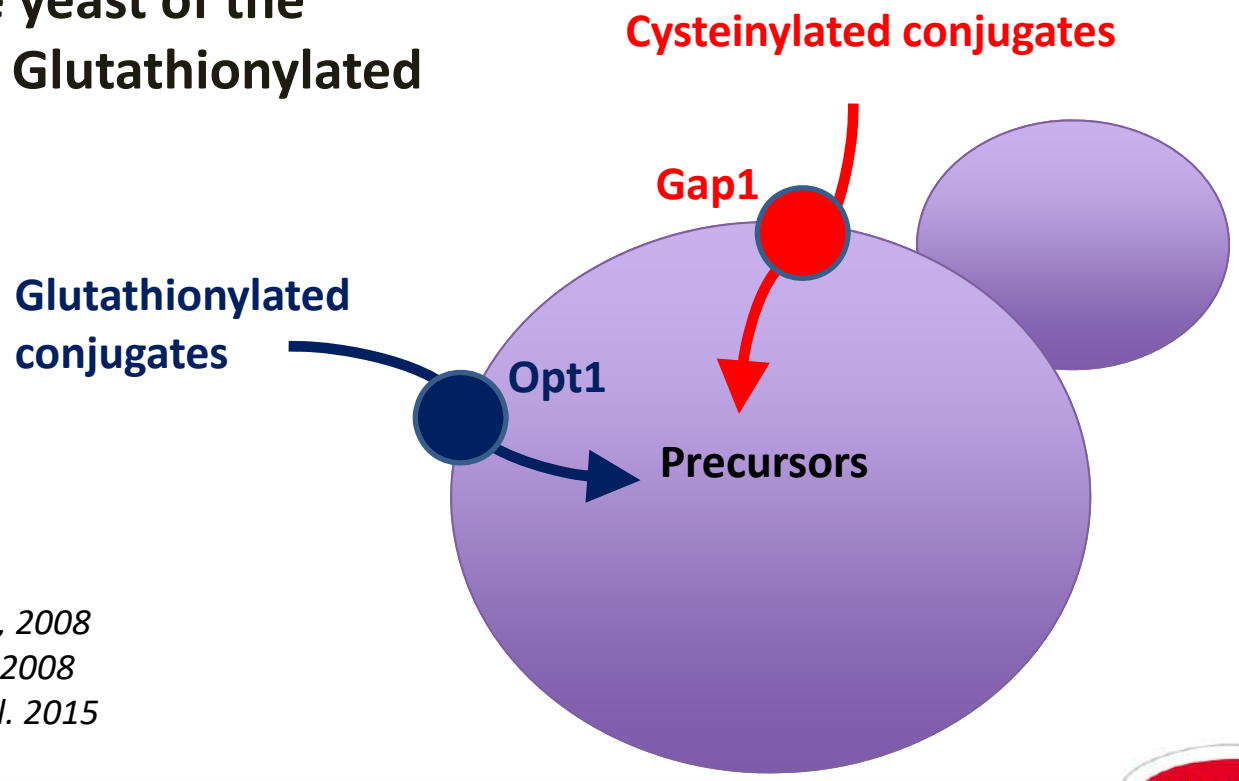



*Cysteinylated* *or* *Glutathionylated*

# Release of thiols from grape precursors
# YEAST : a key role

## Which mechanism? 2 steps :

**1. Uptake into the yeast of the Cysteinylated and Glutathionylated precursors**

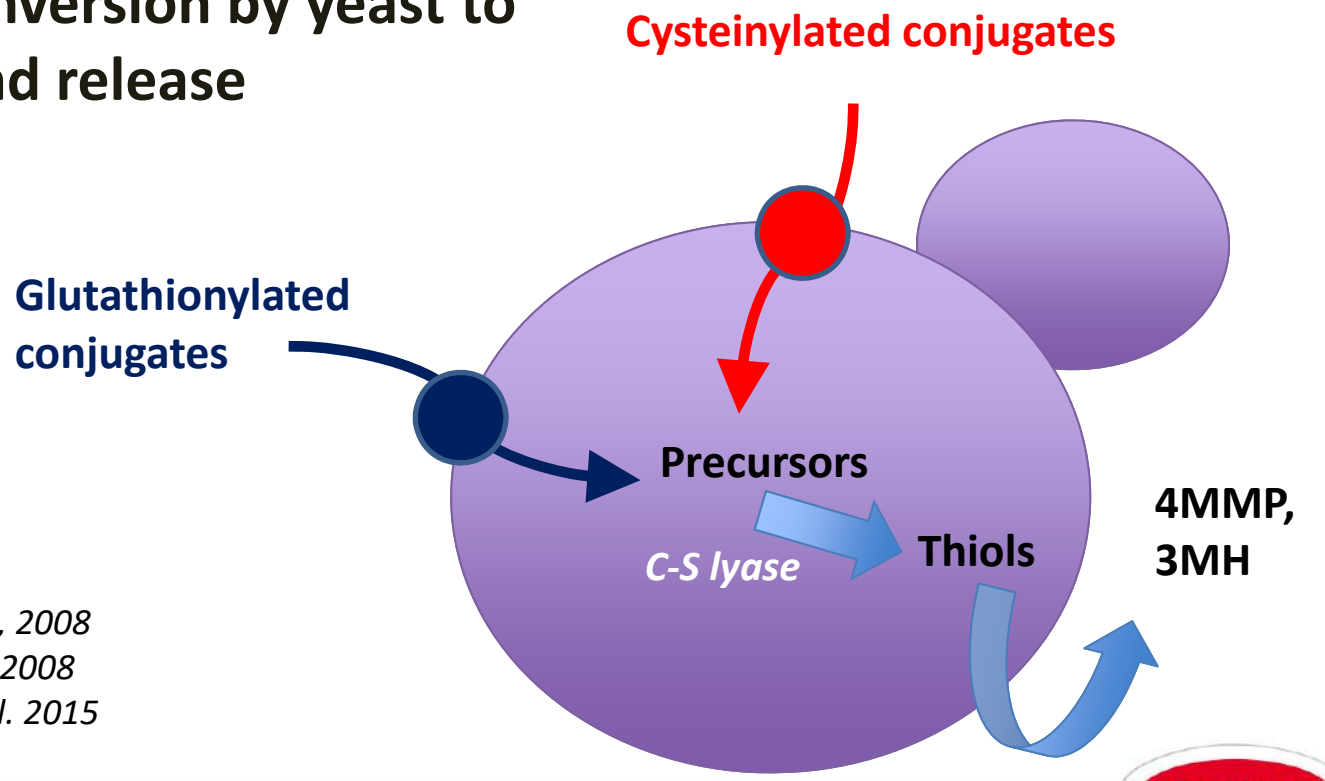



*Subileau et al, 2008*
*Thibon et al., 2008*
*Cordente et al. 2015*

# From grape precursors to volatile thiols
# YEAST : a key role

## Which mechanism? 2 steps :

**2. Precursors conversion by yeast to volatile thiols and release**

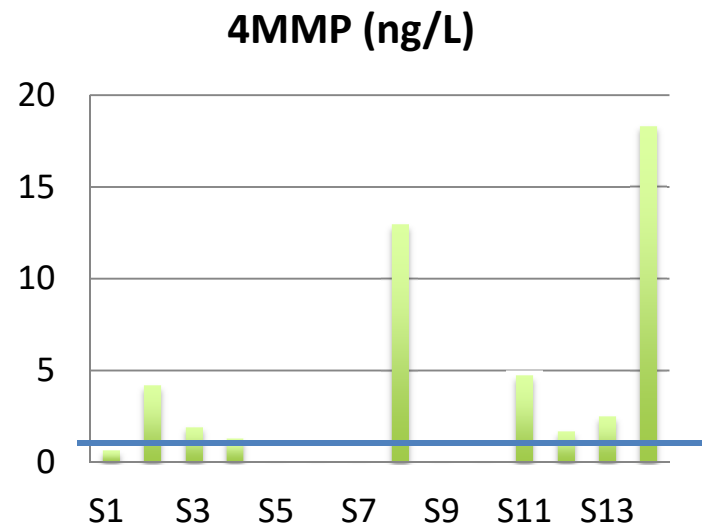


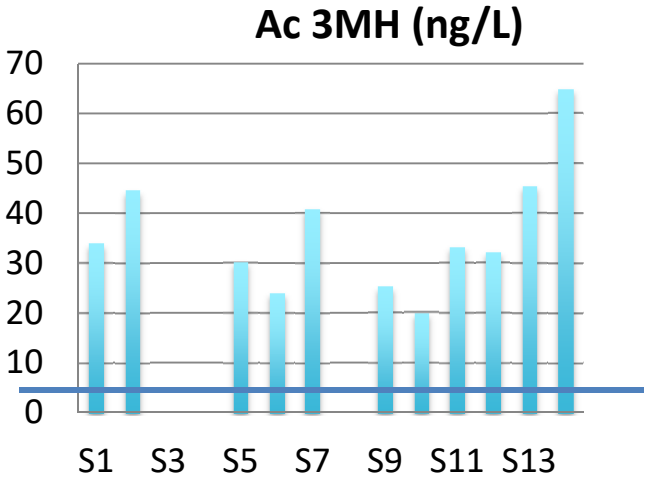

*Subileau et al, 2008*
*Thibon et al., 2008*
*Cordente et al. 2015*

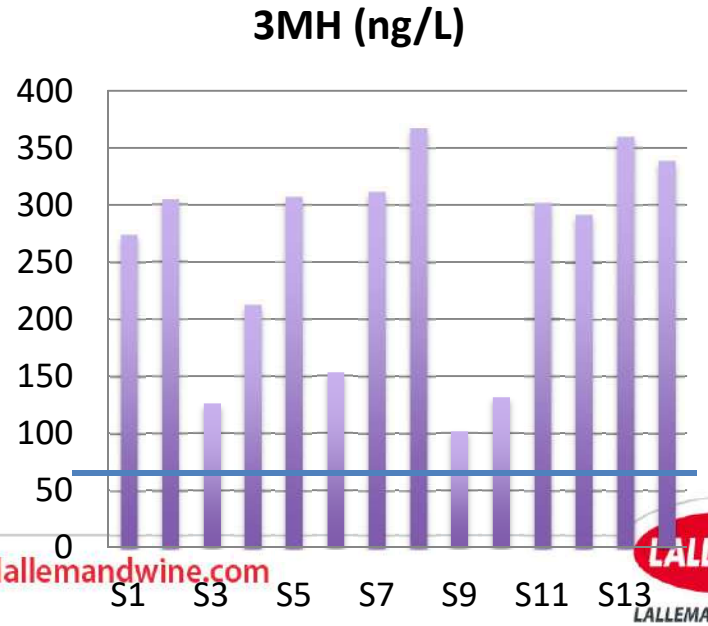
# Release of volatile thiols by yeasts

- **Yeast strain – dependant**

- Correlation with varietal thiols level & sensorial wine profile (*Swiegers et al.*, 2009)




*SVG blanc , Languedoc*
*Lallemand – INRA 2013*



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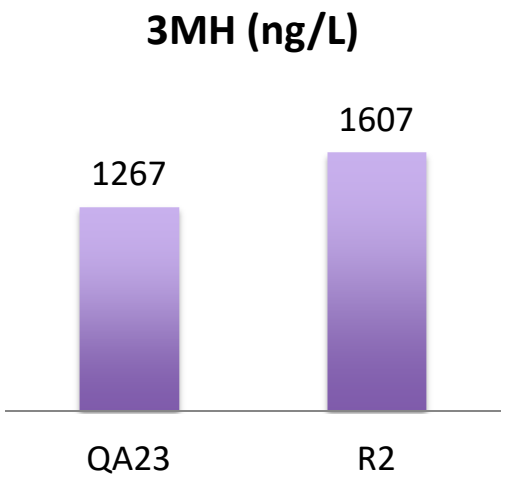
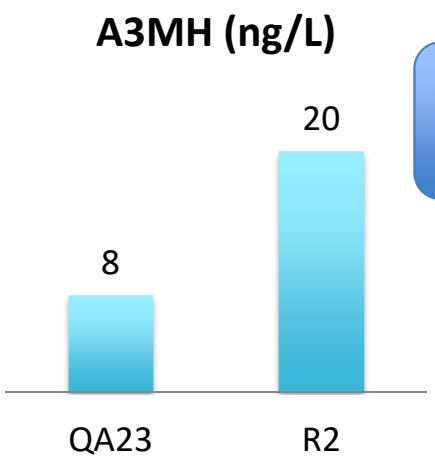
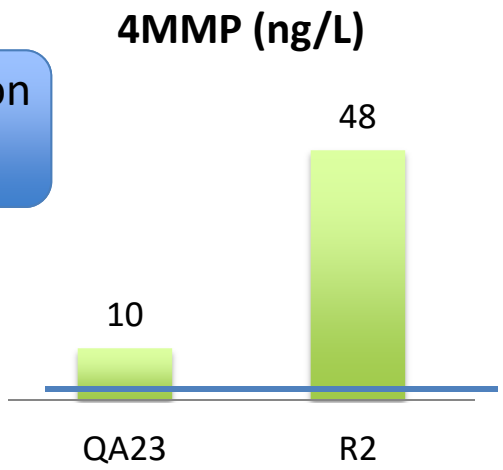
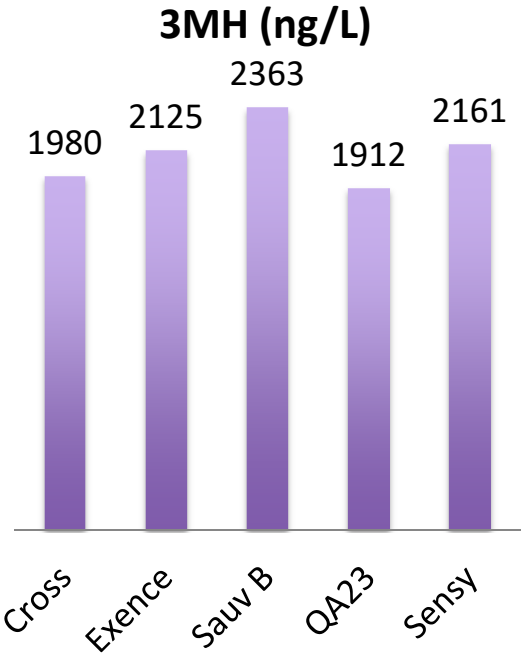
# Release of volatile thiols : → matrix-dependant

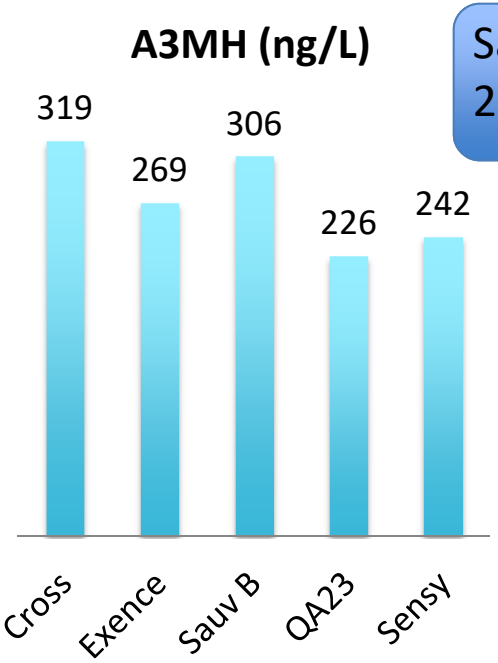


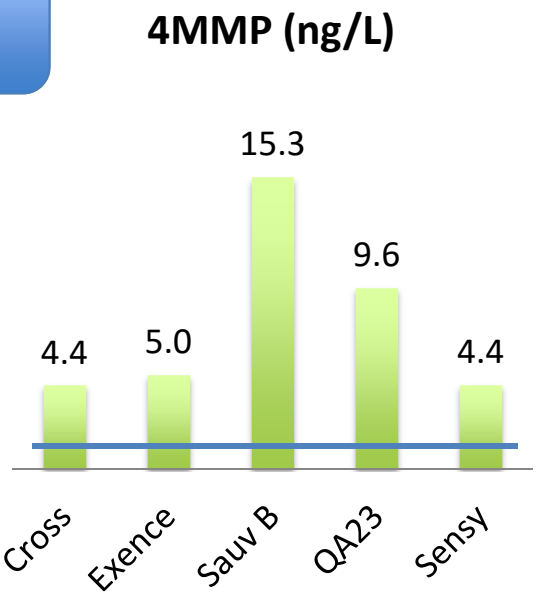

Sauvignon 2016



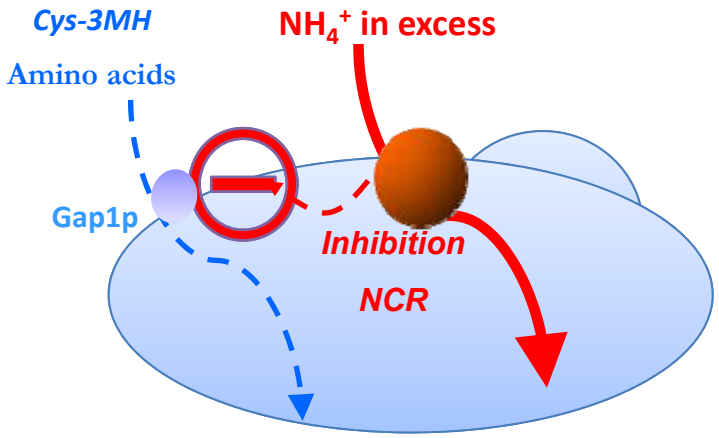



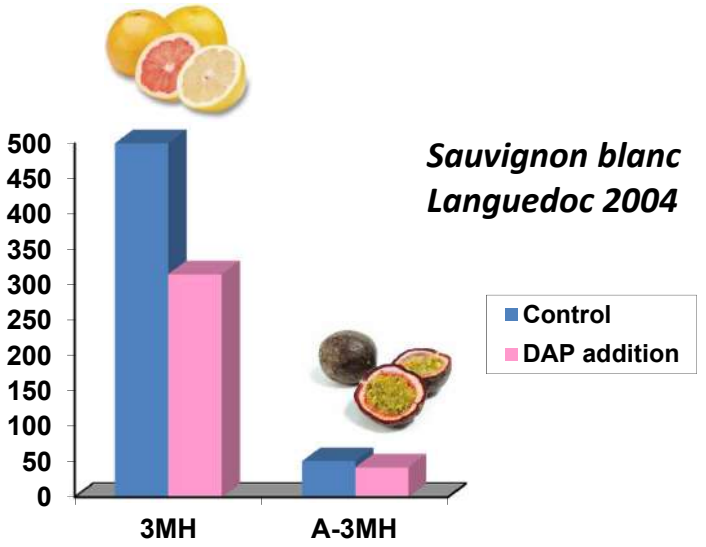



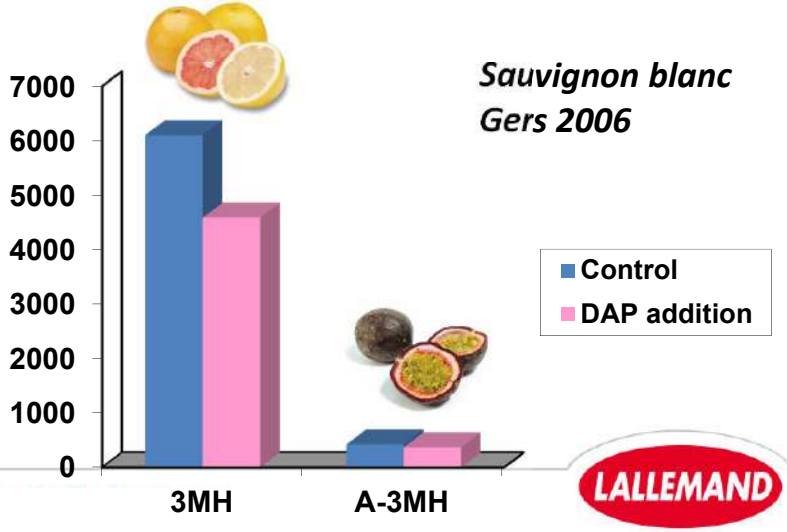

Sauvignon 2017



# Key role of Yeast Nutrition on precursors uptake by yeast & conversion




Release of volatile thiols is governed by **Nitrogen Catabolite repression**:

- In the presence of ammonium, the uptake of amino acids (and Cys-3MH/Cys-4MMP) through Gap1 is repressed

*Subileau & Salmon, FEMS Yeast Res 8 (2008) 771-780*

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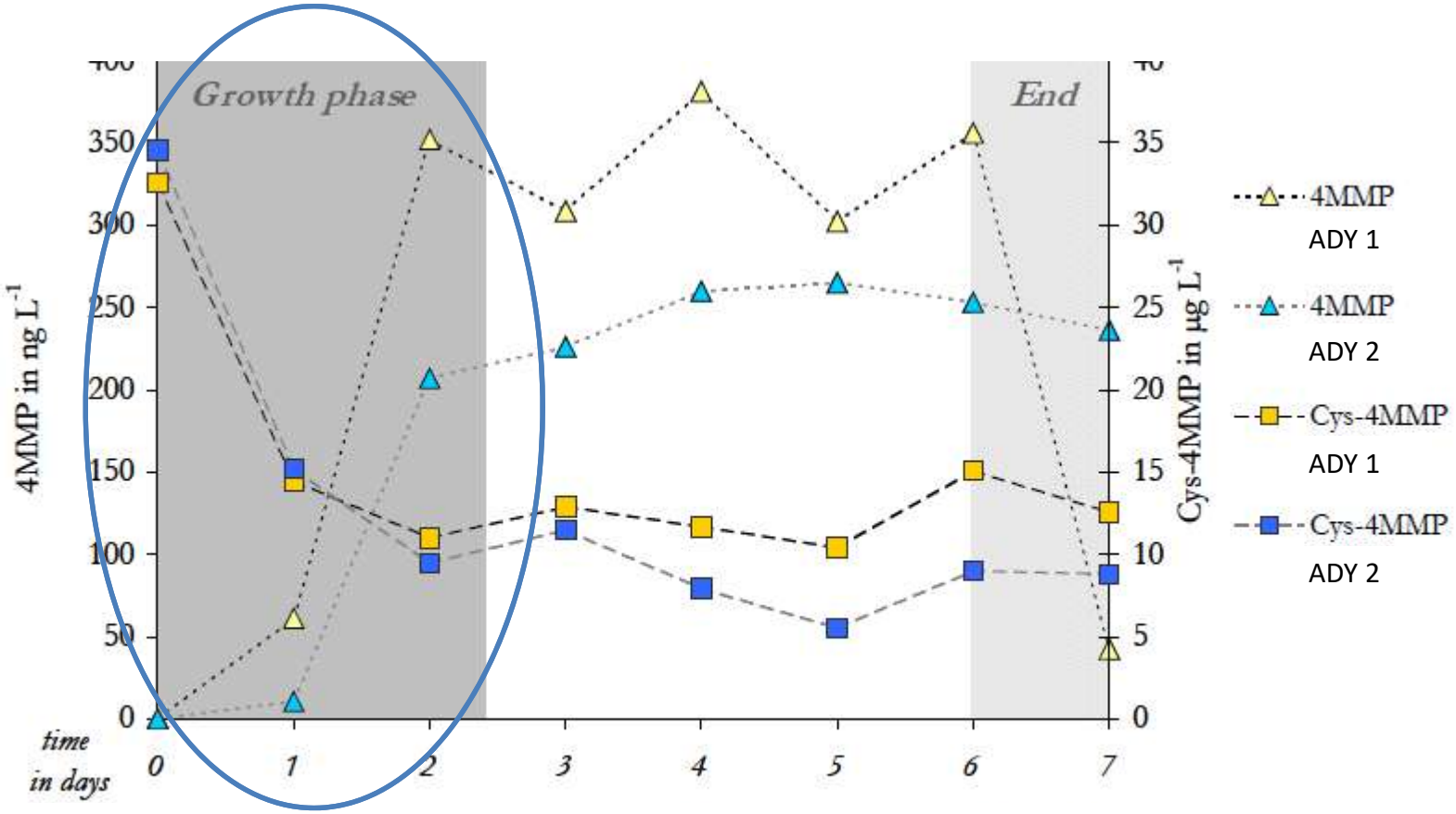
# When are volatile thiols produced?

→ During yeast growth phase:

- Cells multiplication at maximum
- Uptake of the assimilable nitrogen
- High enzymatic activity

# Kinetics of consumption of Cys-4MMP and release of 4-MMP by 2 wine yeasts in SVG blanc




Supplemented with Cys-4MMP (35 µg/L)

*Subileau Thesis (2008)*

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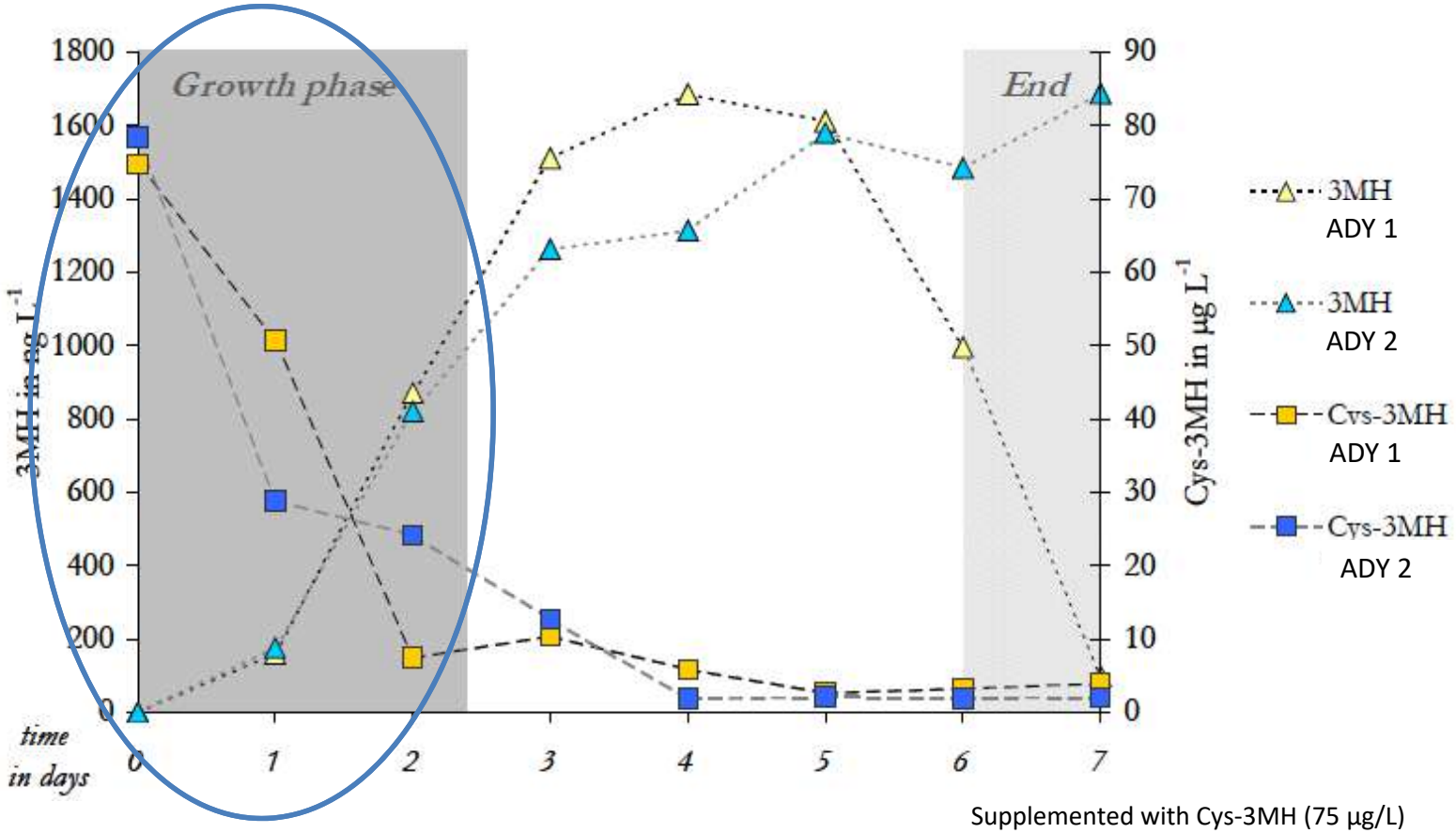
# Kinetics of consumption of Cys-3-MH and release of 3-MH by 2 wine yeasts in SVG blanc




Supplemented with Cys-3MH (75 µg/L)

Volatile thiols are released from their precursors during growth phase

*Subileau Thesis (2008)*

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# Yeast nutrition & micronutrition management

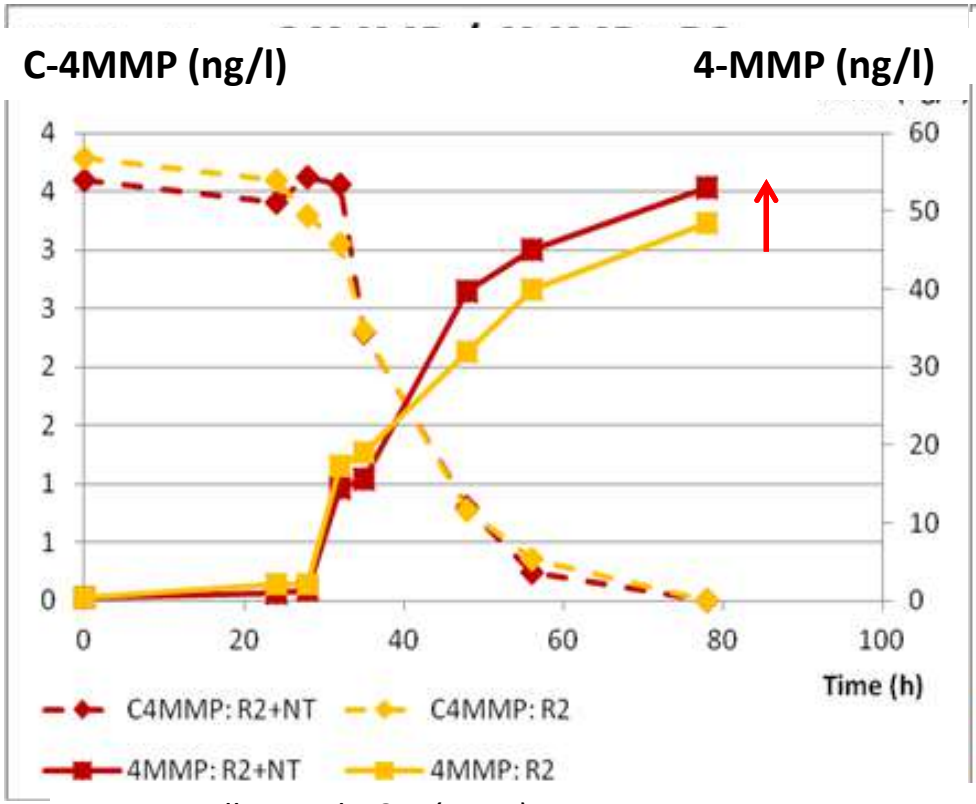
- TAKE CARE of yeast & stimulate its metabolism
- Maintain Yeast viability and Vitality
- OPTIMIZE & REVEAL the aromatic potential of the grapes
- Well balance micronutrition

- **Target on aroma release depending on the grape matrix (precursors)**
- **Development of specific composition : interactions of vitamins, serols, YAN…**
- **Specific Timing of addition**

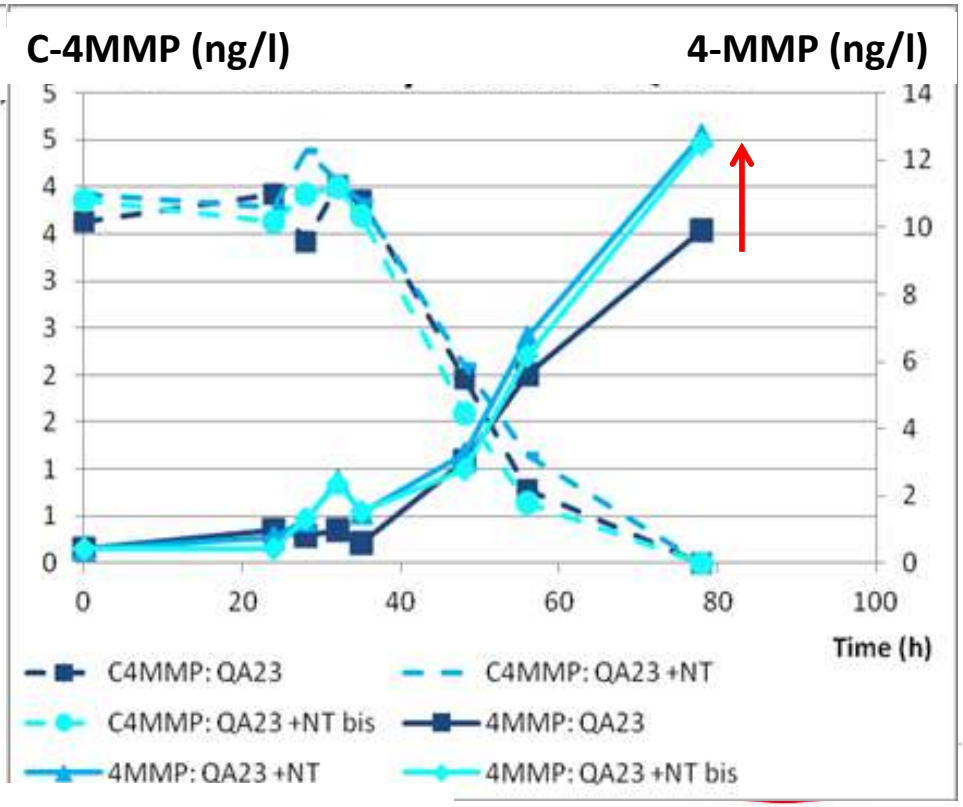
# Release of volatile thiols by yeasts

Kinetics of Consumption of Cys-4MMP and production of 4MMP by 2 wine yeasts in Sauvignon Blanc

⇨ With & without **Stimula Sauvignon** addition at t=0



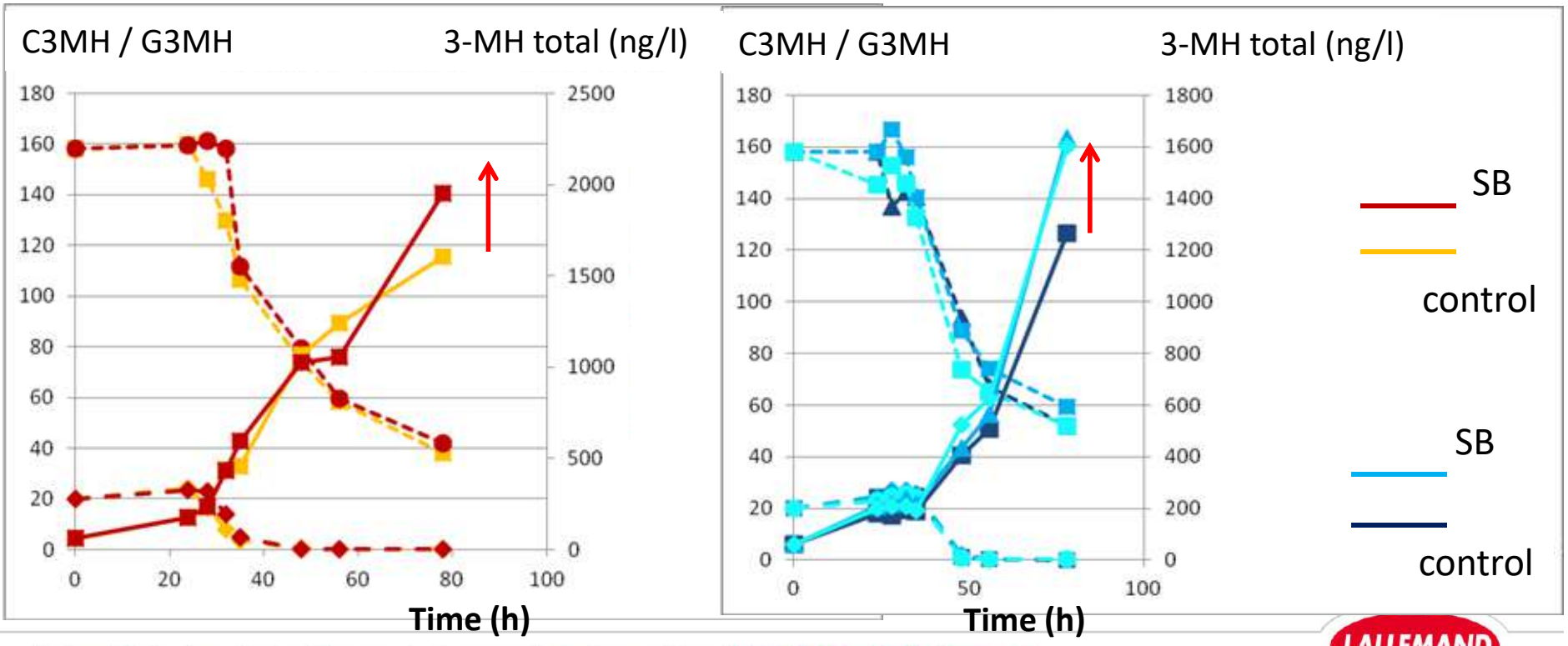

*Lallemand R&D (2016)*



# Release of volatile thiols by yeasts

Kinetics of Consumption of Cys-3MH, G-3MH, and production of 3MH by 2 wine yeasts in Sauvignon Blanc

⇨ With & without **Stimula Sauvignon** addition at t=0




*Lallemand R&D (20016)*

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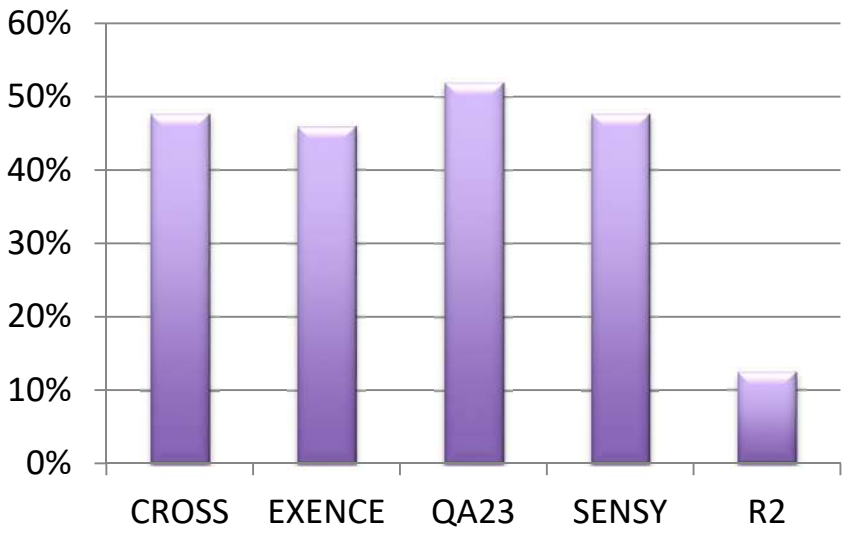
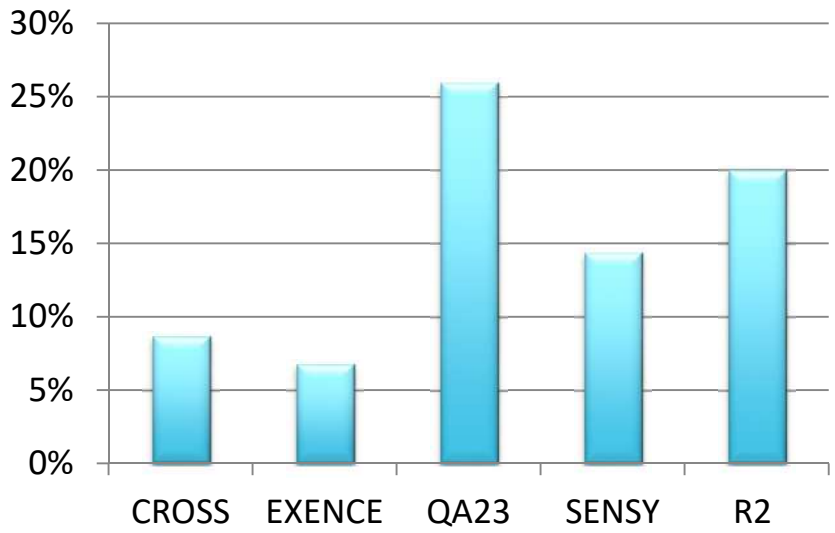
# Precursors conversion rate increases thanks to Stimula Sauvignon addition (t=0)

4MMP increase with Stimula SVG addition




3 MH increase with Stimula SVG addition




- Stimulation of the yeast enzymatic activity
- Yeast strain-dependent
- Index of efficiency? Stimula better adapted for some yeast strain

*Lallemand R&D (2017-2018)*

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# Conclusions

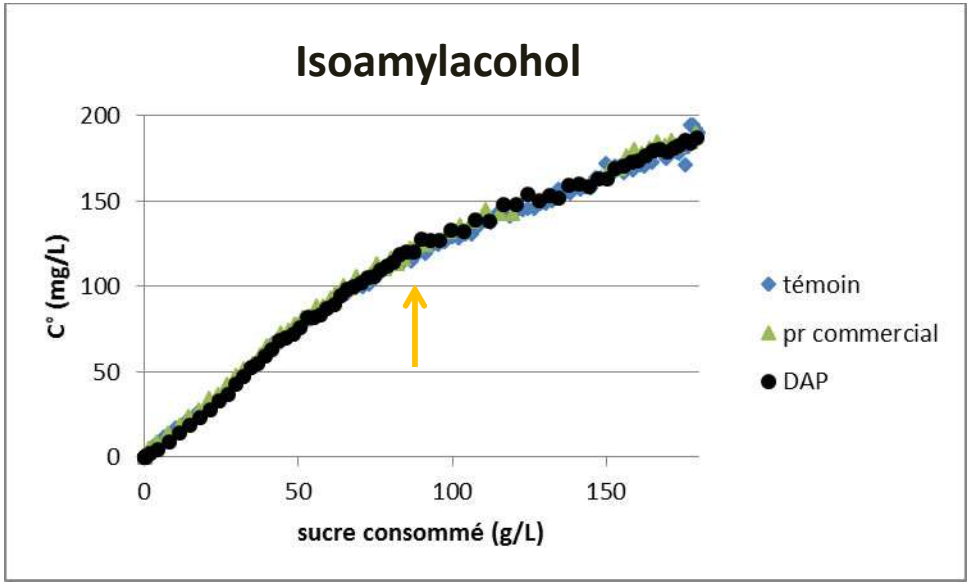
- Precursors uptake: during yeast cell multiplication: growth phase
- Varietal thiols release (4MMP & 3MH) : yeast dependant (beta-lyase activity)

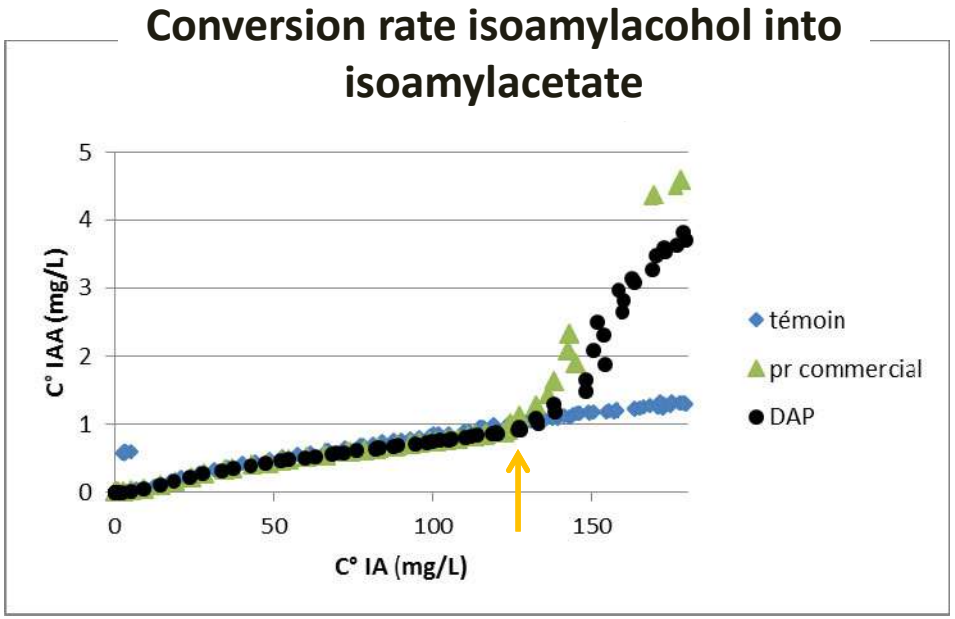
Stimula
Sauvignon blanc

→ to stimulate and enhance the bioconversion of grape precursors into varietal thiols by the yeast

→ addition @ t=0 when yeast consume nitrogen sources & when it's enzymatic activity is at maximum expression level

# Addition @ 1/3 of fermentation: Higher alcohol synthesis kinetics



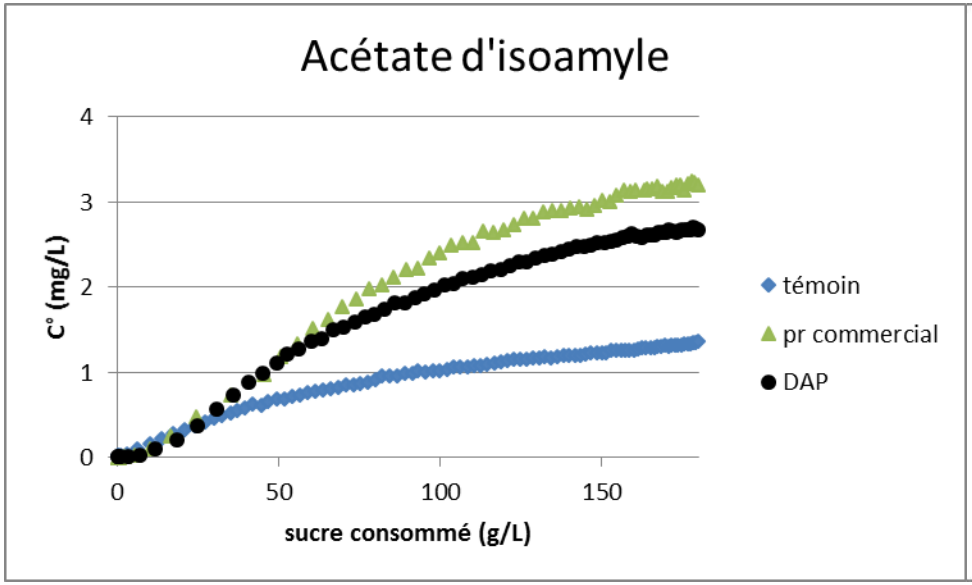




No impact of nitrogen addition at 1/3 on the higher alcohol production

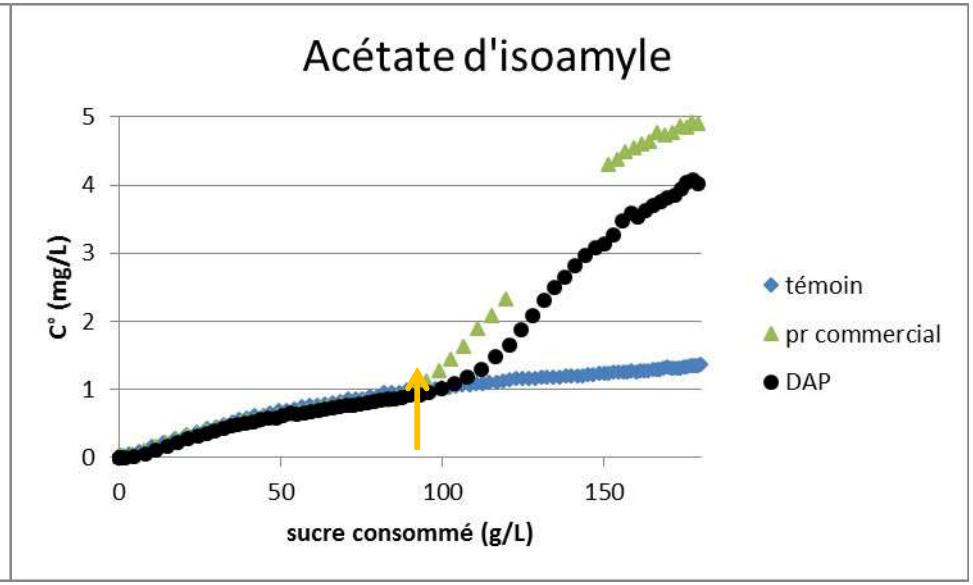
→BUT : big impact on the conversion rate of higher alcohol into esters (ATF1 enzyme regulation)

# Esters synthesis : Impact of timing of nutrient addition (Stimula Chardonnay)

**Addition @ $T_0$**




**Addition @1/3 of AF**




- Nitrogen addition impacts on the esters synthesis
- **Addition @ 1/3 : higher efficiency: +40%** compared to addition at t=0
- Stimula Chardonnay : more efficient than DAP

INRA
Institut National de la Recherche Agronomique

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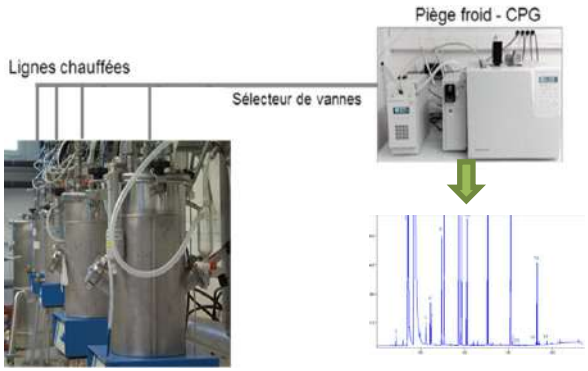
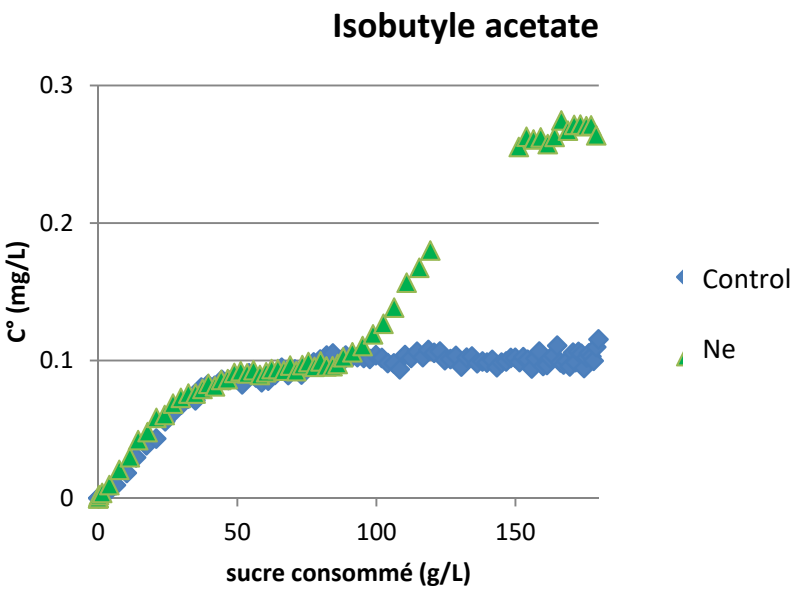
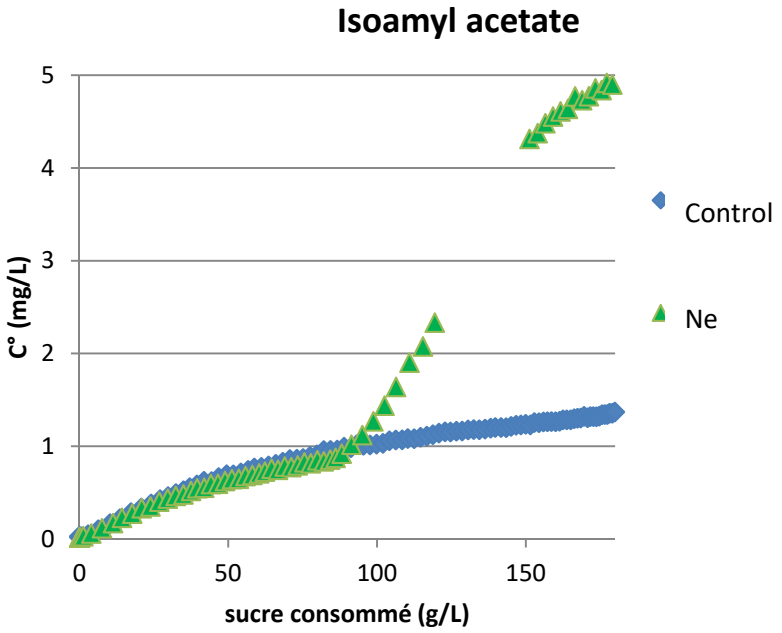
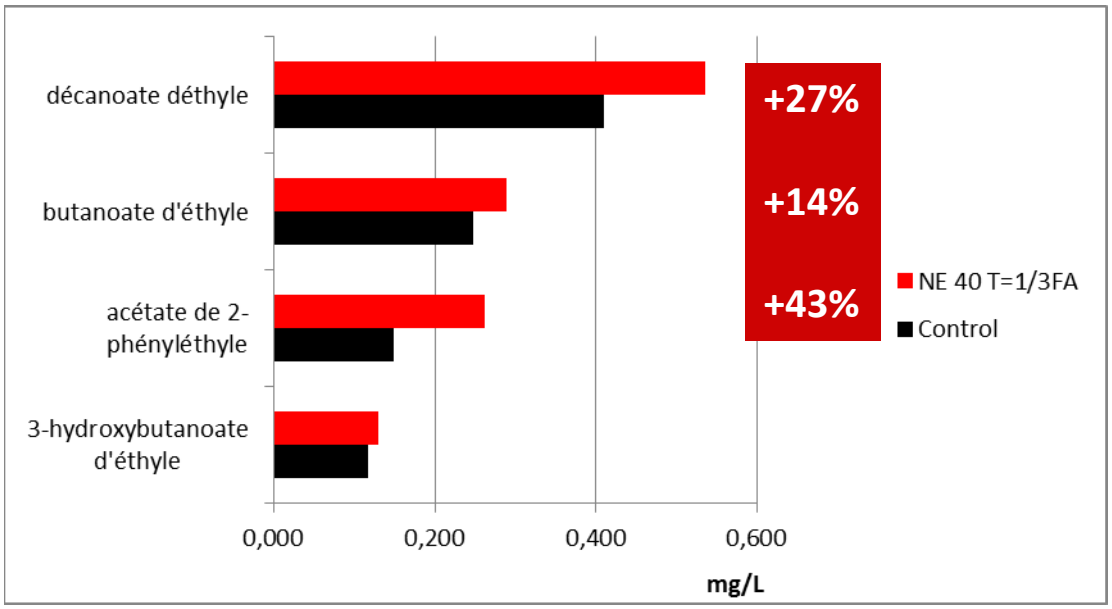
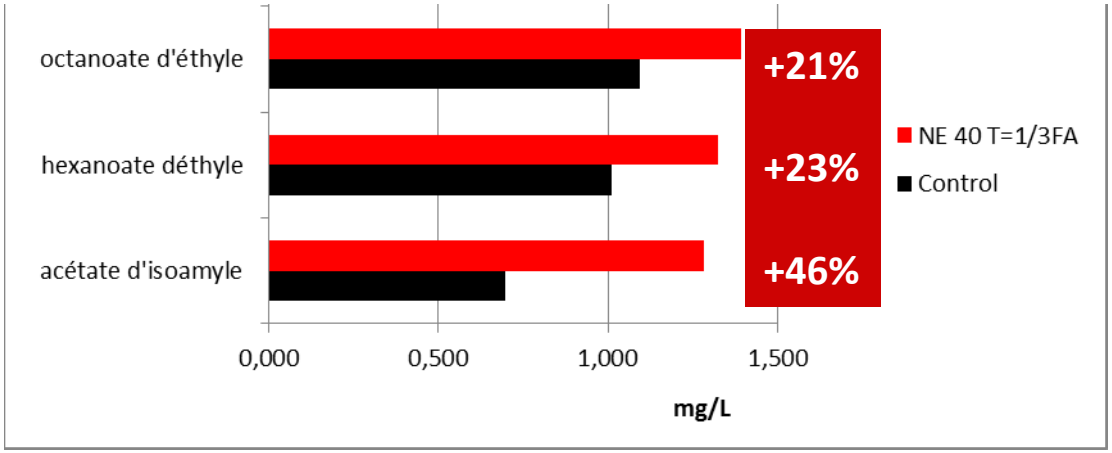


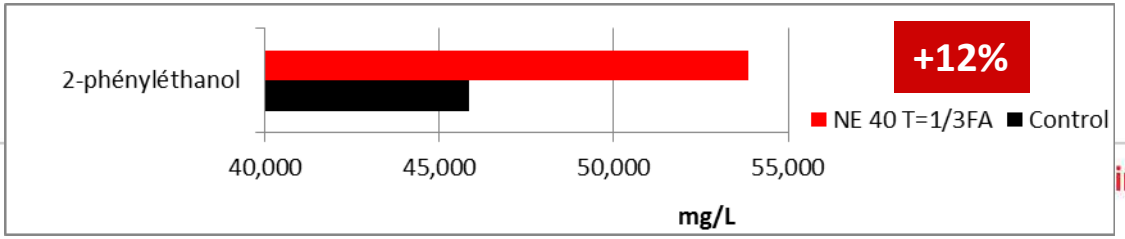

# Impact of Stimula Chardonnay added @ 1/3 on esters synthesis






**Significant increase of esters synthesis with Stimula Chardonnay addition at 1/3 of AF**

**From 12% to 46% depending on aromas**



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# Conclusions

Stimula Chardonnay

- → to stimulate the yeast esters synthesis metabolism
- to add @ 1/3 (after the exponential phase)

# THANK YOU FOR YOUR ATTENTION

# MERCI !!