Interreg

Co-funded by
the European Union

Central Baltic Programme

BALT-FIN-CIDER

Finding the balance in nutrition for Cider

Sigrid G-S (sigrid@lallemand.com)

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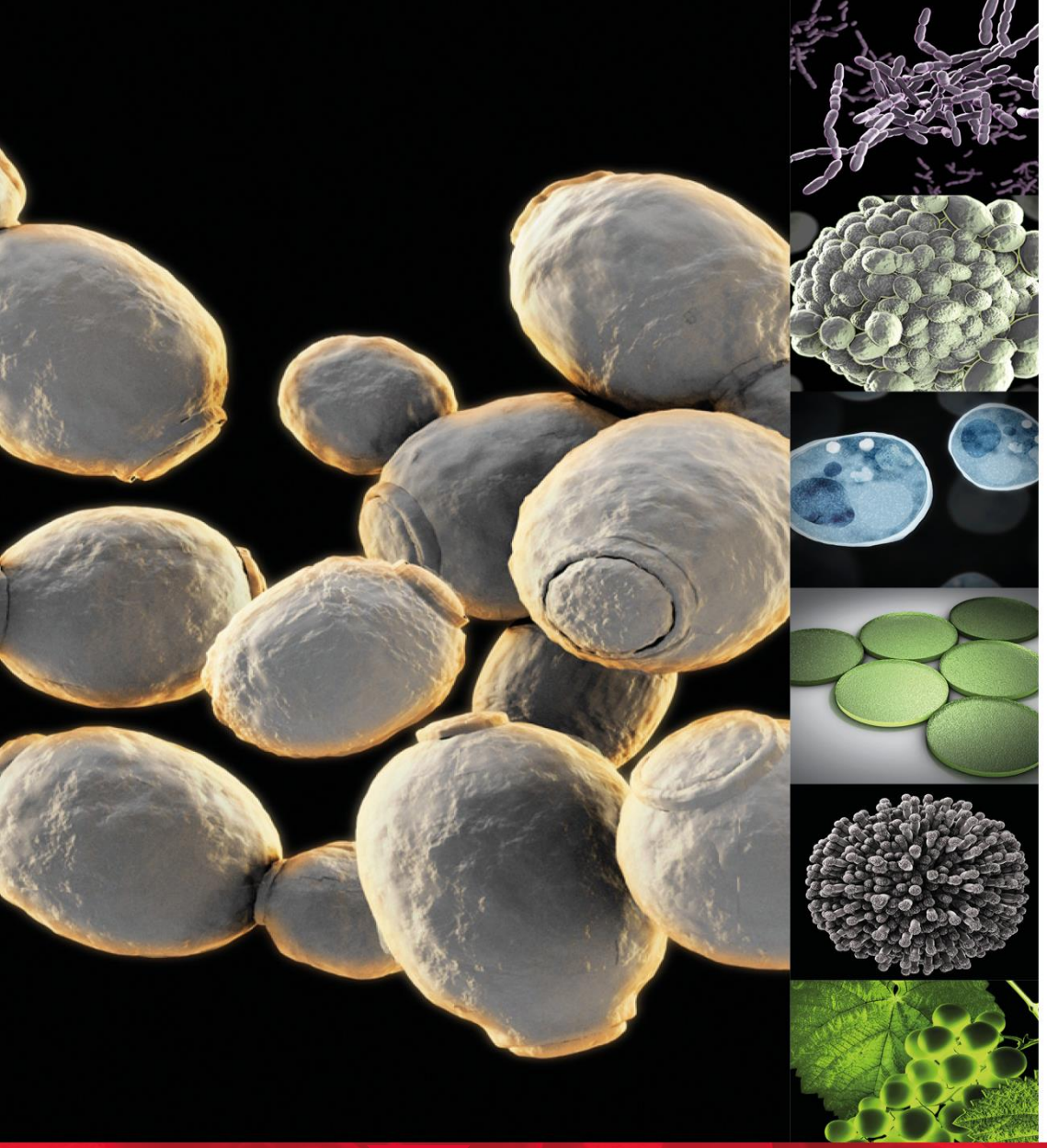
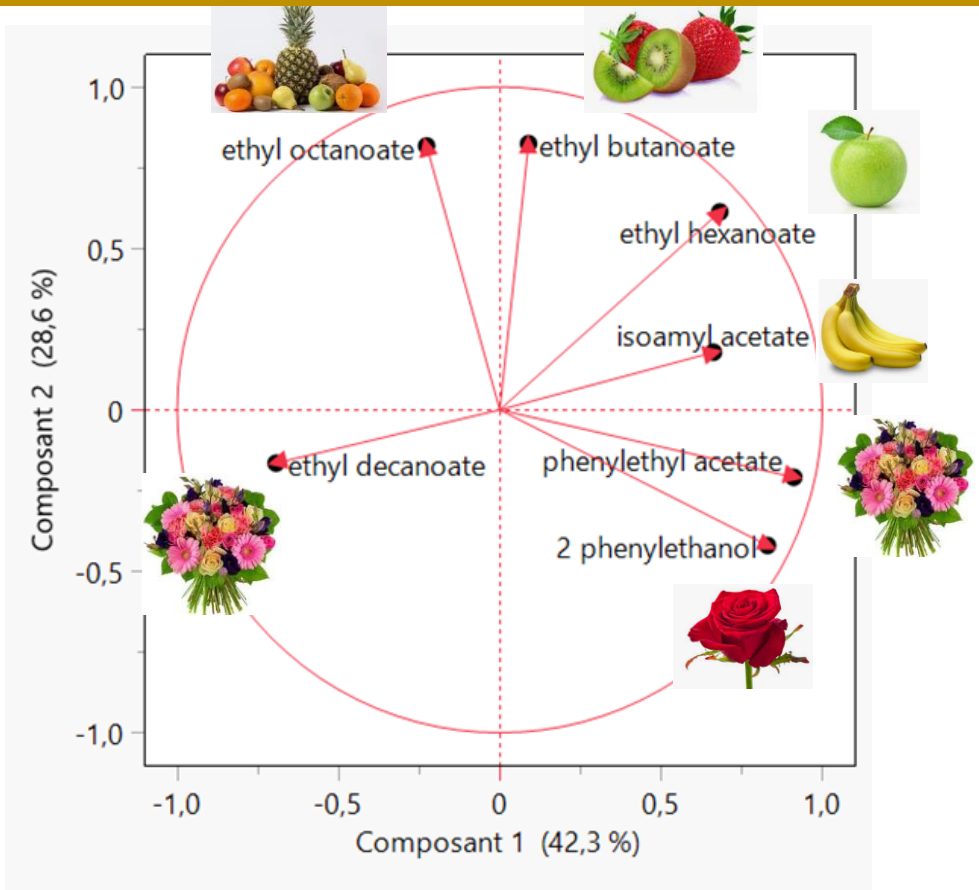
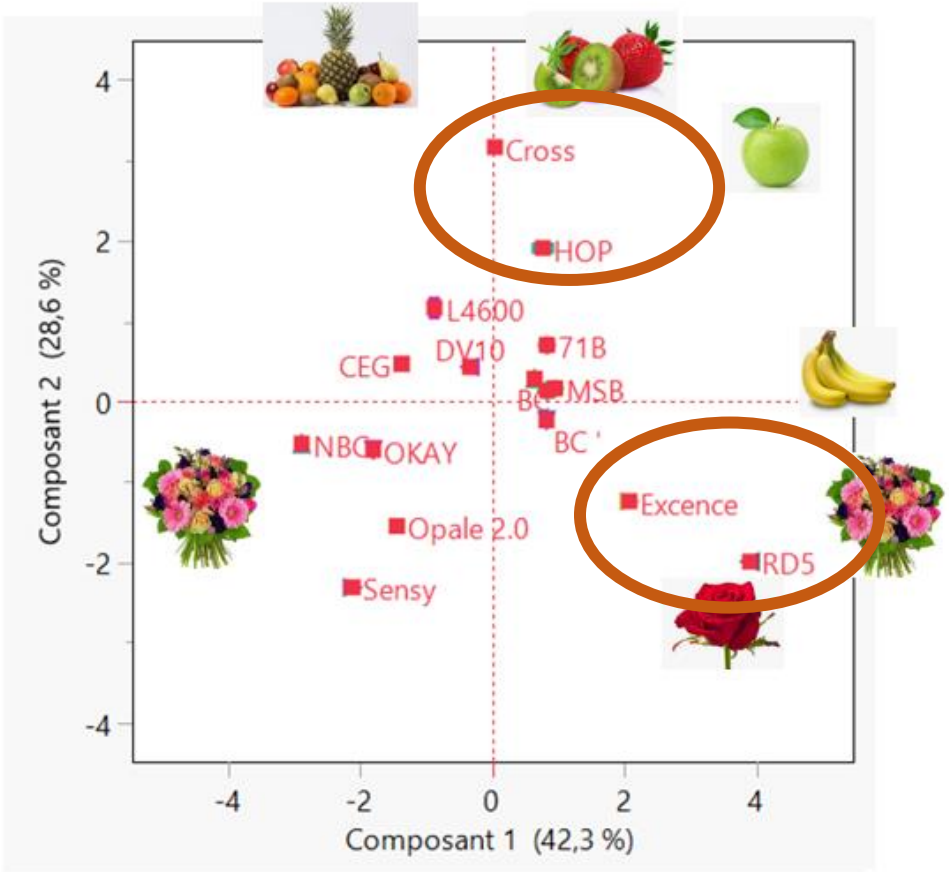
Original by culture



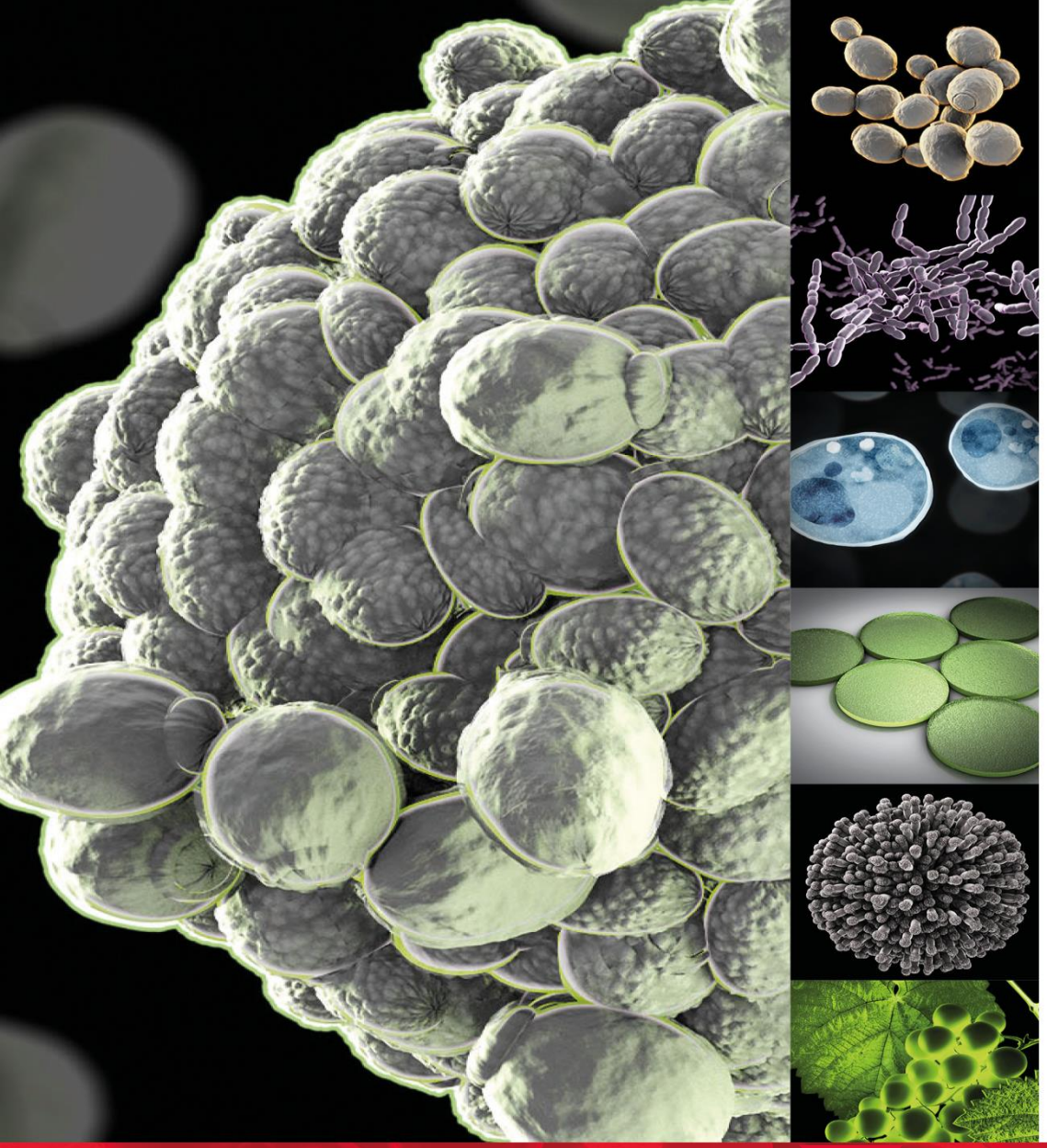
Yeast

Case study: evaluate yeast strains – kinetics & aromatic





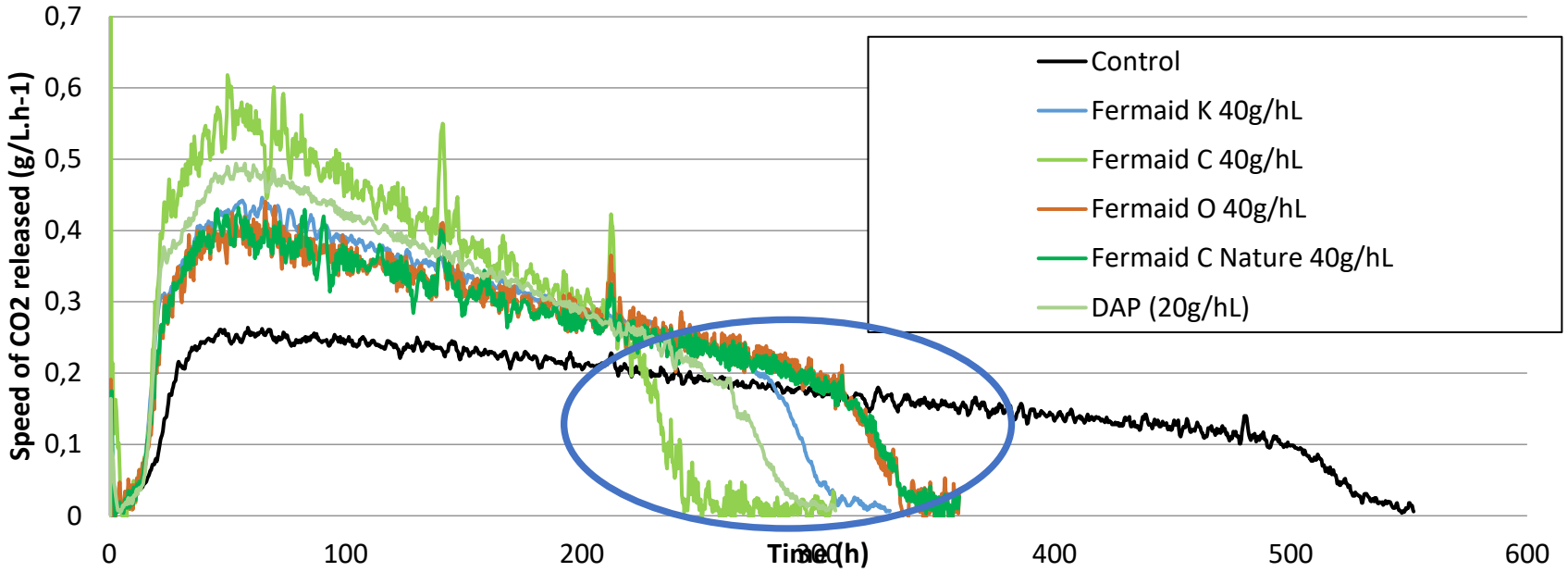
- High strain effect
- Cross Evolution, HOP are more fruity style
- RD5, Excence are more floral, rose



Nutrition

1/ Fermentation and process performance
The nutrition impact

Uvaferm BC: 25g/hL ; 22°C

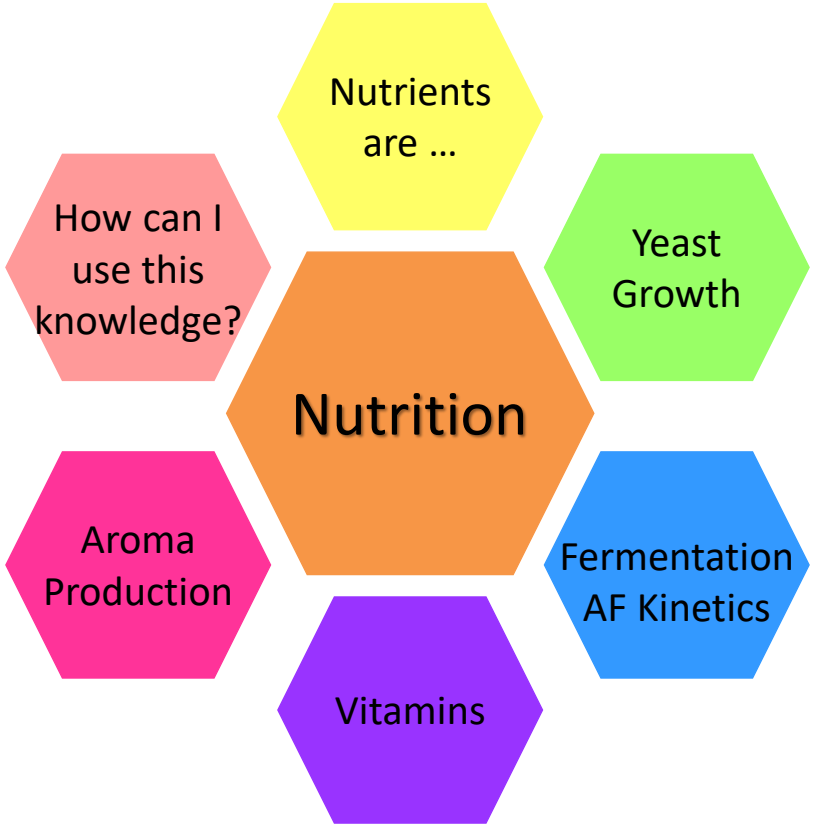


LALLEMAND OENOLOGY **PORTFOLIO**

SYNERGISTIC ACTION TO REVEAL YOUR WINES' INDIVIDUALITY

All is a question of balance

Nutrition is multifaceted



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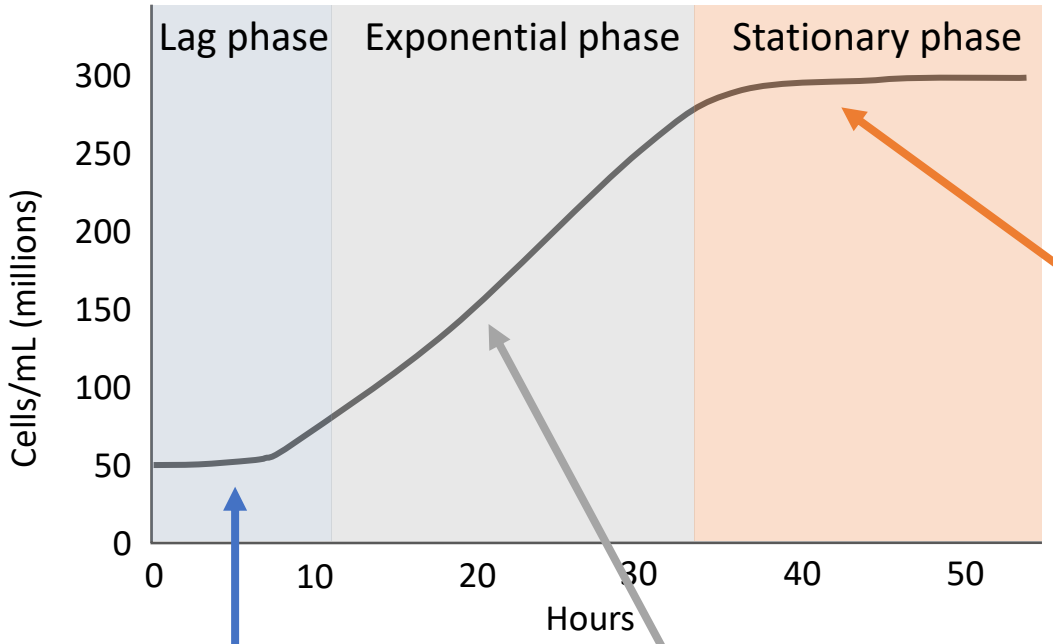
The fermentation – What are the yeast doing?



Phase 3 (stationary phase)

Active yeast population has to collectively work against increasing toxicity (e.g. alcohol & saturated fatty acids) and **complete fermentation**

Phase 1 (lag phase)

Yeast adapting to the environment

(grape must/mash/apple)

Phase 2 (exponential phase)

Yeast focused on energy production from sugar and cell growth to achieve a sufficiently **high** enough population of **actively metabolising** (fit) cells to complete fermentation of all sugar present

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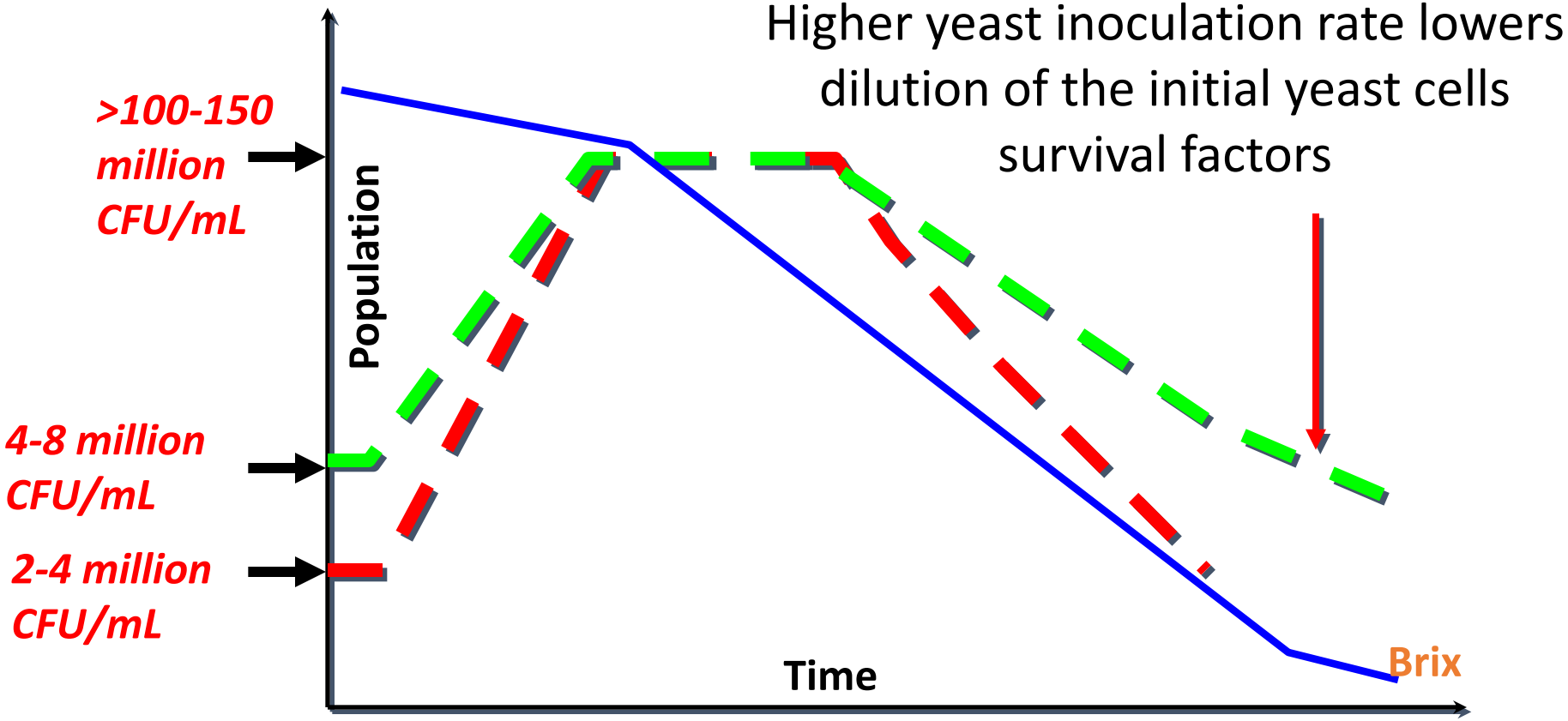
Normal Fermentation Curve



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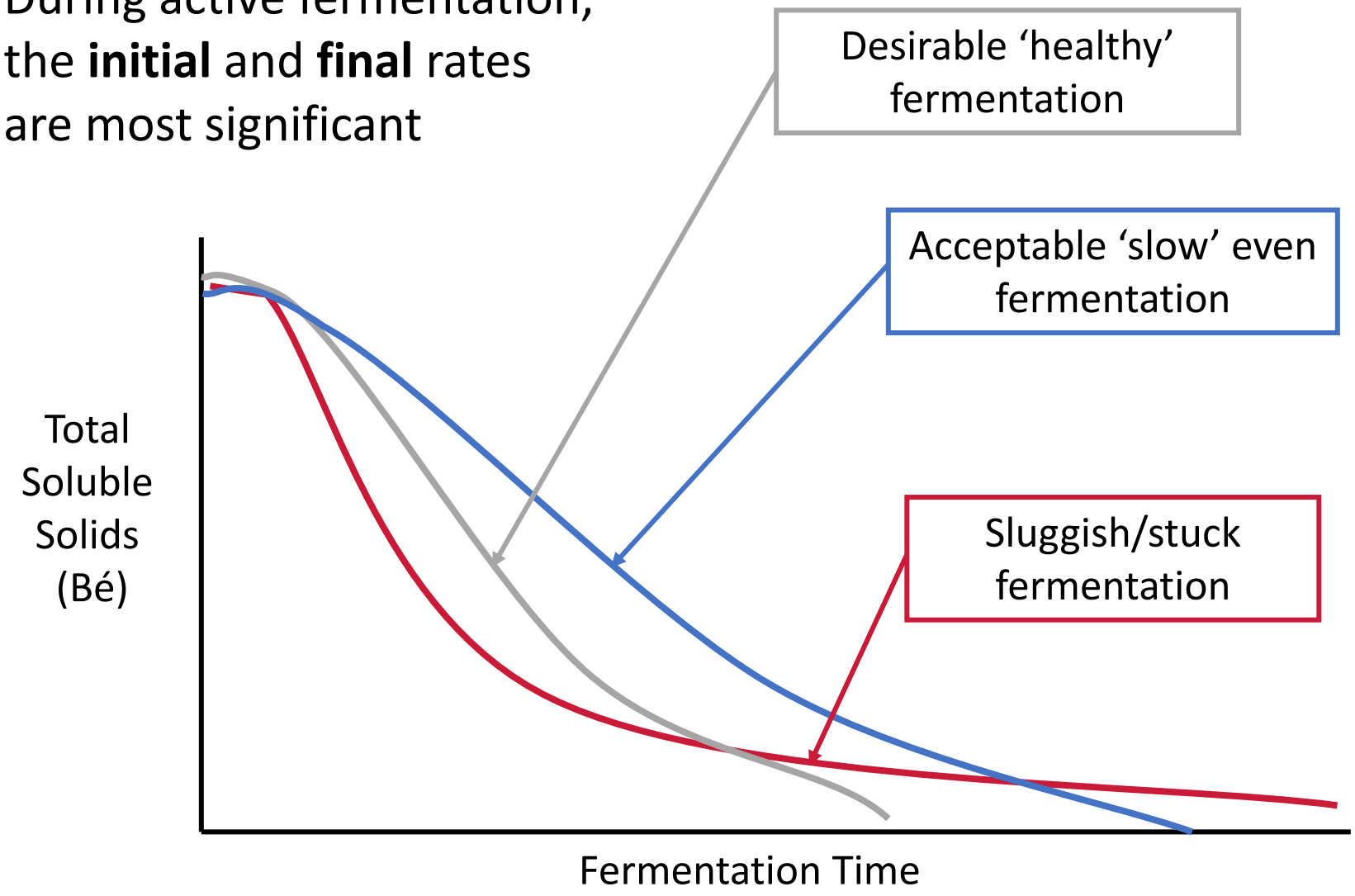
Yeast fermentation patterns

- During active fermentation, the **initial** and **final** rates are most significant



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Yeast Are Exposed to Stress During Fermentation

Physical	*Chemical*	*Biological*	*Microbiological*
• Temperature • Osmotic • Shear	• Ethanol • Starvation • Nutrient Limitation • pH • Metal Ions • Acids	• DNA Mutation • Replicative Aging	• Contaminants

Adapted from Walker (1998) and Smart (2000).

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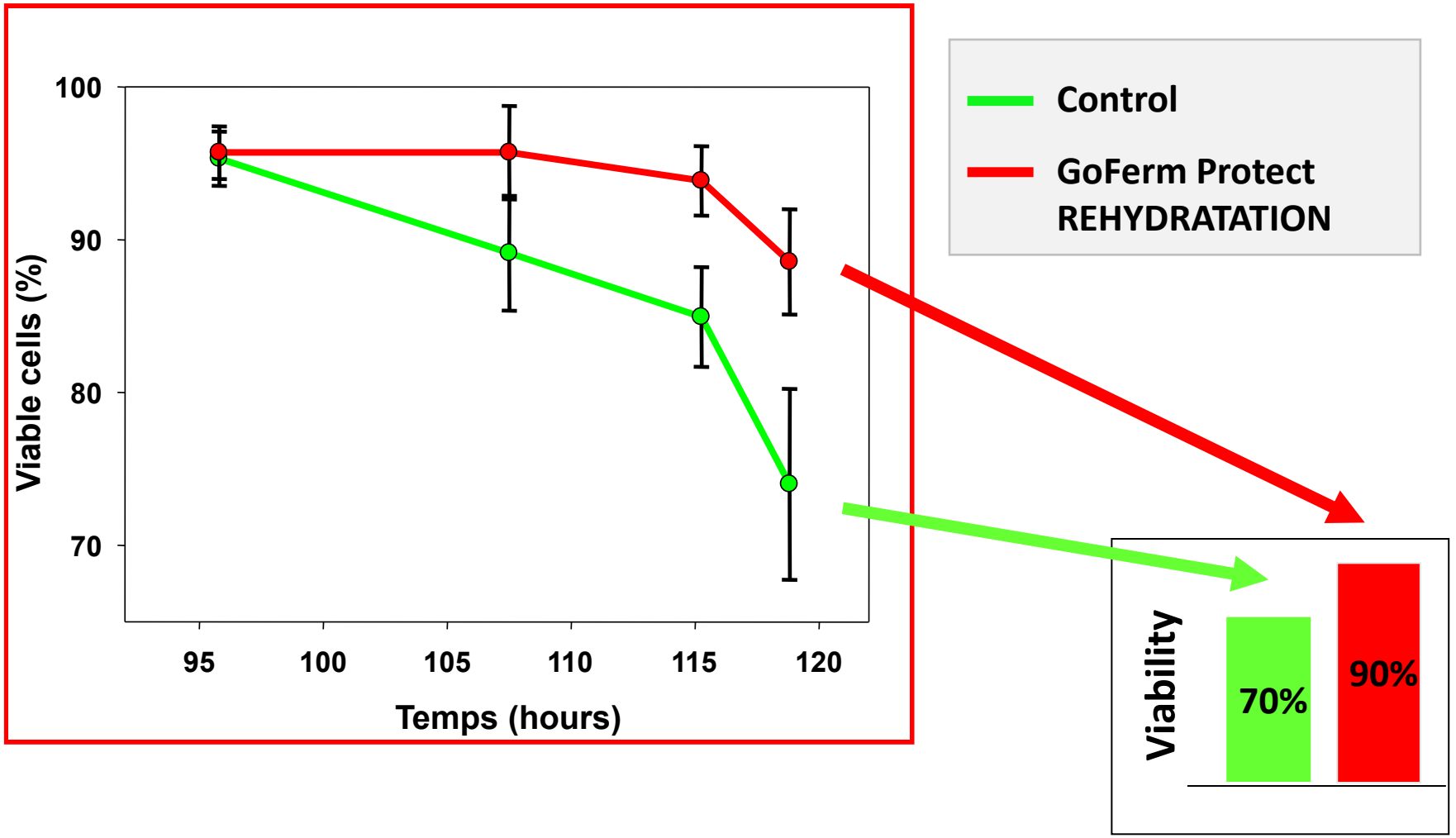
Impact of yeast protection on viability at the end of AF



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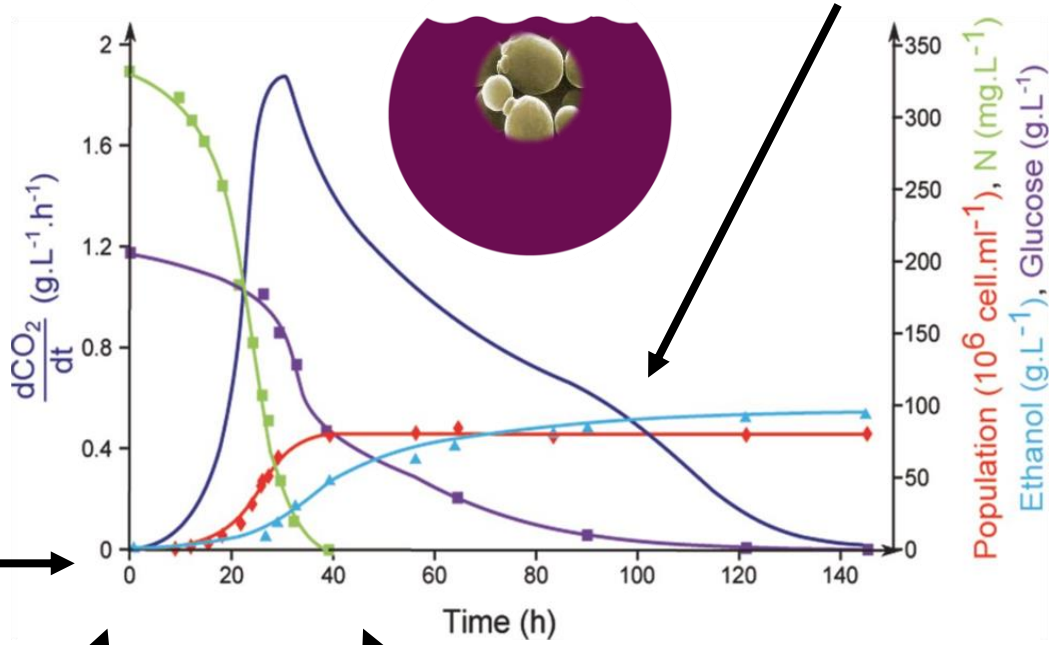
Alcoholic fermentation

A dynamic process

Ethanol stress (up to 15-16% vol)



Sulfur
Anaerobiosis

pH stress
pH (3.0 - 3.6)

Osmotic stress
glucose - fructose
180 - 260 g / L

Nutritional stress
Nutrient deficiencies (nitrogen, lipids, vitamins)

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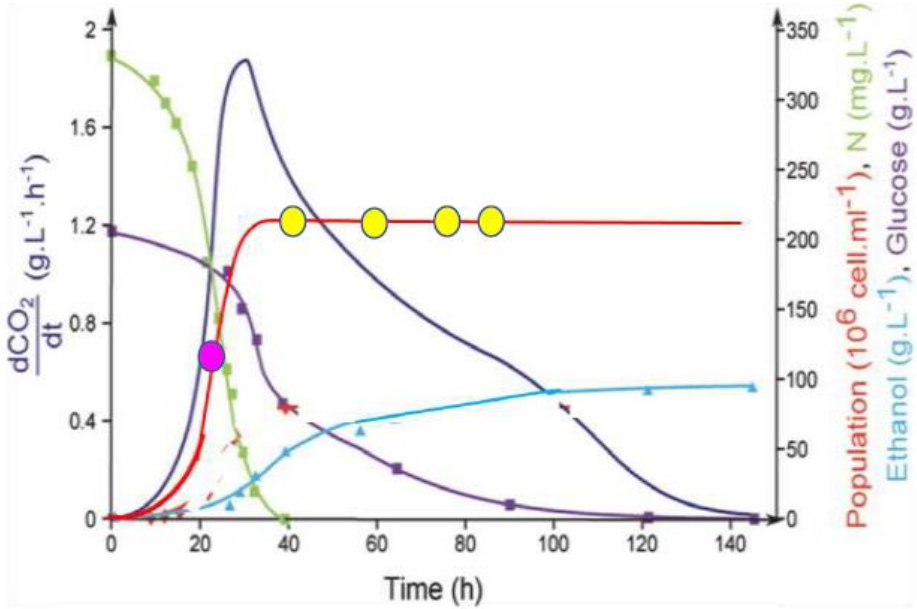
When is vitality important?

- At the mid of the growth phase (multiplication)

Still high osmotic pressure, half of nutritional sources (YAN) consumed, competition with spoilage for vitamins, etc....needs to have strong enzymatic activities



- During stationnary phase

Nutritional sources depleted, still half of the sugar to be uptaken, ethanol toxicity, time for most of the aromatic synthesis

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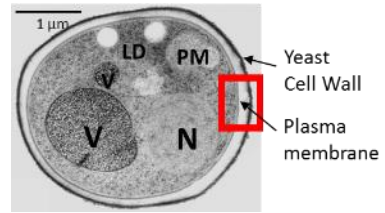
Protection of the cell membrane

- ADY
  - Contain ~ 8% water (reduction in cell volume)
  - This is insufficient for active metabolism
  - ➢ They must be rehydrated to allow reabsorption of the water lost during drying

Beker & Rapoport 1987

- What is the yeast trying to do:
- Important to have time to get back a functioning cell



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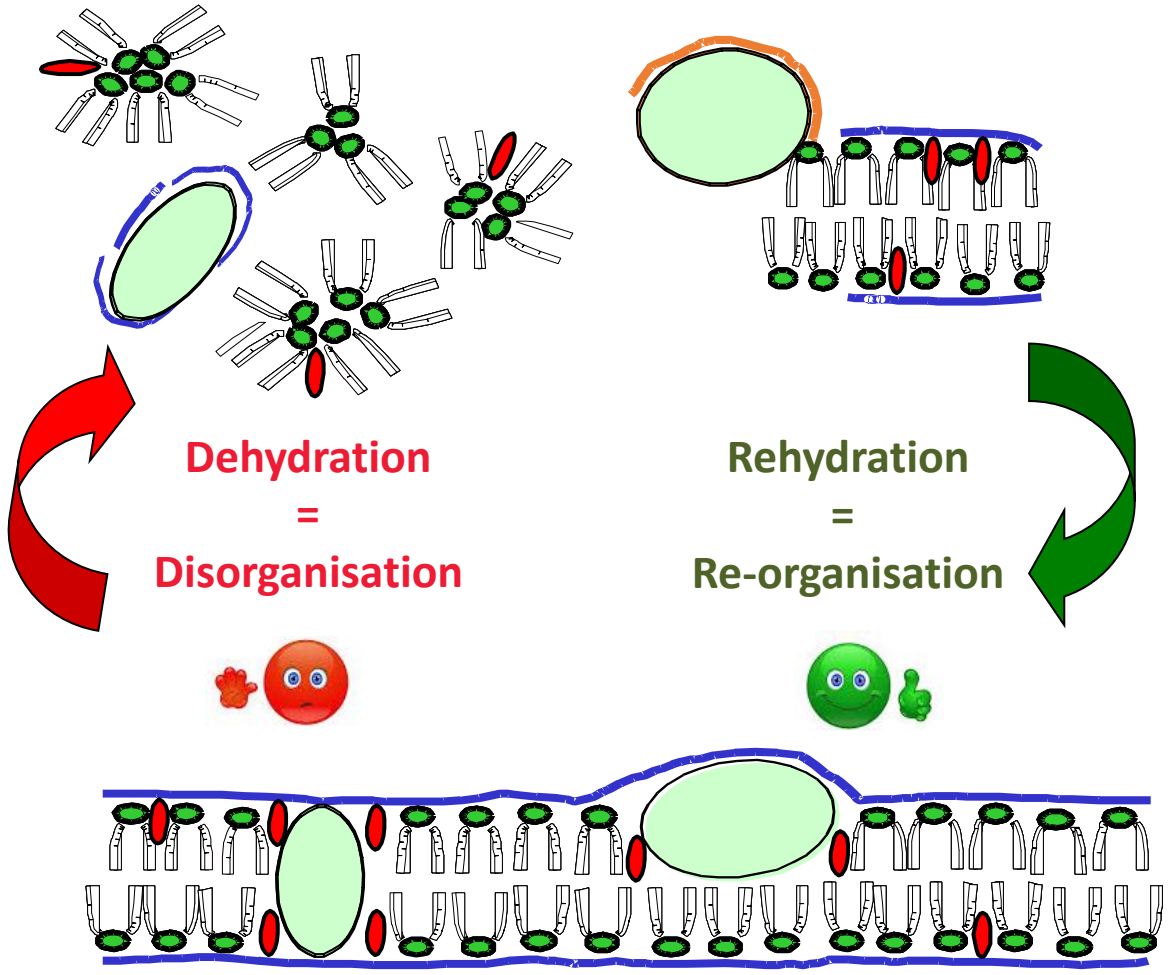
What happens to the yeast cell membrane?



➢ Restoring membrane integrity, cell viability, ethanol tolerance

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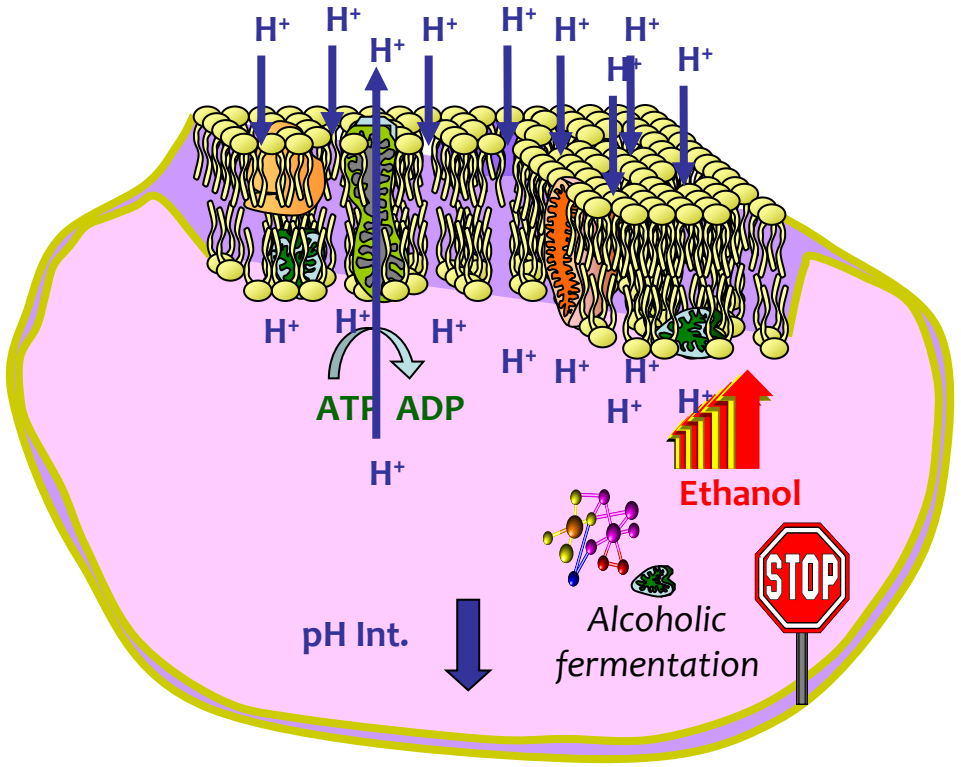
• Restoring membrane integrity, cell viability, ethanol tolerance

- Especially important in the last ½ - ⅓ of AF

- Ethanol ↑
- Membrane integrity is compromised
- Internal acid protons ↑
- ATPase has to work harder to get them out

➢ Cell death

➢ Alcoholic fermentation ↓



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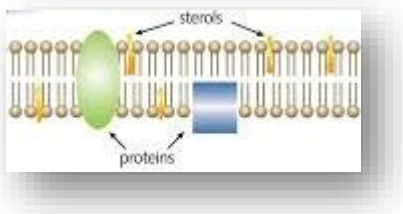
Why is protection necessary?

- Winemaking practices that impact membrane pressures
  - Clarification – low TSS
  - Flotation – low TSS
  - High sugar must – osmotic stress

- These sterols are easily removed during flotation or cold settling



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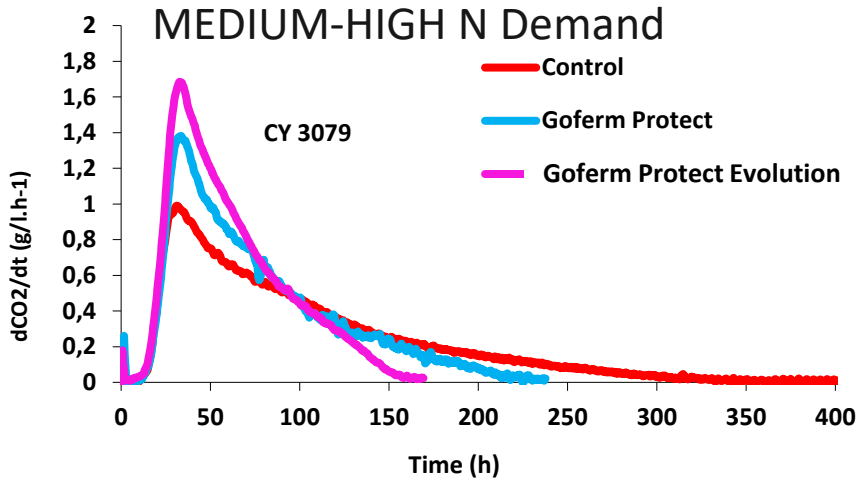
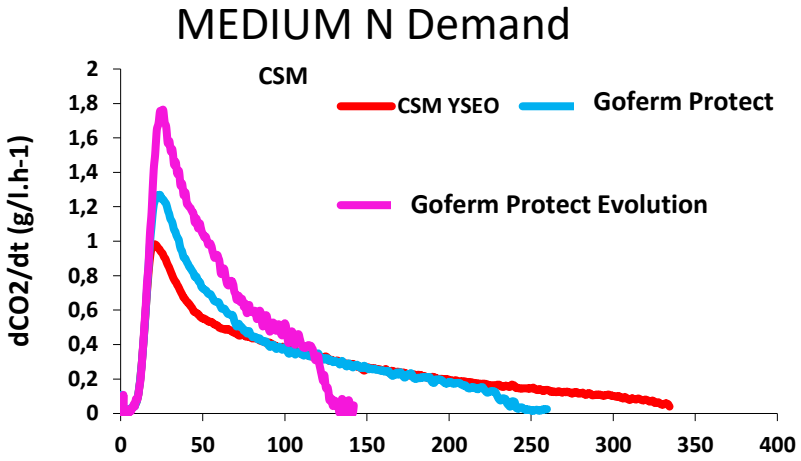
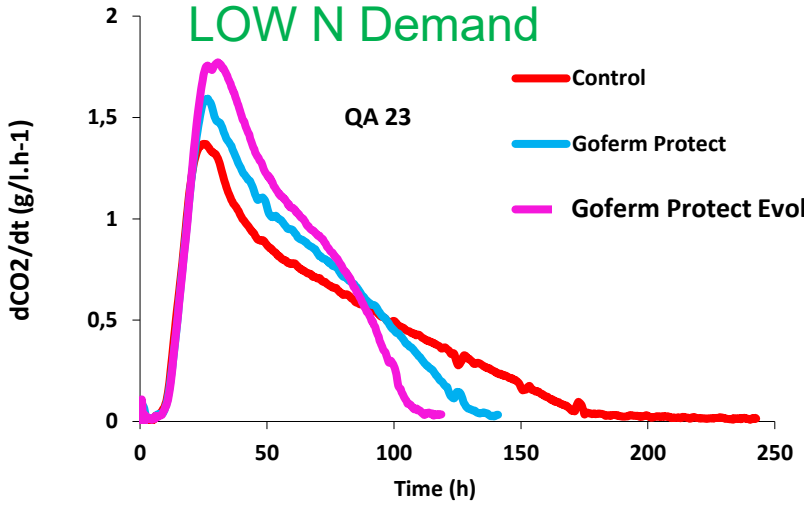
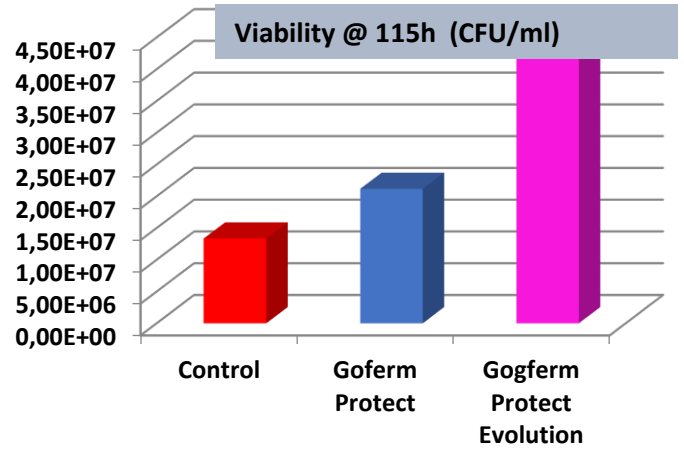
Impact of level of sterols in rehydration

High sterols deficiency conditions at 24°C - Protection effect on AF









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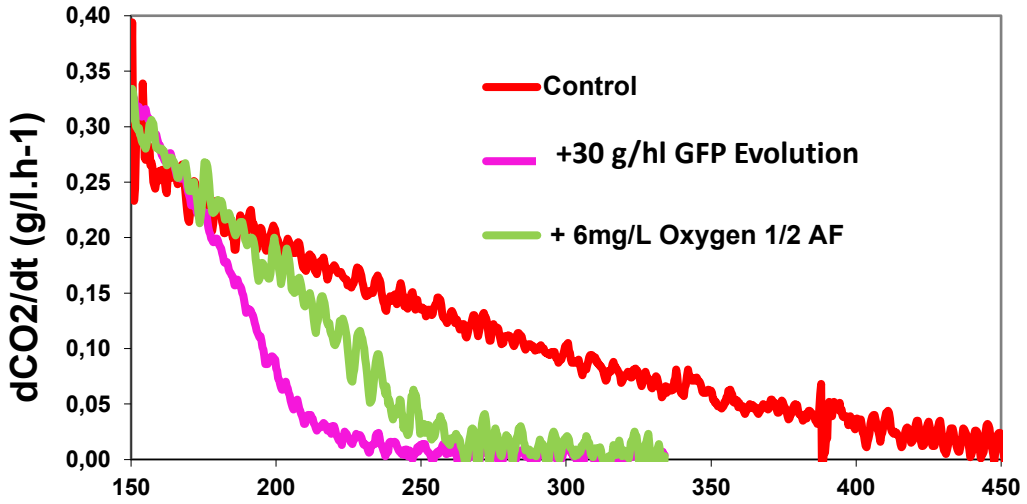
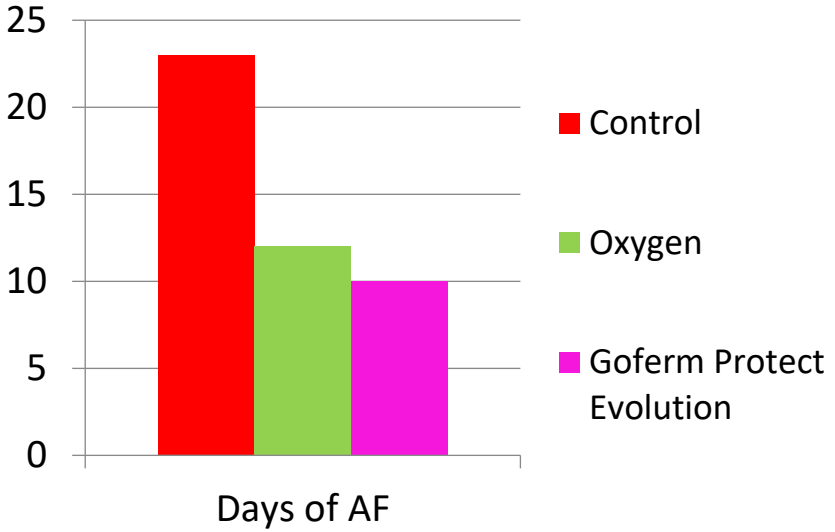
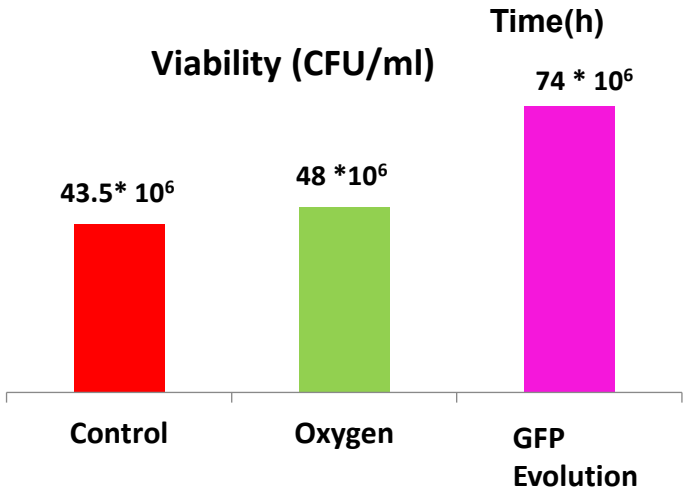
GoFerm Protect Evolution versus Oyxgen addition

Chardonnay, 20°C, high clarification







- **More effective than O2 during AF**
- **Enhancement of fermentation security and fermentation robustness**
- **Significant increase in viability, vitality flavour and aroma.**

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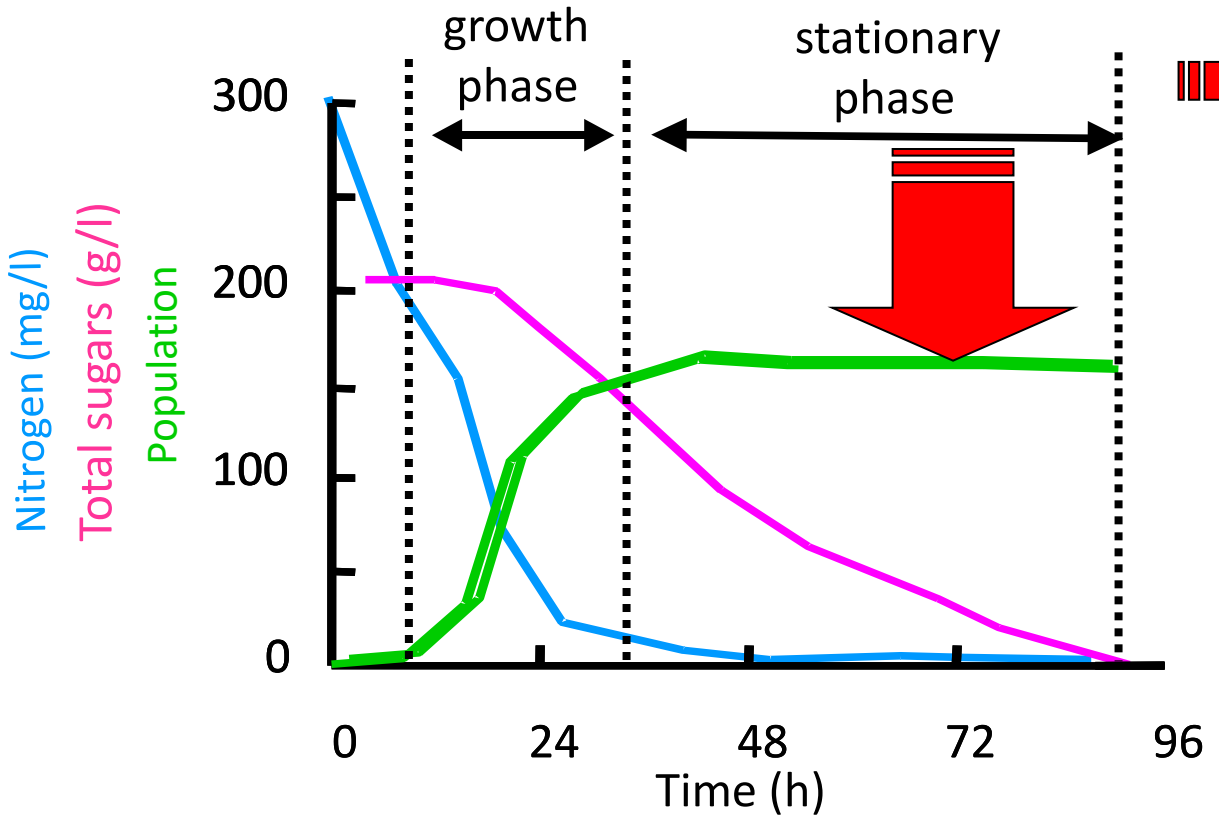
AF: Sterol sources addition best timing



- Yeast cells weaker and weaker due to ethanol toxicity
- necessary to maintain yeast viability
- strongly linked with membrane sterols content

Optimum time to bring assimilable sterols to the yeast:

<u>During their rehydration</u> (= membrane reorganization after the drying process)

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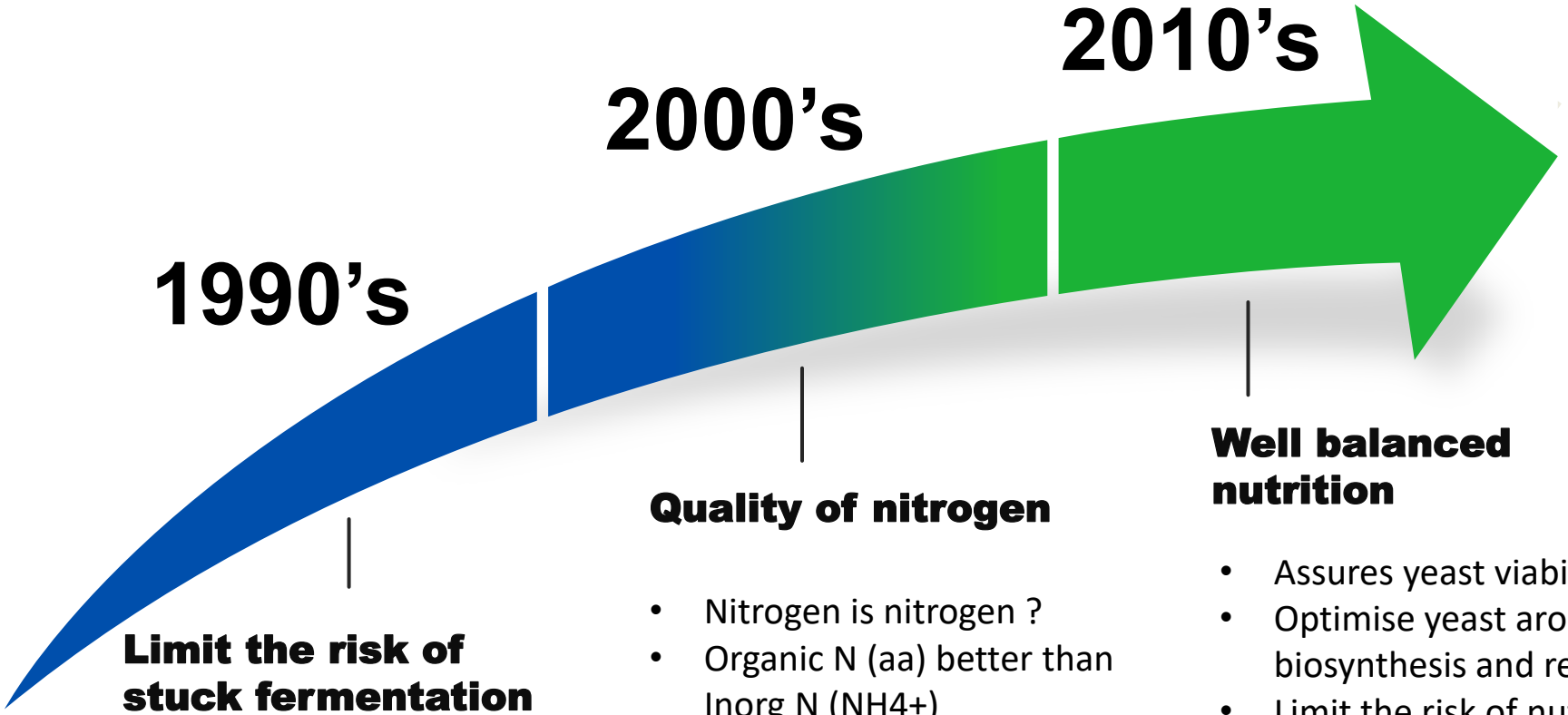
Yeast Nutrition Management
Evolution over the years



Limit the risk of stuck fermentation

- Nitrogen (YAN) is key
  - Defines biomass (X max)
  - Defines impact on fermentation rate (V max)
  - Yeast fermentative activity

Quality of nitrogen

- Nitrogen is nitrogen ?
- Organic N (aa) better than Inorg N (NH_4^+)
- More efficient on fermentation for same YAN

Well balanced nutrition

- Assures yeast viability
- Optimise yeast aroma biosynthesis and release
- Limit the risk of nutritional imbalances

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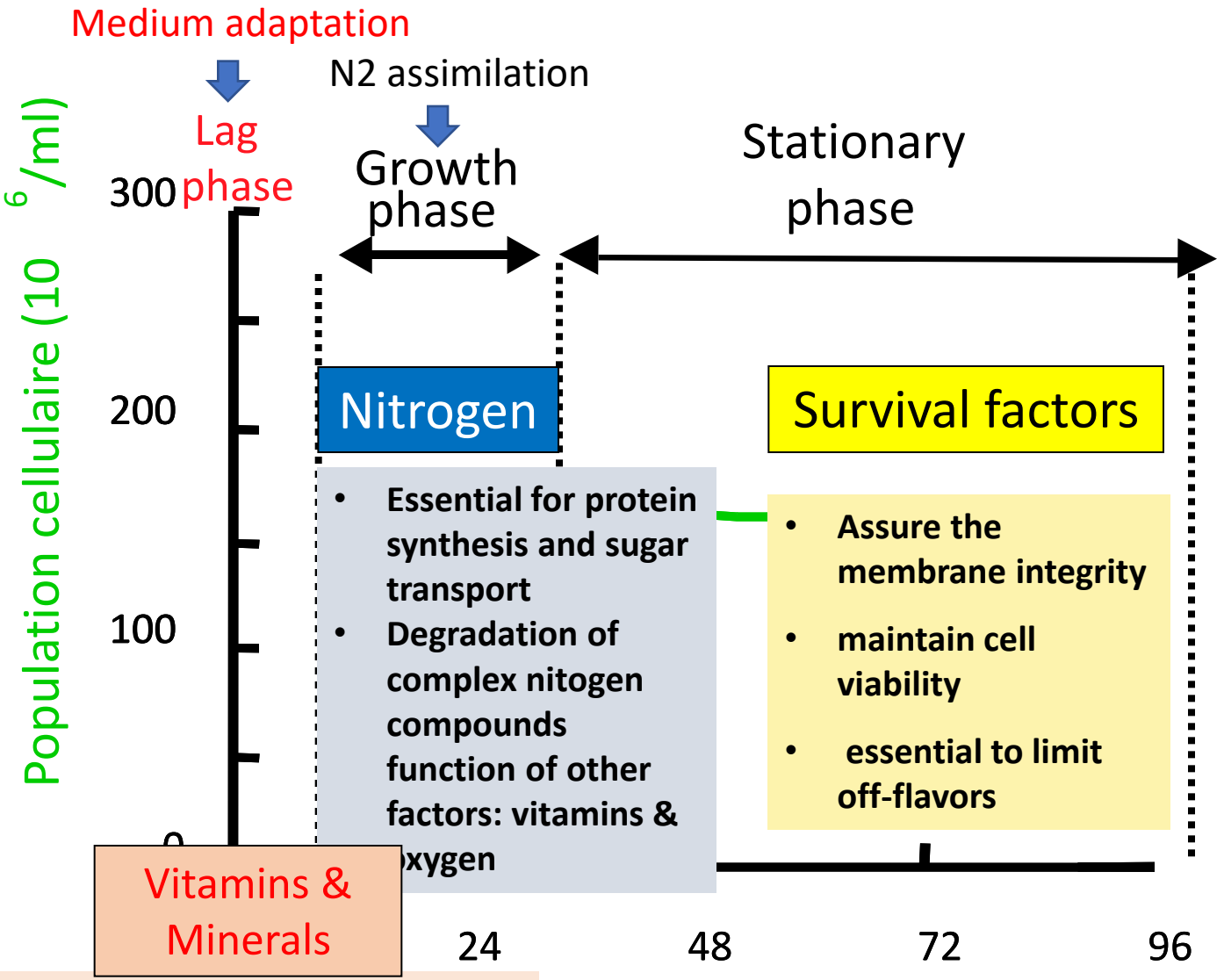
Yeast nutrient requirements depending on fermentation phases



Enzymatic cofactors, growth regulators, prepare the yeast for optimal activity

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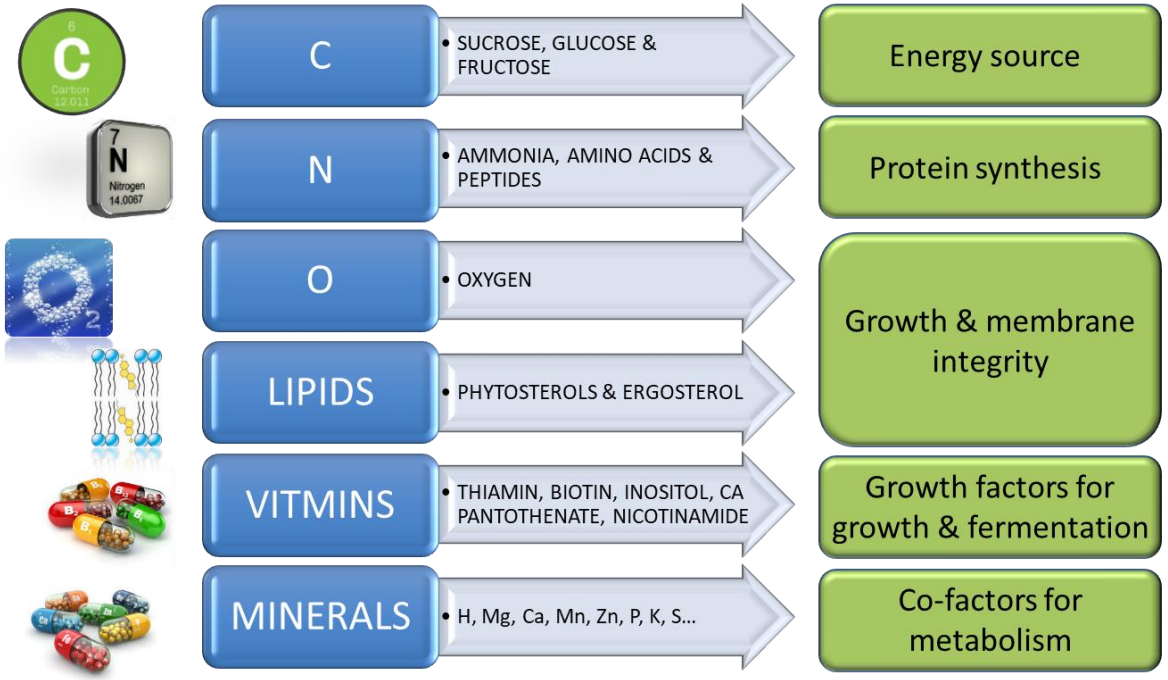
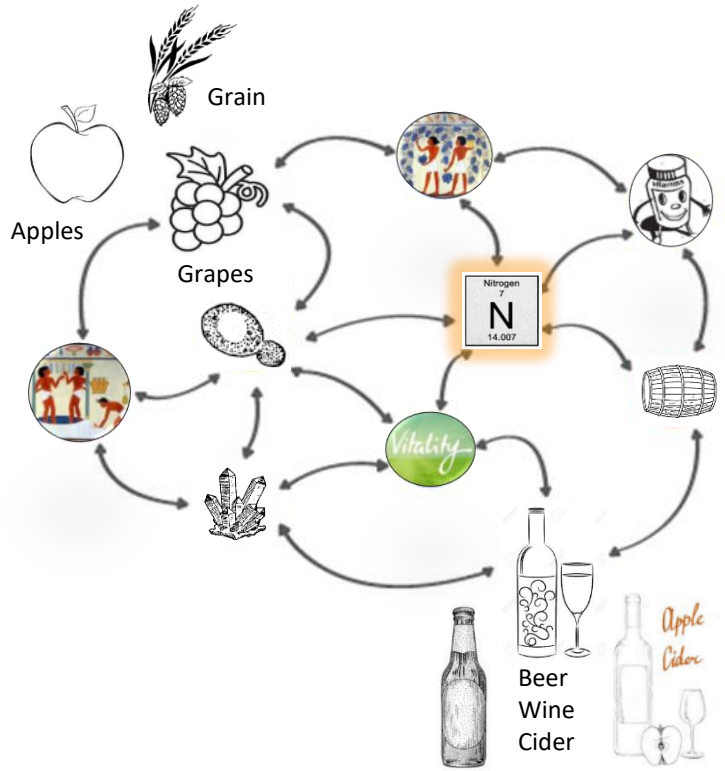
Nutrition

- Key to all factors & aspects of fermentation
- Many contributors
  - Not always straight forward ➢ *Interlinked*
  - Not always defined





➢ Nutrients must be Biologically available and Balanced

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All is a question of balance

Nitrogen, **YAN management** during the AF is important….but **not enough**
The **notion of balance** and synergy between **micro-nutrients & nutrients** is **key**

Minerals

Vitamins

Lipids

Nitrogen

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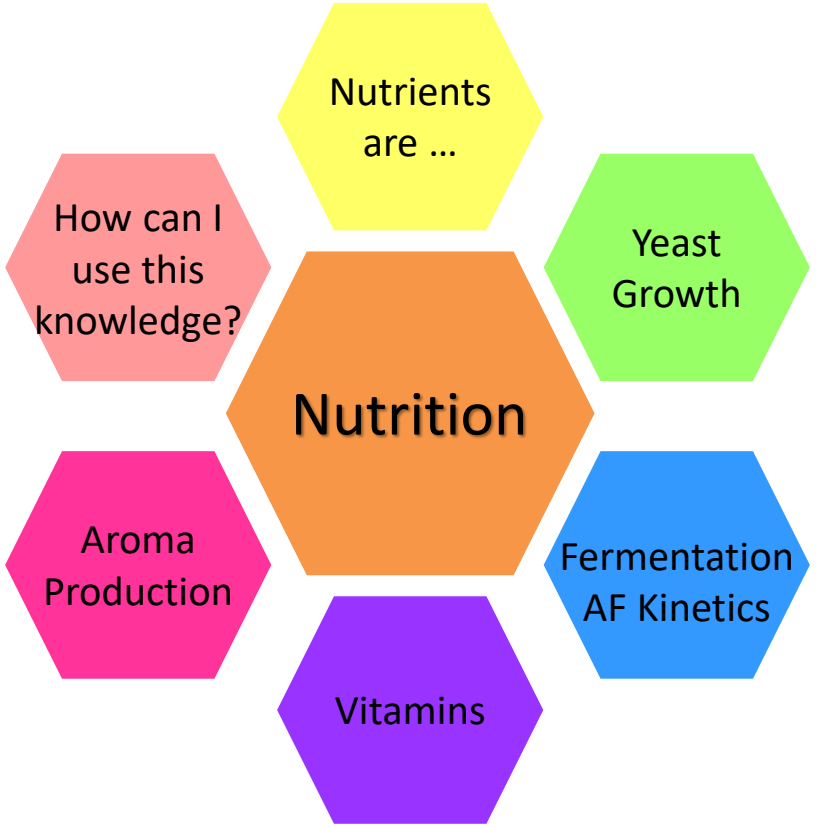
Nutrition is multifaceted



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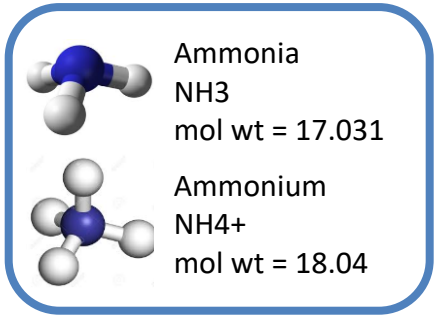
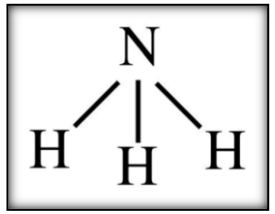
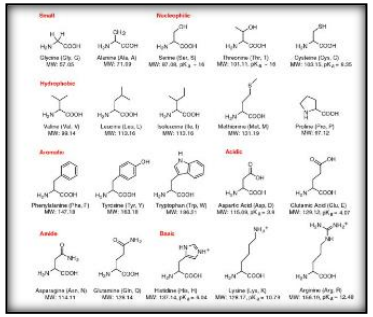
What is assimilable Nitrogen?

- **Ammonia (Inorganic N)**
  - Simple compound
  - Preferentially used
  - Fast = junk food
    - Only lasts for a bit
    - Does not provide lots of nutrition or balanced food





- **Amino acids (Organic N)**
  - Meaning amino acids & peptides
  - Some are more preferentially used than others
    - Easy
    - Intermediate
    - Less so
      - Also equals order of uptake



➢ **Essential for protein synthesis**

Yeast growth & metabolism

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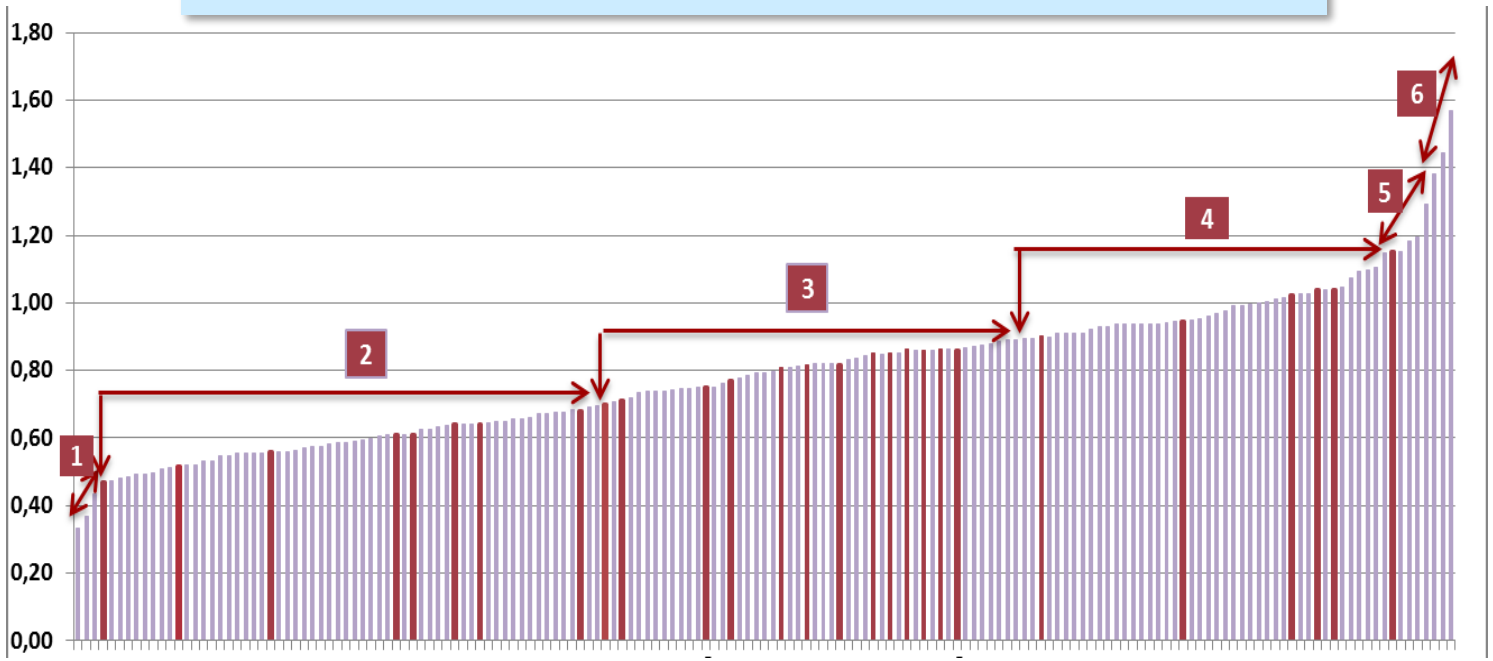
Yeast nitrogen requirements

Yeast nitrogen needs to ferment a nitrogen deficient must (100mg/L YAN)

mg of YAN necessary to consume 1g of sugar (T°: 24°C)



- Some yeasts need 4 times more N than others to ferment a deficient nitrogen must, at the same rate

Julien et al., 2000

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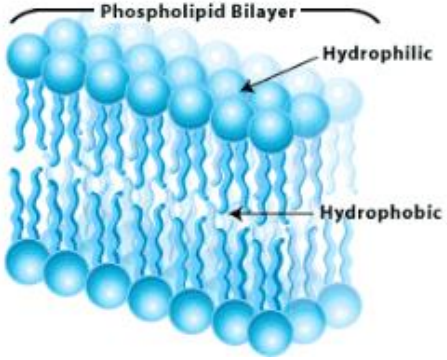
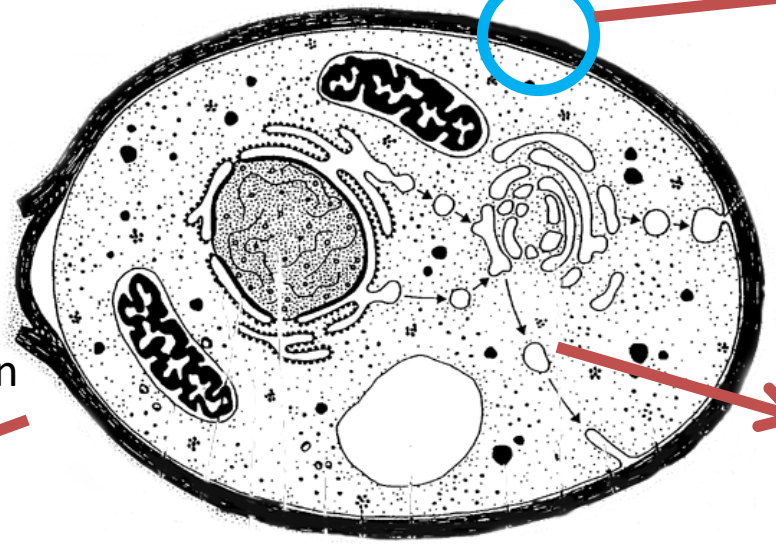
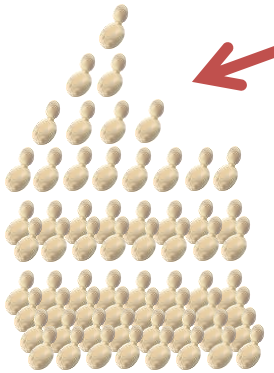
Nitrogen affects numerous aspects of yeast

Cell wall integrity

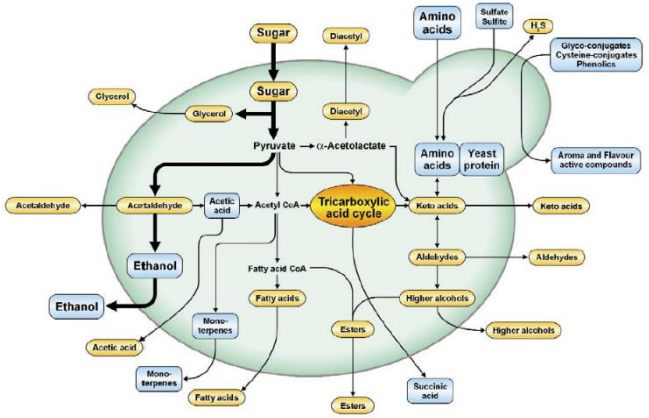




Cell multiplication



Cell metabolism



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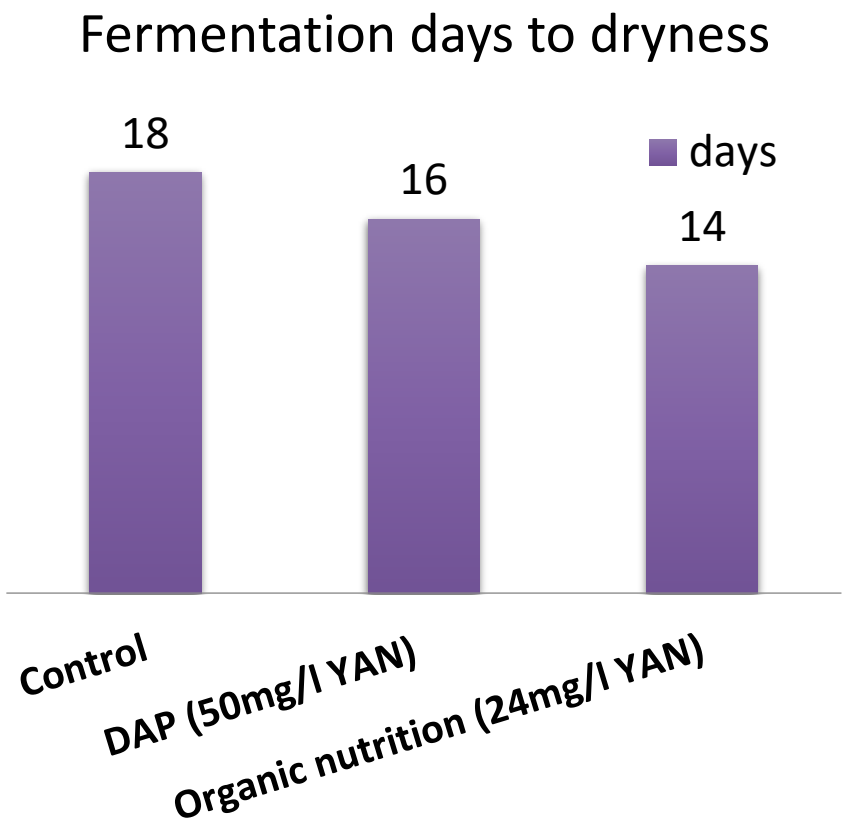
Nitrogen source – Efficiency on fermentation



Adelaide Hills, 2010
Chardonnay

The Australian Wine Research Institute

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

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Analysis method: LC-UV (free amino acids, NH_4^+)

ug/g "as is"	AA		N from AA or NH_4^+	
	NF20 19/3/20 (Thatchers)	NF10 19/3/20 (Thatchers)	NF20 19/3/20 (Thatchers)	NF10 19/3/20 (Thatchers)
Ala	67	87	11	14
Arg	13	12	4	4
Asn	346	1631	73	346
Asp	81	266	8	28
Cys	0	0	0	0
Gln	12	12	2	2
Glu	54	91	5	9
Gly	7	13	1	2
His	0	0	0	0
Ile	8	13	1	1
Leu	2	3	0	0
Lys	4	2	1	0
Met	3	4	0	0
Phe	2	5	0	0
Pro	51	32	6	4
Ser	23	75	3	10
Thr	27	36	3	4
Trp	0	0	0	0
Tyr	5	5	0	0
Val	8	12	1	1
NH_4^+	3	8	3	7
FAN	-	-	122	427
YAN	-	-	124	434

„as is“ – results are based on apple concentrate

YAN (yeast assimilable nitrogen) = FAN + N from NH_4^+, FAN - free amino nitrogen (N from free AA)

this gives 25 ppm and 86 ppm N in the diluted juice (without added nutrients)!

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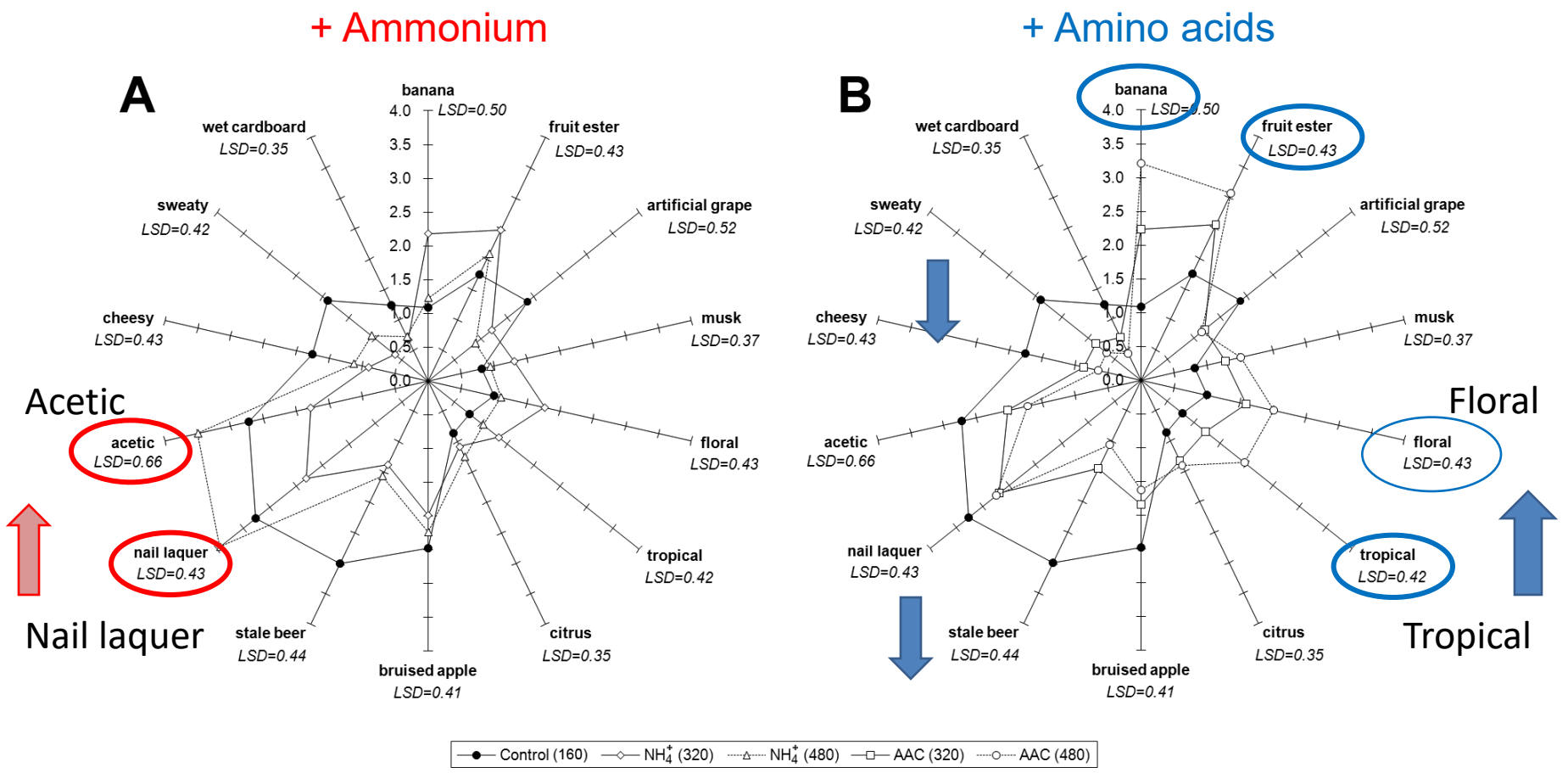
Nitrogen source impacts on wine sensory profile

The Australian Wine Research Institute



Henschke, 2010

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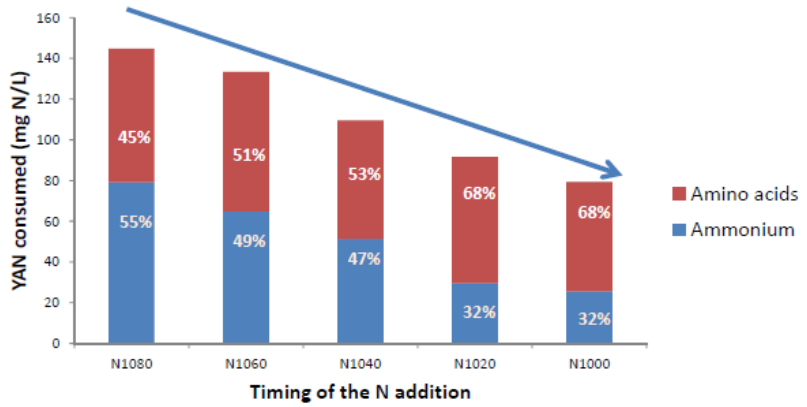
Timing of addition & Type of N source

- N additions during stationary phase improves fermentation rate without affecting yeast growth
- Only additions during exponential growth phase produces increases in yeast cell growth



- Type of nitrogen
  - Ammonium
    - Consumed preferentially during exp phase (growth)
  - Amino acids
    - Consumed preferred sourced during stationary phase

Beltran et al JAFC 53, 996 (2005)

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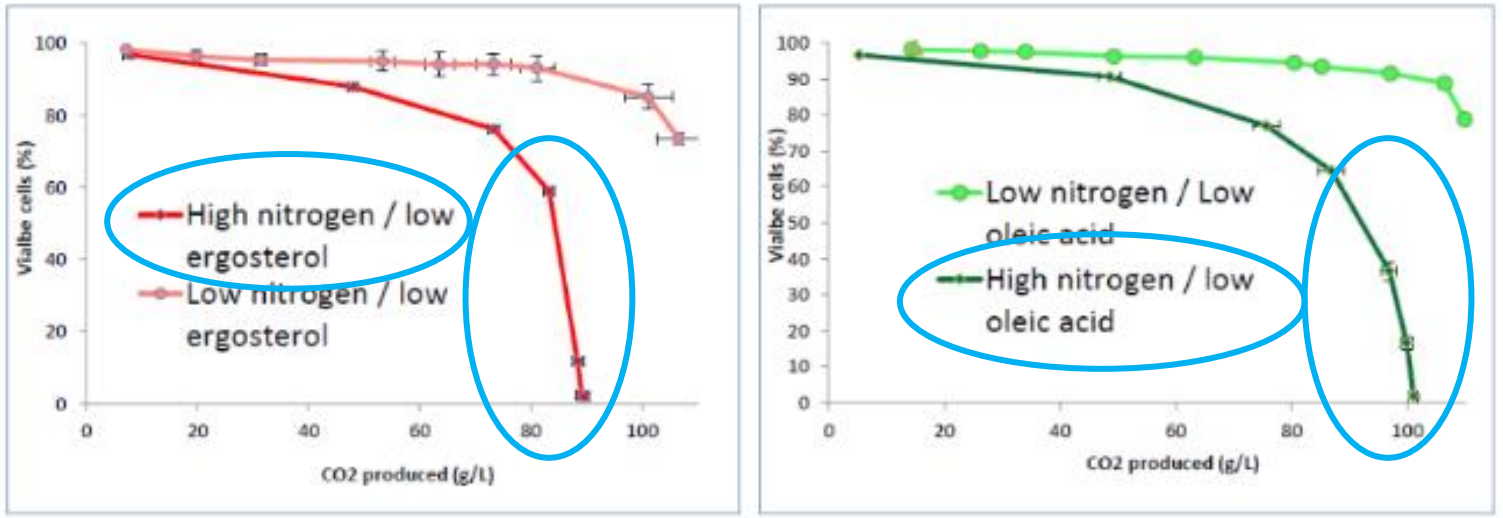
Nutritional imbalances
Impact of lipids on yeast viability



Duc C., 2017
(PhD Lallemand – INRA)

- Lipid source starvation triggers cell death in a nitrogen-dependent manner

➢ *GoFerm Protect Evolution will prevent/limit this risk*

Duc et al (2017) PLOS ONE 12(9): e0184838, 1-22
Duc et al (2019) OenoONE 3, 445-456

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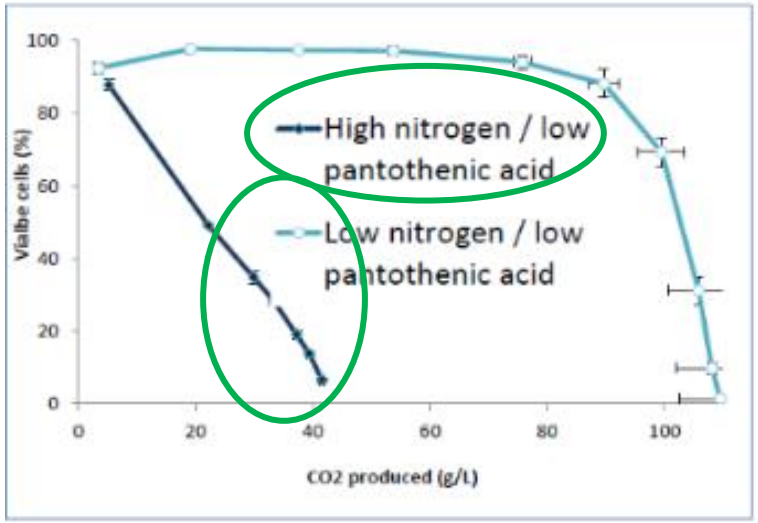
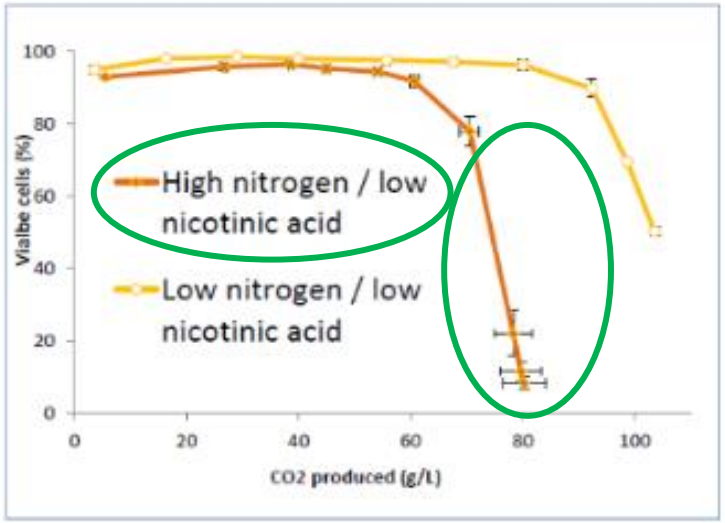
Nutritional imbalances
Impact of vitamins on yeast viability





Duc C., 2017
(PhD Lallemand – INRA)

- Vitamin starvation triggers cell death in a nitrogen-dependent manner

➢ *GoFerm Protect Evoltuion and Fermaid O will prevent/limit this risk*

Duc et al (2017) PLOS ONE 12(9): e0184838, 1-22
Duc et al (2019) OenoONE 3, 445-456

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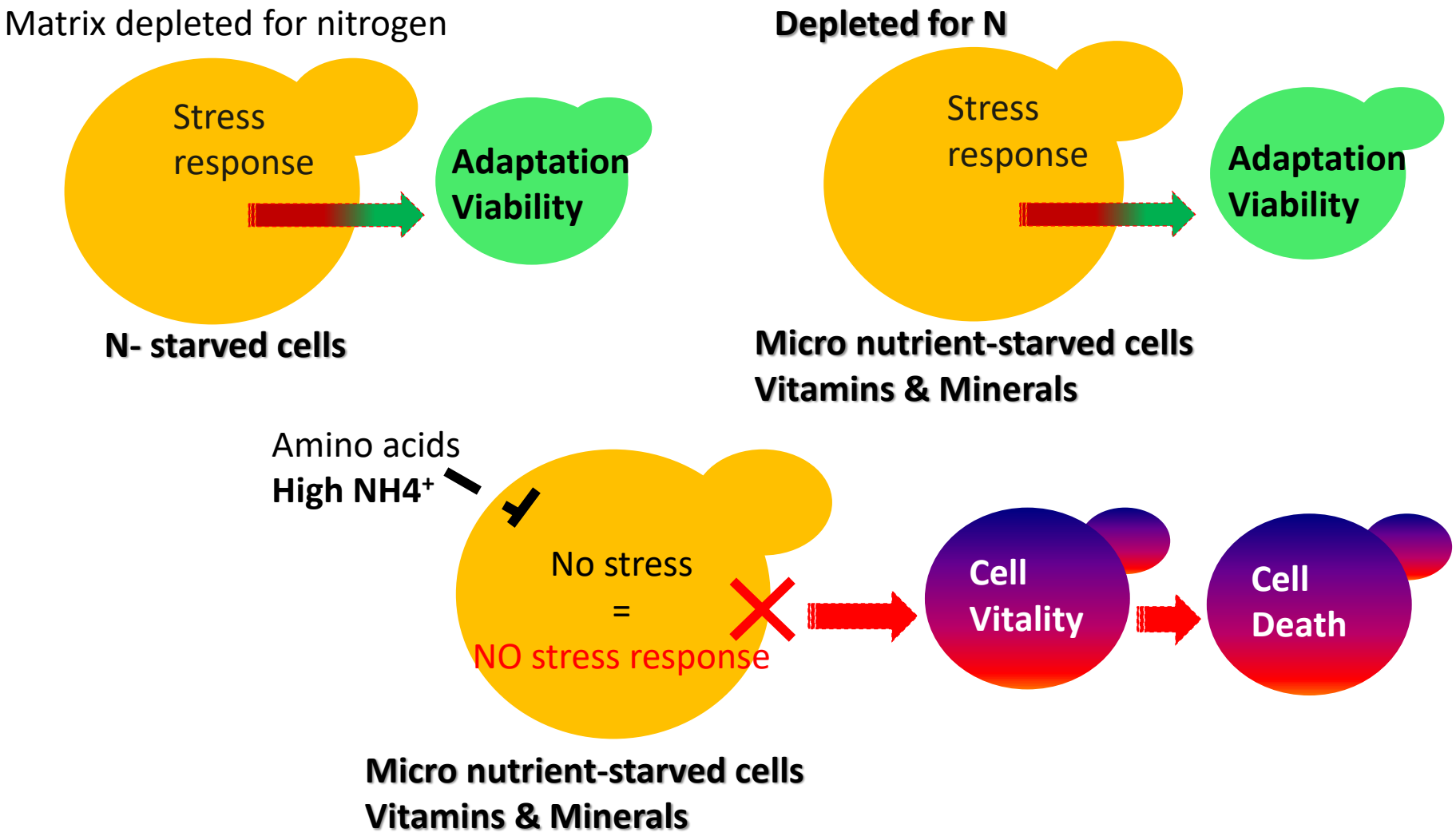
Nitrogen has an important role in signalling a stress response & yeast cell death



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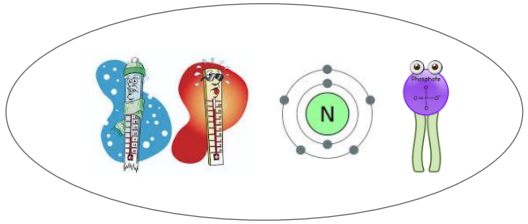
Nitrogen, Temperature & Lipids



- A combined management
  - Nitrogen source (organic vs inorganic)
  - Timing of N addition
  - Temperature
  - Lipids (solids)
- Optimum conditions for production is different for higher alcohols and esters
- *Compromise*
  - Low temp - ↑ esters, but also ↑ fermentation time
  - Low turbidity (lipids) - ↑ esters, but ↑ risk of slow/sluggish AF and acetic acid

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Today : Yeast nutrition management:

1. Focus on nutrients interactions and balance

2. Optimize yeast metabolism

STIMULATION of yeast metabolism :

- **Specific composition (interactions of vitamins, sterols, YAN…)**
- **Timing of addition**
- **Target on aromas depending on the grape matrix (precursors)**

Goal : optimize the aromatic potential of the grape

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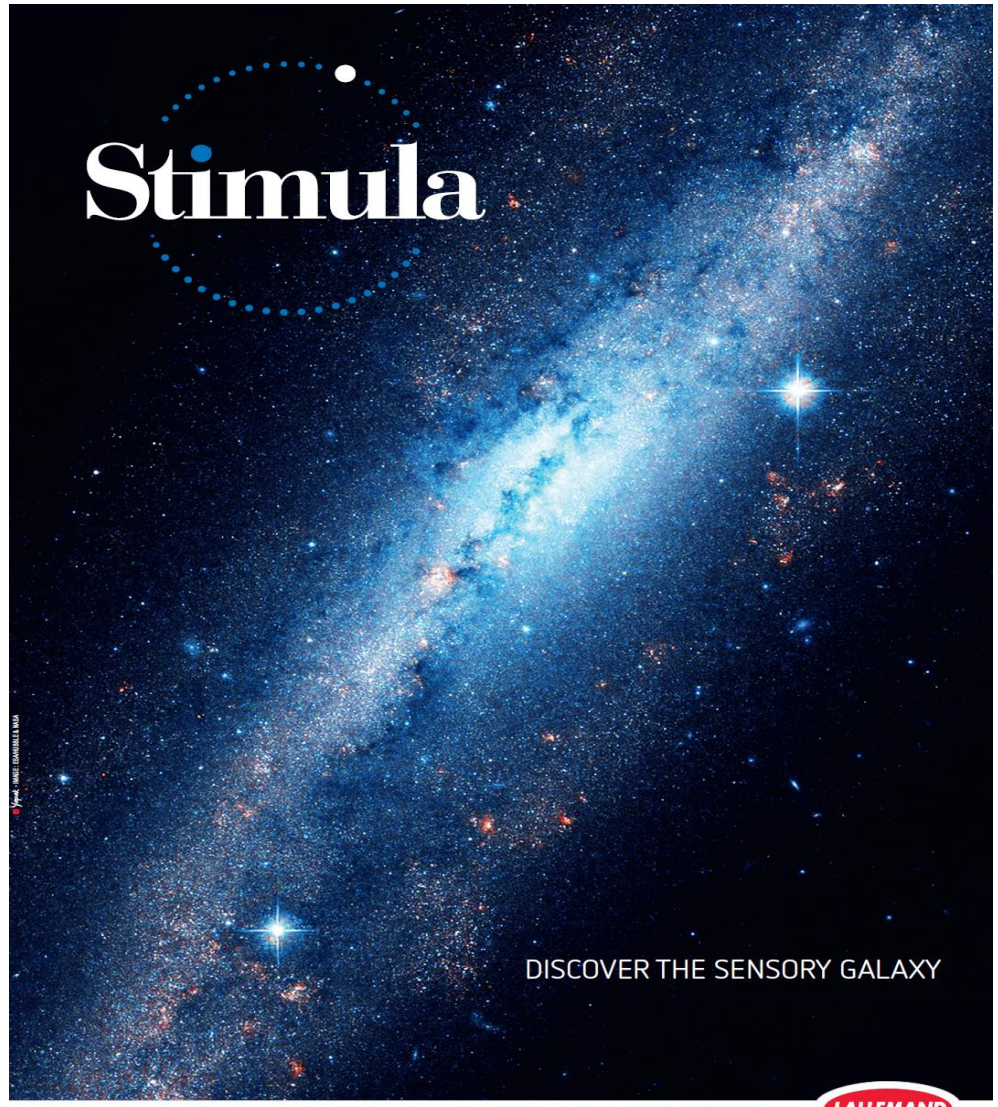

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Stimula
Syrah

A Balance composition

- Different nitrogen sources (100% yeast autolysate): to guarranty a balance nutrition & optimize the aromatic metabolism
- High vitamins content (pantothenate) to avoid H_2S & increase thiols revelation
- High minerals content (Mg) to increase ethanol resistance (higher toxicity with high T°)

Objectives

- To respect and enhance the varietal
- Remove the mask (off-flavors : H_2S)
- To stimulate yeast metabolism of aromas synthesis (better precursors uptake) = Addition at the beginning of the AF
- Nutritionnal balance : free aas, peptides, vitamins & minerals.

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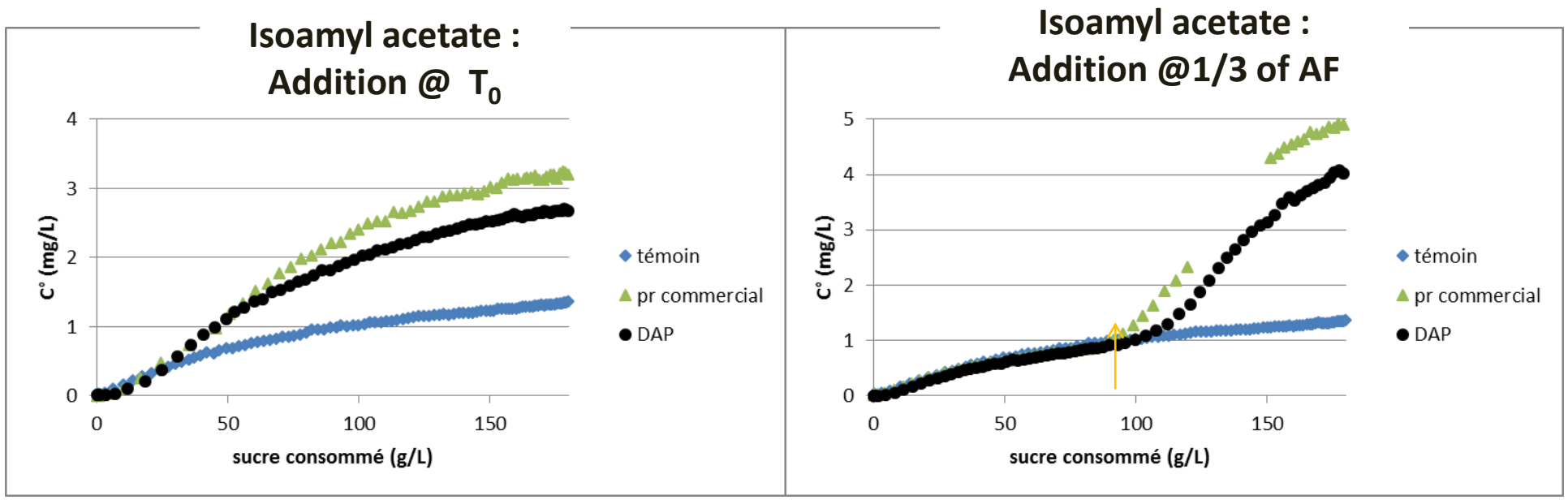
Esters synthesis : Impact of timing of nutrient addition (Stimula Chardonnay)



- 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

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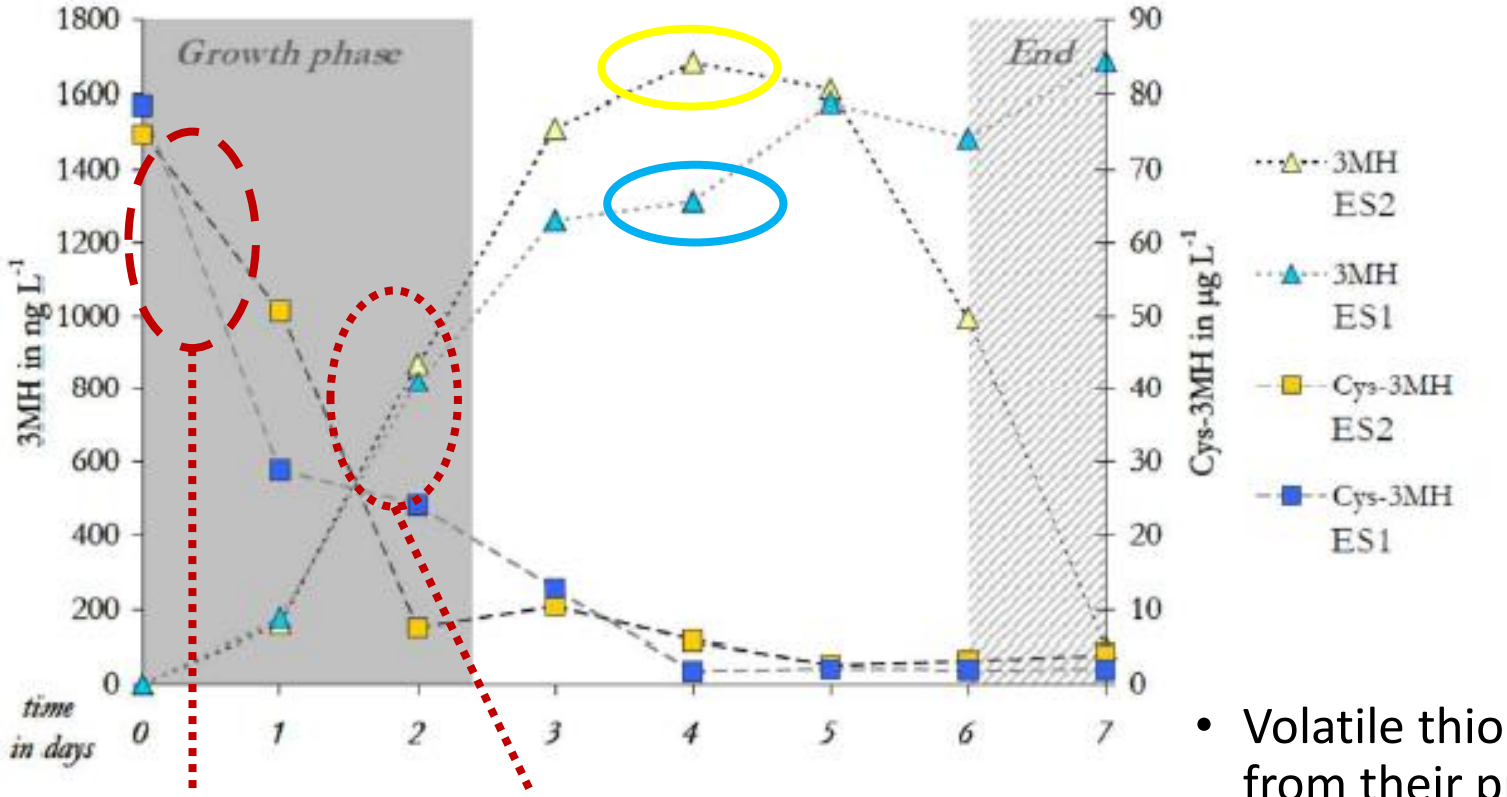
When are thiols released?

Supplemented with Cys-3MH (75 µg/L)



1st third of exp phase
Uptake of thiol precursors

2nd third of exp phase
Release of thiol precursors

- Volatile thiols are released from their precursors during the yeast growth phase
- Release of thiols efficiency is yeast strain dependent

Subileau 2008

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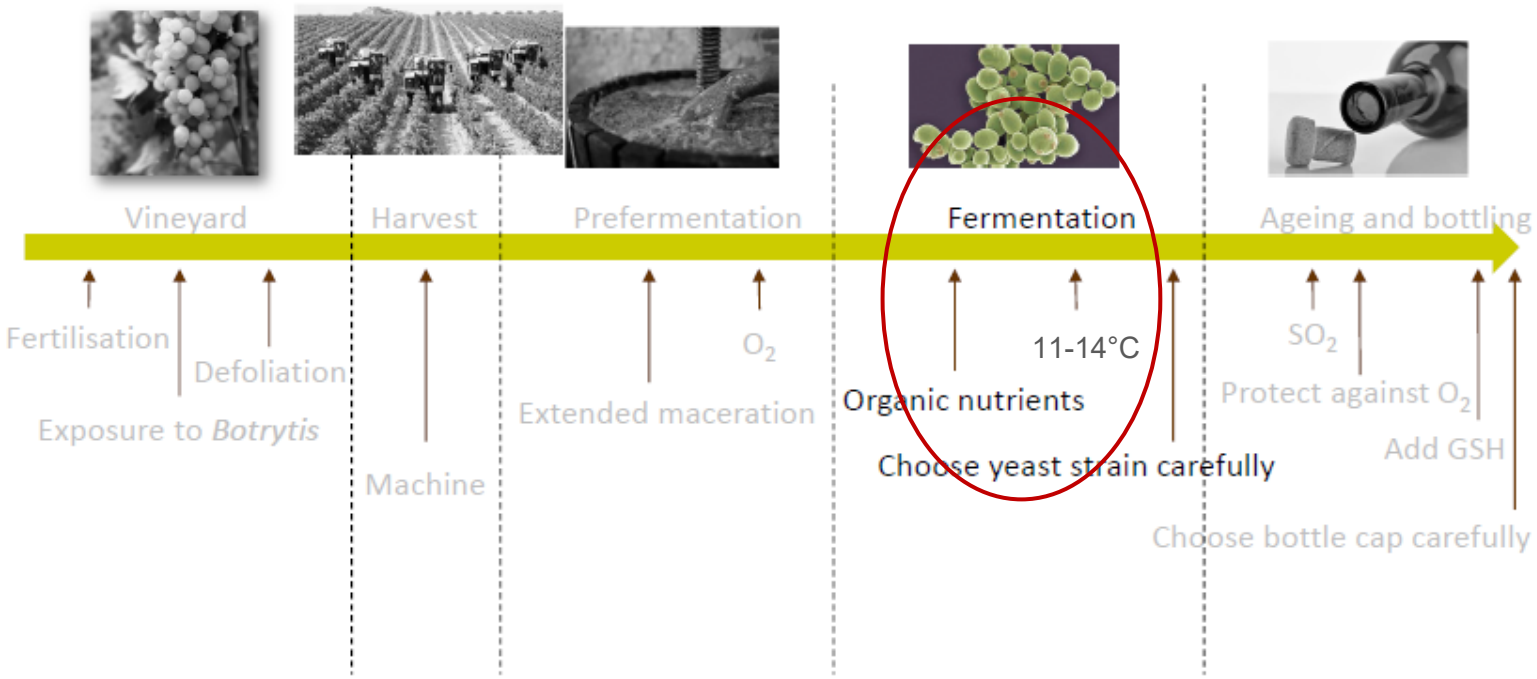
Numerous ways to increase thiol production



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Nutrition is multifaceted

Nutrients are …	More than only Nitrogen : ***All are interlinked*** NH3 – Amino acids – Vitamins – Minerals – Lipids (sterols)
Yeast Growth	N requirements are strain dependent NH3 = growth Amino acids = finishing AF & 2° metabolites
Fermentation AF Kinetics	Micronutrients (vitamins & lipids) are very important
Vitamins	Important for yeast metabolic functions Combination with N is important
Aroma Production	NH4 can inhibit the uptake of thiol precursors
Biggest take home message	Nutrition is multifaceted : Rational strategies **KEY** – Complex and balanced

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SYNERGISTIC ACTION TO REVEAL YOUR WINES' INDIVIDUALITY

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Bactiless

Reduce the risk of spoilage bacteria

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PREVENTING SULFUR OFF-ODORS DURING FERMENTATION

Cider has a strong propensity to develop sulfur off-odors which are largely attributable to hydrogen sulfide (H_2S). There are several variables and potential sources of these off-odors. This guide has been created to determine critical control points for preventing sulfur off-odors before they develop.

TERMS USED TO DESCRIBE SULFUR OFF-ODORS INCLUDE:

H_2S	Rotten Egg	Reduced	Burnt Rubber	Onion	Skunky	Garlic	Funk

HOW ARE THESE ODORS CREATED?

There are several potential sources of sulfur that end up creating off-odors including:

1. **Elemental sulfur from orchards or storage**
2. **Sulfur dioxide (sulfite) additions to prevent spoilage or oxidation in juice**
3. **Sulfur from sulfur-containing amino acids**
4. **Naturally occurring sulfates in apples**

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PREVENTING SULFUR OFF-ODORS

The key to preventing sulfur off-odors during fermentation is **compensating appropriately for your juice's nutritional shortcomings** and **maintaining a low-stress environment for yeast**:

JUICE	FERMENTATION
Understand and compensate for nutritional shortcomings	*Maintain a low-stress environment for yeast*

- Yeast nutrition
  - Healthy cells – vitality & viability
  - Organic & Complex nutrients (Fermaid O & Fermaid K)
- Yeast Handling
  - Dominate the microbial competition
    - Inoculate early
    - Protect (GoFerm Protect Evolution)
    - Manage temperatures

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- Sulfur off-odors treatment:
  - Reduless
    - Specific Yeast Derivative with fixed copper to reduce sulfur off-odors
    - Alternative to chemical copper treatments that damage the fruit character and create harsh flavour sensations

  - Noblesse
    - Specific Yeast Derivative nutrient that contributes to an overall roundness and rebalancing of flavours
    - Can help reduce undesirable aggressive characters or sensations of dryness

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- Building positive mouthfeel
  - Mannolees
    - a unique blend of mannoproteins and vegetal polysaccharides
    - Mannolees can offer a degree of stability, soften aggressive sensations, enhance body, add length, and maintain fruity characters
    - 100% soluble
    - Add 24 hours before bottling the cider

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What is Mousy taint?

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Sensory threshold – wine pH adjustment

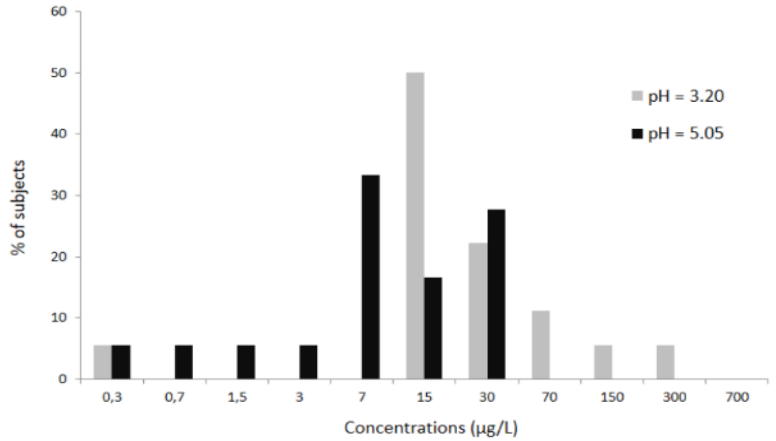

FIGURE 3. Individual detection threshold distribution for APY before and after pH adjustment (n = 18).

Compound	pH	Sensory threshold (µg/L)	R2
APY	3.2 5.05	54.9 8.6	0.91 0.91

- The absolute threshold (group threshold) for APY in red wine (Gamay) was reduced by a factor of 6.4 when the pH was increased.

Tempère et al (2019) OenoOne 2, 95-105

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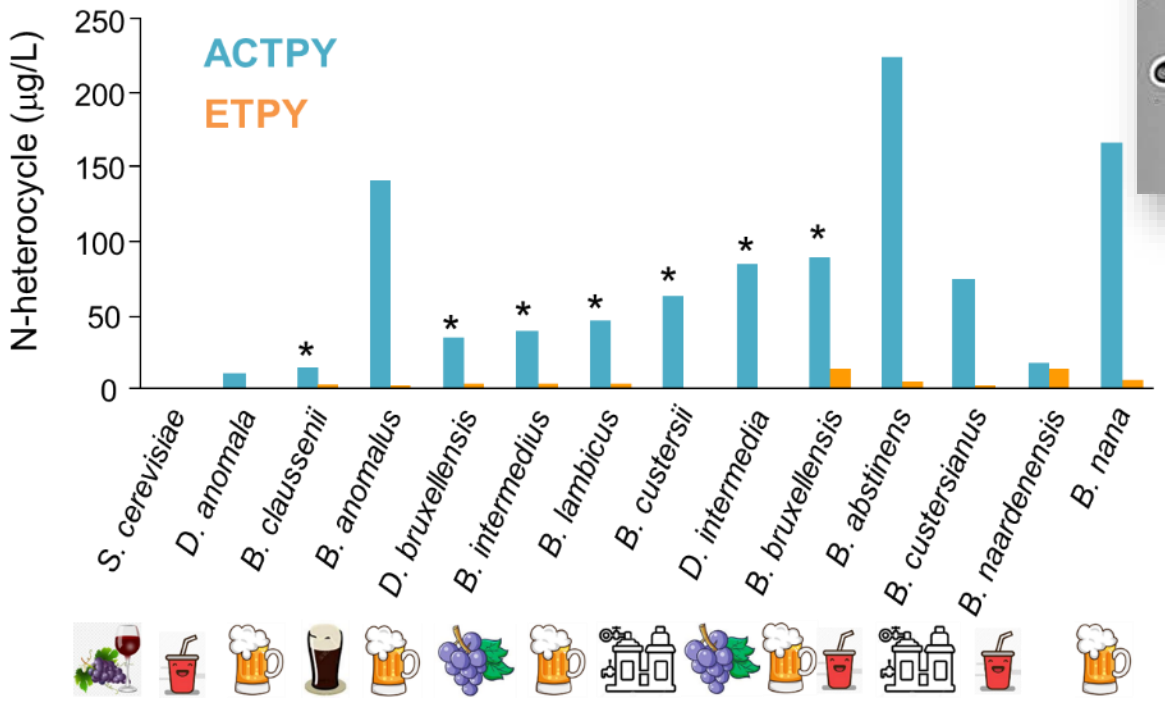
Microbiology of Mousy taint – Yeast

- Only species within *Brettanomyces* & *Dekkera* have been shown to produce mousy taint compounds
  - Main species is *Brettanomyces bruxellensis*



* Species associated with wine

Grbin & Henschke (2000) AJGWR 6, 255-262

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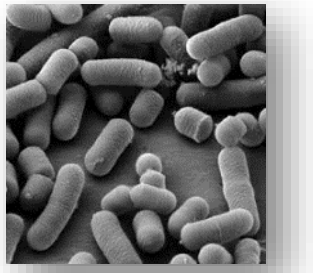
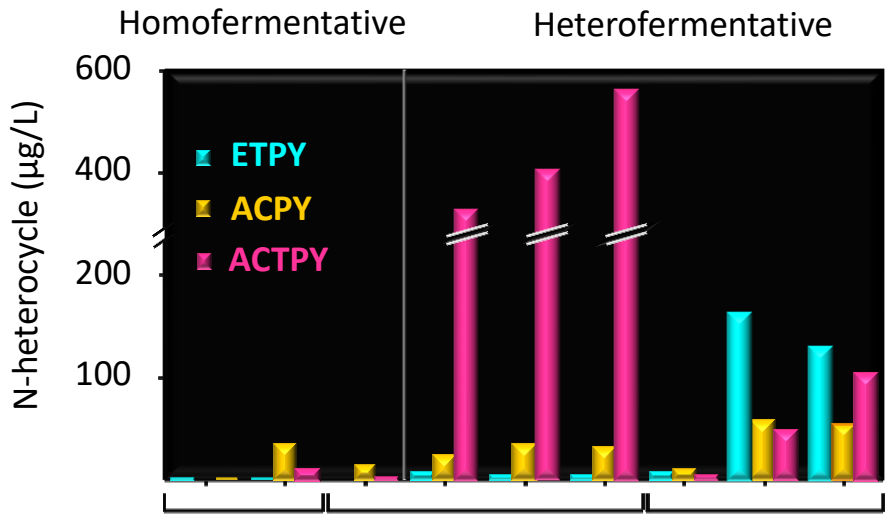
Microbiology of Mousy taint – Bacteria

- LAB species have been shown to have the capability to produce mousy taint compounds
  - ➢ Bacteria are the main culprit for production of mousy taint compounds

- Heterofermentative LAB – main producers
  - *Lactobacillus hilgardii*
  - *Lactobacillus brevis*
  - *Oenococcus oeni*
- Homofermentative LAB – to a lesser extent
  - *Pediococcus*
  - *Lactobacillus plantarum*





Costello & Henschke 2001 AJGWR 7, 160–167

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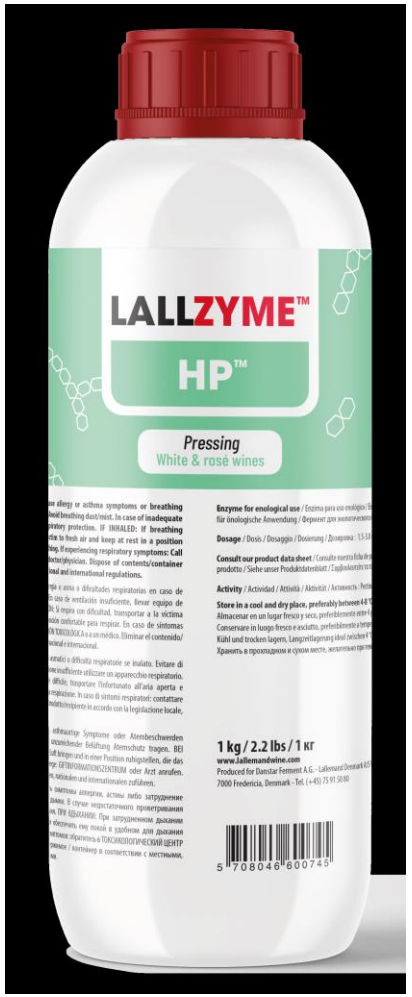
Fruit Pressing & Clarification

- Main factors influencing pectinase activity
  - Temperature – optimum 8 – 12 C
  - Treatment time – optimum 1 – 2 hours
  - Dosage – higher dosage => faster depectinization



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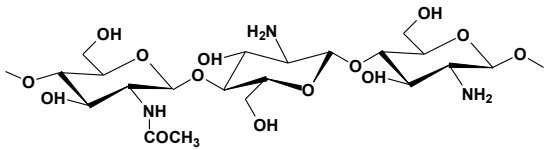
In conclusion …

- Clarification
  - https://scottlab.com/preventing-and-treating-sulfur-off-odors-in-cider
- Yeast nutrition
  - Healthy cells – vitality & viability
  - Complex & Organic nutrients (Fermaid AT & Fermaid O)
- Fermentation
  - Malic acid reduction
- Oxidation protections
  - Specific Inactivated Yeast
- Manage spoilage
  - Chitosan products



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