Impact of fermentation conditions on the ester production by wine yeast

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Agenda

- **Context and objectives**
- **Material and method**
- **Characterization of 12 *Saccharomyces* strains**
  - Analytically
  - Aromatically
  - Sensorially
- **Fermentation conditions optimization for 2 selected strains: Impact of T, YAN and Yeast hulls addition on:**
  - Analytical profile
  - Aromatic profile
    - With a first approach on production/concentration kinetics
  - Sensorial profile
- **Conclusions and perspectives**

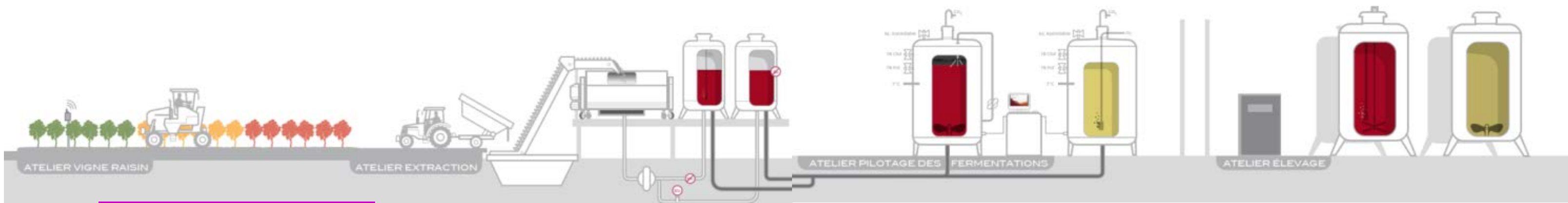
Context and Challenges

How to increase ester production during white wine fermentation?

- Select the yeast
- Adjust the fermentation conditions
- Select the appropriate nutrients

Vivelys's transversal approach

Build with each of our clients the most effective production line to produce wine profiles & volumes that are adapted to the market.



Fruit maturity: Dyostem®

Micro-oxygenation: Visio® series

Fermentation management: Scalya®

O2CM: Cilyo®

Ageing: Keryan® & Batonneur®

Indigeneous yeasts selection

Active yeasts room

Boisé: Oak chips and staves

Sensory analysis: Siryel®

Grape profile → Wine profile

VINEYARD

Characterize terroir

Qualify grape profile

Manage harvest

VINIFICATION

Secure and optimize alcoholic fermentation

Control wine profiles

AGEING

Optimize wine profiles

PRODUCT DEFINITION

QUALITY CONTROL & PRODUCT CONFORMITY

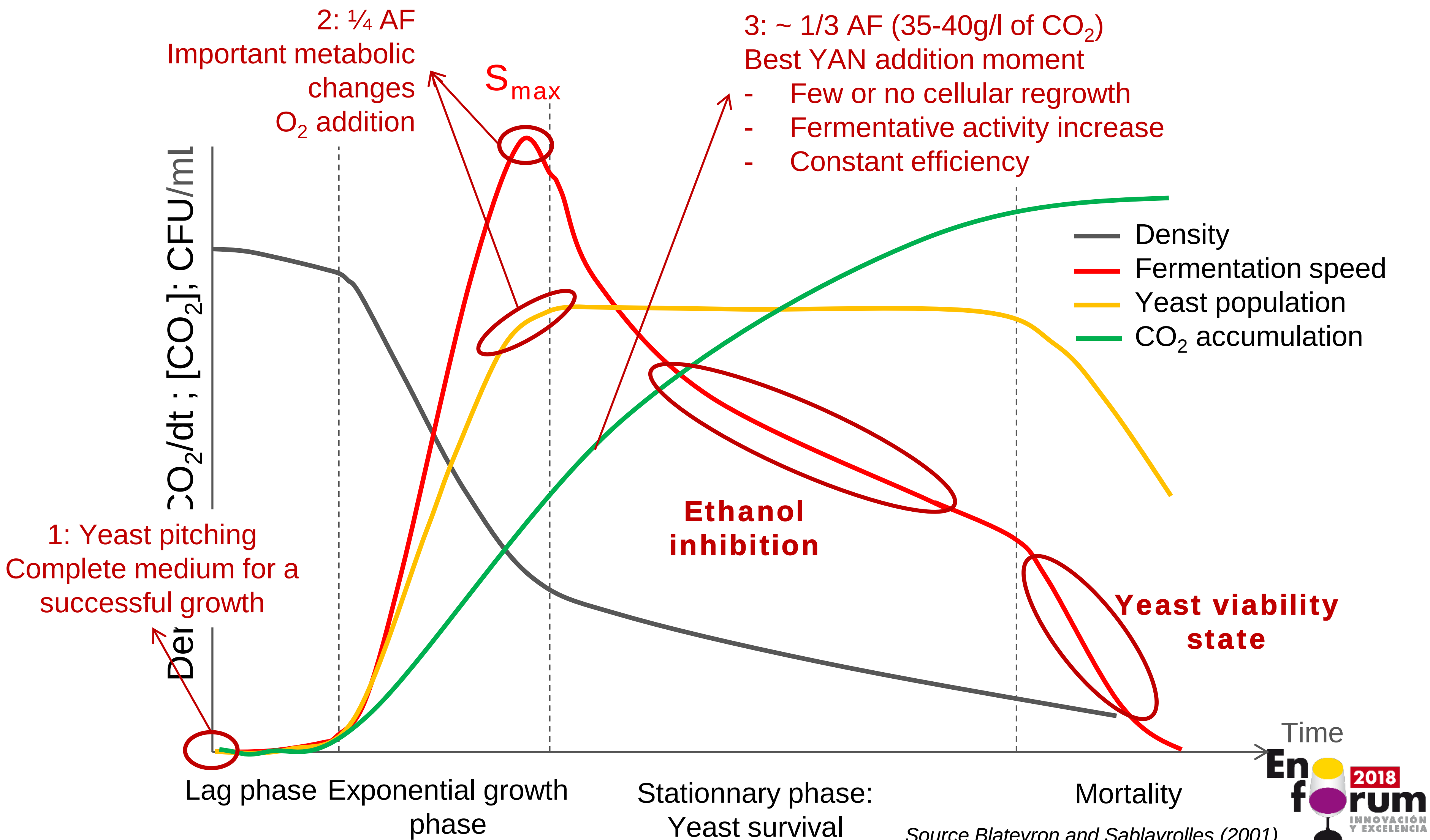
Objectives

1. Analytically and aromatically characterize 12 *Saccharomyces* strains supplied by Fermentis on a non-deficient must.

2. Optimize fermentation conditions to increase the production of aromas (especially esters) for 2 selected strains.

Material & Method

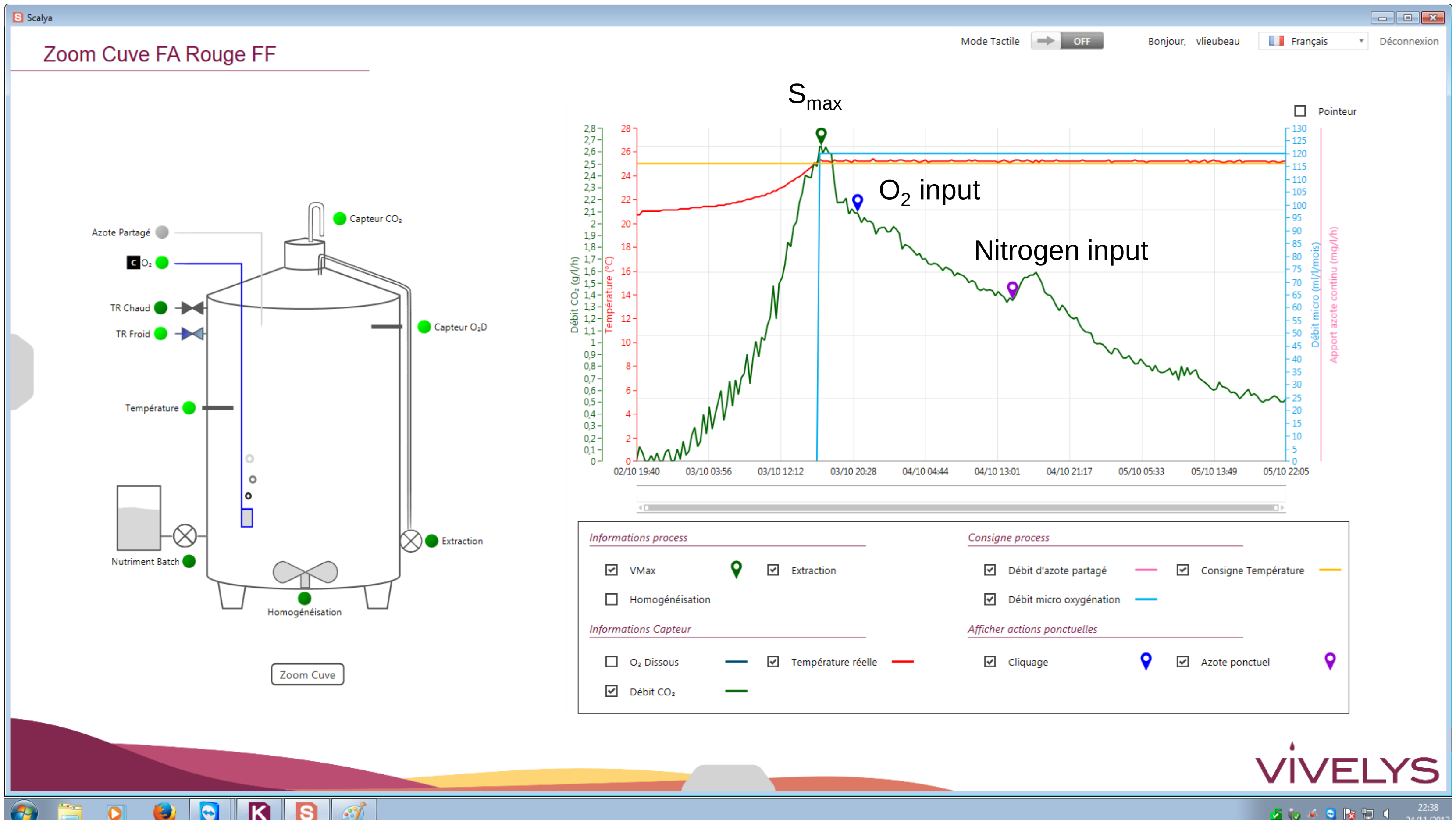
Basics of the experimental work: V_{max} principle



Source Blateyron and Sablayrolles (2001)

Material & Method

SCALYA® fermentation management tool



Material & Method

Experimental cellar

Specific storage area

Cigar-shaped stainless steel tanks with mid-tank sampling

CO_2 sensors on top

Material & Method

Analyses

All the wines produced were analyzed

- **Chemically**
- **Analytically** (Aroma)
- **Sensorially** (Internal and Expert Jury)

Material & Method

Fermentation recipe

Temperature:

4 temperature stages : 16-14-16-18° C

Oxygen input:

A 5mg/L pulse at the Smax

Nitrogen input:

Nutrients were added to obtain a 1:1 ratio Yeast Assimilable Nitrogen (YAN) to fermentable sugars, to prevent a deficiency from affecting yeast performance. We kept the same intake for all strains.

The addition was made in the form of DAP at 35% of fermentation progress.

Characterization of the 12 strains

Experimental conditions for the 12 strains

- Turbidity adjustment to 150 NTU

- Assimilable nitrogen and residual sugars titration

- Homogeneization through nitrogen bubbling

- Inertage and filling of the tanks at 80L

Flash-pasteurized Chardonnay → Mix in buffer tank → 10hL → Strain 1, Strain 2, Strain 3, Strain 4, Strain 5, Strain 6, Strain 7, Strain 8, Strain 9, Strain 10, Strain 11, Strain 12

- Turbidity measurement of each tank for any readjustment

- Nitrogen bubbling for deoxygenation then inerting of the top of the tank with CO2

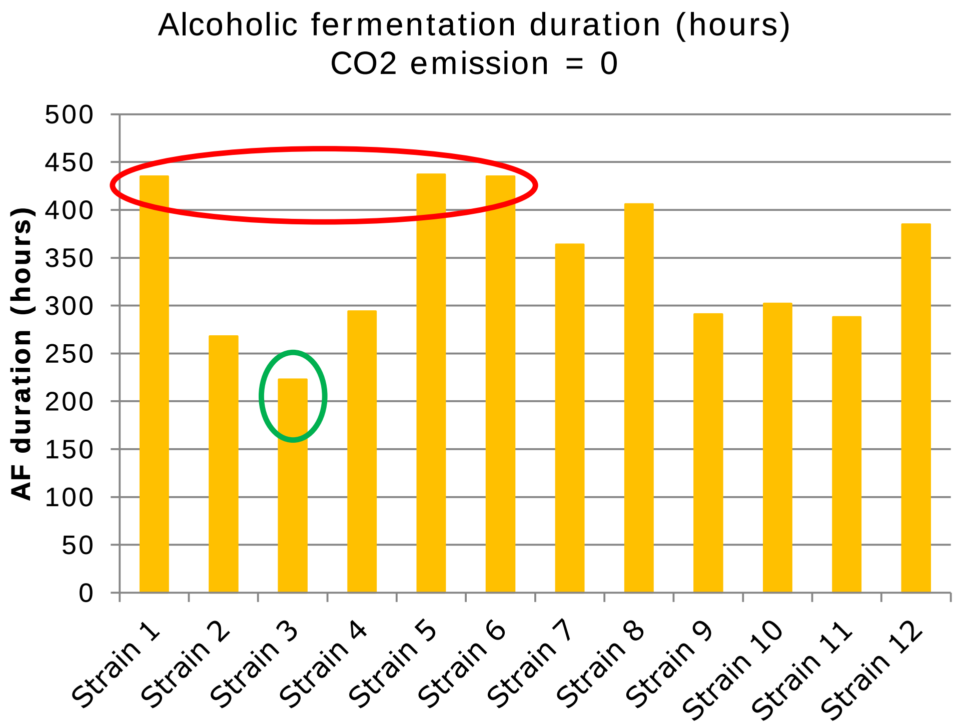
- Yeasting at 20g/hL

- Nitrogen intake at 35% of advancement rate

Sugar	207 g/L
pH	3,45
Total Acidity	3,22 g/L
Malic Acid	3,7 g/L
Volatile Acidity	0 g/L
Free SO_2	0 mg/L
Total SO_2	24 mg/L
Assim. Nitrogen	165 mg/L

Characterization of the 12 strains

Comparison of the 12 strains kinetics

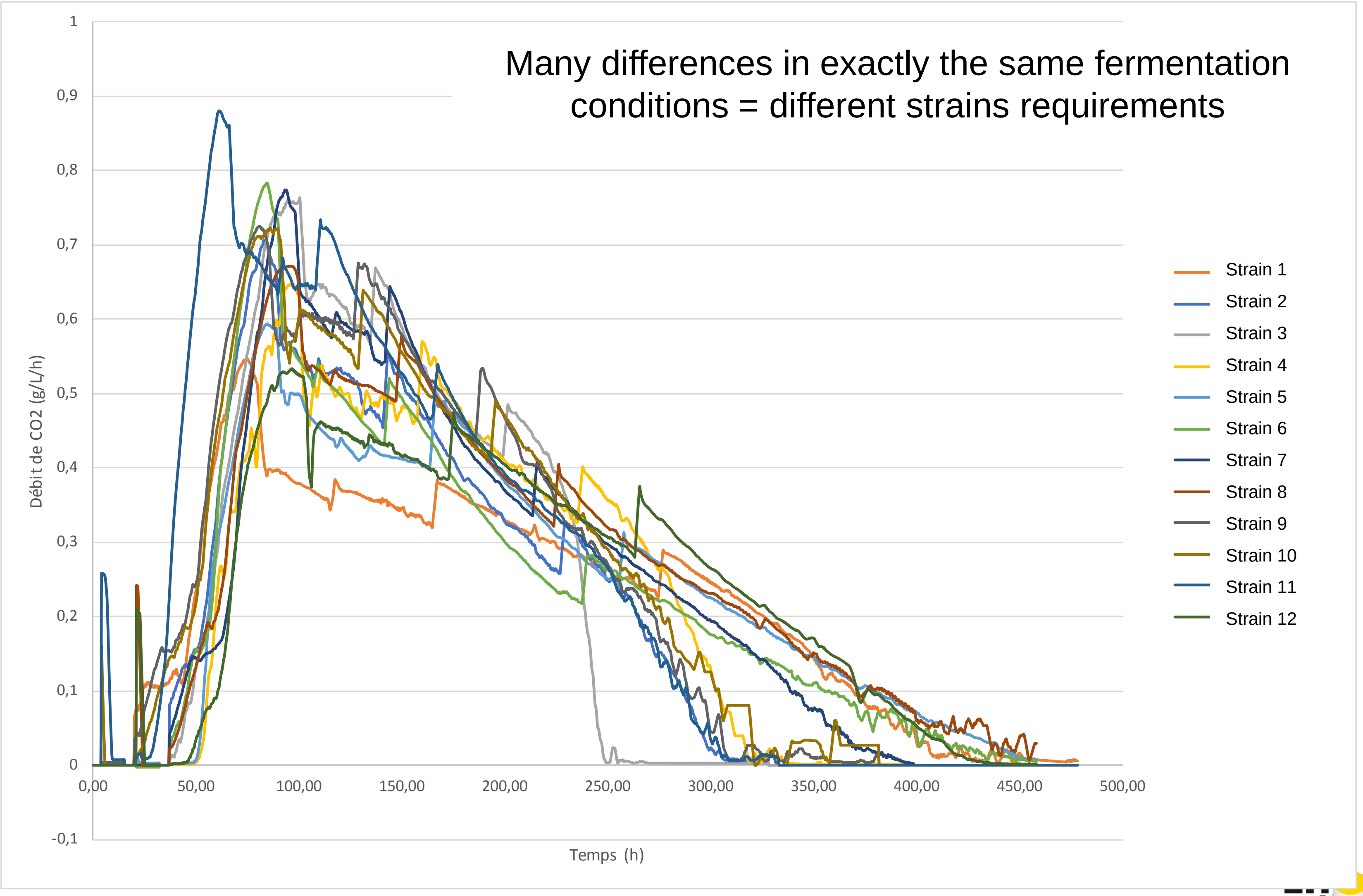


Strain	AF duration in hours
1	435
2	268
3	223
4	294
5	437
6	435
7	364
8	406
9	291
10	302
11	288
12	385

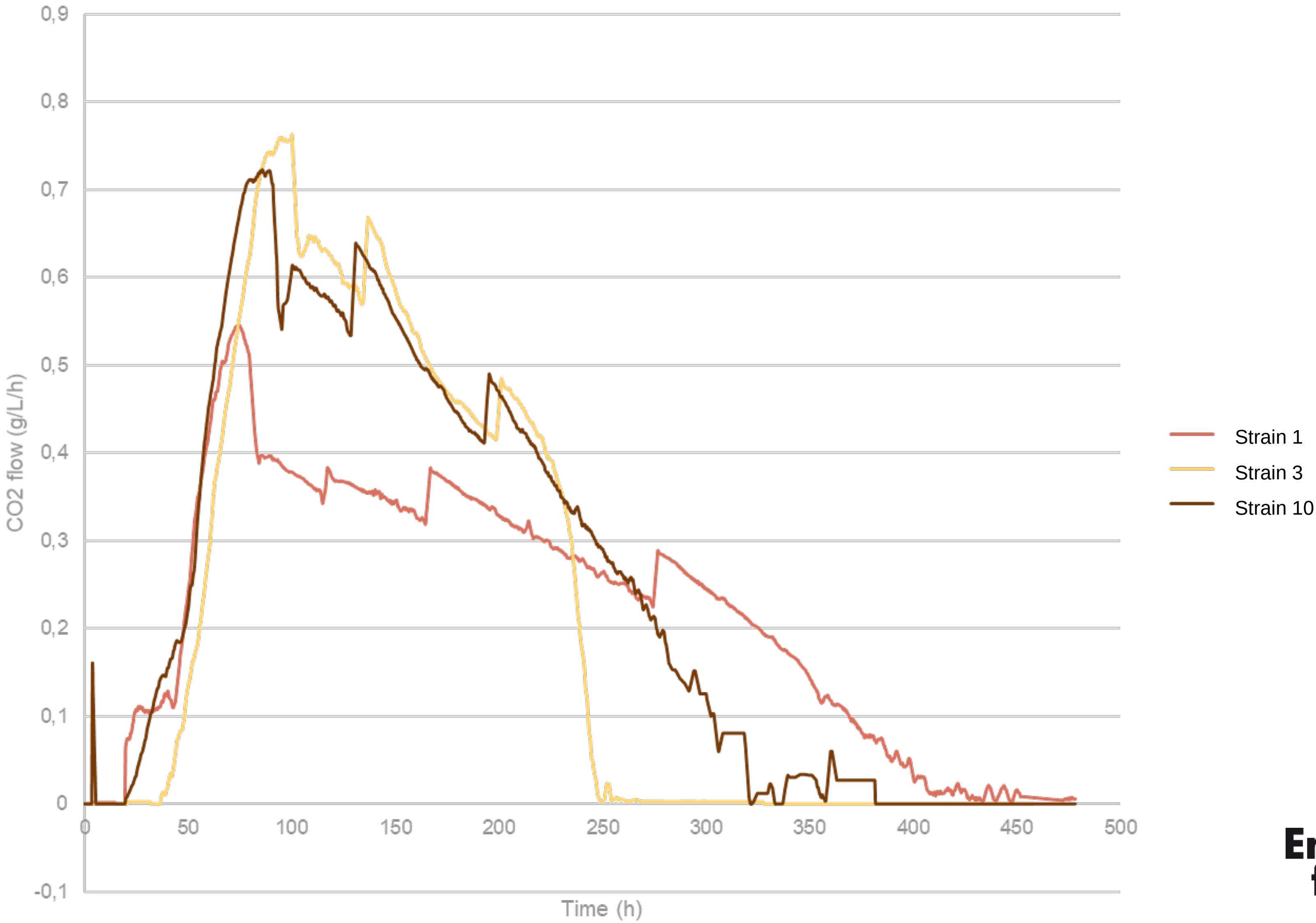
The duration of fermentation varies a lot between the different strains.

Strain #3 ends its fermentation in 223 hours, whereas strains #1, #5 and #6 need twice this amount of time (437 hours).

Characterization of the 12 strains

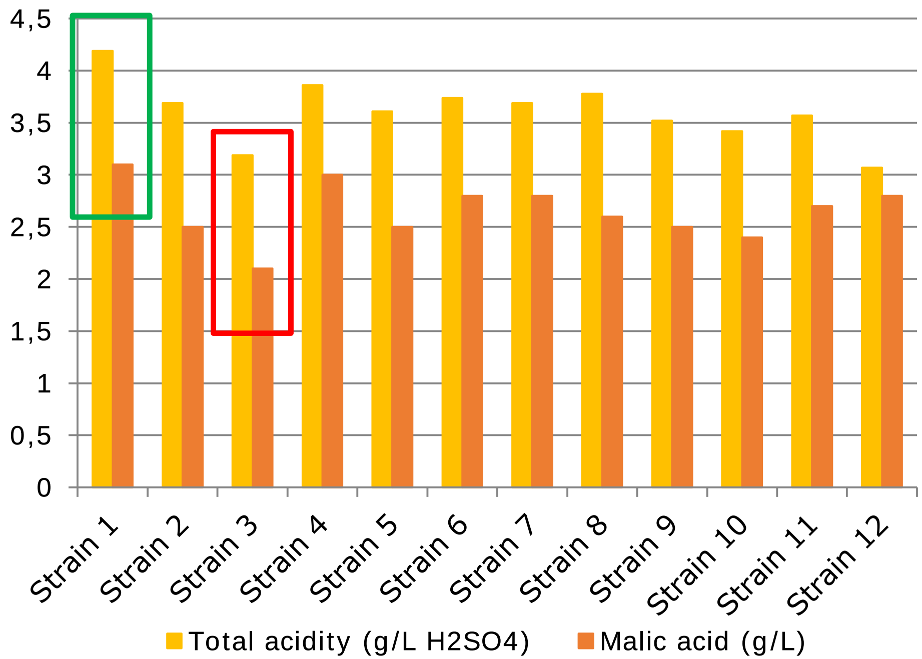


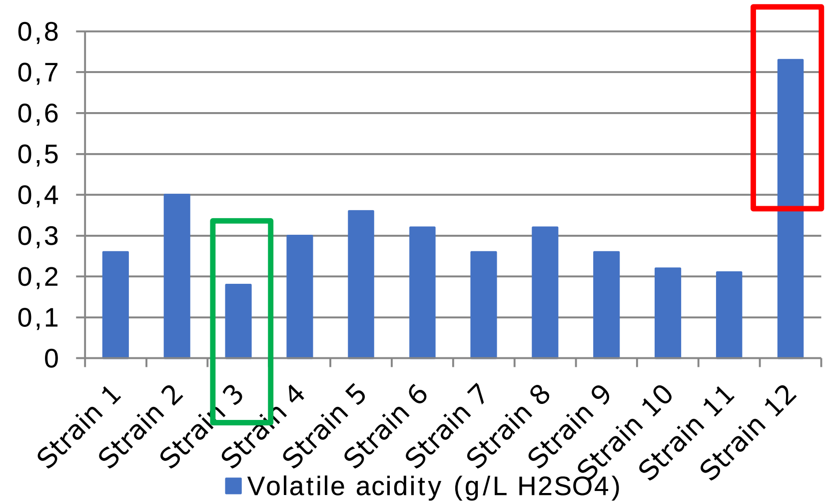
Characterization of the 12 strains

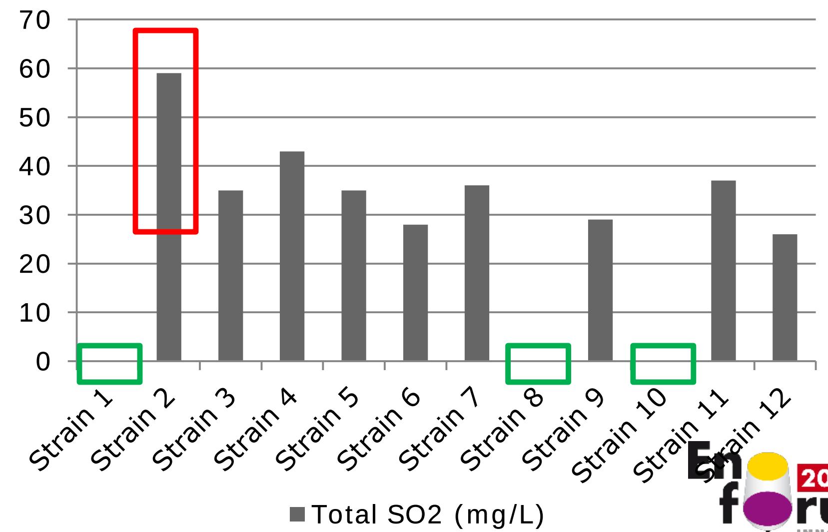


Characterization of the 12 strains

Classic chemical parameters (end of alcoholic fermentation)







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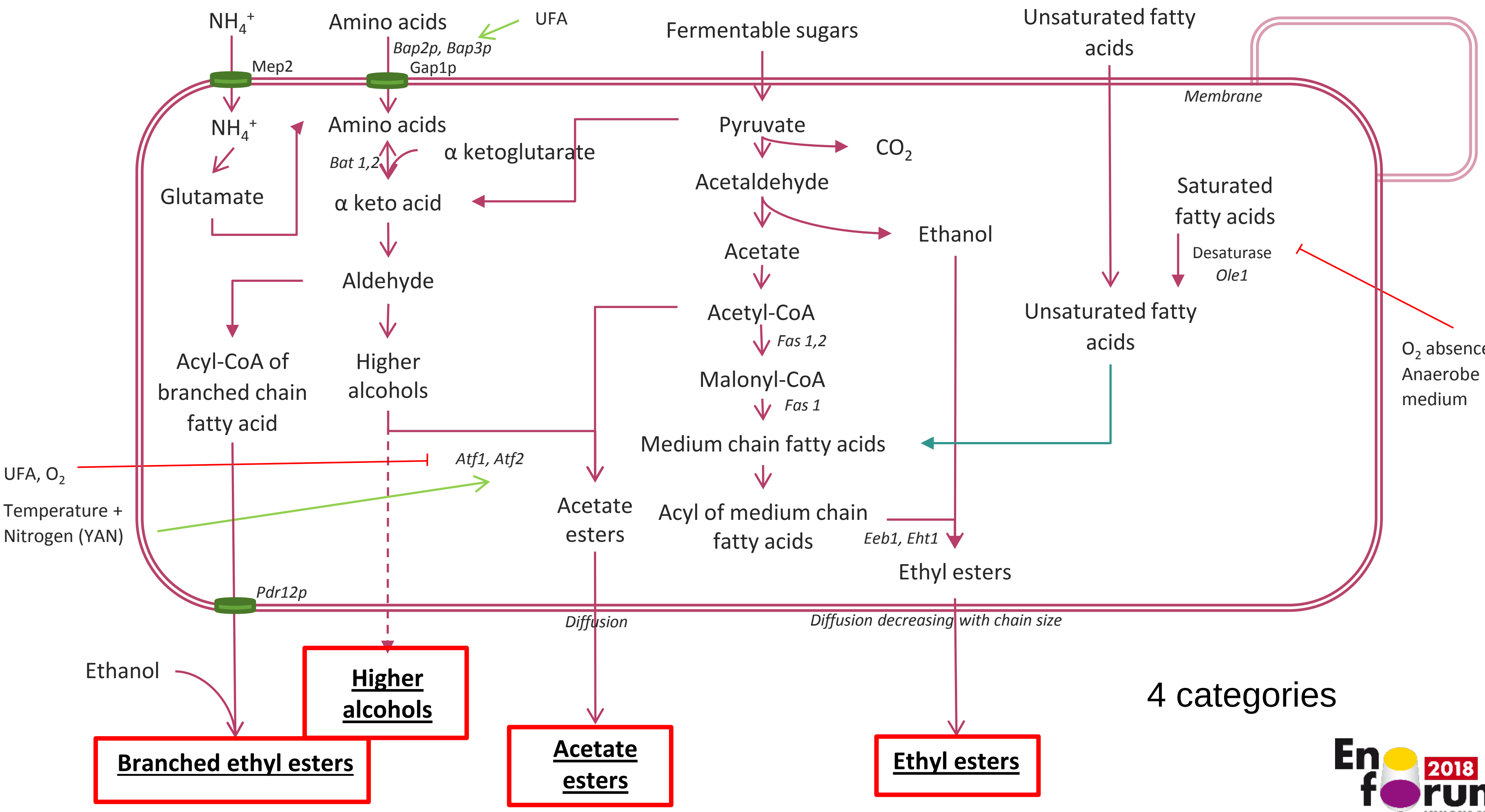
Characterization of the 12 strains

Aromatic analyses basics:

Interest focused on fermentation aroma = Higher alcohols and esters



4 categories

Characterization of the 12 strains

Aromatic analyses basics:

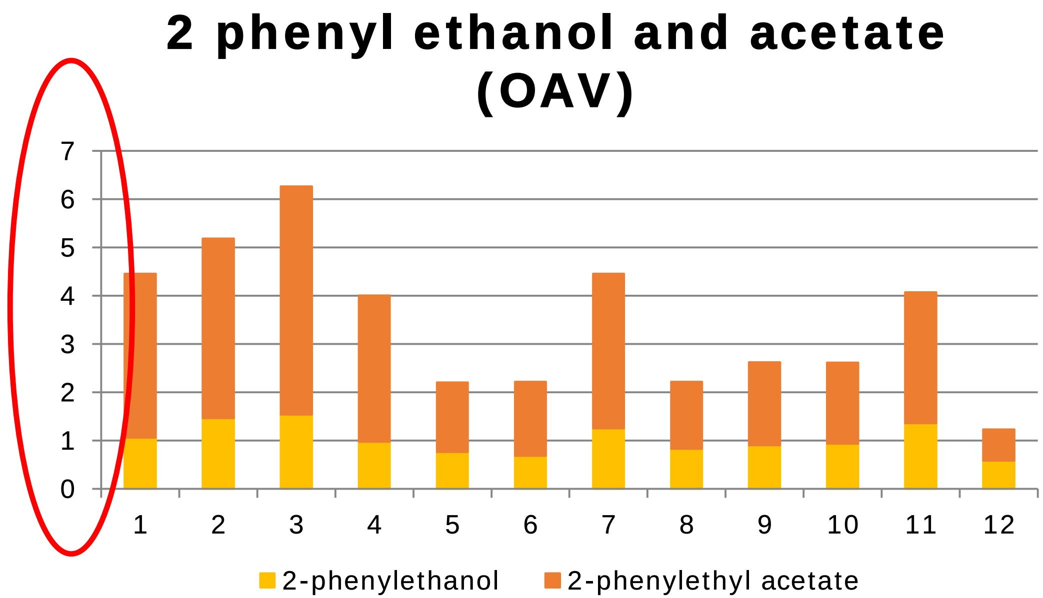
Precursors and metabolic pathways of main higher alcohols and esters: 4 categories

Compounds of interest		Metabolism	Precursors and catalysts				Toxicity
			Amino acids	Carboxylic acids	Alcohol	Catalyst	
2-phenylethanol	**OH**	Amino acid degradation	Phe	Amino acids, sugars, α-ketoacids and aldehydes		Enzymes	
Hexyl acetate	**Acetate**	**Grape C6 alcohols**	/	Acetic acid	**Hexanol** (Fatty acid alcohol)	Atf1, Atf2p Alcool Acyl Transferases Iah1 Esterase	
Isoamyl acetate		Amino acid degradation	Leu		Isoamylic alcohol		
2-phenylethyl acetate		Amino acid degradation	Phe		2-Phenylethanol		
Ethyl decanoate	**Ethyl (F)**	Fatty acid degradation	/	Decanoic acid	Ethanol	Eht1p, Eeb1p EtOH Acyltransferases	☠☠☠
Ethyl hexanoate		Fatty acid degradation, **grape acids**	/	Hexanoic acid			☠
Ethyl octanoate		Fatty acid degradation, **grape acids**	/	Octanoic acid			☠☠
Ethyl butanoate		Fatty acid, **Amino acid degradation**	/	Butanoic acid			☠
Ethyl 2-hydroxypropanoate	**Ethyl (Post F)**	**Cell carbon metabolism**	/	Lactic acid	Ethanol	**Malolactic fermentation**	
Ethyl 3-hydroxybutanoate		Amino acid degradation	Leu ?	3-hydroxybutyric acid		Strong alcohols	
Ethyl 2-methylbutanoate		Amino acid degradation	Ile	2-methylbutanoic acid			
Ethyl 2-methylpropanoate		Amino acid degradation	Val	2-methylpropanoic acid			
Ethyl 2-hydroxyisocaproate		Amino acid degradation	Leu	2-hydroxyisocaproic acid			

Characterization of the 12 strains

Aromatic analyses results:

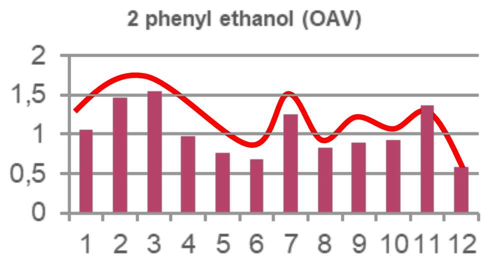
Results presented for molecules presenting a concentration only above their perception threshold (Odor Active Value = Concentration/Perception threshold)



- Different production by the strains but slightly significant in terms of flavor impact
- Correlation between both compounds as expected and slight differences in their ratio

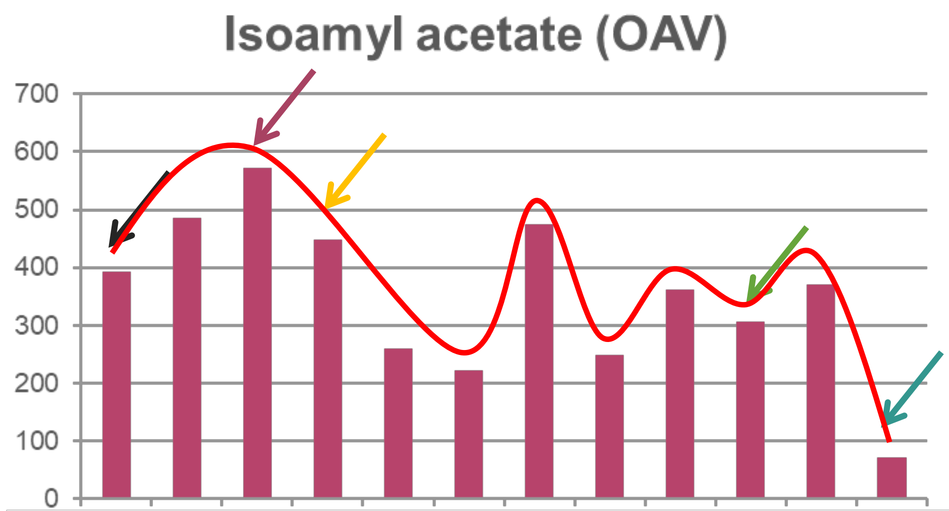
Characterization of the 12 strains

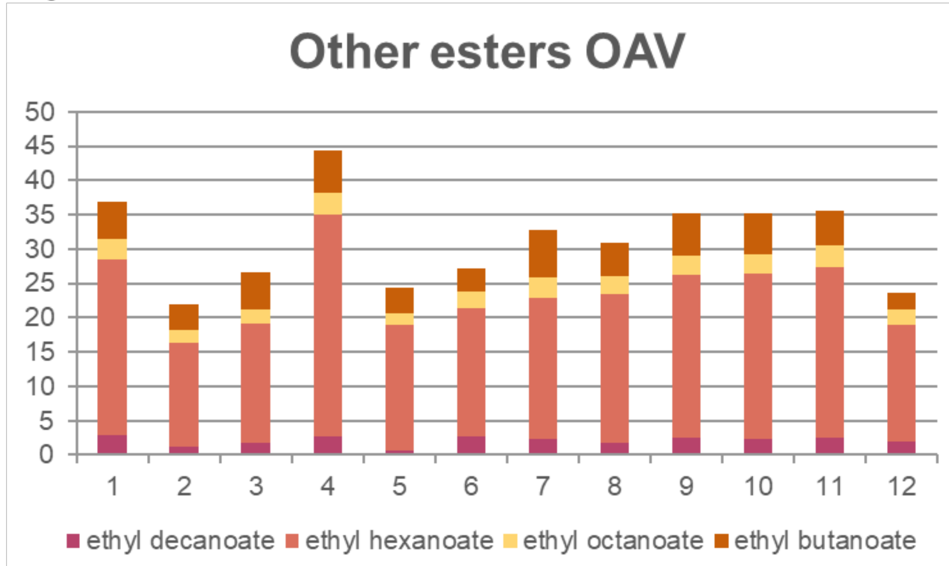
Aromatic analyses results:



Acetate following higher alcohol…
along the same pathway.

Medium acetate / Medium ethyl esters
High acetate / Low ethyl esters
High acetate / High ethyl esters
Low acetate / High ethyl esters
Low acetate / Low ethyl esters





Characterization of the 12 strains

Sensorial analyses basics:

Identification of 4 main fermentative profiles issued from aromatic molecules descriptors

AMYLIC

Isoamyl acetate	Banana, candy, tropical fruit

LACTIC

Ethyl butanoate	Reduction, Parmesan, Baby vomit, Fermented pineapple, Rancid

FRUITY

Ethyl decanoate	Soap, Pear
Ethyl hexanoate	Ripe strawberry, Pear, Badian, Anise, banana
Ethyl octanoate	Fruity, Soap, Pear, White flowers
Hexyl acetate	Stone fruit, Apricot, Pineapple
Ethyl 2-methylpropanoate	Ripe strawberry, Pear, Badian
Ethyl 3-hydroxybutanoate	Green, fruity, Grapes
Ethyl 2-hydroxyisocaproate	Black fruits (blackberry), Fresh fruits

~~Ethyl 2-methylbutanoate~~	Green, Apple, Lemon, Yoghurt, Fruity cheese
~~Ethyl 2-hydroxypropanoate~~	Lactic, Fresh fruits, Grape berry, Pineapple, Mango, Cherry

FLORAL

2-phenylethanol	Overblown rose, Violet, Caramel, Floral, Linden
2-phenylethyl acetate	Fresh rose, Yellow rose, Bell pepper, Pharmacy

~~Not detected on young wines (synthesized during ageing)~~

Above perception threshold

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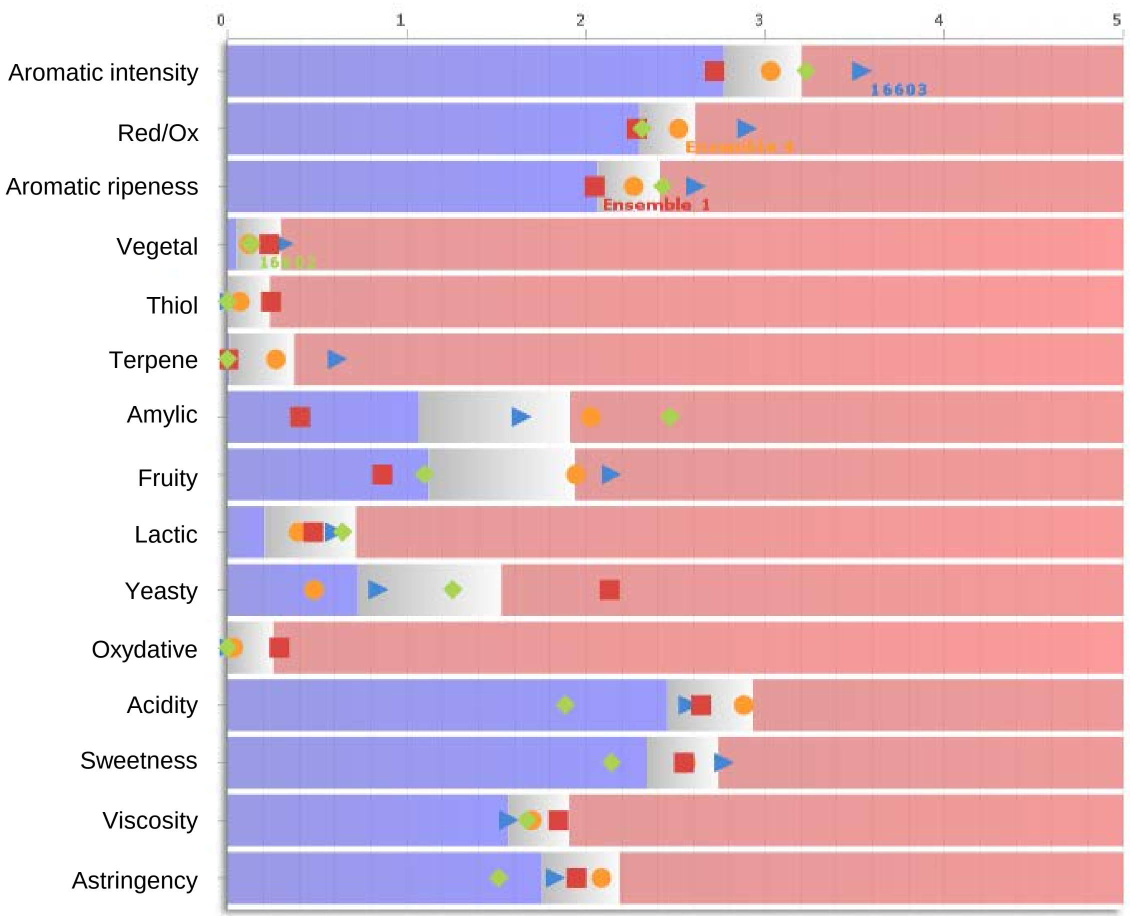
Characterization of the 12 strains

Sensorial analyses:

Simple ANOVA (alpha risk 5%) – well correlated with analytical results



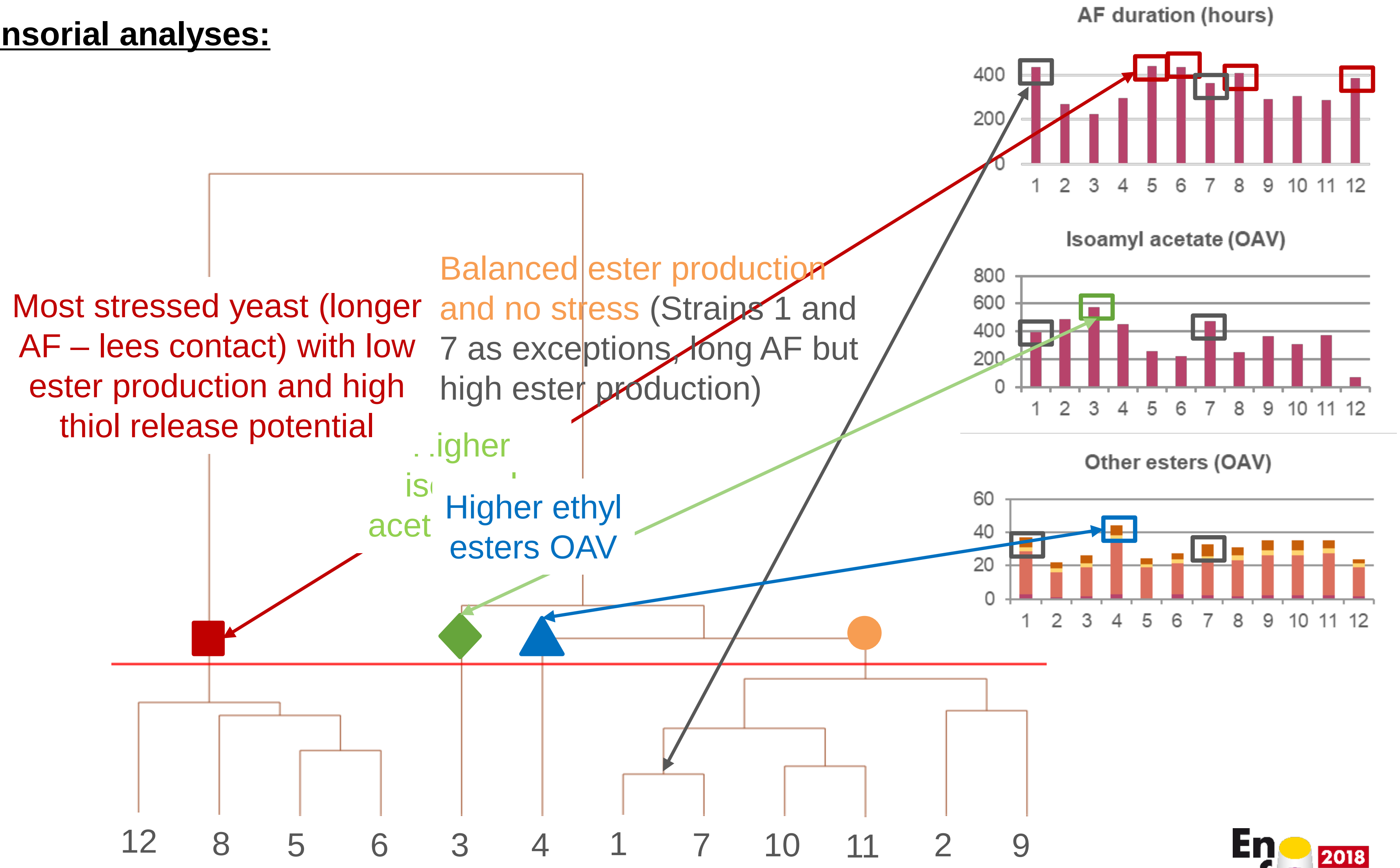
Aromatic intensity, Red/Ox and aromatic ripeness differences

Strain 3 : More amylic
Strain 4 : More fruity
Group 1 : Yeasty
Group 4 : Amylic/Fruity

- Group 4 (strains 1, 2, 7, 9, 10, 11)
- Group 1 (strains 5, 6, 8, 12)
- Strain 3
- Strain 4

Characterization of the 12 strains

Sensorial analyses:



Strain selection

Choice of strain 3: BC S103 and strain 10: HD S62

BC S103

- The fastest
- The most robust
- Low volatile acidity
- Medium SO_2 production
- High 2-phenylethanol
- Very high isoamyl acetate
- Low ethyl esters

HD S62

- Medium fast
- Robust
- Medium volatile acidity
- No SO_2 production
- Low 2-phenylethanol
- Low isoamyl acetate
- High ethyl esters

Fermentation conditions optimization

The objective of this second step is to study the impact of 6 fermentation conditions on the kinetics and production of fermentation aromas.

1. **<u>Control:</u>** Vivelys' fermentation recipe, already used in step 1 (12 strains)

2. **<u>Conditions of temperature:</u>**
    1. Standard recipe +4° C (20-18-20-22° C)
    2. Standard recipe -4° C **(12-10-12-16° C)**

3. **<u>Conditions of nitrogen:</u>**
    1. Excess of DAP → YAN/S = 1.45
    2. No addition of DAP → **YAN/S = 0.8** (ideal ratio to secure AF for most strains)

4. **<u>Condition of fatty acid source:</u>**
    1. Addition of yeast hulls → 20g/hl

Fermentation conditions optimization

Experimental conditions for the 2 selected strains

Flash-pasteurized Chardonnay

Mix in buffer tank

10 hL

Sugar	207 g/L
pH	3,45
Total Acidity	3,16 g/L
Malic Acid	3,66 g/L
Volatile Acidity	0,1 g/L
Free SO_2	0 mg/L
Total SO_2	20 mg/L
Assim. Nitrogen	165 mg/L

- Turbidity adjustment to 150 NTU
- Assimilable nitrogen and residual sugars dosage
- Homogeneization through nitrogen bubbling
- Inerting and filling of the tanks at 80L

- Turbidity measurement of each tank for any readjustment
- Nitrogen bubbling for deoxygenation then inerting of the top of the tank with CO2
- Yeasting at 20g/hL
- Nitrogen intake according to the recipe

BC S103 Standard

BC S103 +4° C

BC S103 -4° C

BC S103 +135ppmYAN

BC S103 without YAN

BC S103 + Hulls

HD S62 Standard

HD S62 +4° C

HD S62 -4° C

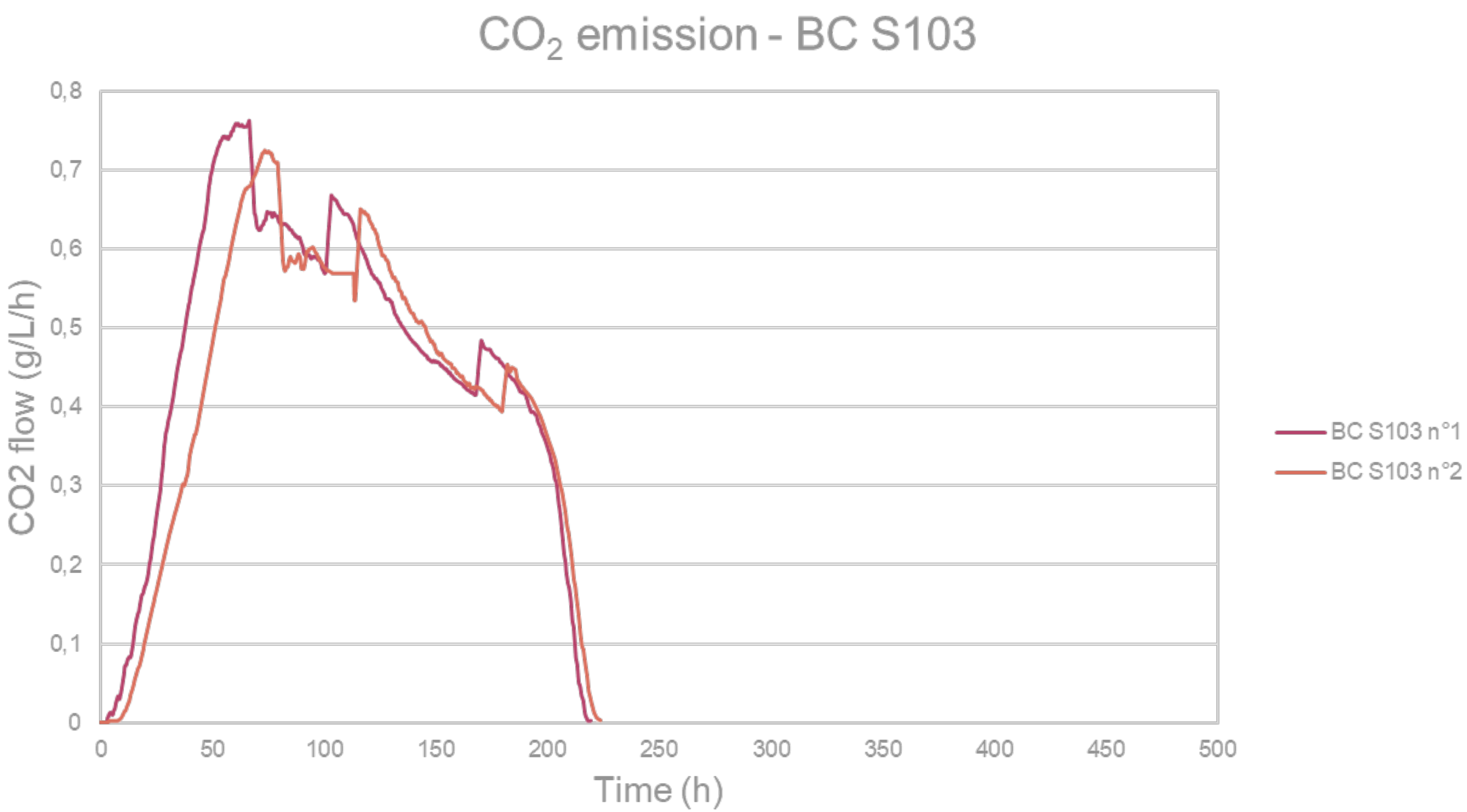
HD S62 +135ppm YAN

HD S62 without YAN

HD S62 + Hulls

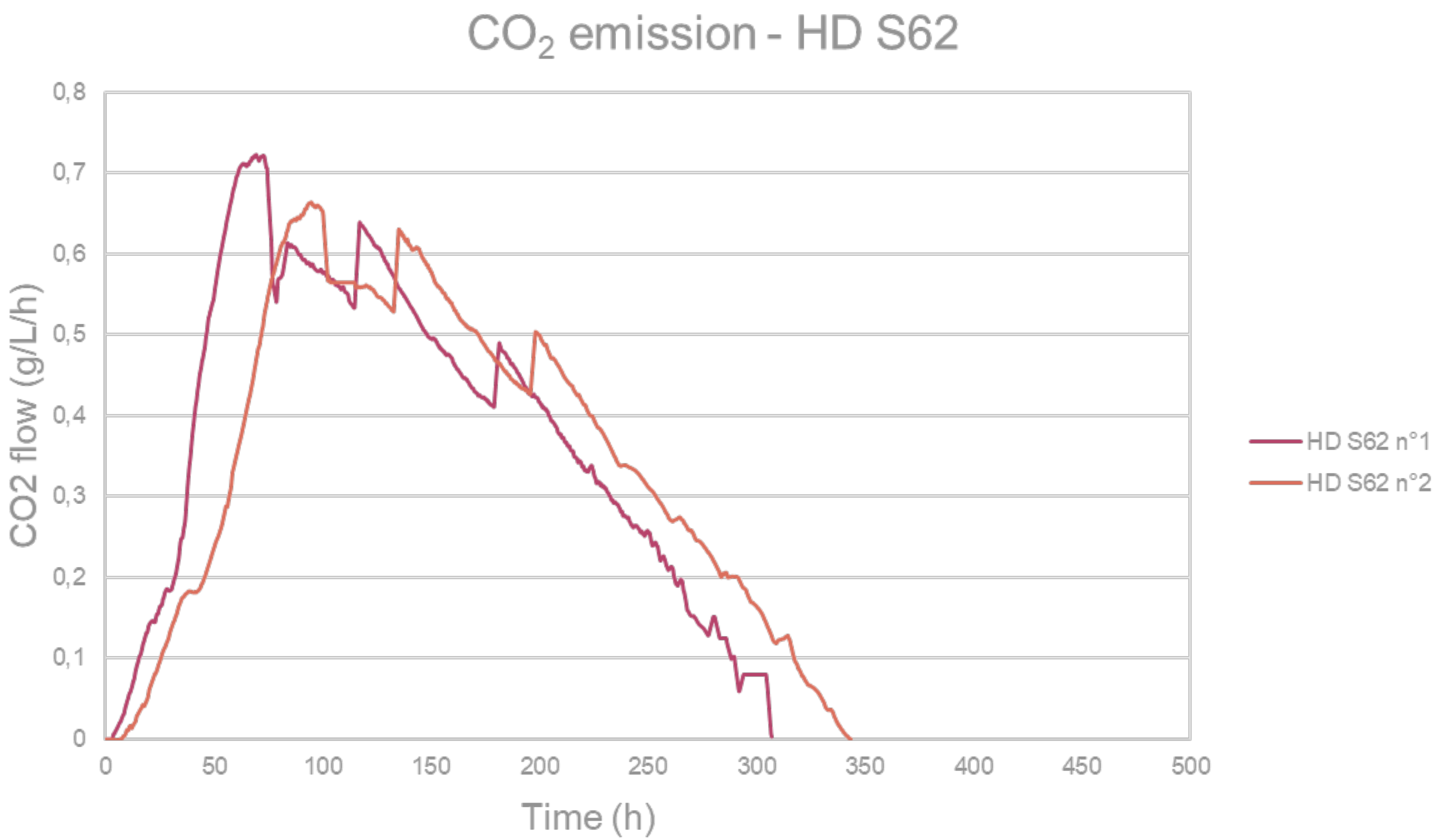
Fermentation conditions optimization

Kinetics superposition – Trial 1 & Trial 2 – Standard recipe



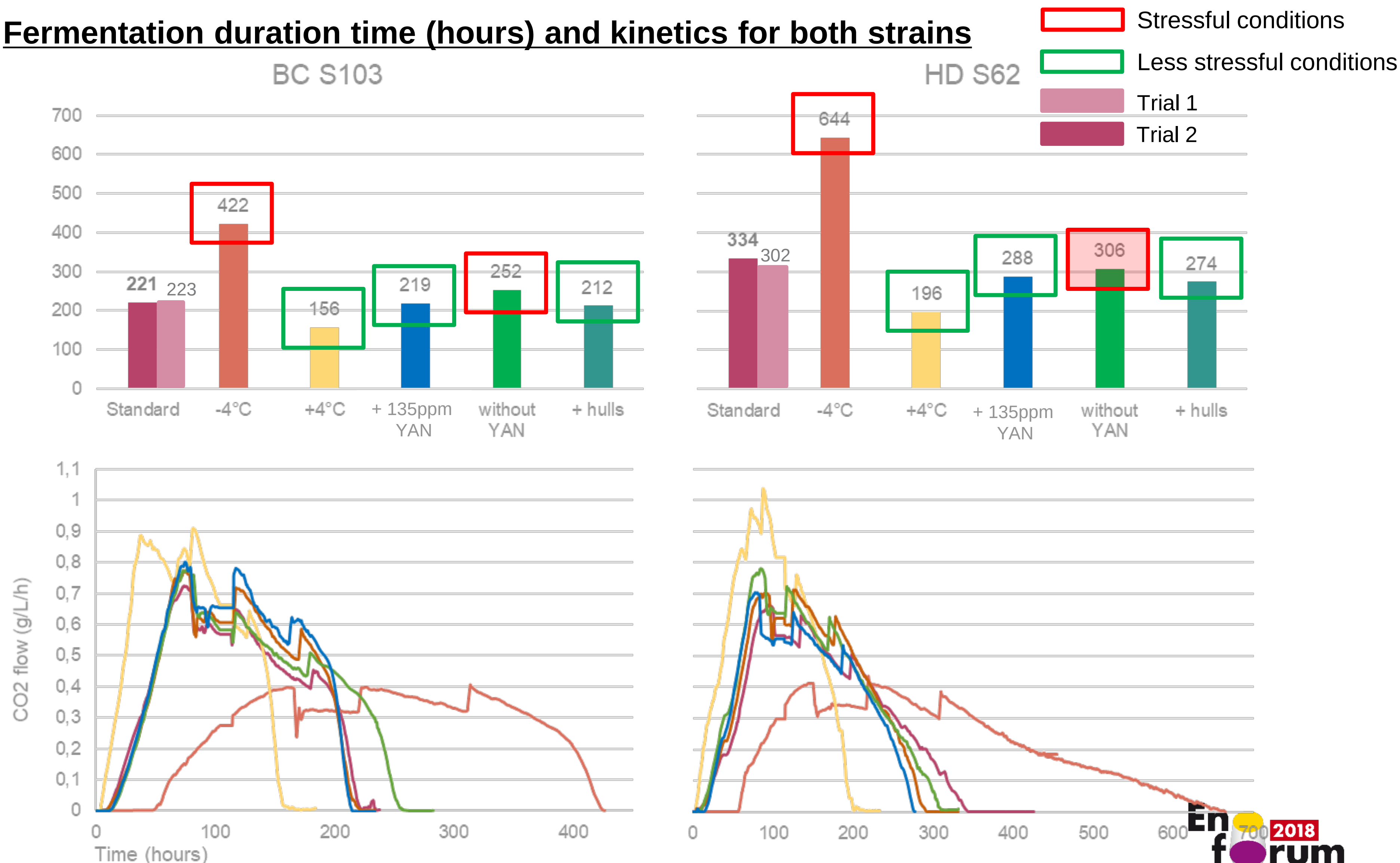
Fermentation conditions optimization

Kinetics superposition – Trial 1 & Trial 2 – Standard recipe



Fermentation conditions optimization

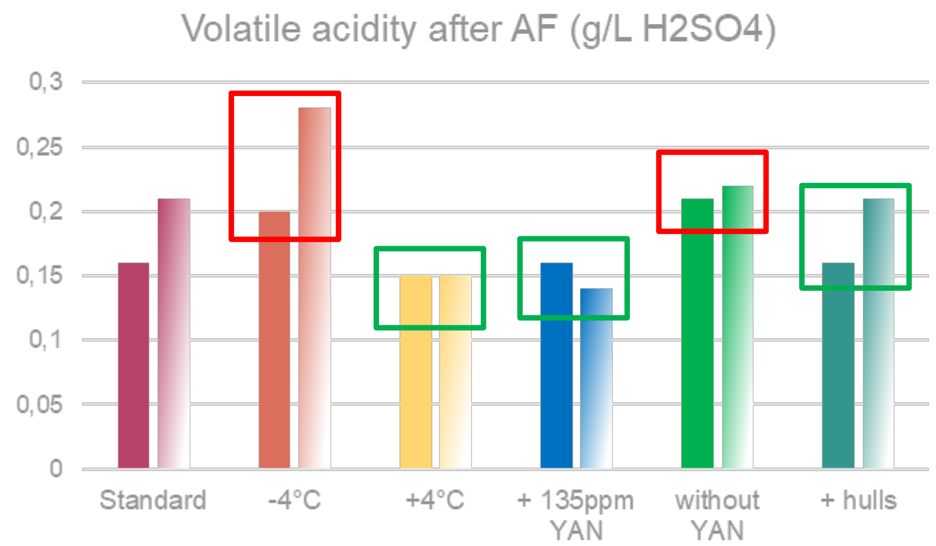
Fermentation duration time (hours) and kinetics for both strains

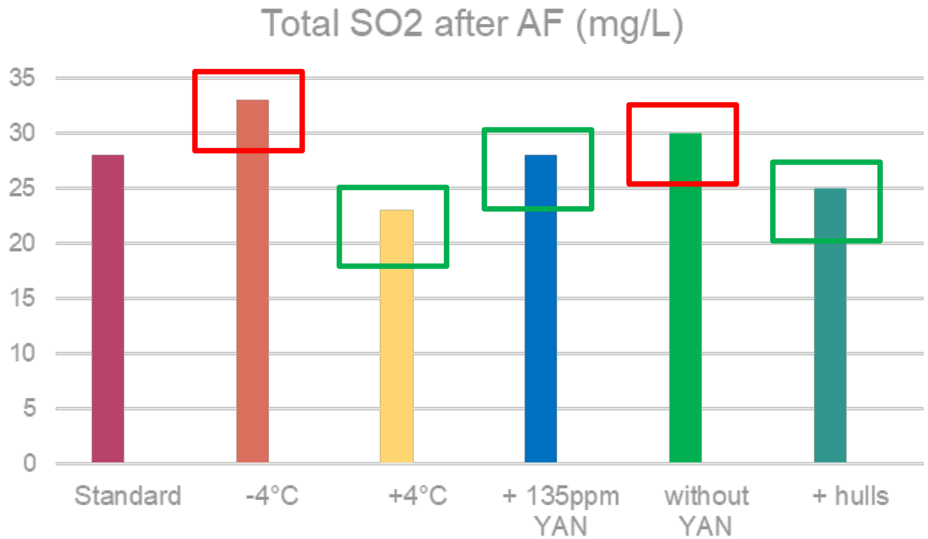


Fermentation conditions optimization

Volatile acidity and sulfites for both strains

Stressful conditions
Less stressful conditions
BCS103
HDS62





Fermentation conditions optimization

Aromatic analyses: comparisons with stage 1

Same profiles with the same molecules above threshold

Evolution between 1 and 2: slight decrease of phenyl ethanol and acetates, increase of ethyl esters, especially C8 and C10

Fermentation conditions optimization

Aromatic analyses: extensions compared to stage 1

Higher alcohol/ester production/concentration kinetics

Concentrations of volatiles in a liquid

Chemical balance
Biosynthesis/Degradation

Physical phenomenon of
Evaporation

Analyses all along the fermentation of the standard recipes as a first approach

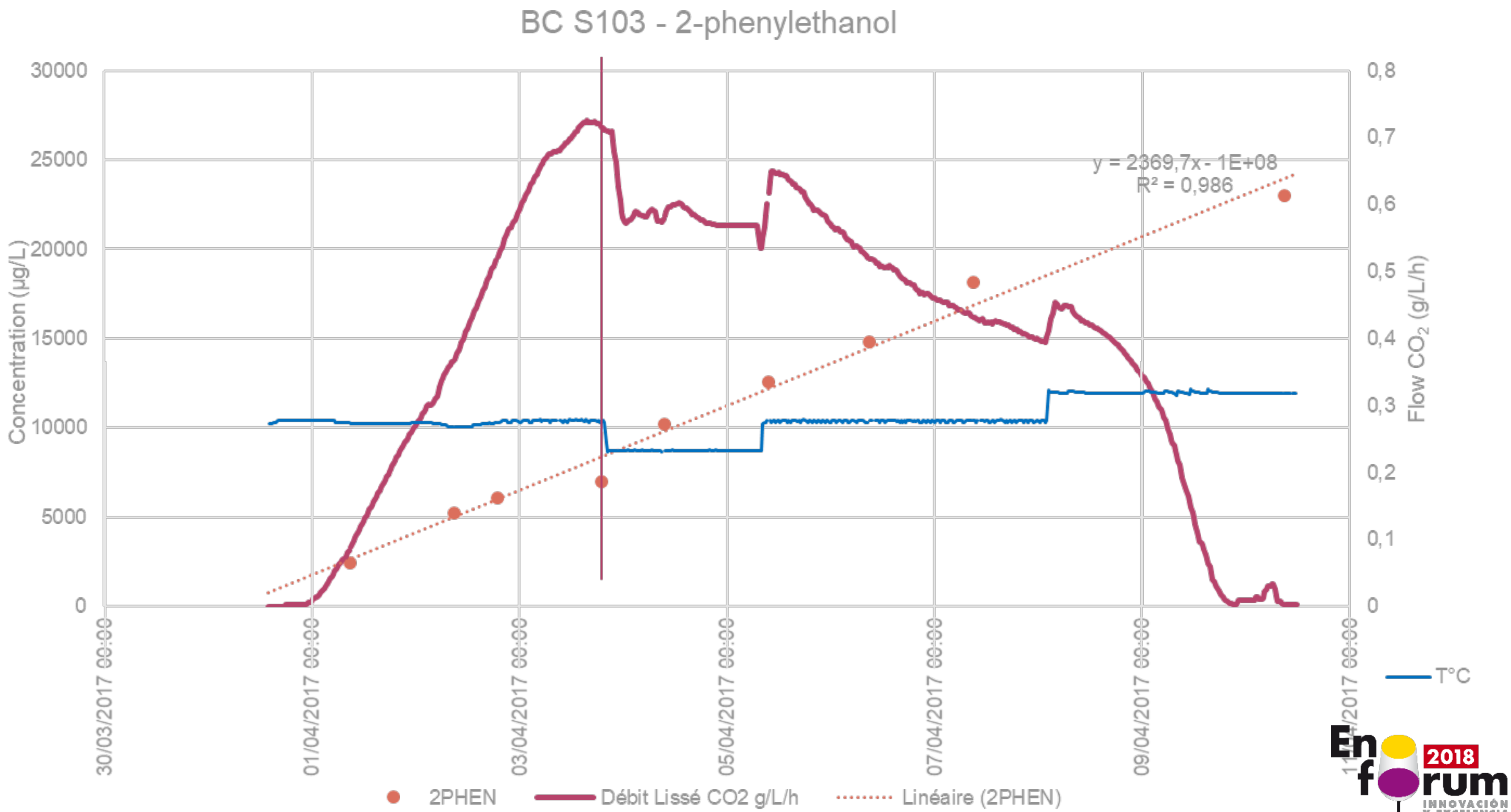
Samples have been taken every day and frozen before final analyses

6 production/concentration kinetic profiles have been identified depending on the compounds

Fermentation conditions optimization

Aromatic analyses:

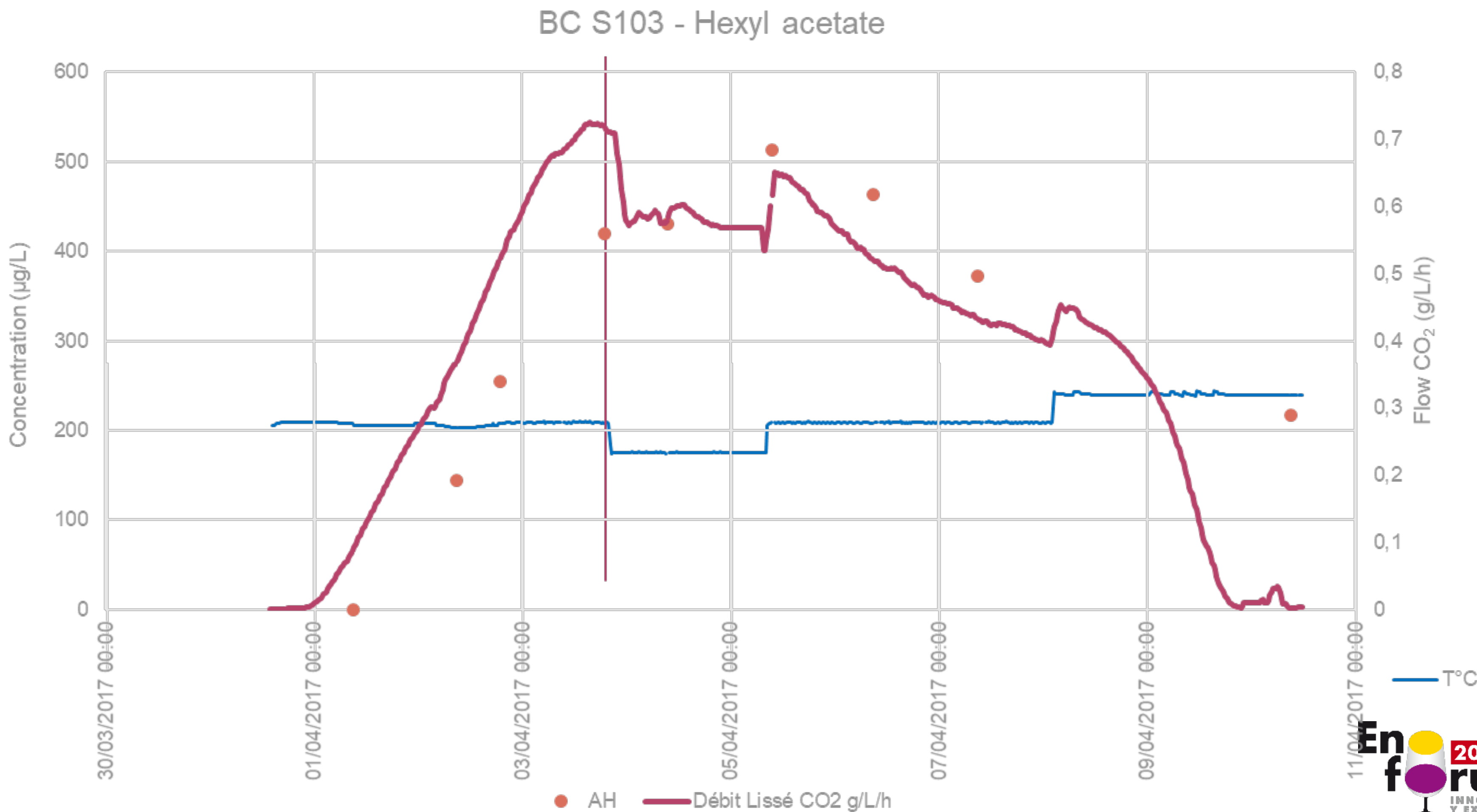
Profile 1: concentration increase almost independent from the fermentation progress with a slow down at temperature drop / nitrogen uptake (more accentuated for HD S62) – stable towards temperature



Fermentation conditions optimization

Aromatic analyses:

Profile 2: concentration seems to follow the fermentation speed curve / yeast fermentation activity: production during yeast growth and part of stationnary phase boosted by nitrogen uptake then sensitivity to temperature



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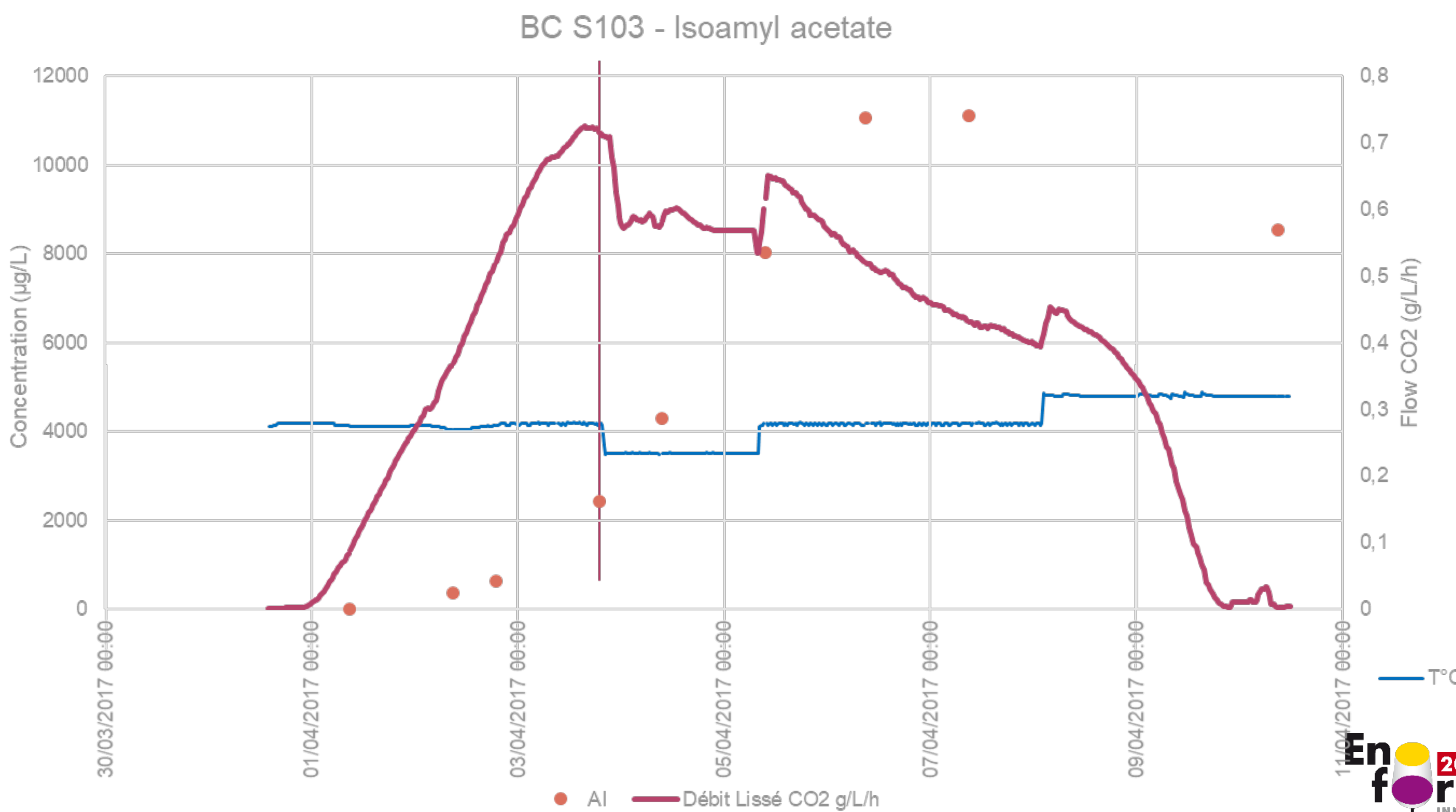
Fermentation conditions optimization

Aromatic analyses:

Profile 3: concentration follows yeast growth with an increased lag phase, continues increasing during stationnary phase and decreases late with temperature increase



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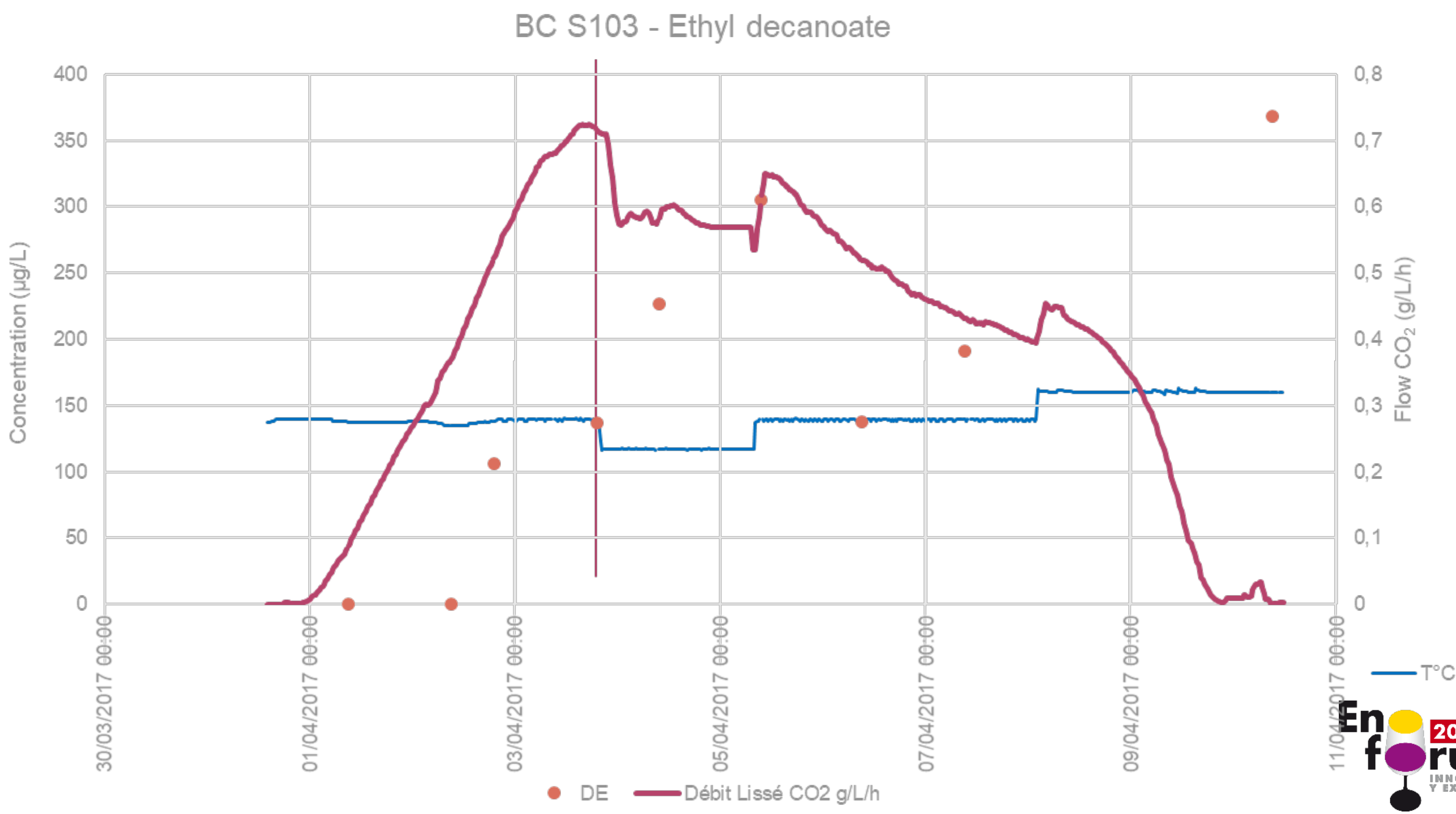
Fermentation conditions optimization

Aromatic analyses:

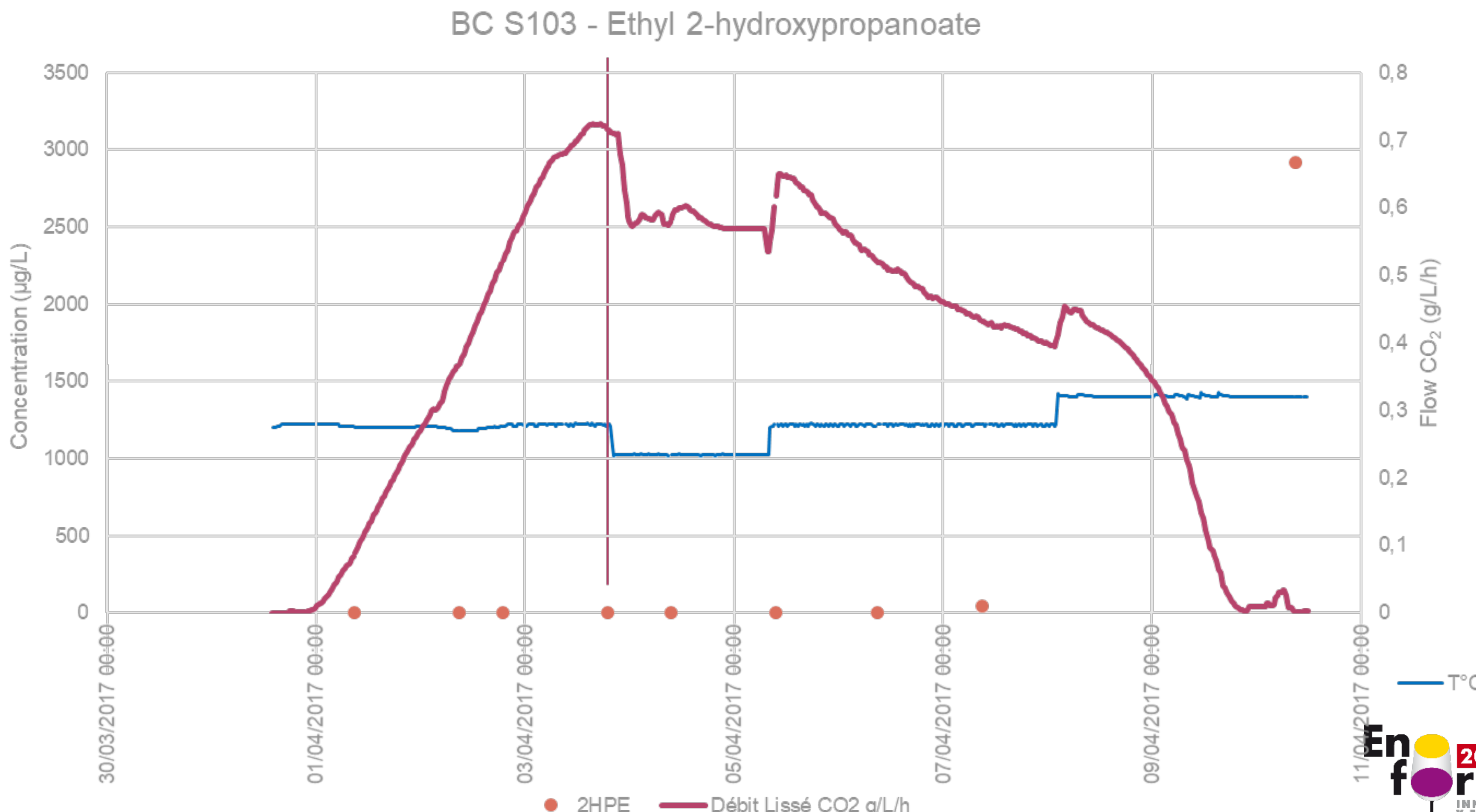
Profile 4: concentration follows yeast growth (with a possible lag phase), increases during stationnary phase boosted by O_2 and/or nitrogen then drop and increase again with temperature



Fermentation conditions optimization

Aromatic analyses:

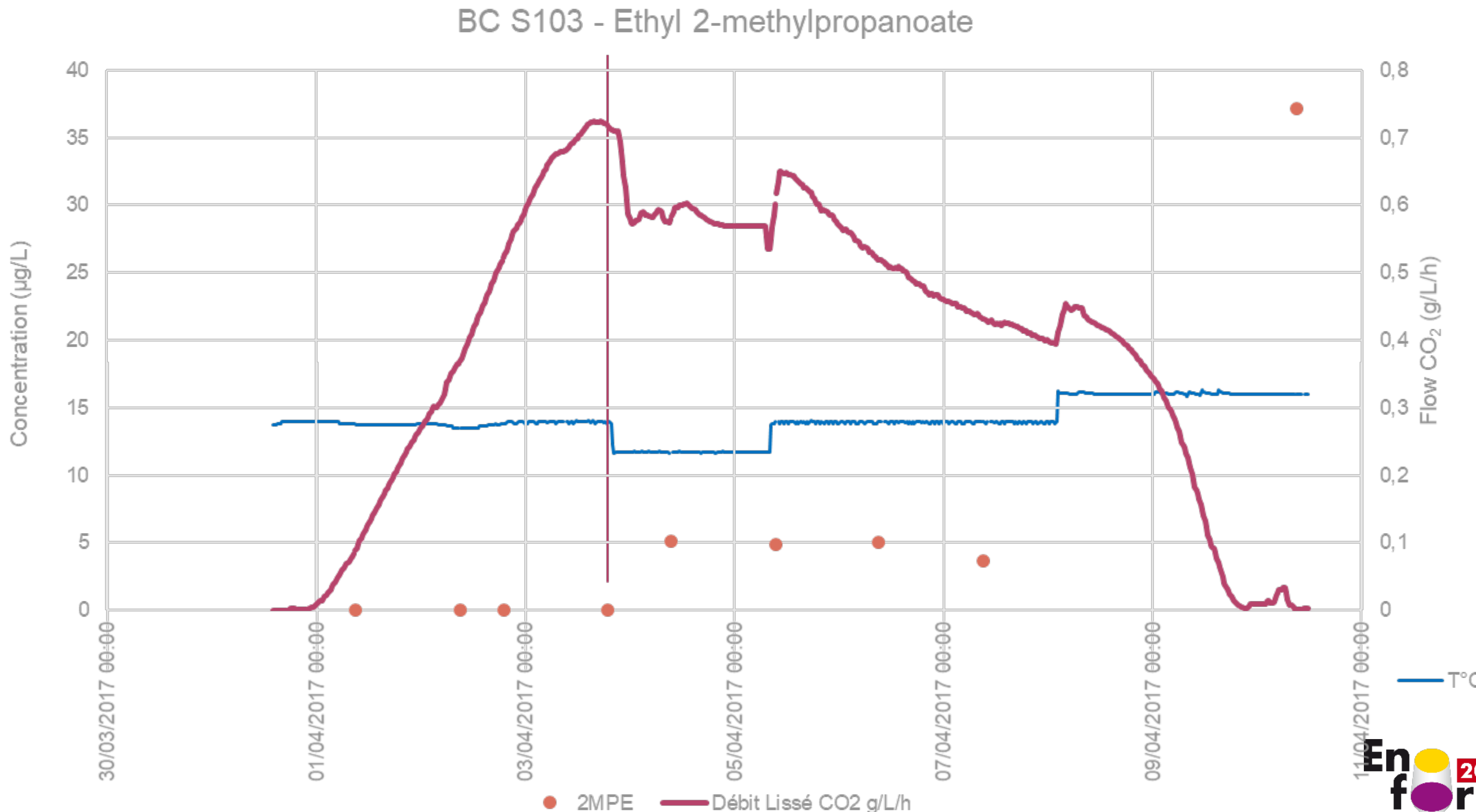
Profile 5: Production towards the end of the fermentation or after (during/after yeast mortality)



Fermentation conditions optimization

Aromatic analyses:

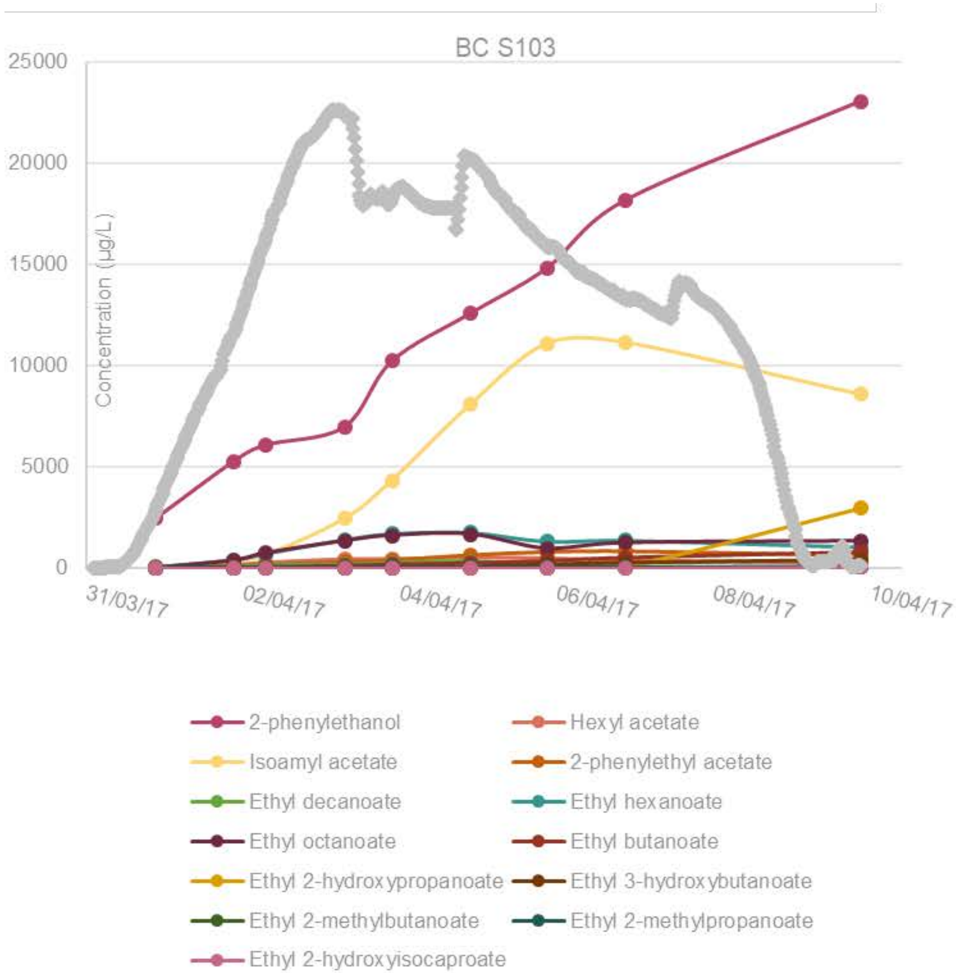
Profile 6: Production at (or just after) S_{max} (O_2 addition/YAN absence/stationnary phase) and then towards the end of the fermentation or after (during/after yeast mortality)

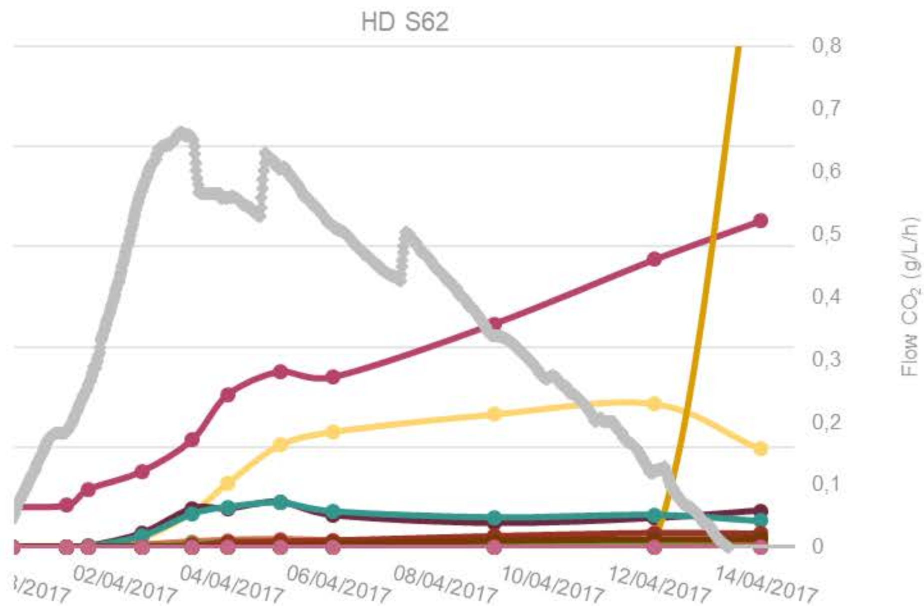


Fermentation conditions optimization

Aromatic analyses:

Comparisons between strains:





Same profiles!

Fermentation conditions optimization

Aromatic analyses: Possible explanations

Profile	Compounds of interest		Metabolism
1	2-phenylethanol	OH	Amino acid degradation
2	Hexyl acetate	Acetate	**Grape C6 alcohols**
3	Isoamyl acetate	Acetate	Amino acid degradation
3	2-phenylethyl acetate	Acetate	Amino acid degradation
1 / 3 (S 62)	Ethyl butanoate	Ethyl (F)	Fatty acid, **Amino acid degradation**
2	Ethyl hexanoate	Ethyl (F)	Fatty acid degradation, grape acids
4	Ethyl octanoate	Ethyl (F)	Fatty acid degradation, grape acids
4	Ethyl decanoate	Ethyl (F)	Fatty acid degradation
1 bis	Ethyl 3-hydroxybutanoate	Ethyl (Post F)	Amino acid degradation
5	Ethyl 2-hydroxypropanoate	Ethyl (Post F)	Cell carbon metabolism
5	Ethyl 2-hydroxyisocaproate	Ethyl (Post F)	Amino acid degradation
5	Ethyl 2-methylbutanoate	Ethyl (Post F)	Amino acid degradation
6	Ethyl 2-methylpropanoate	Ethyl (Post F)	Amino acid degradation

Close to profile 3 but hexanol substrate present at early stages from grapes

Delayed after corresponding higher alcohol production and less stable towards temperature

Production coming also from the catabolism of amino acids (like higher alcohols and acetates) then sensitivity to temperature

Hexanoic acid substrate present at early stages from grapes (hexanol) then sensitivity to temperature ?

Sensitivity to temperature. Linked to chain length?

Production related to ethyl butanoate production

Corresponding branched carboxylic acids coming from amino acid catabolism released through autolysis and blocked before by a repressed active transport

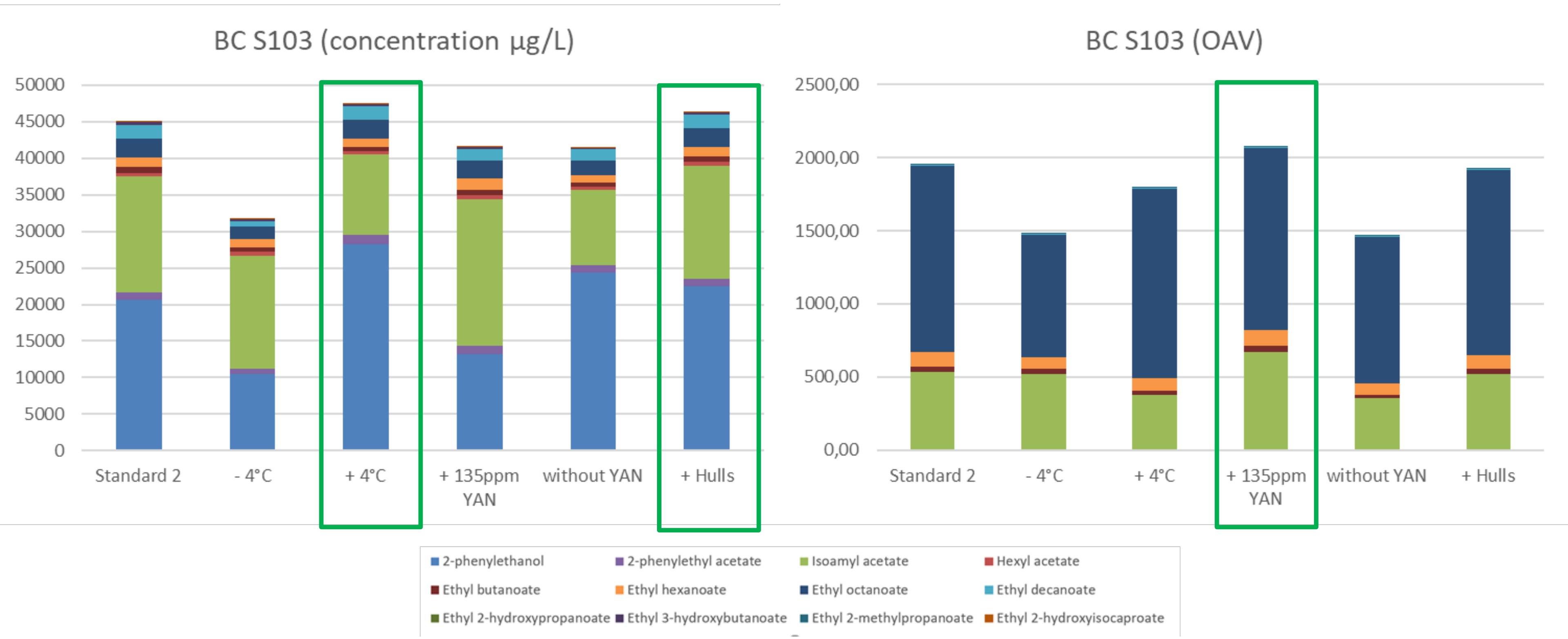
Chemical esterification during ageing

Production of precursor isobutyric acid from leucine in early stage of AF?

Fermentation conditions optimization

Aromatic final analyses: impacts of temperature, YAN and hulls on aromas

Concentrations *vs* OAV



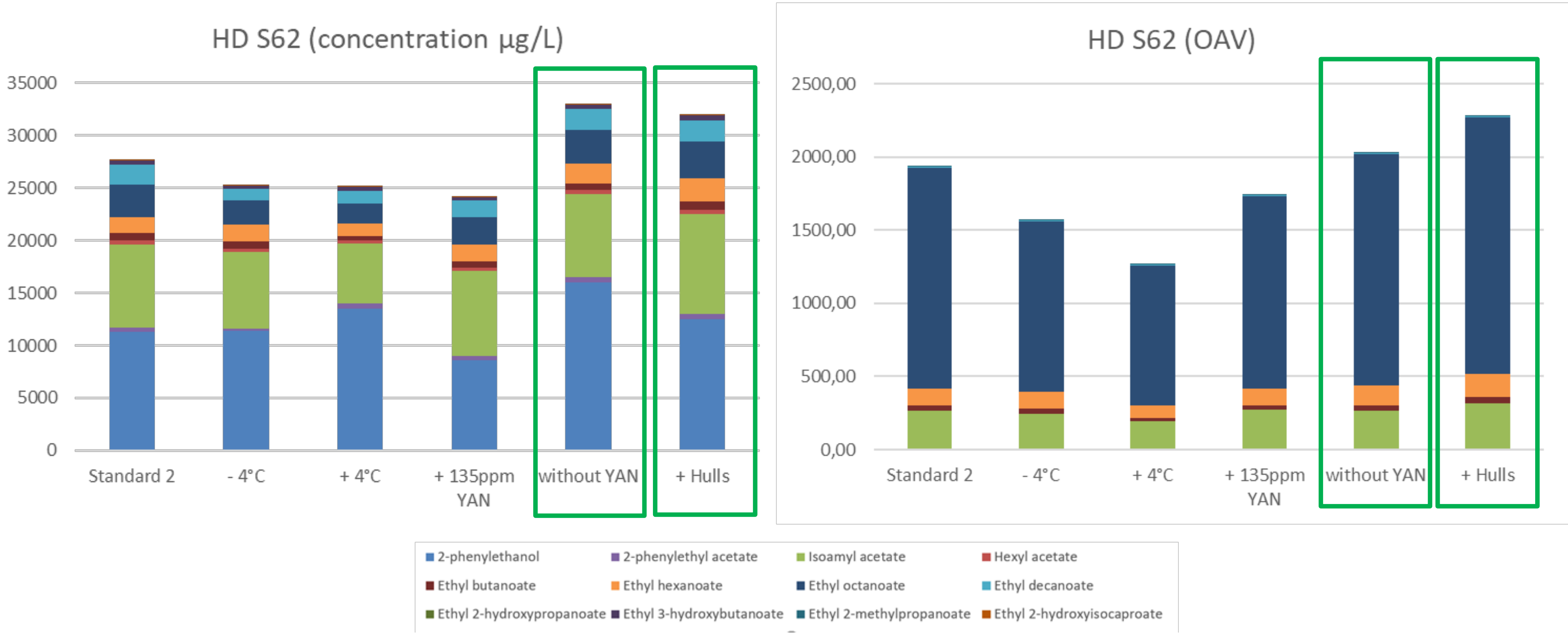
Very high perception threshold for 2-phenylethanol, very low for ethyl octanoate

→ Results expression in OAV shows which conditions favors the synthesis/concentration of the most perceived molecules at sensorial level: **+135ppm of YAN for BC S103**

Fermentation conditions optimization

Aromatic final analyses: impacts of temperature, YAN and hulls on aromas

Concentrations *vs* OAV



→ **Without YAN addition and with yeast hulls addition for HD S62**

Fermentation conditions optimization

Aromatic final analyses: impacts of temperature, YAN and hulls on aromas

Strain differences – Variation compared to standard recipe

Aromatic compounds	BC S103				
	Temperature		YAN		Hulls
	- 4°C	+ 4°C	Sufficient	Excess	Addition
2-phenylethanol	**-50%**	**37%**	**18%**	**-36%**	**9%**
2-phenylethyl acetate	**-29%**	**26%**	**-3%**	**13%**	**4%**

Aromatic compounds	HD S62				
	Temperature		YAN		Hulls
	- 4°C	+ 4°C	Sufficient	Excess	Addition
2-phenylethanol	**0%**	**20%**	**41%**	**-24%**	**10%**
2-phenylethyl acetate	**-33%**	**18%**	**29%**	**-2%**	**38%**

2-phenylethanol: **expected impact, increased for BC S103**

- T° C (alcohol synthesis), YAN (negative correlation) and Hulls (membrane fluidity for amino acid uptake)

2-phenylethyl acetate: **expected impact except for YAN with HD S62**

- T° C and YAN favors atf1 and atf2 expression, Hulls: same effect than previously
- The difference with HD S62 could be due to the big impact of YAN on the precursor 2-phenylethanol

Fermentation conditions optimization

Aromatic final analyses: impacts of temperature, YAN and hulls on aromas

Strain differences – Variation compared to standard recipe

Aromatic compounds	BC S103				
	Temperature		YAN		Hulls
	- 4°C	+ 4°C	Sufficient	Excess	Addition

HD S62				
Temperature		YAN		Hulls
- 4°C	+ 4°C	Sufficient	Excess	Addition

Ethyl butanoate: **Metabolism difference between BC S103 and HD S62 except for temperature**

- Expected impact only for BC S103: YAN (amino acid degradation), Hulls (negative correlation with FA supply)
- It seems that the hulls impact on improving fermentation conditions for HD S62 is overtaking the other effects

Fermentation conditions optimization

Aromatic final analyses: impacts of temperature, YAN and hulls on aromas

Strain differences – Variation compared to standard recipe

Aromatic compounds	BC S103					HD S62				
	Temperature		YAN		Hulls	Temperature		YAN		Hulls
	- 4°C	+ 4°C	Sufficient	Excess	Addition	- 4°C	+ 4°C	Sufficient	Excess	Addition

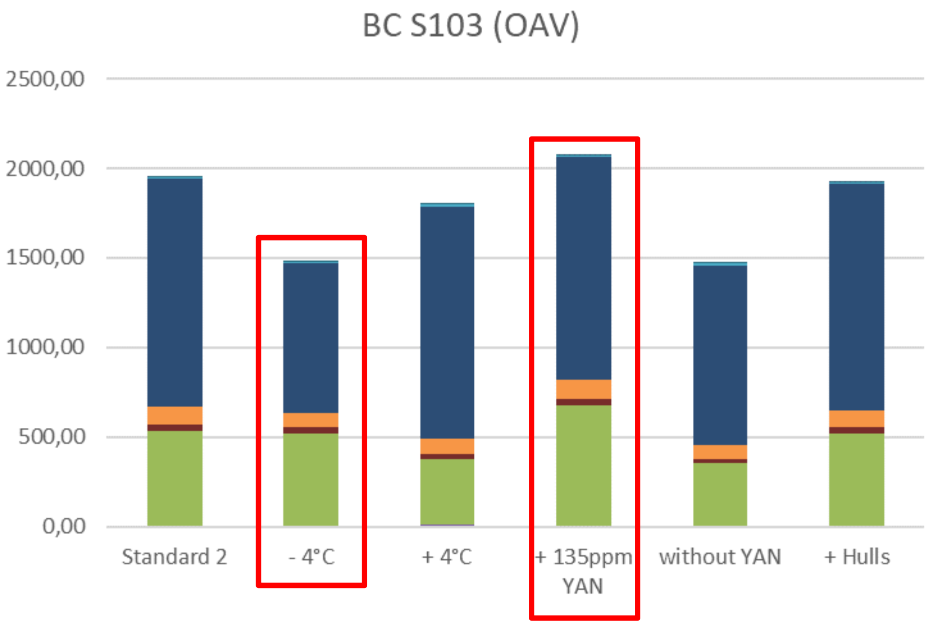
Ethyl octanoate and decanoate: Metabolism difference between BC S103 and HD S62 except for temperature

- Same than hexanoate but effects increased by temperature

Fermentation conditions optimization

Links with sensorial analyses

Evaluation by Vivelys internal panel



Not directly related to aromatic intensity but…

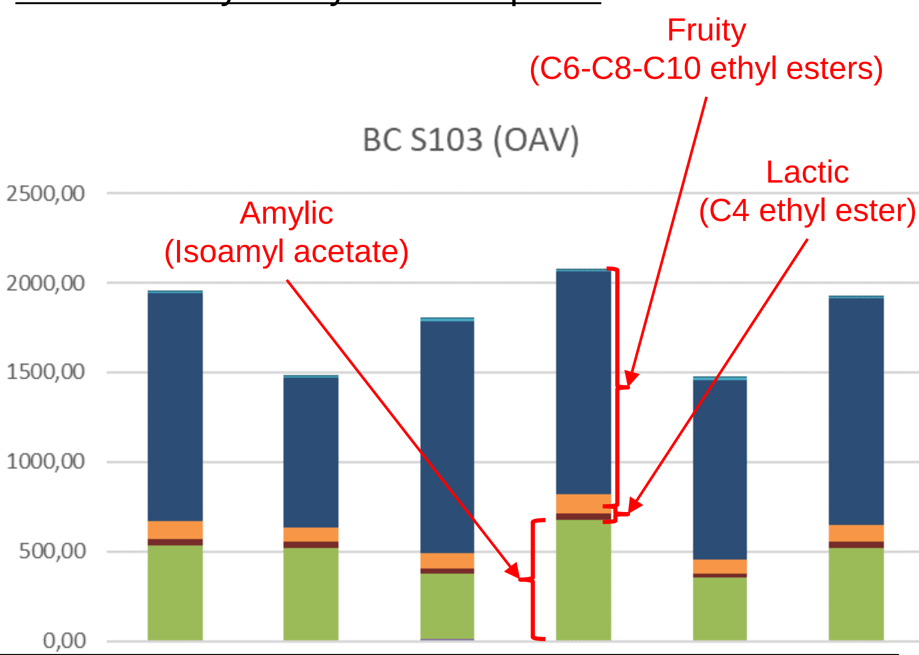
+ YAN and + 4° C

Standard and - 4° C

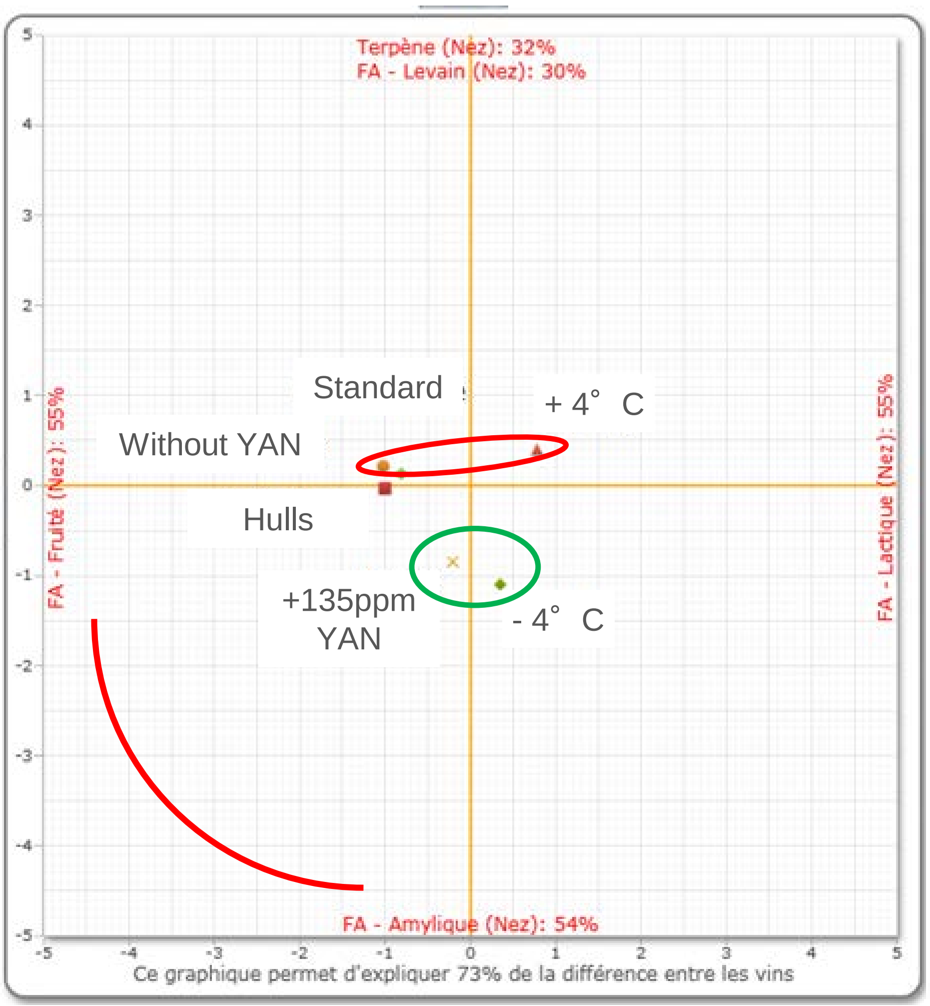
Fermentation conditions optimization

Links with sensorial analyses

Evaluation by Vivelys internal panel



	Standard	- 4° C	+ 4° C	+ 135ppm YAN	without YAN	+ Hulls
Fruity	71%	63%	78%	66%	75%	71%
Amylic	27%	35%	21%	32%	24%	27%
Lactic	2%	2%	2%	2%	2%	2%



Isoamyl acetate as aromatic enhancer

Quite well correlated with aromatic descriptors and ratios!

Fermentation conditions optimization

Links with sensorial analyses

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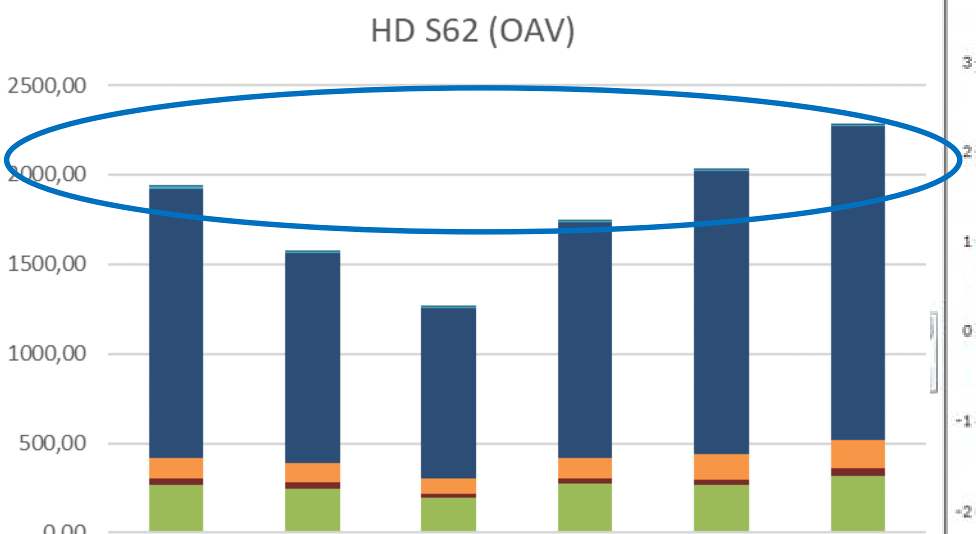
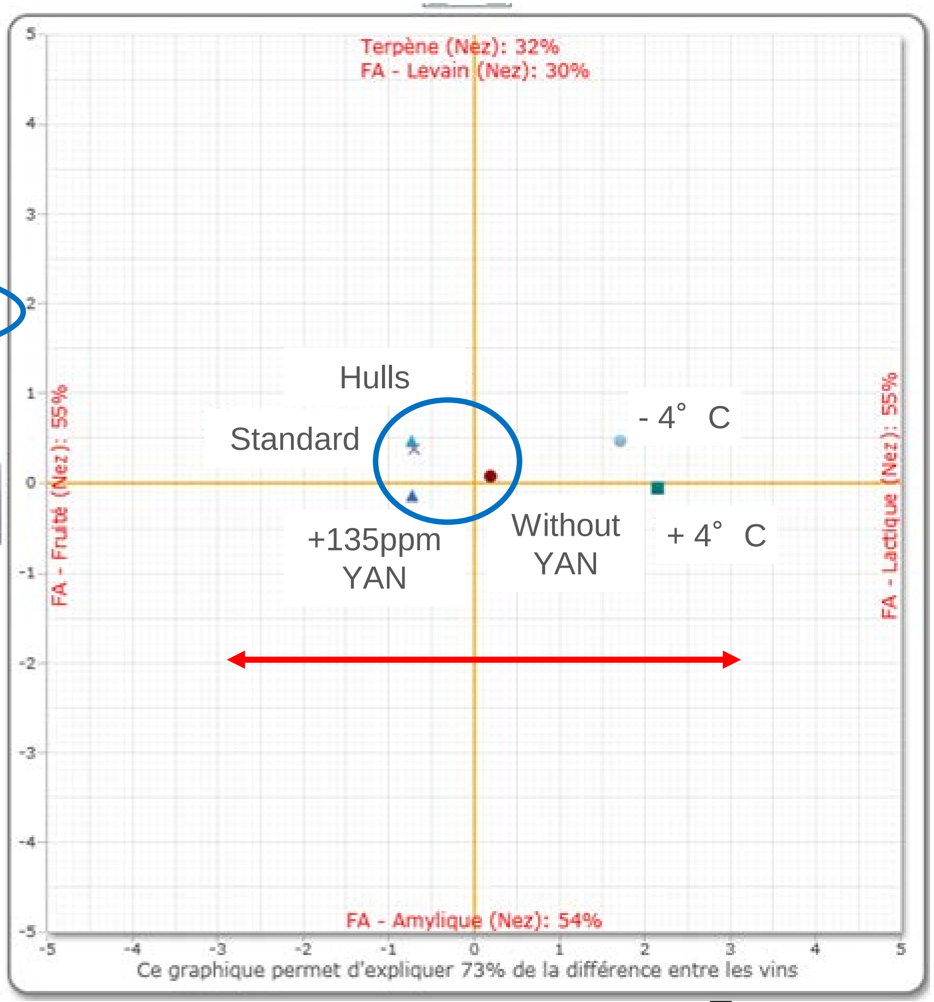


	Standard	- 4° C	+ 4° C	+ 135ppm YAN	without YAN	+ Hulls
Fruity	84%	82%	83%	83%	85%	84%
Amylic	14%	16%	15%	16%	13%	14%
Lactic	2%	2%	2%	2%	1%	2%



Differences towards lactic/fruity axe mainly related to the concentration of fruity ethyl esters

Isoamyl acetate seems to be a key flavor enhancer

Conclusions

Examples of profiling: Synthesis between analyses and sensory

Synergies between parameters linked with a selected yeast to maximize the chances of targeting the desired profile

AMYLIC profile

- BC S103
- Low temperature
- **YAN addition**
- No yeast hulls addition

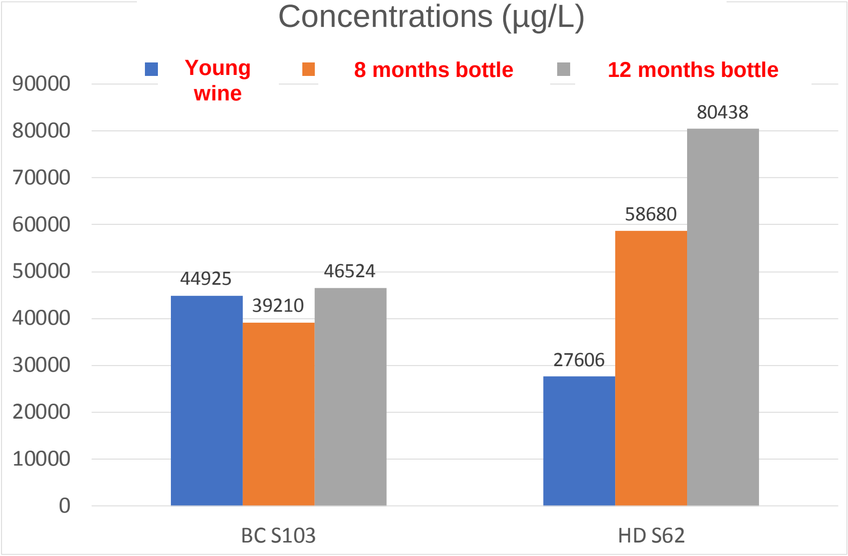
FRUITY profile

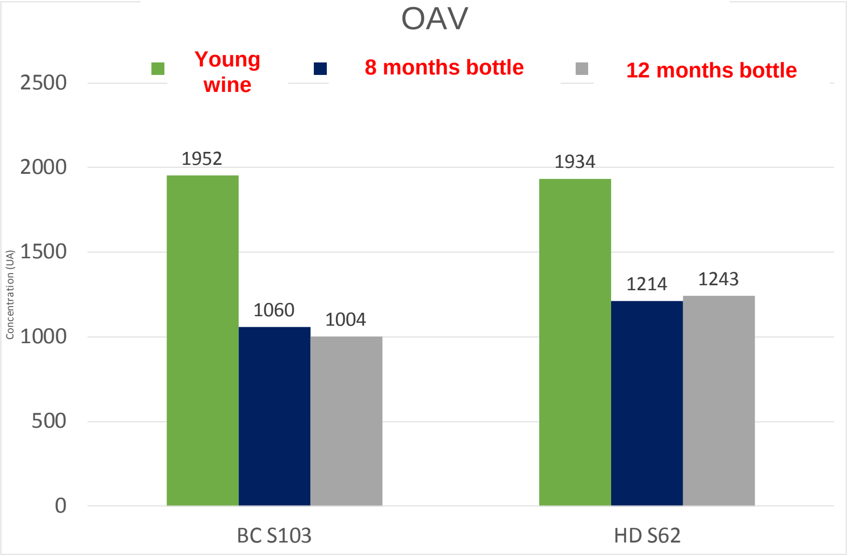
- HD S62
- Low to medium temperature (standard recipe)
- No YAN addition
- **Yeast hulls addition**

Perspectives

Next steps

- Analyze aroma production/concentration kinetics depending on the conditions
- Compare mineral nitrogen and organic nitrogen supplementation
- Study the effect of O_2
- Study the evolution of the aromas over time…





- Other aromas
- Etc…

Gracias!

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