Solutions for winemaking
LAMOTHE-ABIET

LAMOTHE-ABIET
FERMENTATION BOOKLET

LAMOTHE-ABIET FERMENTATION BOOKLET

Who are we?

With 140 years of experience in enological development, Lamothe-Abiet is inspired by tradition in order to envisage the future.

Here at Lamothe-Abiet, we have worked tirelessly since 1878 to produce innovations that make use of our expertise in wineries across the globe, faithful to the expression: “today’s progress will be tomorrow’s tradition”.

Our mission: to provide winemakers around the world reliable, effective and affordable solutions to get the best out of their grapes, and to make the wine that they wish to produce.

Our trade: to understand the needs of our clients, to anticipate changes, and to develop well-adapted enological products. We always endeavour to innovate.
This booklet is a tool produced for winery workers and winemakers.

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LAMOTHE-ABIET
ALCOHOLIC FERMENTATION

I. Enological yeast

A. Definition

1. What is a yeast?

Yeasts are single-celled fungi which carry out the alcoholic fermentation, transforming sugars into alcohol.

2. What is an active dried yeast (ADY) ?

Active dried yeast (ADY) are found in many different agrifood industries (cheese, beer, bread-making, etc.). In the wine world, they appeared in the 70s and quickly spread with great success. Nowadays, an estimated 3000 tonnes of enological ADY are sold every year. The rate of inoculation reaches nearly 100% in certain regions.

On the market, there are around 300 "strains" available from a large choice of suppliers. However, certain strains have numerous brand names.

The production method is standardised: a liquid biomass is produced in a fermentor using a pure strain. This biomass is then concentrated to obtain a paste, called a yeast cream. This yeast cream is then dried and packaged.

Only one exogenous product is used to stabilise the ADY: Sorbitol monostearate (E490). It is a fatty acid ester used to protect the yeasts' cell membranes during the drying process. We are currently working towards removing it completely from our production process.

3. So, ADY or natural yeast?

A spontaneous alcoholic fermentation (without adding ADY) is generally carried out by several unselected yeast strains. They may come from the grape, but also from equipment or the winery. The risk of alterations and/or faults is greater with natural yeasts. Professor Denis Dubourdieu used to say: "not adding yeast is choosing not to choose, to leave everything to chance."
There are numerous advantages to using ADY:

- Safeguard the alcoholic fermentation
- Avoid faults linked to the indigenous flora
- Promote certain organoleptic criteria
- Ease of use (can be stored for a long time at room temperature)

B. Selection of enological yeast

1. Mass selection

Mass selection involves isolating a yeast strain, generally in the vineyard or the winery. In this case, the yeast undergoes asexual reproduction, and all of its genetic characteristics are retained in its offspring since the DNA comes from one single parent. This may include certain negative characteristics which may be part of the strain. This process is similar to grafting plants: the new plant will be genetically identical to the parent.

2. Breeding

The second mechanism involves sexual reproduction by yeast sporulation. Therefore, in the case of yeast selection through breeding, two natural yeasts (from mass selection) are encouraged to sporulate under specific conditions. The spores produced by each parent are then selected and crossed in order to produce a hybrid descendant. The phenotypic characteristics of these hybrid strains, which have both parents' genes, are studied in order to choose yeast strains with the most positive characteristics (alcohol tolerance, aromatic profile, SO_2 , volatile acidity, H_2S...). This technique enables the positive markers of different strains to be associated within a single yeast strain.

To go further : GMO and Breeding - the same proceed ?

A genetically modified organism, or GMO, is a term used to describe a living being whose genome has been deliberately modified by human intervention, using genetic engineering techniques (genetic modification by gene isolation, cloning, sequencing, excision...). For breeding, two yeasts reproduce naturally through sexual reproduction. The offspring with the best fermentary and organoleptic capacities are then selected.

Why carry out breeding and not mass selection?

Breeding can help to speed up the search for enologically interesting strains, as opposed to random sampling. For fermentation, yeast produced through breeding have much better capacities, since they do not come up short for any major characteristics (H_2S, volatile acidity, ethanol yield, SO_2). Strains produced through breeding are capable of revealing large amounts of varietal aromas, as well as producing large quantities of polysaccharides, bringing volume to the wine. This technique enables strains to be selected which have perfect fermentary capacities and much more specialised organoleptic characteristics. All of the yeasts in our **Excellence®** range are produced by breeding and developed at the ISVV (Bordeaux Institute for Vine and Wine Science).

II. What are yeast requirements?

A. The essential nutrients

1. Sugars

Yeast mainly use carbon in organic form: sugars, alcohols and acids. Generally speaking, all yeast species use hexoses (fructose and glucose) as a source of energy. The two sugars are transported inside the cell by the same protein, except that it has a greater affinity for glucose, which explains why yeasts preferentially use glucose during fermentation.

2. Nitrogen

Nitrogen in the musts is an essential element for yeast fermentation. It plays a major role in yeasts' metabolism and in the synthesis of biomass as well as of enzymes and membrane transporters, which are essential to their functioning. Nitrogen plays a role on the fermentation kinetics and also on its completion. In this way, simply for a successful fermentation, it is necessary to have the right amounts of nitrogen in the must (Sablayrolles et al., 1996).

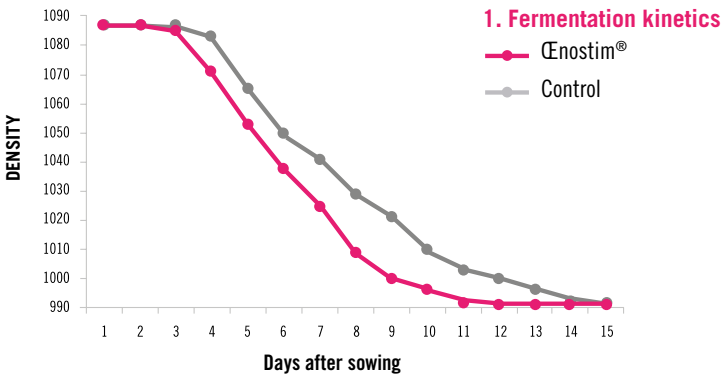
Through nitrogen metabolism, and in particular that of amino acids, aromatic compounds, with a direct effect on the wine's profile, are produced. Certain amino acids, when assimilated by the yeast, lead to the formation of higher alcohols and consequently to their respective acetate esters. Others play a role in the revelation or preservation of certain amino aroma precursors (cysteinylated precursors of varietal thiols). Must's nitrogen composition can thus modify the wine's aromatic profile.

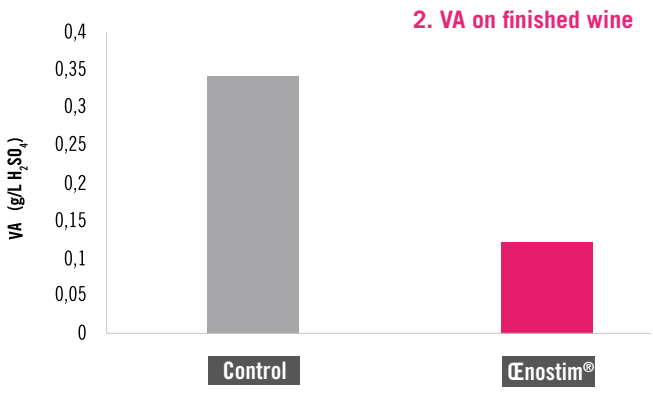
3. Vitamins and minerals

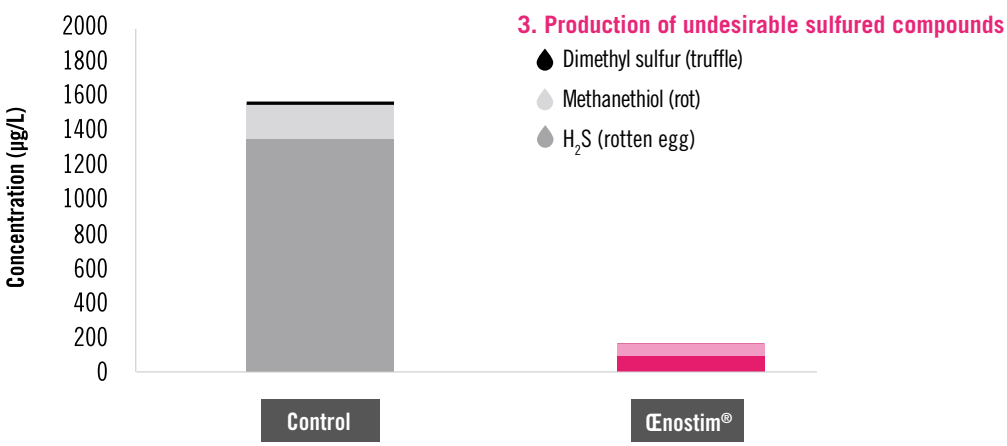
Vitamins and minerals supply essential enzymatic cofactors that improve yeast metabolism and thus produce cleaner smelling wines. Particular care should been taken if there is Botrytis or other moulds on the grapes. In these cases, the concentrations of vitamins and minerals is greatly decreased. It is therefore recommended to supply the yeasts with all the nutrients that they need during rehydration. If there is a deficiency, the inoculated yeast risk to be compromised, which could lead to stuck fermentations.

B. How to make yeast more resistant during the fermentation?

Yeast rehydration is one of the most important steps of the fermentation. If this step is not carried out correctly, there is a risk of ending up with low yeast populations in the must which will have difficulty in ensuring a clean and complete fermentation. Adding yeast derivatives during rehydration consistently helps to improve the yeasts condition. These derivatives supply them with a mix of vitamins, minerals, amino acids as well as lipids and sterols. Sterols play a role in maintaining the integrity and the fluidity of the yeast membrane, increasing its resistance to ethanol. This has a significant impact on the fermentation kinetics, especially during the second half of the fermentation when the ethanol concentration increases. All of the elements lead to improved yeast metabolism, and ensure a complete fermentation, with cleaner aromas.







Using a rehydration product such as **Œnostim®** improves the yeast's rate of establishment, gives better fermentation kinetics, and helps to avoid the production of undesirable compounds and thus gives cleaner aromas.

C. Differences between organic and mineral nutrition in enology

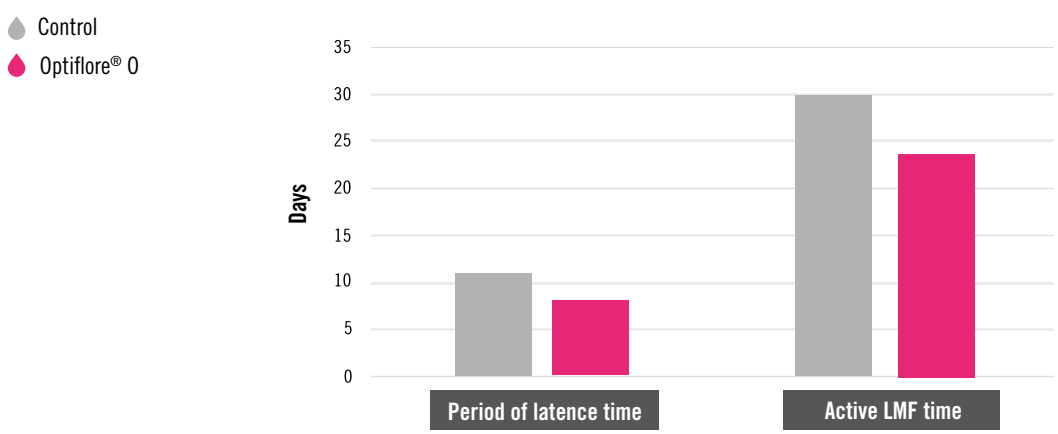
Nitrogen in mineral form, usually as DAP (ammonium phosphate) or SAP (ammonium sulfate), is nowadays widely used to ensure fermentations come to completion. This type of nutrient is preferred by the yeast and rapidly consumed to give a quick boost to the population. Mineral nitrogen is useful for adjusting the level of nitrogen in musts which are deficient in assimilable nitrogen. However excessive and poor usage can lead to undesirable consequences in the resulting wine. Using too much mineral nitrogen can lead to an excessive increase in the biomass, resulting in:

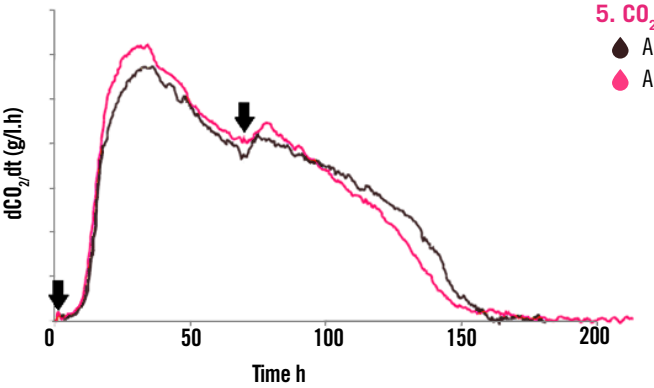
- The production of H_2S characterised by notes of reduction ranging from egg to cabbage
- An induced deficiency: with an excessive population, the remaining nutrients are not sufficient to feed this biomass
- This deficiency can lead to sluggish and/or stuck fermentations
- Excessive heat production can be a problem
- A stimulating effect on nitrogen catabolic repression (decreasing the production of aromatic thiols)

On the other hand, "organic" nitrogen can be added mainly via yeast derivatives. These derivatives are rich in amino acids, vitamins, minerals and oligoelements. Adding **Optiflore® O** has a better quality stimulatory effect than ammoniacal nitrogen. Despite adding less assimilable nitrogen, the inactivated yeasts are more effective thanks to the vitamins, minerals and oligoelements that they contain. An addition of 10g/hL **Optiflore® O** (5mgN/L in amino acid form) is equivalent to an addition of 15 to 20 mgN/L in ammoniacal form (see graphic below). This type of nutrient is assimilated much more progressively. There are several positive impacts:

- Yeasts and malolactic bacteria which feed only on amino acids are fed
- No effect of nitrogen catabolic repression
- Increased aromatic complexity
- H_2S production inhibited

4. Micro-nutrients input essential for bacterium





5. CO_2 released depending on the nitrogen nutrition

- Amine nitrogen (10 g/hL derivated yeasts=5 mgN/L)
- Ammoniacal nitrogen (10 ghL DAP=20 mgN/L)

D. Managing your nitrogen nutrition

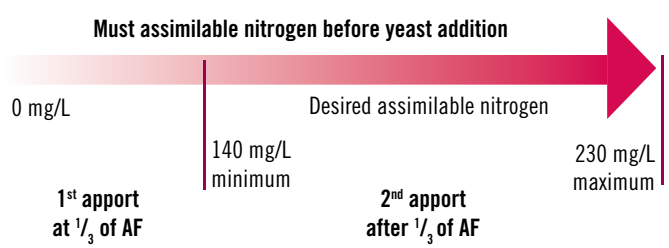
A must's nitrogen nutrition is dependent of several parameters. Beyond the initial assimilable nitrogen, other elements should be taken into account according to the type of wine produced. For red wine production, the main factors are potential alcohol, the strain of yeast used, fermentation temperature, and the pH. For white and rosé wines, the main parameters that must be understood are the potential alcohol, the yeast strain, fermentation temperature, the turbidity, and oxygen addition during fermentation. Optimize your yeast nutrition using our online diagnostic tool:

On our website in the tab LA Solutions / Toolbox

On our Oenosolutions mobile app, available on the Appstore and Google Play Store

Generally speaking, it is essential to have at least 140 mg/L assimilable nitrogen in your must before the start of the AF. If the required nitrogen addition is small, the use of organic nitrogen at the beginning of the AF is sufficient. If more is required, it is recommended to use some mineral nitrogen at the beginning of the AF, in order to boost the selected yeast's biomass. Afterwards, organic nitrogen should be favoured during the AF, and if still more is required, some mineral nitrogen can be added as well.

6. Nitrogen additions



- If requirement <60 mg/L, add 10-30g/hL Optiflore
- If requirement > 60 mg/L, add Vitaferment

- If requirement <30 mg/L, add 20-40 g/hL Optiflore
- If requirement > 30 mg/L, add 20-40 g/hL Optiflore and add some Vitaferment is needed

E. Specific nutrition

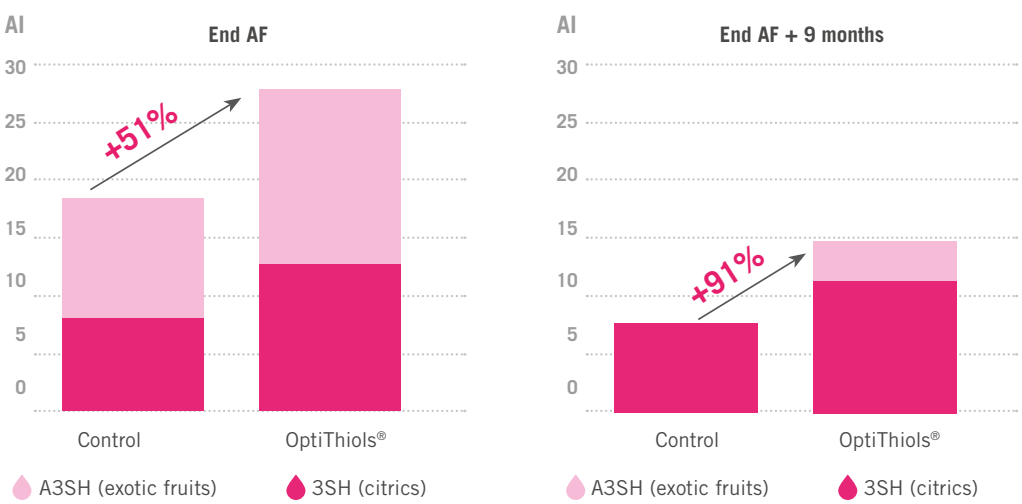
The aromatic profile is a vital parameter for the end consumer since it is reflects the terroir of the wine. It is therefore essential for the winemaker to fully control the fermentations in order to producer a clean wine, without faults, whose flavours reflect its terroir.

1. OptiThiols®

Varietal thiols (in particular 4MSP, A3SH and 3SH) have been identified as key compounds in the aroma of young wines from various varieties. 4MSP has an odour of boxwood and cassis bud, whereas A3SH and 3SH give white and rosé wines fruity notes with odours of passionfruit and grapefruit. Thiols are produced during the alcoholic fermentation thanks to certain yeast enzymatic activities which release volatile thiols from thiol precursors present in the must. Thiols are very easily oxidisable, it is therefore essential to optimize their production during the fermentation. Internal trials carried out from 2012 have shown the positive impact on the revelation of these varietal aromas when specific yeast derivatives are added.

A specific formulation, based on inactivated yeasts rich in reducing molecules (cysteine, homocysteine, glycine-cysteine, glutamylcysteine, N-acetylcysteine, glutathione), was then developed. The product is specifically formulated for this application, and its use is directly correlated to the optimization of thiol production. Trials have shown that the timing of its addition has an effect on the products effectiveness. It is therefore recommended to add the yeast derivatives during the pre-fermentation phase, just before yeast addition (during cold stabilization or after clarification). It should also be noted that the dosage is an important parameter to take into account. It is recommended to use a dosage of 30g/hL. The use of **Optithiols®** has two advantages: it enables the proportion of varietal thiols to be increased at the end of the alcoholic fermentation. These are then better preserved over time, thanks to the reducing compounds that it is made up of.

7. Aromatic index (AI)
[volatile thiols] / perception threshold

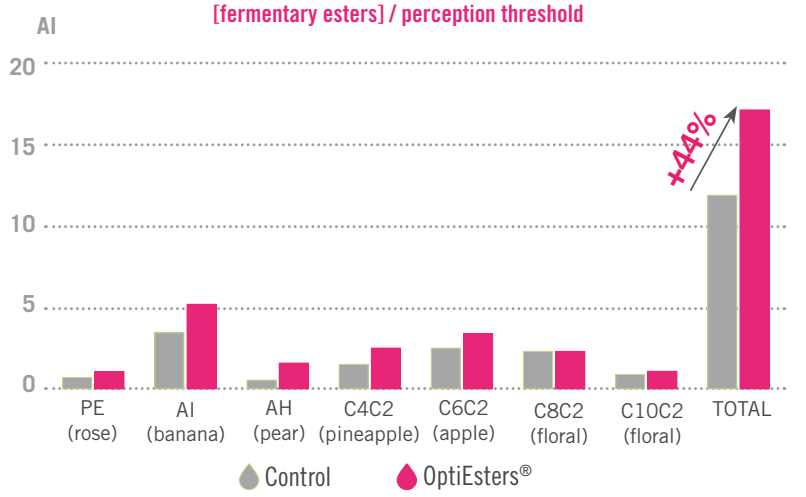


2. OptiEsters®

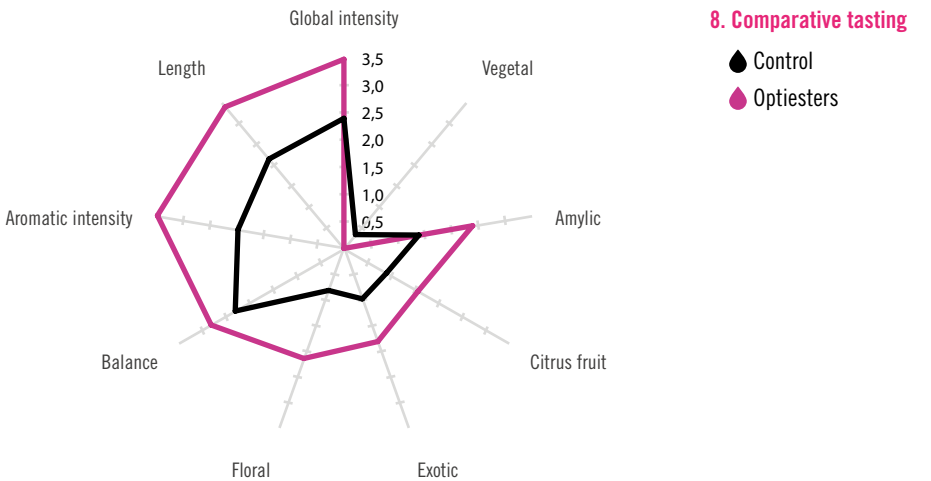
For certain white and rosé wines, a more generalised fruity profile is desired, with notes ranging from banana to strawberry, with pear and/or floral notes. These notes are part of a family of aromas called esters. They are mainly due to the yeast's secondary metabolism and are produced during the alcoholic fermentation (Sumby et al, 2010). Fermentary esters are split into two categories: the acetate esters of higher alcohols, and the ethyl esters of fatty acids (Saerens, Delvaux, Verstrepen, & Thevelein, 2010). The main acetate esters found in wines are isoamyl acetate (banana), hexyl acetate (pineapple, peach), and 2-phenylethyl acetate (floral notes). The ethyl esters have aromas of white-fleshed fruits such as apple or pear (Scheider & Subileau, 2008).

Production of these aromatic compounds depends directly on certain yeast enzymatic activities, and on these enzyme's substrates. These substrates fall under two categories: fatty acids and amino acids. It has been shown that adding these during fermentation leads to a significant increase in the concentration of fermentary esters at the end of the AF. Therefore, several different types of inactivated yeasts were tested internally in order to select the most effective. Trials carried out in wineries revealed on formulation which showed particularly strong results. The addition of **Optiesters®** during alcoholic fermentation significantly increases the concentration in the resulting wines' fermentary esters. Their aromas are judged to be more fruity and of greater quality.

8. Aromatic index (AI)
[fermentary esters] / perception threshold



8. Comparative tasting



III. Our solutions

A. White and rosé yeasts

In enology, yeasts play an critical role in the final result of the wine. This is why it is essential to choose them well. A programme initiated by Lamothe-Abiet, and carried out in partnership with the University of Bordeaux, has enabled us to meet winemaker's increasing need to know how to find a specific yeast for different organoleptic results in white and rosé wines. This resulted in three yeasts, produced using the breeding technique, to better meet these needs.

1. FTH

Excellence® FTH is a yeast that helps to reveal varietal aromas, particularly thiols. Therefore, **Excellence® FTH** is the best choice on varieties with high concentrations of thiol precursors, such as Sauvignon blanc, if this objective is a "thiol" aroma profile. Varietal thiols are liberated during the first few days of the alcoholic fermentation by Saccharomyces cerevisiae thanks to its beta-lyase activity. This enzyme is particularly active in **Excellence® FTH**, giving wines with intense aromas of tropical and citrus fruits, with notes of boxwood.

2. TXL

Excellence® TXL is POF(-), which refers to its low cinnamyl decarboxylase activity. All yeasts express this enzyme, some to a higher degree than others. It enables yeasts to break down cinnamic acids to produce vinyl phenols. These vinyl phenols mask other aromas and are also substrates for Brettanomyces for the production of 4EP and 4EG through its metabolism.

Excellence® TXL is also URE2(-). The ure2 protein inhibits the transcription of genes necessary for the metabolism of organic sources of nitrogen during fermentation. Being URE2(-) means that **Excellence® TXL** can assimilate more complex forms of nitrogen, even under high concentrations of mineral nitrogen. The production of aromatic thiols is greatly improved.

Finally, **Excellence® TXL** is classified as SSUR-1(+), which indicates that it is resistant to sulfites. This improves its implantation in the must, giving a short latent phase and a faster start to the fermentation.
Excellence® TXL brings out typical varietal characteristics, whilst giving the wine greater volume on the palate.

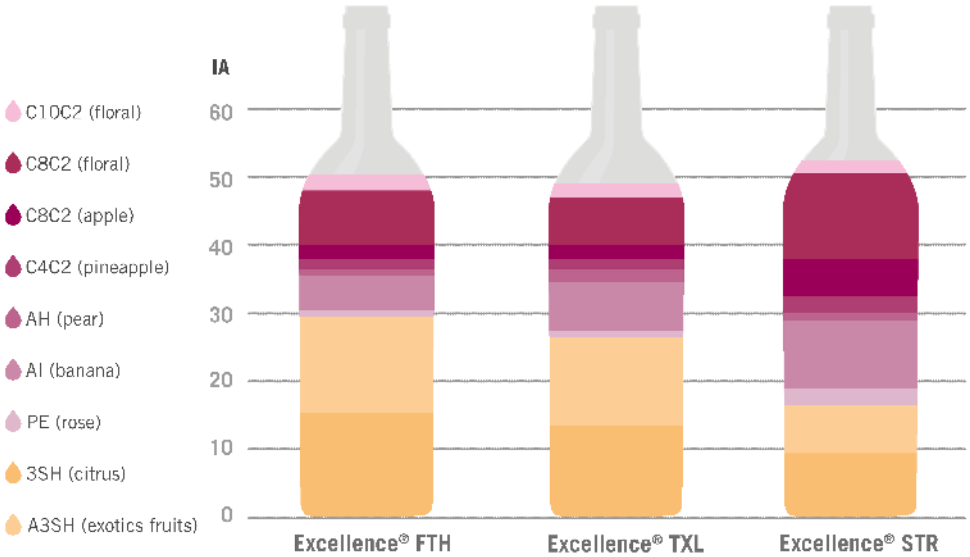
3. STR

Excellence® STR is also POF(-), ensuring wines which are cleaner on the nose. This yeast is characterized by its high production of two types of fermentary esters: acetate esters of higher alcohols (banana, pineapple, peach) and ethyl esters of fatty acids (apple, pear). **Excellence® STR**'s high performance in revealing these aromas can be explained the high activity of certain enzymes belonging to this yeast.

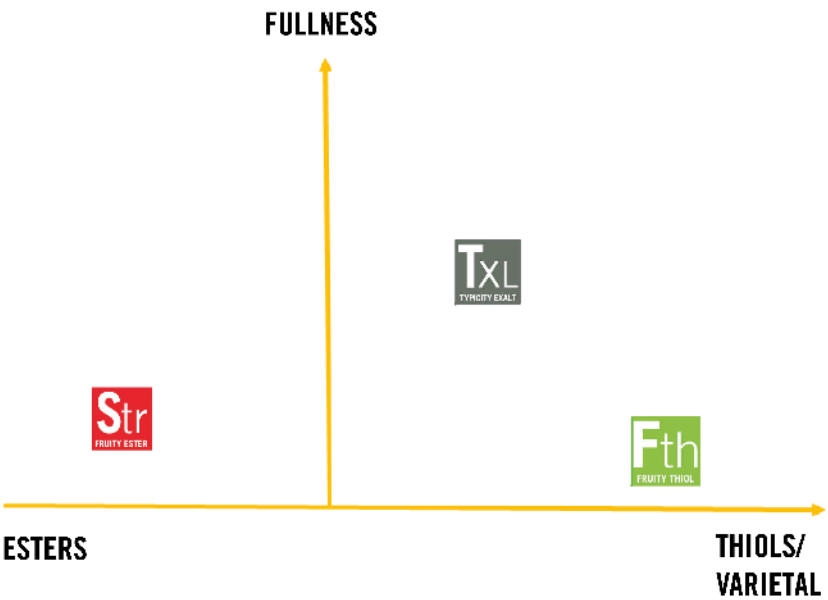
1. Aromatic index (AI)
[thiols] [fermentary esters] / perception threshold

Trial conditions:

- Sauvignon Blanc, 2016
- Pessac Léognan, Bordeaux
- TAV : 14 % vol
- pH = 3.48



2. Yeats profile



B.Red wine yeasts

1. XR

Excellence® XR is one of the first yeasts on the market to have been produced by breeding at the University of Bordeaux. It was selected for its low production of volatile acidity and fermentary esters. It produces high quantities of polysaccharides, which are rapidly liberated due to fast lees autolysis. All of the factors make this yeast the reference for high quality Bordeaux wines. Wines produced with **Excellence® XR** are round and powerful, with ripe fruit notes.

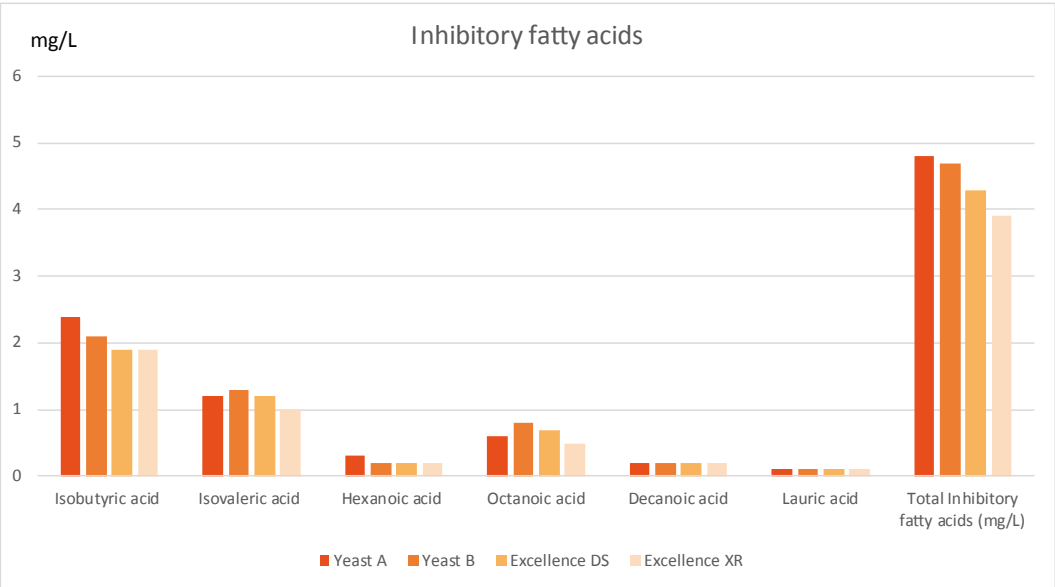
2. DS

Excellence® DS was selected on the same criteria as **Excellence® XR**. The main differences being that it produces greater quantities of fermentary esters, with less volume from the production of polysaccharides. It has been shown to obtain wines with more intense and stable color.
Thus, tannin quality, structure and the aromatic profile are completely different when using **Excellence® XR** and Excellence® DS.

3. MLF compatibility

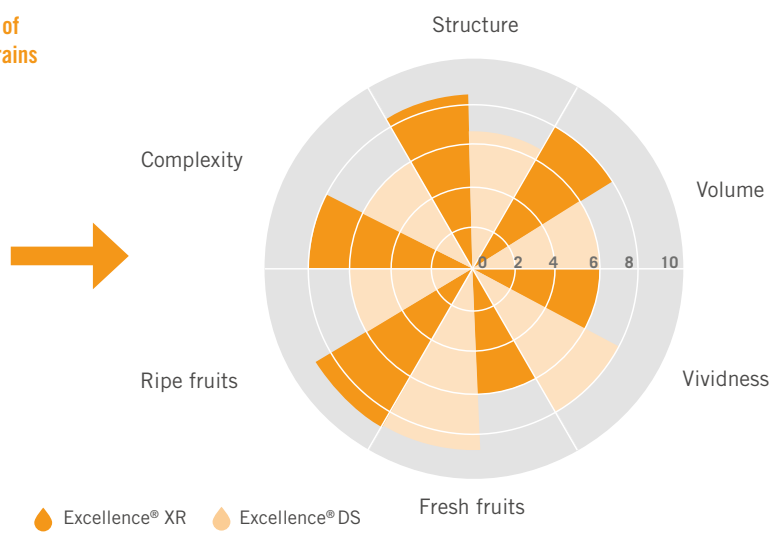
Major factors in the selection of yeasts for red wines are ethanol tolerance and the low production of inhibitory fatty acids, to enable the alcoholic and malolactic fermentations to be easily completed. Over the past few years wines have higher alcohol contents. In response to this stress, yeasts tend to produce inhibitory fatty acids during the AF which can lead to a stuck MLF. Modern yeasts are selected to combat these changes and are tolerant at high alcohol contents (>15.5%). The figure below shows the different amounts of inhibitory fatty acids produced by **Excellence® XR**, **Excellence® DS** and two other commercial strains. The low production of inhibitory fatty acids increases the ease and speed of the MLF.

3. Inhibitory fatty acids



4. Comparison of organoleptic profiles of Excellence® XR and Excellence® DS strains

Trial characteristics :
Excellence® DS and Excellence® XR
- Cabernet sauvignon - 2015
- Pessac-Léognan, Bordeaux
- TAV 13.6% vol – pH= 3.6



C. Specific characteristics of the Excellence® range

1. Resistance to copper

With more and more vineyards converting to organic agriculture, the use of synthetic phytosanitary products is decreasing. On the other hand, the use of copper based treatments is increasing, resulting in musts that are ever more concentrated in this element. Copper is known to be anti-fungal and, when in excess, its presence in the must can alter the yeasts' viability and thus the fermentation kinetics, leading even to stuck fermentations. Due to this, Lamothe-Abiet has carried out an internal study in order to characterize the copper resistance of some of its yeast, especially from the **Excellence®** range. For this, alcoholic fermentations were carried out in the laboratory with the same must. Copper (in CuSO45H2O form) was added to some modalities at concentrations from 2 to 5 mg/L.

No significant differences were shown on the AF kinetics. None of the modalities of the **Excellence®** range contained residual sugars at the end of the AF. This demonstrates the yeasts' excellent resistance to the eventual presence of copper in musts, even at high concentrations (5 mg/L).

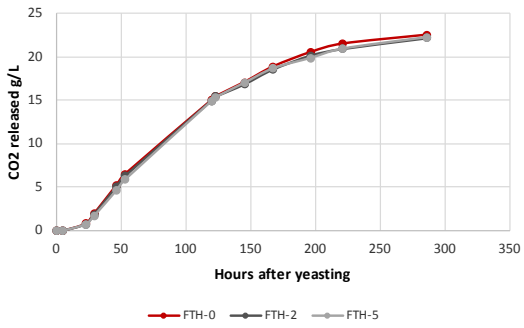
2. Net SO2 production

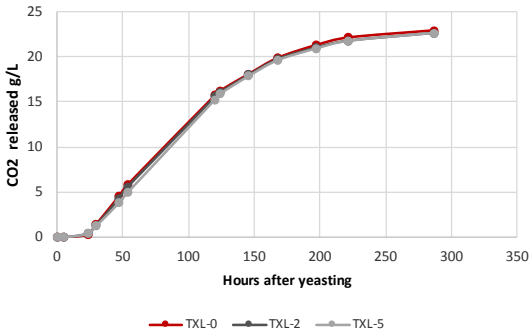
Under real winemaking conditions, Saccharomyces cerevisiae uses and produces SO_2 during the alcoholic fermentation. This production can be from a few mg/L to over 100 mg/L according to the fermentation conditions and the strain used.

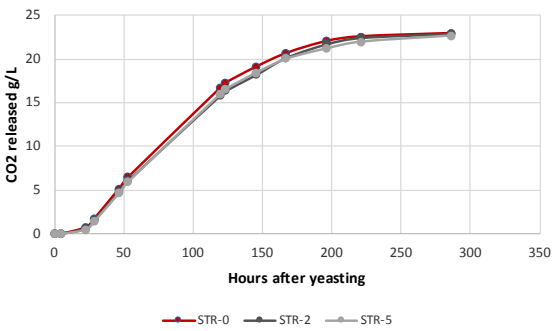
Lamothe-Abiet has carried out internal studies in order to better characterise the «SO_2 footprint» of its yeasts. The study was carried out using 4 different musts whose free SO_2 varied from 7 to 33 mg/L and the total SO_2 from 35 to 67 mg/L. The objective was to observe the «SO_2 footprint» of these strains: whether they tend towards a net consumption or production of SO_2. This study showed that all the strains of the **Excellence®** range have a net consumption. The concentrations of SO_2 present in the wines are lower than the initial concentrations in the musts.

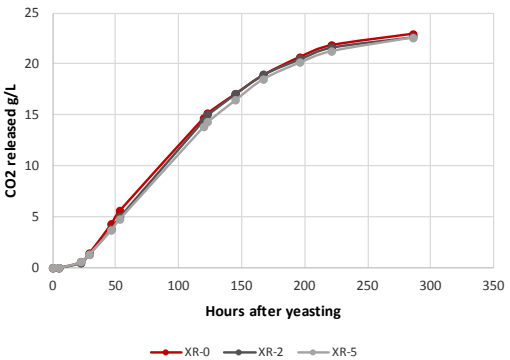
Therefore, by controlling the fermentation conditions (yeast nutrition, rehydration conditions, AF temperature, etc.) and by choosing a suitable yeast, it is possible to produce wines with less than 10mg/L total SO_2.

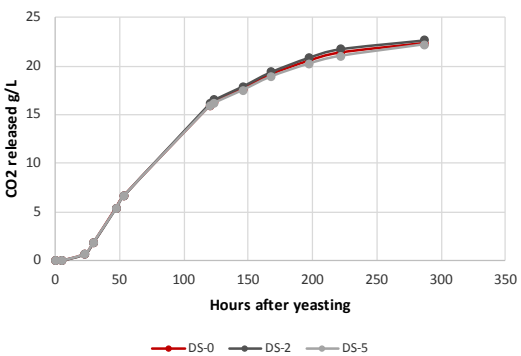
5. CO_2 released according to the copper content of the must











D. Excellence® Bio-Nature: bioprotection yeast

1. The concept of bioprotection

Sulfites have been, and still are, widely used in enology for their large range of action. Indeed, sulfites have the advantage of combining antioxidant, antiseptic and antioxidasic effects (Blouin, 2014). From a microbiological point of view, sulfite addition destroys all or part of the indigenous flora and thus limits the development of undesirable microorganisms (Lonvaud-Funel et al, 2010). Over the last ten years, an alternative practice has proven itself to be reliable: bioprotection. Instead of eliminating the indigenous flora, and thus creating a microbiological vacuum open to contamination, bioprotection enables microbiological balances to be controlled (Immelé, 2010). It involves implanting a selected microorganism which colonises the ecological niche, thus inhibiting the proliferation of spoilage microorganisms such as Brettanomyces, acetic bacteria, or non-Saccharomyces yeasts which may have a negative effect on the future wine (Lonvaud-Funel et al, 2010 ; Bartolini et al, 2010).

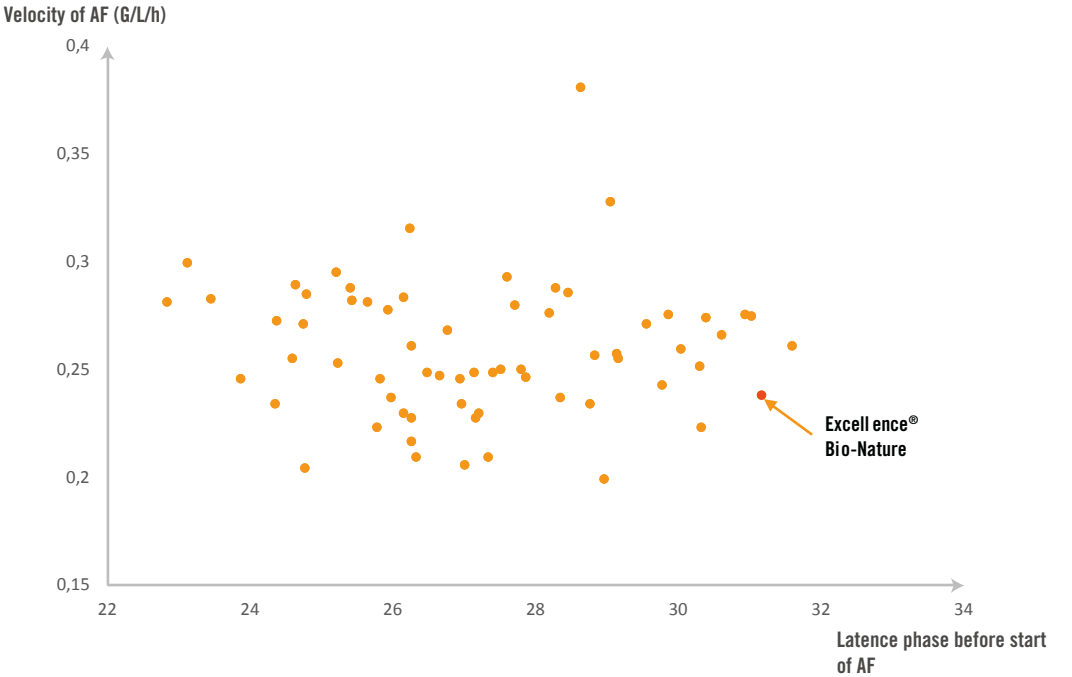
The selected microorganism must have certain characteristics in order to fulfil all the objectives of correct bioprotection:

- It must have a low fermentary capacity to avoid fermentation starting during the pre-fermentation phases;
- It must be resistant to low temperatures in order to withstand pre-fermentary cold soaks or stabilisation;
- It must be able to quickly colonise the medium in order to inhibit the proliferation of other microorganisms;
- It must be compete with the Saccharomyces cerevisiae inoculated for the fermentation of the sugars;
- It must not create any faults for the future wine.

2. Selection of Excellence® Bio-Nature

An identified Metschnikowia pulcherrima was selected by a joint research programme with the two universities, including the University of Bordeaux. This strain is particularly interesting due to its high aptitude to implant itself and its very low fermentary activity. These characteristics are essential for white and rosé production for the clarification phases as a spontaneous start to the AF does not leave the winemaker enough time to separate the clear juice from the lees. This Metschnikowia pulcherrima strain has been selected from a strain bank, from which each strain was studied for its fermentary kinetics, enzymatic potentials, production of volatile acidity, H_2S, etc...

6. Fermentary capacity of Excellence® Bio-Nature



Excellence® Bio-Nature is the strain with the best capacity for bioprotection. As shown on graph X, it has a low fermentation speed, as well as a long latent phase.

Criteria	*M. pulcherrima*
H_2S Production	Low
Volatile acidity production	Medium
POF Character	POF-
Must implantation	Fast
Fermentary capacity	Low (2-3.5 % vol)
Production of ethyl acetate	Low
Orgnaloleptics	No faults in AF
SO_2 resistance	Up to 4 g/hL
Temperature resistance	Up to 2 °C
pH resistance	Up to 3

3. Trial results

Trial conditions:

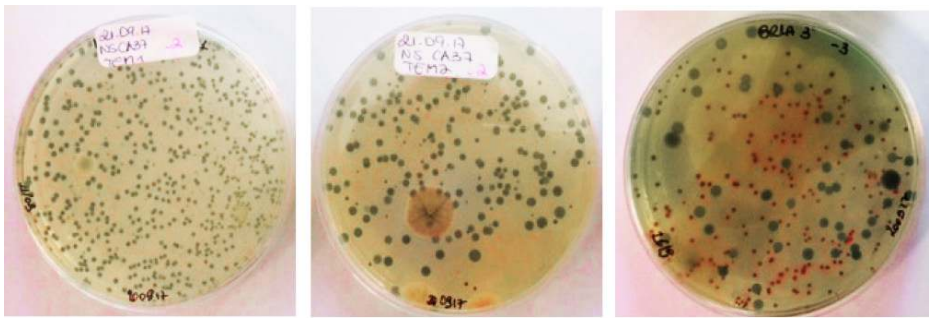
- Chenin, Vallée de la Loire, 2017
- ABV 12.8% vol, pH 3.2

Modalities:

- Starting must = Starting must, before sulfite or yeast addition
- SO_2+ = Sulfite added at 5 g/hL on grapes at harvest
- Bio-Nature = 5g/hL d'Excellence® Bio-Nature on grapes at harvest

Non-*saccharomyces* populations

Green colonies = Hanseniaspora Uvarum | Red colonies = Metschnikowia pulcherrima



Starting must | SO_2 + | Bio-Nature

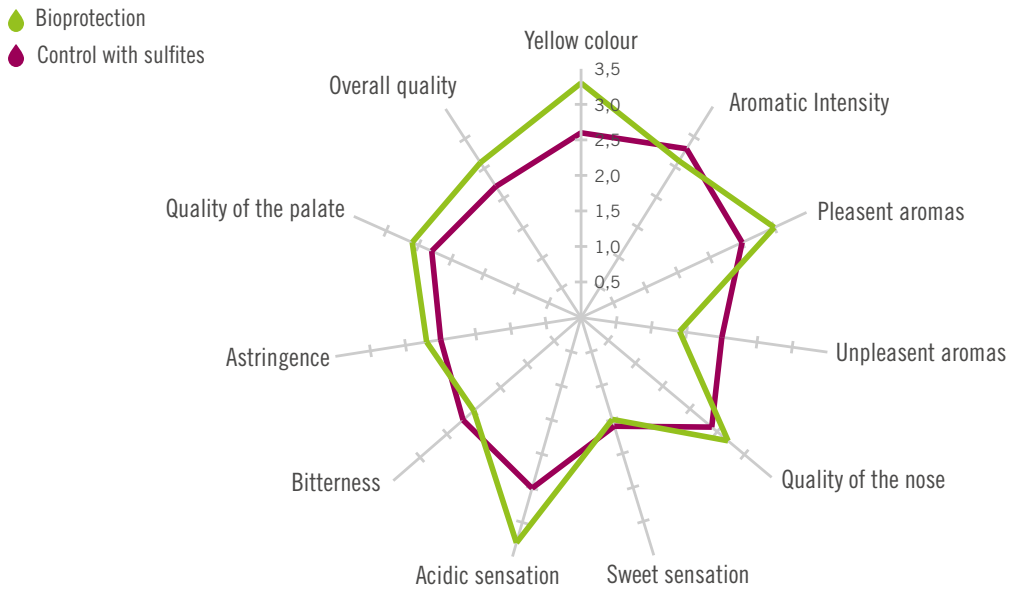
Post AF analyses

	SO_2+	Bio-Nature
Free SO_2 (mg/L)	0	0
Total SO_2 (mg/L)	26	7
Acetaldehyde - Ethanal (mg/L)	24	0
Estimation of the TL35* (mg/L) Post AF	104	80

** quantity of sulfite to add to wine to attain 35mg/L free SO_2*

Almost the entire wild population is made up of *Hanseniaspora uvarum*, very recognisable by its large green colonies. Adding sulfur to the grapes at a concentration of 5g/hL was not enough to completely destroy this wild flora. The modality inoculated with **Excellence® Bio-Nature** has entirely colonised the environment since its population is greater than that of the wild population.

7. Comparative tasting at the end of AF



As well as removing the need to add sulfites to the grapes at harvest, bioprotection with **Excellence® Bio-Nature** helps to reduce the production of substances that combine SO_2, as well as giving more complex wines.

IV. Aromatic optimisation

Lamothe-Abiet offers its expertise for optimising thiol and fermentary ester aromas. The methods shown have proved themselves around the world.

Fermentary esters

Optimal turbidity = 50 – 100 NTU
Optimal AF temperature = 14-16°C

TO KNOW

• The production of fermentary esters depends directly on the strain of yeast used. Certain enzymatic activities specific to the yeast are essential for an optimal revelation of acetate esters and ethyl esters of fatty acids. Excellence® STR was selected for this very reason.

Novoclair® Speed

Usage: on the grapes as soon as possible
Benefits: Fast depectinisation of must in cold clarification or flotation

GreenFine® Range

Usage: after pressing
Benefits: Clarification of must
Removal of polyphenols
Colour management

Volatile thiols

Optimal turbidity = 150 – 200 NTU
Optimal AF temperature = 18°C

Vinozym® FCE G

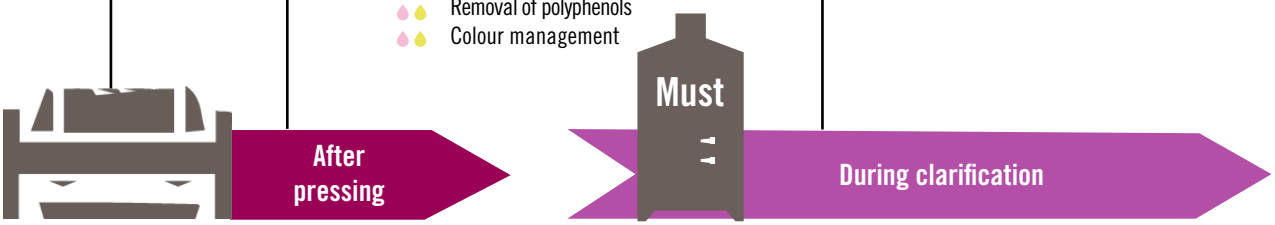
Usage: on the grapes as soon as possible
Benefits: Depectinise must
Extract aroma precursors

GreenFine® Range

Usage: after pressing
Benefits: Clarification of must
Removal of polyphenols
Colour management

OptiThiols®

Usage: before AF
Benefits: Stimulates the synthesis of thiols during AF
Better preservation of thiols after AF



OenoStim®

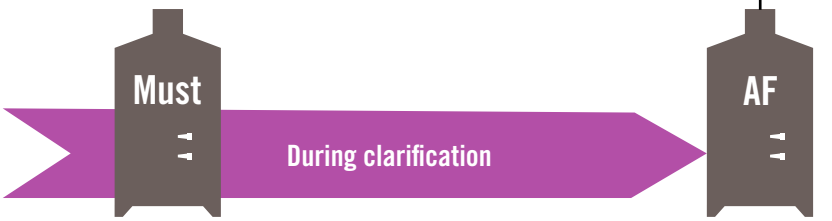
Usage: in rehydration water for the yeast
Benefits: Optimised fermentation kinetics
Better implantation of selected yeast

Excellence® STR

Usage: yeast addition
Benefits: Synthesis of fermentary esters
Good fermentation kinetics

OptiEsters®

Usage: at the end of the first third of AF
Benefits: Stimulate synthesis of fermentary esters during AF



OenoStim®

Usage: : in rehydration water for the yeast
Benefits: Optimised fermentation kinetics
Better implantation of selected yeast

Excellence® FTH / TXL

Usage: yeast addition
Benefits: Reveal aroma precursors
Good fermentation kinetics

Oenozym® TH

Usage: start of AF
Benefits: Reveal aroma precursors of 4MSP, 3SH and A3SH

Optiflore O

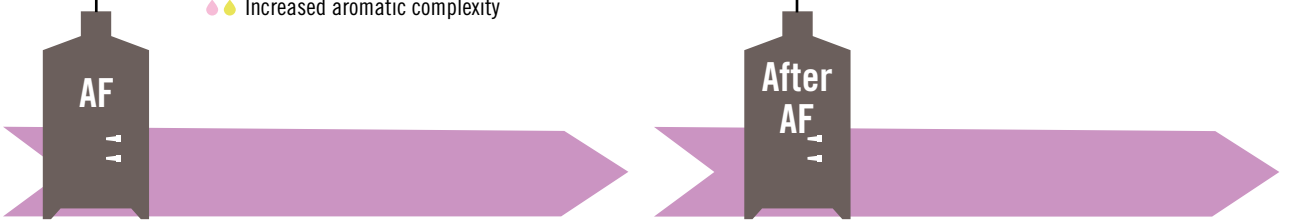
Usage: after first third of AF
Benefits : No effect on nitrogen catabolic repression
Increased aromatic complexity

Aroma Protect®

Usage: after AF or during maturation
Benefits: Protection of thiol aromas thanks to high concentration in glutathione

Oenozym® TH

Usage: during maturation
Benefits: Reveal aroma precursors 4MSP and 3SH



V. How to manage stuck fermentations?

When there is a sluggish or stuck fermentation, the risks of faults increase. It is therefore necessary to carry out a specific protocol for restarting the fermentation, in order to ensure that all the wine finishes dry.

1
TREATMENT OF THE STUCK TANK (100 HL)

Decant sheltered from air and eliminate the lees
Treat with sulfite at 2 g/hL
Detoxify wine :
- Flor'Protect® at 40 g/hL (4 kg)
- Actibiol at 40 g/hL (4 kg)

Homogenise the tank in a closed circuit
Keep the temperature to 20 °C
Wait 48 hours, then rack the entire volume and remove 5% (5 hL)

2
BASIC WINE FOR THE «PIED DE CUVE» (5 HL)

Adjust the ABV to 6-7% vol.
Adjust the sugar concentration to 20-30 g/L
Add 20 g/hL of Vitaferment or Vitaferment PH (100 g)

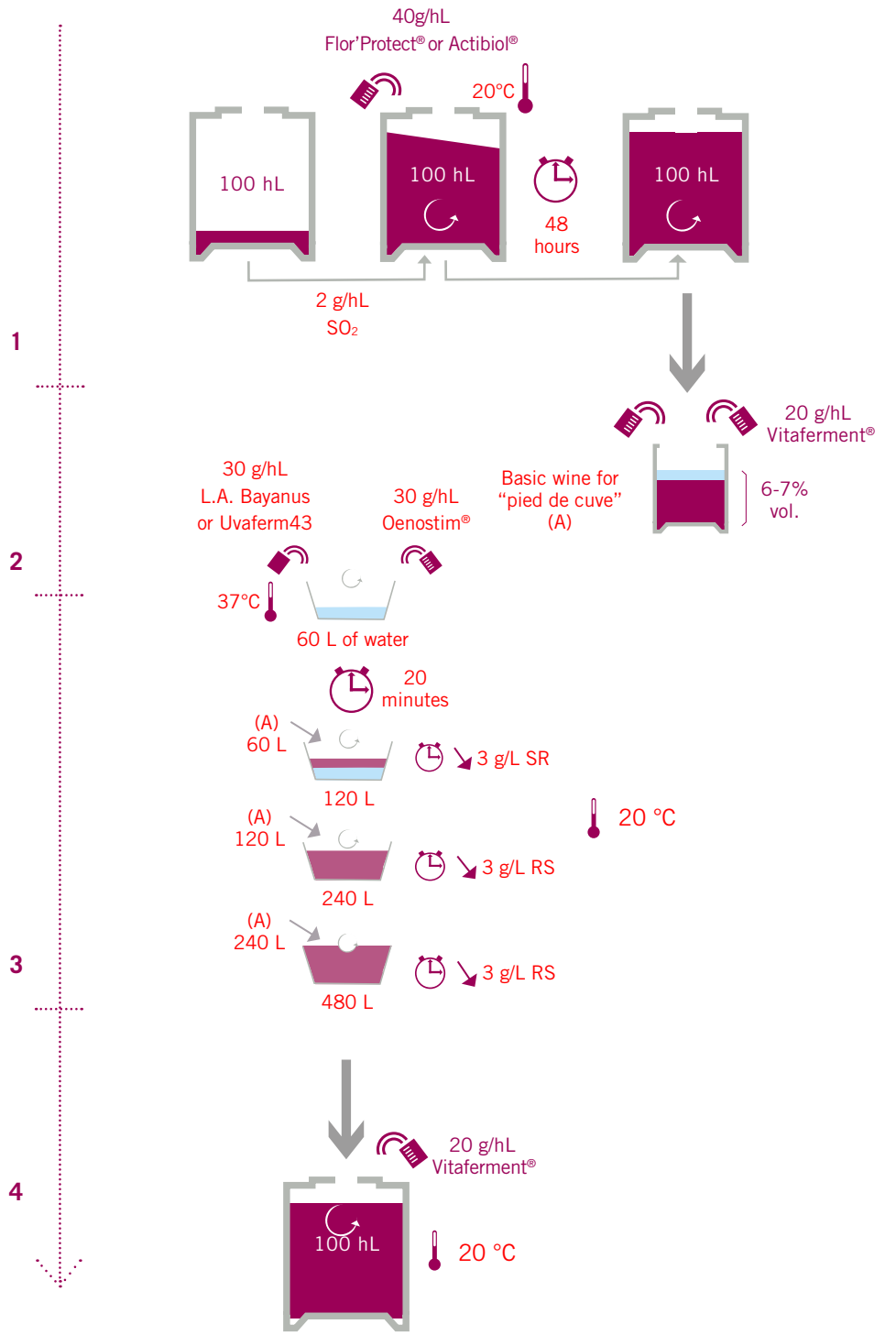
3
REHYDRATION AND ACCLIMITISATION OF YEAST

Rehydrate in 60 L of water at 37 °C with L.A. Bayanus or Uvaferm yeasts, 43 to 30 g/hL (3 kg), along with Œnostim®, 30 g/hL (3 kg)
After 20 min, double the volume (add 60 L) from the «pied de cuve» tank.
Immediatly after the AF start (controled with a mustimeter), make a large ventilation
When the residual sugars (RS) concentration is less than 3 g/L, double the volume of the starter by adding 120 L from the «pied de cuve» tank. When the concentration is again less than 3g/L, again double the volume of the starter by adding 240 litres from the «pied de cuve» tank.
Keep aerating until the residual sugars fall below 3g/L.
Keep the temperature at 20 °C

4
REHYDRATION AND ACCLIMITISATION OF YEAST

Incorporate the «pied de cuve» to the stuck tank
In the worst cases, the 5% volume can be still doubled before incorporation to the tank
At the moment of the «pied de cuve» incorporation, treat the stuck tank with 20 g/hL of Vitaferment (2 kg)
Keep the temperature at 20 °C

PROTOCOLE FOR 100 hL



LAMOTHE-ABIET
MALOLACTIC FERMENTATION

I. What is malolactic fermentation?

Malolactic fermentation is the transformation of L-malic acid, naturally present in the grape, into L-lactic acid and CO_2 thanks to the activity of certain lactic bacteria, especially of the species *Oenococcus oeni*.

II. What are its effects on the wine?

The biggest effect of the malolactic fermentation is the deacidification of the wine. Once finished, the acidity is reduced and the pH increased. The average decrease in the total acidity is 0.4 g/L H_2SO_4 per gram of L-malic acid that is transformed.

The decrease in acidity also influences the wine's organoleptic profile. L-malic acid, often associated with vegetal notes, is transformed into L-lactic acid which is less aggressive.

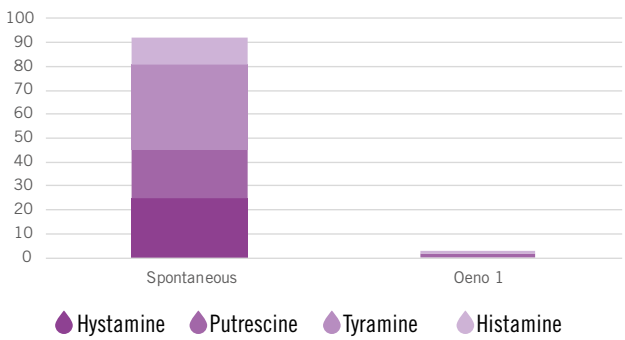
As well as the effect on the acidity and the wine's organoleptic profile, MLF improves the wine's microbiological stability. This is because the bacteria reduce the amount of available nutrients (nitrogen, vitamins, residual sugars) for the development of other spoilage microorganisms.

III. Why inoculate with selected lactic bacteria?

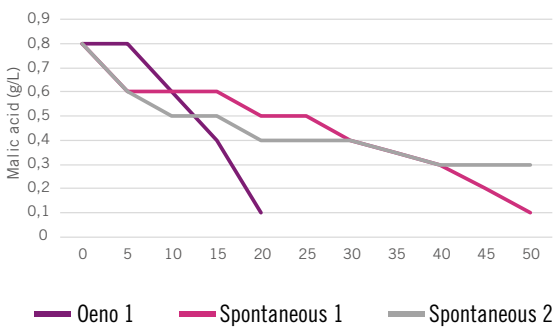
Selected lactic bacteria are generally extracted from wines with specific parameters (high ABV, low pH, high SO_2, low temperatures) in which they have naturally developed and completed the MLF. The main benefit of using selected lactic bacteria is to bypass the growth phase of wild lactic bacteria which is unreliable and unpredictable. The goal is to quickly occupy the environment. MLF generally occurs after the alcoholic fermentation. Using selected lactic bacteria, MLF can take place during or at the end of the AF. We call this co-inoculation. When it starts after the AF, it's called sequential inoculation. A chapter is devoted to co-inoculation, a very interesting practice from a technological and economic point of view.

Not inoculating with selected lactic bacteria is leaving things to chance. Certain wild lactic bacteria have a low fermentation capacity or even the ability to produce biogenic amines. Histamine is the most monitored biogenic amine since it is responsible for allergic reactions and headaches. Putrescine (which gives an odour of putrefaction) is another particularly monitored biogenic amine. It has a perception threshold of around 60 mg/L in wine, but at lower concentrations can mask the wine's aromas.

1. Production of biogenic amines (mg/L)



2. MLF kinetics



IV. The malolactic fermentability index*

For MLF to take place, certain conditions that are favourable to the lactic bacteria's metabolism are required. The index of malolactic fermentability allows an estimation of wine's capacity to carry out the MLF. For this, several parameters come into play to determine this indicator:

- The pH: this is one of the most important parameters. If the pH is too low, the lactic bacteria's metabolism can not be activated;
- The alcohol content (ABV): the higher the ABV, the harder it is for the lactic bacteria to transform L-malic acid to L-lactic acid;
- The temperature: when wines are too cold, it is difficult to start the MLF;
- Total SO_2: the more SO_2, the harder it is for the MLF to take place;
- The concentration of amino acids: bacteria almost exclusively metabolise amino acids. The wine must have enough of them;
- The concentration of malic acid: an insufficient concentration of malic acid can make it difficult for the MLF to start properly.

There are other parameters which can also play a role in the fermentability index, but these are the most important. Lamothe-Abiet has developed an application that can calculate the fermentability index of your wine using these parameters.

Evaluate the ease of carrying out malolactic fermentation on your wines :

On our website in the tab LA Solutions / Toolbox

On our Oenosolutions mobile app, available on the Appstore and Google Play Store

** based on the work of Renouf V (2013). La fermentation malolactique dans les vins : Mécanismes et applications pratiques. Lavoisier, Paris*

V. The benefits of co-inoculation

A. Principle

Early co-inoculation helps the malolactic fermentation to take place much more quickly than with sequential inoculation. It also has a significant impact on the overall quality of the wine.

This is because it is easier for lactic bacteria to become established at the beginning of the alcoholic fermentation (AF) than at the end. The environment is less hostile to their development since there is a lower alcohol content, fewer extracted polyphenols, more available organic nutrients, and fewer inhibitory fatty acids produced by the yeast.

B. Benefits

1. Technical

At the end of the AF, the "ecological niche" is fragile. This microbiological vacuum favours the development of undesirable microorganisms such as *Brettanomyces bruxellensis*, which can produce ethyl-phenols, and/or acetic bacteria which increase the wine's volatile acidity. The unsulfited wines are prone to oxidation, which can create undesired development or losses of aromas. As well as this significant benefit, the practice of co-inoculation has other benefits:

- Decreased diacetyl production: diacetyl is an aromatic compound produced by lactic bacteria. This compound has a buttery aroma. Below a certain threshold (< 5mg/L), it lifts the wine's aromatic profile. Above this threshold, this molecule masks other aromas and the subtleties of the wine's organoleptic profile. Co-inoculating can help to maintain the fruity profile of the wine;
- Fewer compounds that combine SO_2 : the concentration of ethanal is always lower in co-inoculated wines than for those sequentially inoculated. Thus, the addition of sulfur during maturation is more effective for a co-inoculated wine;
- Fewer substrates for *Brettanomyces*: co-inoculation helps to significantly decrease the amount of cinnamic acids after the MLF. This decreases the risk of ethyl-phenol spoilages;
- In case of *Brettanomyces* development, ethyl-phenol production is always lower when MLF has already finished, hence the importance in doing this as early as possible.

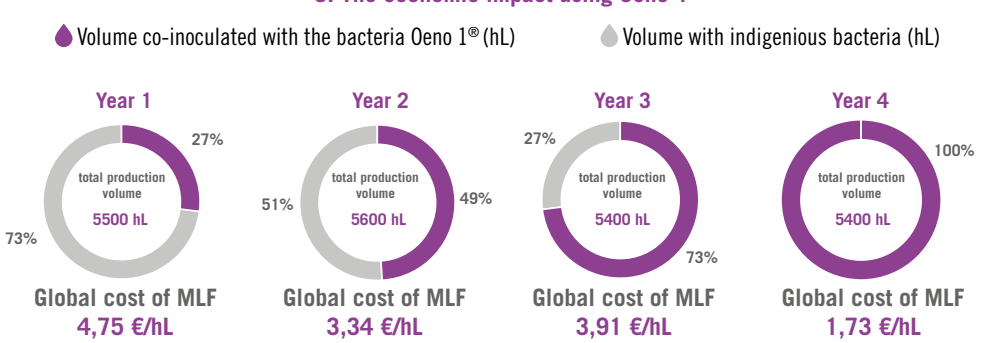
Several criteria must be respected to properly carry out a co-inoculation: select a yeast that produces few inhibitory fatty acids, ensure that the nitrogen nutrition is well managed, and that the temperature is properly controlled (do not go above 30°C), and of course use an adapted lactic bacteria.

2. Economic

Co-inoculation also has economic benefits :

- Better organisation of winery workers;
- Reduced fermentation time;
- Lower cost of wine analyses;
- Possibility to put the wines on the market more quickly (en primeur tastings, bulk sales, quick commercialisation);
- Optimise energy costs (heating of tanks).

3. The economic impact using Oeno 1®



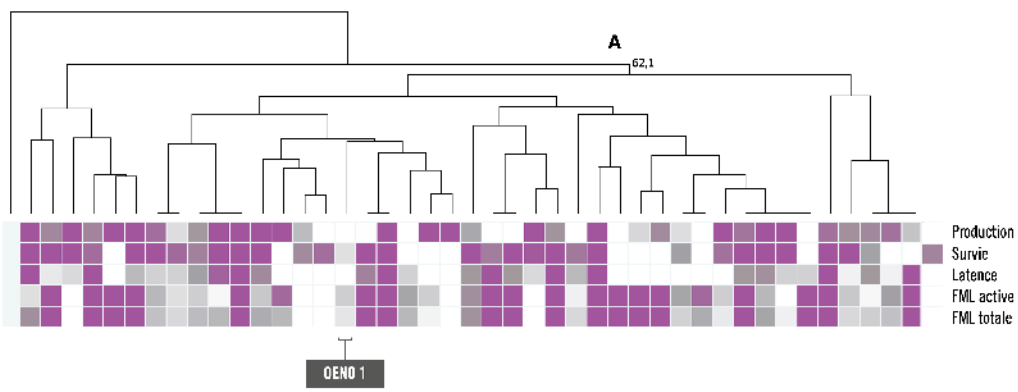
VI. Our solutions

A. Oeno 1

1. The selection

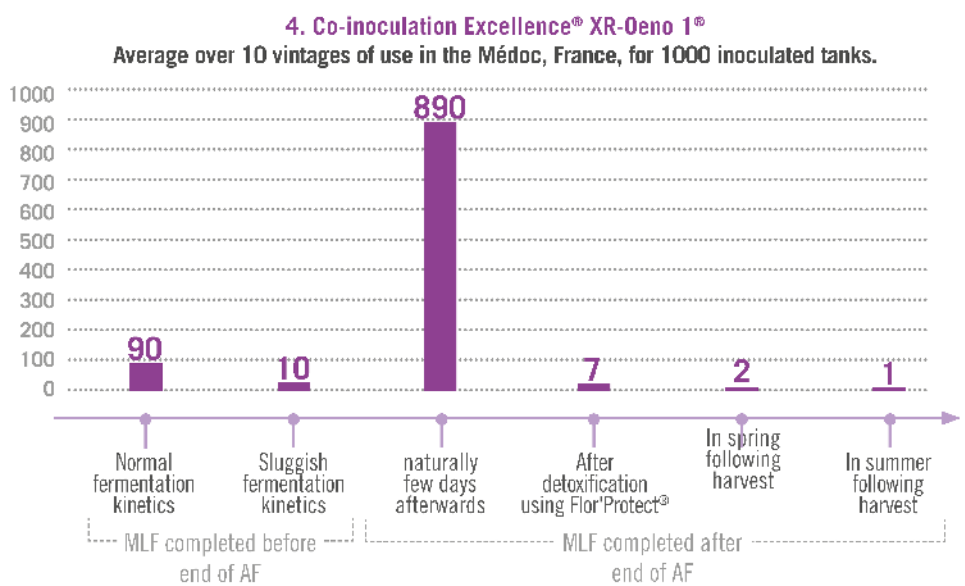
Oeno 1® is a strain of lactic bacteria which has been selected for 5 variables of technological interest from a bank of over 40 different lactic bacteria:

- Ease of production
- Survival rate > 75%
- Lag phase < 7 days
- Length of active MLF < 14 days
- Total MLF length < 23 days



The lighter the value, the more it is favourable. The darker the value, the less it is favourable. It is interesting to note that **Oeno 1®** comes out particularly well in this study and has favourable values for the 5 variables of interest.

2. The results



Over 10 vintages, co-inoculation with **Oeno 1®** enabled the MLF to occur without problem in over 99% of cases.

	Year 1	Year 2	Year 3	Year 4
Co-inoculation / Total	27%	49%	73%	100%
Electricity cost in €	18781	13959	12013	0
Oeno 1® cost in €	2592	4752	6748	9331
Analysis costs to monitor barrel MLF in €	900	0	450	0
Labour cost for barrel work in €	3840	0	1920	0
Total cost in €	26113	18711	21131	9331

It is interesting to note that the reliability and effectiveness of **Oeno 1®** can reduce production costs. Co-inoculation allows savings to be made in electricity, analyses and labour.

B. Oeno 2

Oeno 2 is a lactic bacteria which is recommended for late co-inoculation and sequential co-inoculation. A malolactic activator is provided with it to improve its resistance, thus ensuring that the MLF goes smoothly.

VII. Other factors of interest

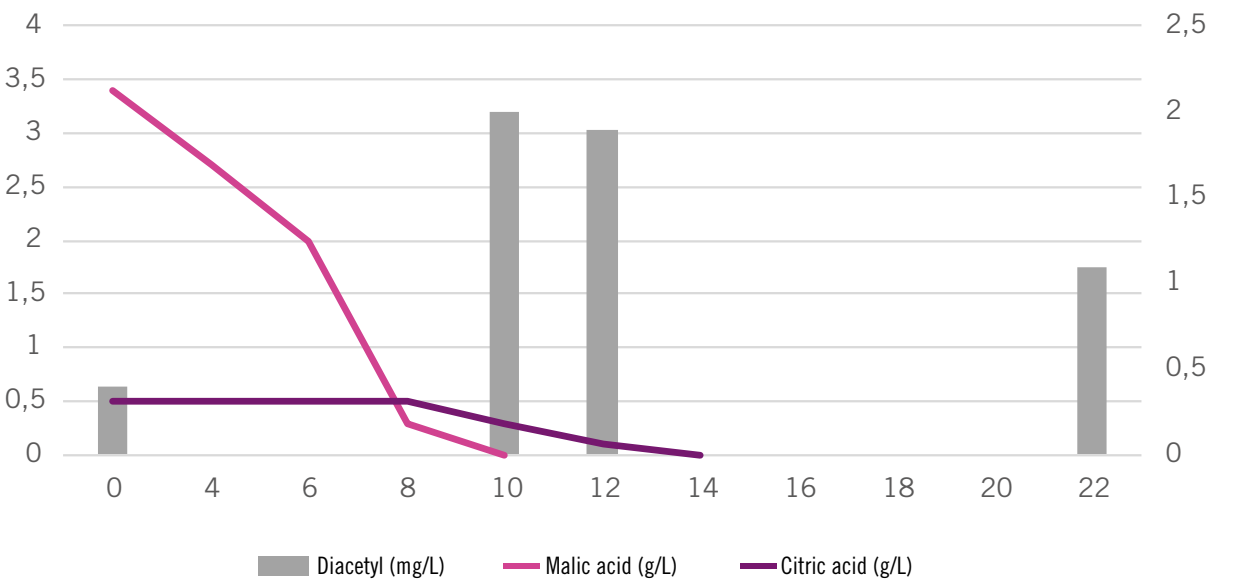
A. Diacetyl

During the malolactic fermentation, lactic bacteria are able to metabolise citric acid.
This citric acid can be broken down to several molecules:

- Acetic acid
- Lipids
- Acetoin derivatives including diacetyl. This molecule has a buttery note in wines. It has been shown that just a few mg/L of diacetyl (about 2 to 3 mg/L for white wines, 5 mg/L for red wines) can have a positive effect on the bouquet. At higher concentrations, the buttery aroma becomes too dominant and decreases the wines' quality.

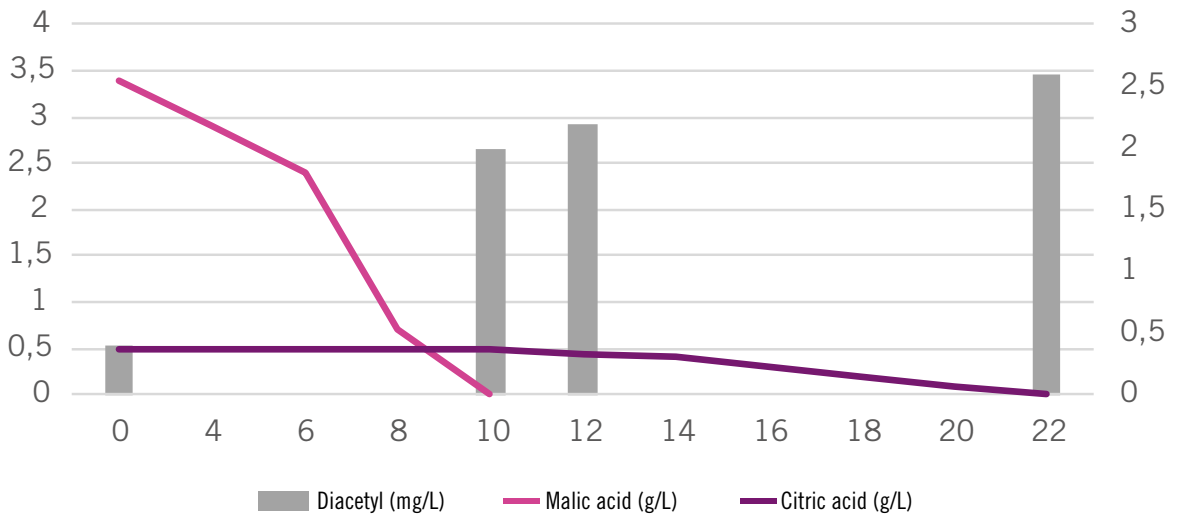
For our bacteria, **Oeno 1®** metabolises citric acid very quickly and produces a very small amount of diacetyl (2.5 mg/L). The peak of production coincides with the end of the MLF. Over the days following the end of the FML, **Oeno 1®** is able to reduce diacetyl to acetoin and/or butanediol, which have a neutral odour.

5. Diacetyle - Oeno 1®



Oeno 2's metabolism is slower and able to produce diacetyl in moderate to high concentrations. The peak of diacetyl production is roughly 10 days after the end of the MLF.

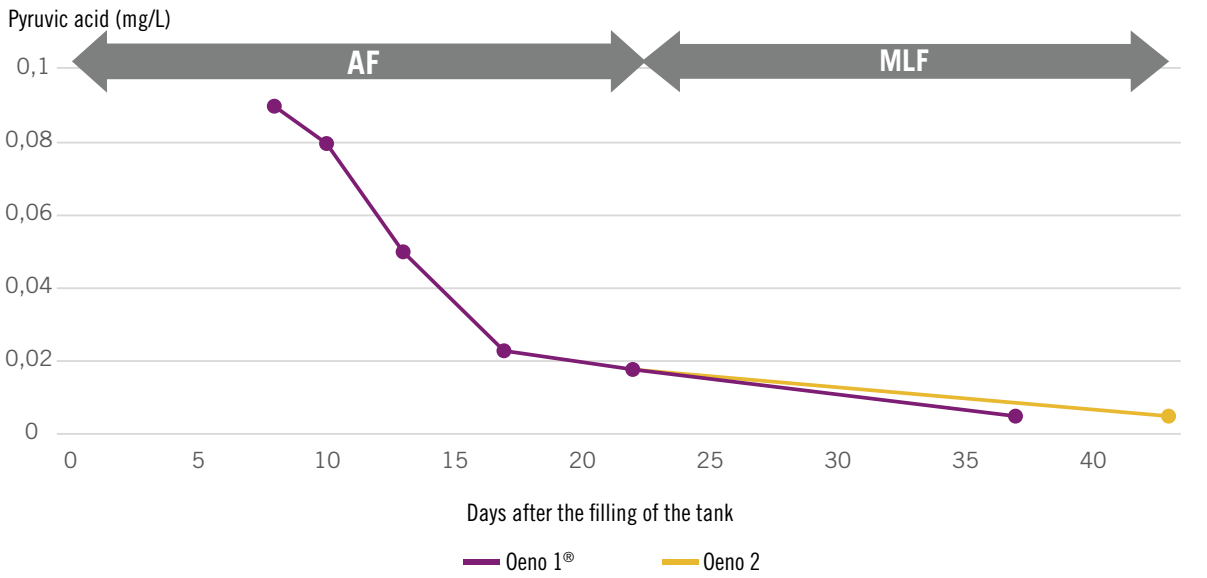
6. Diacetyle - Oeno 2



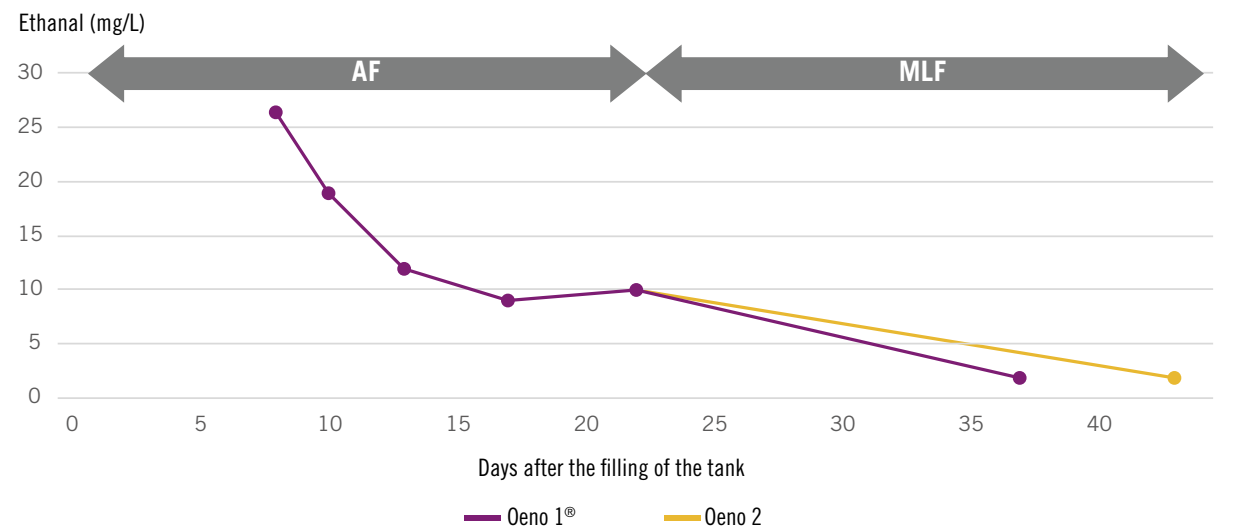
B. Compounds that combine SO_2

It is well known that, during the malolactic fermentation, lactic bacteria are able to consume certain compounds that combine SO_2. Ethanal and pyruvic acid are two compounds that have a strong capacity to combine SO_2. **Oeno 1®** and **Oeno 2** consume SO_2 combining compounds in a similar fashion. After the MLF, no pyruvic acid or ethanal remains. Therefore, if sulfur is added during maturation, the rate of combination is low and the SO_2 added will be more effective, leaving more free SO_2 in the wine.

7. Pyruvic acid consumption by Oeno 1® and Oeno 2 bacteria



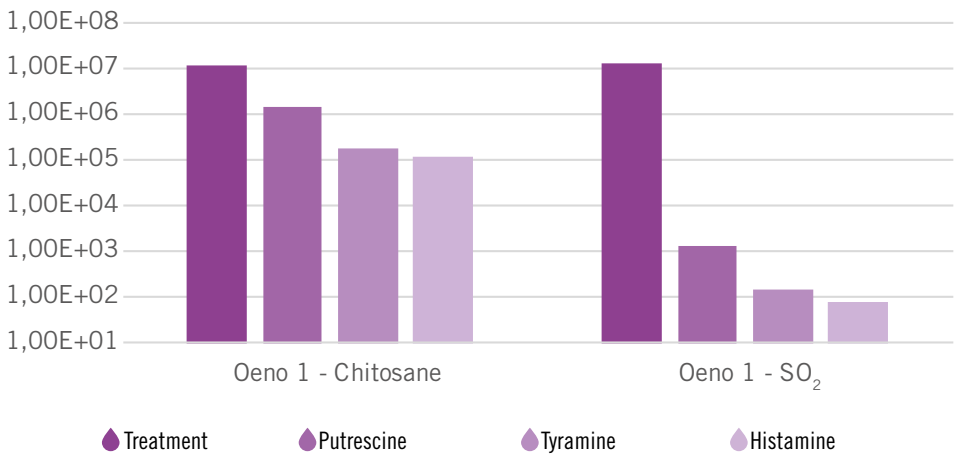
8. Ethanal consumption by Oeno 1® and Oeno 2 bacteria



C. Our bacterias's resistance to microbiological stabilisation

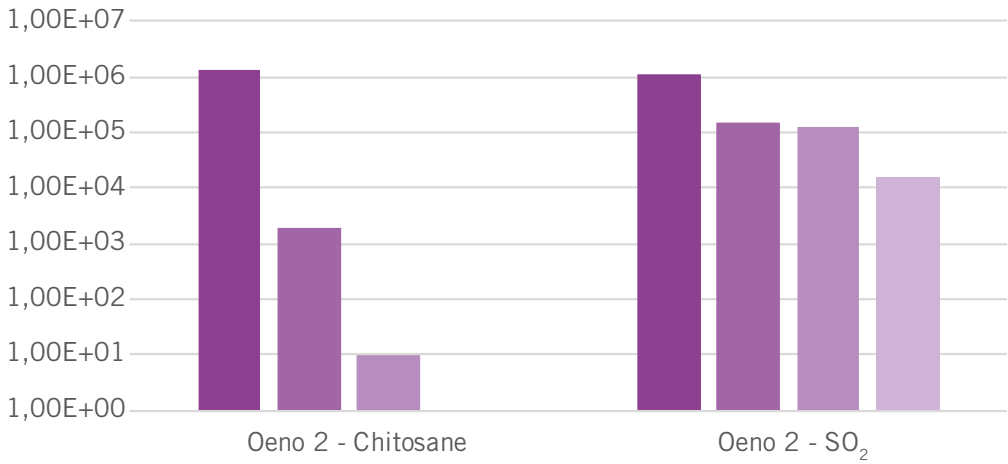
Oeno 1® and **Oeno 2** bacteria react completely differently to microbiological stabilisation treatments. **Oeno 1®** is sensitive to SO_2 since its population drastically decrease after sulfite addition at 4 g/hL. When chitosan is added at 10 g/hL, its population remains relatively stable over time.

9. Resistance of Oeno 1 to different types of microbiological stabilisation



Oeno 1®'s resistance to different types of microbiological stabilisation.
Oeno 2 bacteria reacts differently. It is resistant to SO_2 but chitosan significantly reduces its population in the wine.

10. Resistance of Oeno 2 to different types of microbiological stabilisation



Oeno 2's resistance to different types of microbiological stabilisation.

This information can be of interest for maturation without sulfites or with low sulfites. The presence of a large selected lactic bacteria population during maturation can give a natural protection of the environment, preventing spoilage microorganisms through competition.

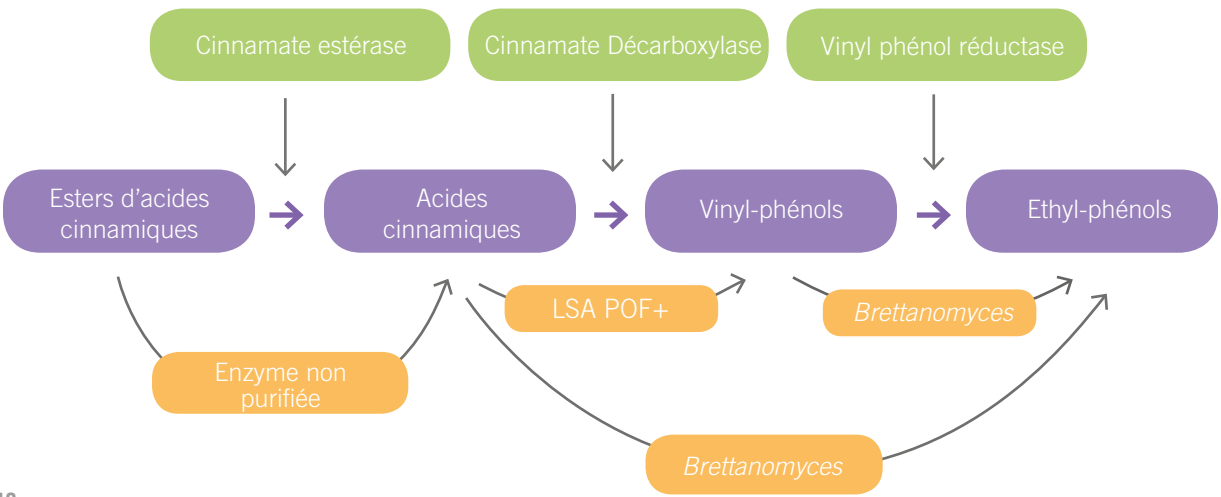
D. Cinnamate esterase activity

Cinnamoyl esterase activity has been detected in certain O.oeni strains. It is responsible for the liberation of free hydroxycinnamic acids during the MLF. The main hydroxycinnamic acids in wine are naturally found in the must, predominantly in the form of tartaric acid esters (see table below). In free form, they have a wide range of beneficial enological properties (antioxidant, colour stabilisation). However, they are mainly considered precursors to vinyl- and ethylphenols, molecules which mask wines' fruity aromas, given a "phenolic" character. Even though the use of a "cinnamate esterase positive" strain is not entirely linked to the appearance of B. bruxellensis spoilages, this property is nowadays considered as a risk factor in certain sensitive cases and it recognized as an essential criterion for the characterisation of malolactic starter bacteria. The responsible genes and enzymes have not yet been identified for O. oeni. Therefore, the cinnamate esterase activity is indirectly diagnosed, based on the evolution of reaction substrates and/or products.

Hydroxycinnamic acids		Vinylphenol	Ethylphenol
Esterified	Free		
Coutaric acid	p-coumaric acid	4-vinylphenol	4-ethylphenol
Fetaric acid	Ferulic acid	4-vinyl guaiacol	4-ethylguaiacol
Caftaric acid	Caffeic acid	4-vinylcatechol	4-ethylcatechol

Coumaric and ferulic acids are respectively precursors of 4-vinylphenol/4-ethylphenol and 4-vinylguaiacol/4-ethylguaiacol, described as the main markers of spoilage. Despite a significant quantity of caffeic acid in musts, 4-ethylcatechol is not analysed in case of spoilage. Its concentrations remain low (<100 µg/L) in the large majority of wines, below the perception threshold (between 400 and 800 µg/L).

This spoilage is mainly caused by the *Brettanomyce bruxellensis* species which has enzymes reduced to ethylphenols. Thus, any chemical or biological process which hydrolyses the esterified hydroxycinnamic acids increases the amount of available precursors.



We decided to investigate this parameter on our strains with internal trials. The objective of these trials was to define the "cinnamate esterase" profile of the **Oeno 1®** and **Oeno 2** strains under enological conditions.

The results below are from experiments carried out in our experimental centre during the 2018 vintage on a Cabernet Sauvignon wine. After the AF, the **Oeno 1®** and **Oeno 2** strains were inoculated to 5L of wine. The MLF were carried out at 18°C in duplicate. The analyses of the hydroxycinnamic acids after the AF and 10 days after MLF are shown below.

	After AF	10 days after MLF		Control wine**
		Oeno 1*	Oeno 2*	
Coumaric acid (mg/L)	0	0	0	0
Ferulic acid (mg/L)	0	0	0	1.9
Caffeic acid (mg/L)	1.5 ± 0,5	2.35 0.5	1.6 ± 0.1	12.2

In the absence of coumaric and ferulic acids, the wine was treated with an enzyme that has a cinnamate esterase activity in order to evaluate the presence of precursors (Control wine). The results show that fetaric and caftaric acid are present in the wine but that coutaric acid is not.

The results and their standard deviations, showing low concentrations of ferulic and caftaric acid, indicate an absence of cinnamoyl esterase activity for the **Oeno 1®** and **Oeno 2** strains.

Thus, by using **Oeno 1®** and **Oeno 2**, the production of substrates for the production of volatile phenols will be lower and the risks of deviation greatly limited

VIII. Winemaking protocols

In summary, our studies demonstrate the following information:

	OENO 1	OENO 2
Diacetyl production	Medium	Medium to high
Citric acid metabolism	Fast	Slow
Phenolic acid production	Negative	Negative
SO_2 resistance	Low	High
Chitosan resistance	Medium	Low

These data allow two different production protocols to be envisaged:

Protocol without SO_2

Oeno1 co-inoculation:

- Less phenolic acids
- Less volatil phenols production if contaminated with *Brettanomyces bruxellensis*

After MLF - *Brettanomyces* analysis

If no *Brettanomyces*,
redo analysis after 1 month

If *Brettanomyces* present, **Killbrett®** treatment:

- **Oeno 1®** resistant to chitosan (bioprotection during maturation with **Oeno 1®** after treatment)

Protocol with SO_2

Co-inoculation with Oeno 2:

- Fewer compounds that combine SO_2
- Less phenolic acids
- Less volatil phenols production if contaminated with *Brettanomyces bruxellensis*

Check MLF completion

Quickly **treat with SO_2**:

- Prevent *Brettanomyces*
- Decrease diacetyl production
- **Oeno 2** resistant to SO_2 (bioprotection during maturation with **Oeno 2** after sulfite addition)

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