1ST EDITION

WINEMAKER HANDBOOK

LAFFORT

l'œnologie par nature

Dear Winemakers,

For more than 125 years, **LAFFORT®** have been working for a precision enology, partnering our innovative Research & Development center in Bordeaux with the most prestigious wine research institutes. The fruits of our work have led to a wide range of developments in natural and preventive enology. **LAFFORT®** are also a team of practical winemakers, well trained, and from diverse regions throughout the world. Our specialty is to understand and facilitate the fermentation, finishing, and stabilization of all wines.

In our first Winemakers Handbook, we bring to you a synthesis of our research and practical implementation, a summary of the most common questions that are asked of us. Adding to the knowledge of the **LAFFORT®** USA team, there are multiple articles from our branches in France, Australia, and South Africa, and we draw on the knowledge from the 60 countries that Laffort is distributed.

Whether it be tools for optimizing aromatics in rosé wines, a reminder of the exact process for a fermentation restart, the best practices for dealing with rot in grapes, or introducing **BIO**Protection into your vineyard and cellar practices, we offer you the **LAFFORT®** toolbox. You may use this Handbook to answer many of the most common and detailed questions about winemaking ingredients and processing aids, remembering that even more information a simply a text, email, or phone call away. Indeed, this book will not replace your **LAFFORT®** Technical Representative, who lives and breathes a tremendous depth of knowledge of winemaking.

In 2021, **LAFFORT®** aim to obtain ISO 26000 certification, reflecting the implementation of our approach to Corporate Social Responsibility, a collaborative project that will allow sustainable development of our company while operating in an economy that is more respectful of our planet. We are committed to adopting responsible behavior and doing everything possible to ensure that our activities address the issues that we have defined as a priority, namely the environment, social equity, and economic viability.

We thank you for the trust you have placed in **LAFFORT®** and all its teams, and wish you all the best for this 2021 vintage and beyond.

Shaun RICHARDSON
General Manager, LAFFORT USA

YEAST

YEAST

ARTICLE

YEAST SELECTION BACK TO BASICS

Offering some fundamental advice on how to select the best yeast for your wine is Joana Coulon,manager of microbiology at BIOLAFFORT, and Alana Seabrook, technical manager and LAFFORT® AUSTRALIA.

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INTRODUCTION

Nowadays, choosing a yeast for fermentation is a simple as flicking through a shiny catalogue or a website and finding all of the desirable attributes. However, not so long ago the choice of commercial yeast was non-existent and winemakers were forced to rely on their own resources to ensure fermentation went through to completion. But now the choices almost seem endless…where to start? What is important? How relevant are all of these so-called desirable attributes in a winelike environment? The intention of this article is to navigate through the factors around yeast attributes and work out what is key for the winemaker.

ORIGINS

Saccharomyces cerevisiae (S. cerevisiae) is arguably a domesticated species and is often found in human environments. It is associated with numerous fermented beverages and can be traced back to 3200BC (Cavalerri et al. 2003). Fermentation activities probably due to this microorganism were even detected in neolithic poteries (6000-7000BC) in China (McGovern et al. 2004). Nowadays *S. cerevisiae* are found in cellars and on grape berries (Mortimer and Polsinelli 1999) but they are thought to originally inhabit forests on tree bark (Wang et al. 2012), transported by insects to colonise highly fermentable ecosystems.

S. *CEREVISIA*E VS S. BAYANUS

In past decades, *S. bayanus* was associated with not being able to metabolise galactose (Gal-) and *S. cerevisiae* was Gal+ (Barnett 1992). *S. bayanus* then became a generic term to denominate strains of *S. cerevisiae* that were not able to metabolise galactose. Today, the species *S. bayanus* exists, but no longer refers to the Gal (-) group originally described. Genetically it is very distinct from what we now know as *S. cerevisiae* and not associated with oenology (used primarily for brewing). In 1953 it was observed that what was anciently called '*S. bayanus*' (which belongs, in fact, to the *S. cerevisiae* species) had better fermentation abilities and was often associated with the end of AF (Peynaud and Domercq 1953). This is now no longer relevant to oenology (Frezier 1992). Hence the 'Gal-' criteria is not best suited to designate strong fermentation ability strains among *S. cerevisiae*.

SENSORY IMPACT

Every yeast strain will possess a different spectrum of enzymatic activities that influence the sensory profile of the wine. Some yeast strains are natural isolates from regions renowned for the production of a particular wine. This means they were identified during a fermentation as being the yeast responsible for the fermentation. Often this is perceived as a way of identifying a yeast strain that will produce the sensory profile desired. But, unfortunately, a strain isolated from a Sangiovese in Chianti may not impart the same sensory profile on an Australian Sangiovese with identical winemaking processes. Moreover, the concept of a 'terroir' strain still remains questionable. Indeed, even though regional strains can be found, the link between the origin of a strain and the organoleptic signature of the corresponding fermented wine is still a debate (Borlin 2014, Knight and Goddard 2015). In addition, these strains would also be tested for alcohol tolerance, fermentation kinetics, YAN demand and temperature sensitivity. This is where crossing yeast strains with ideal attributes becomes important when considering different environments for a particular desired outcome.

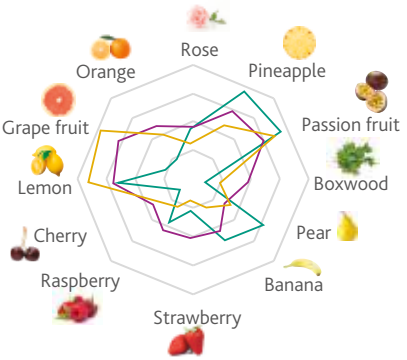

Figure 1. The aroma profile of three different yeast strains.

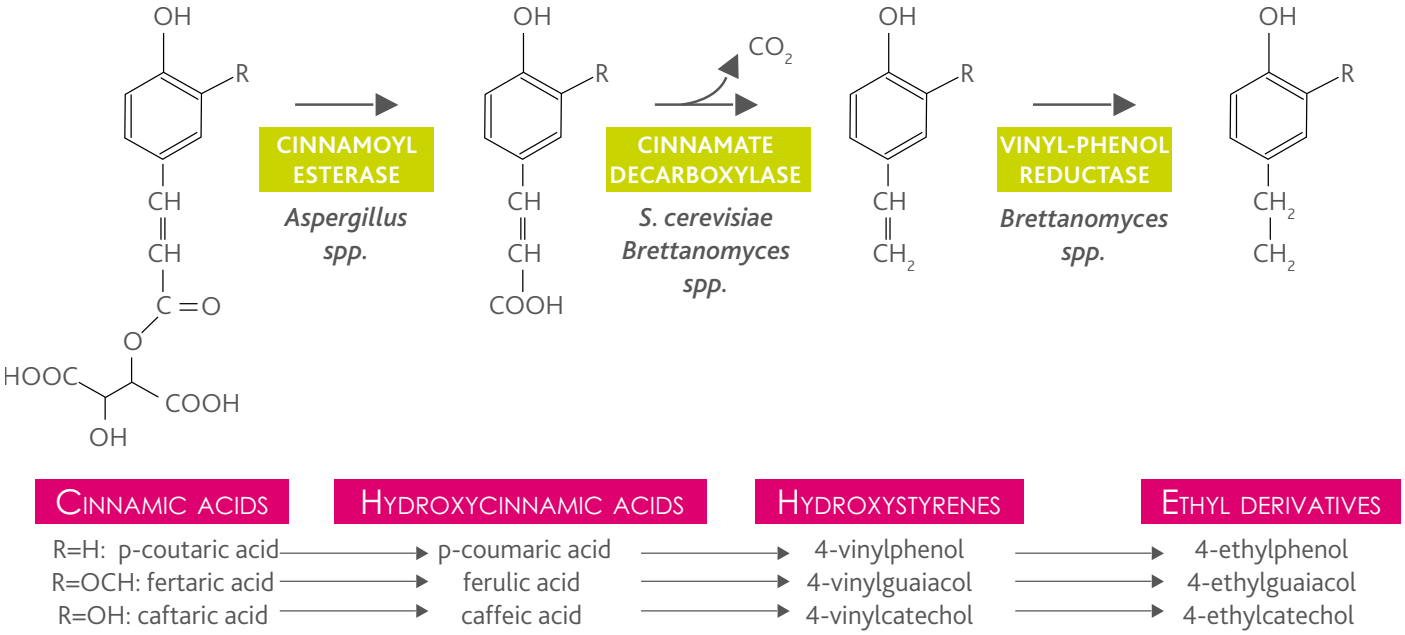

Figure 2. Conversion of cinnamic acids to Ethyl derivates (Sourced from **BIOLAFFORT®**, France).

Most commercial yeast strains will present an indication of the types of aroma compounds produced and the sensitivities/tolerances. Do they make a big difference sensorially? Absolutely. Depending on the wine in question, particular aroma compounds are critical. For example, in Sauvignon Blanc thiols are key aroma compounds. The volatile form of these compounds is produced by yeast and many yeast will not produce these key thiols. Esters, important for many fruit aromas in both red and white wines, are converted by yeast into a more volatile form making the selection of yeast key. Some aroma compounds are present in grapes and are linked to a sugar; a yeast may produce enzymes that cleave off this sugar to rendering the aroma volatile. Understanding that these enzymes have temperature sensitivities and that the aroma compounds themselves are volatile and may be susceptible to oxidation is critical. Figure 1 demonstrates the aroma profiles of three different yeast strains in the same wine. Understanding the desired profile is ideal when choosing a yeast strain, but really the only way to know what sensory profile a yeast strain will produce is by trialing it on the desired grapes in those particular conditions.

KILLER CHARACTER

Yeasts that have a 'killer factor' are deemed to be positive in oenology, as their presence suggests they can outcompete other yeast strains and species by producing a killer toxin. A yeast may contain virus like particles that allow:

- K(+) Killer toxin production.
- R(+) Immunity factor production.

If a yeast contains both it is able to produce a killer factor and an immunity factor; it is a killer strain, whilst K(-)/R(+) is a neutral strain and K(-)/R(-) is a sensitive strain. However, the killer factors produced by yeast would appear not to be relevant to winemaking conditions due to the pH of must/wine and the presence of polyphenolic compounds in red wines (Guiterrez et al. 2001).

So, is this really relevant to oenology or is it a marketing tool? Research suggests that killer factors are likely inhibited in wine-like conditions thereby making them less critical factors in selecting a yeast strain.

ABILITY TO PRODUCE VINYL PHENOLS – THE POF CHARACTER

Saccharomyces cerevisiae strains may be characterised as either POF+ (ability to produce vinyl phenols), or POF- (not able to produce vinyl phenols). This means that they can produce vinyl phenols from hydroxycinnamic acids which are naturally present in grapes (Figure 2). This is of concern as some yeasts are able to convert vinyl phenols into ethyl-phenols. Of winemaking concern is the production of 4-ethyl-phenol and 4-ethyl guaiacol by *Brettanomyces bruxellensis* which can be detrimental to wine quality. Other yeast species are able to produces these compounds, but *B. bruxellensis* is very good at it and able to tolerate winemaking conditions that many other species are not able to tolerate.

How important is choosing a POF- or POF+ yeast? In white wine vinyl phenols have a detrimental sensory impact at 750 ug/L (1:1 ratio of 4-vinyl phenol + 4-vinyl guaiacol) (Chatonnet et al. 1993). Choosing a yeast with POF- character becomes even more relevant in white wines when commercial enzyme preparations with cinnamate esterase activity are present, as *Aspergillus* spp produces enzymes that convert cinnamic acids into hydroxycinnamic acids. If these precursors are present, they will also serve as a substrate for ethyl phenol production should *B. bruxellensis* be allowed to grow. But the primary concern in white wines is the detrimental sensory effect of the vinyl phenols which can not only taint but mask varietal aromas (Chatonnet et al. 1993).

In red wines the POF+ character is strongly inhibited by phenolic acids present, making it more critical to white wine production (Chatonnet et al. 1993). In red wine the major risk is the presence of *B. bruxellensis*. Whilst Pichia spp has been known to produce ethyl phenols pre-fermentation (Barata et al. 2006), its spoilage potential is only a fraction of that of *B. bruxellensis*. If this is allowed to proliferate due to low levels of molecular SO_2, the presence of residual glucose/fructose, or lack of sterility in bottle, the taint will likely form. It is unlikely that using a POF+ strain will increase the amount of substrate. Ultimately control of *B. bruxellensis* is key (Malfeito-Ferreira 2018) to preventing the production of ethyl phenols.

S. CEREVISIAE AND SO_2 PRODUCTION AND CONSUMPTION

All strains of *S. cerevisiae* both consume and produce SO_2. How much they consume and produce is strain dependent and also relies on must condition and composition. Some will consume more than they produce whilst others will produce more than they consume. The starting concentration of SO_2 will impact the final amount present at the end of alcoholic fermentation. Work conducted by **BIOLAFFORT®** France, based on standardised fermentation conditions set up by Peltier et al. (2018) demonstrated the maximum and minimum levels of SO_2 post fermentation on five different must, 34 strains each in triplicate (Table 1). It means that the influence of yeast strain alone can alter the levels of SO_2 post fermentation from a 23% decrease in total SO_2 to a maximum of 77% additional TSO_2 from the starting amount of TSO_2 in the must.

Apart from initial SO_2 added in the vineyard or at picking, the main precursor of SO_2 is sulfates (Jiranek et al. 1995). Production of methionine and cystine is regulated by the input of sulfates and output of SO_2. In the presence of amino acids, SO_2 consumed by yeast will go on to form cystine and methionine, important aroma precursors.

Yeasts also produce SO_2 binding compounds, that is compounds that bind to free SO_2, rendering the SO_2 bound as opposed to in the free molecular form. The higher the amount of SO_2 binding compounds present the more SO_2 will be required to achieve a desired molecular SO_2. Low consuming SO_2 strains (which consume less SO_2 than they produce) usually can be correlated to high levels of SO_2 binding compounds (Table 2) (data sourced from **BIOLAFFORT® R&D**).
What does all this mean in terms of yeast selection? Every yeast strain commercially available and spontaneously found in nature will consume SO_2 and produce SO_2. The amount of SO_2 produced will depend on how much is in the must initially, the strain selected and the quantity of sulfates (precursors) present in the must.

	TOTAL SO_2 (mg/L or PPM)		
	Must	Minimum at the end of AF	Maxiumum at the end of AF
Cabernet Sauvignon 2015	35	39	56
Merlot 2014	37	38	53
Merlot 2015	46	39	55
Sauvignon Blanc 2014	34	38	61
Sauvignon Blanc 2015	67	48	85

Table 1. Maximum and minimum levels of total SO_2 using 34 strains in triplicate on 5 musts (derived from Peltier et al 2018).

	RESULTING SO_2 (AND CL35*)	
	Initial total SO_2: 30 ppm	Initial total SO_2: 70 ppm
Low SO_2 consuming strain	+ 39 ppm TSO_2 (150 ppm SO_2 CL35)	+ 45 ppm TSO_2 (181 ppm SO2 CL35)
High SO_2 consuming strain	+ 3 ppm TSO_2 (100 ppm SO_2 CL35)	+ 3 ppm TSO_2 (141 ppm SO_2 CL35)

*CL35 is the amount of SO_2 required to archive 35ppm FSO_2

Table 2. Amount of SO_2 A) Produced and; B) Required to add to achive 35 ppm of FSO_2 (CL35 value) with a low and high SO_2 consuming strain (**BIOLAFFORT** R&D).

YEAST ASSIMILABLE NITROGEN (YAN) DEMAND

When choosing the right yeast strain, often YAN demand is a factor. What does this mean? A yeast strain with a high YAN demand indicates a strain that will produce more biomass (more yeast cells) with a given amount of nitrogen (Figure 3). Conversely a low nitrogen-demanding strain will produce less biomass with the same level of nitrogen. This has downstream implications; if there is a higher number of cells in a ferment, they will likely need more YAN to support them through the fermentation. The two critical points in fermentation for YAN addition is in the first 24 hours of inoculation for biomass production, and a third of the way through ferment when maximum population has been achieved (this is dependent on how much sugar is in the must and the nitrogen demand of the strain) to sustain the population through alcoholic fermentation.

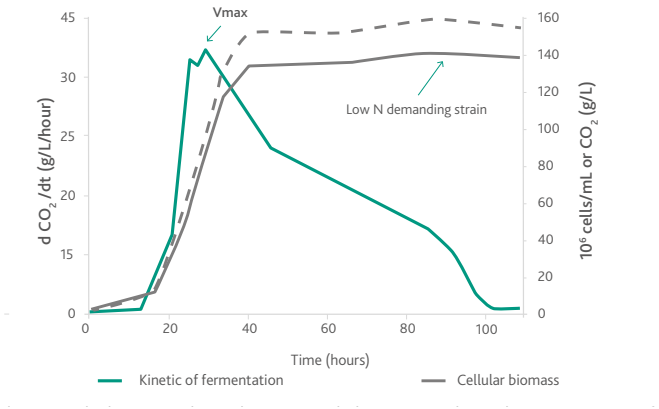

Figure 3. Graph demonstrating the difference between a high nitrogen demanding strain and a low nitrogen demanding strain in terms of cell biomass and fermentation kinetics (personal communication Marina Bely, University of Bordeaux).

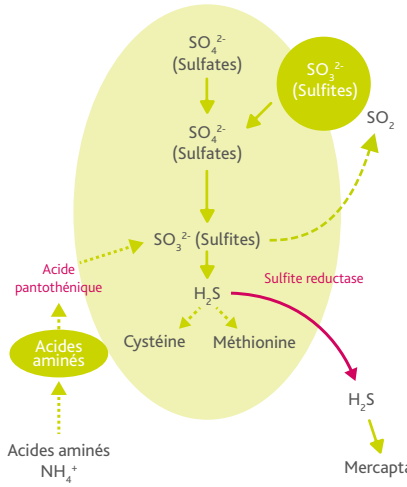

Figure 4. Schematic representation of the SO_2 pathway in and out of the yeast cell.

H_2S PRODUCTION

Hydrogen sulfide (H_2S) production by yeast can not only mask fruit aromas, but its production can hinder the formation of key aroma compounds (Mestres et al. 2000). In the absence of key amino acids, the SO_2 or sulfates taken up by the yeast will be converted to H_2S and released (Figure 4). In the presence of key amino acids, the H_2S formed by yeast can go down the cystine and methionine pathway, important aroma precursors. The timing of and type of YAN supplementation is critical to managing H_2S (Mendes-Ferreira et al. 2010).

AFFINITY FOR FRUCTOSE

In perfect ripening conditions, the ratio of the fermentable sugars glucose and fructose is 1:1. As grapes head towards over-ripeness the ratio can change to favour fructose over glucose (Kliewer 1967, Shiraishi 2000). *Saccharomyces cerevisiae* metabolises glucose more easily than fructose (Guillaume et al. 2007). Because glucose is the preferred sugar by yeast, fructose is often the main sugar left in a stuck or sluggish fermentation. A higher fructose-to-glucose concentration in stuck wines is the consequence and not the cause of a stuck fermentation. The limiting factor is the transportation of sugar into the cell regulated by a gene called HXT3 (Luyten et al. 2002), and in the presence of ethanol it is even harder for yeast to take up fructose (Berthels et al. 2007).

Yeasts that have a better chance of taking up fructose have been identified to have a particular form of the HXT3 transporter that has a higher affinity for fructose (Guillaume et al. 2007). It is linked to an alternative form of the HXT3 gene, encoding for the corresponding transporter. Not all yeast strains have this, hence why it is important to choose a robust strain with both high tolerance to alcohol and affinity towards fructose when dealing with high alcohol and/or stuck fermentations.

But despite some yeast strains containing both forms of HXT3, the yeast will always take up glucose as a preference. However, having the alternative form helps to better assimilate fructose. Within the Laffort range, **ACTIFLORE® BO213** contains two alleles of the HXT3 gene enhancing affinity for fructose and is, in itself, tolerant to 18% v/v alcohol. Other strains do not have this allelic form present at all and would struggle coping with high levels of sugar as well as alcohol.

CONCLUSIONS

It is evident that there is a lot of information to assess before picking a yeast strain for a particular wine.

FOR WHITE WINES:

- Aromatic characteristics determined by enzymatic activities are important in the wine style; of relevance to white winemaking are thiol production, ester production and terpene release.
- Choosing a POF- strain and using an enzyme preparation purified from cinnamate esterase activity to minimise the formation of vinyl phenols before they reach a critical level that has a detrimental sensory impact.
- Alcohol, pH and temperature tolerances should be taken into account.

- Starting SO_2 levels and yeast strain production of SO_2.
- Understanding YAN and correct supplementation to ensure support for biomass production based on starting YAN, potential alcohol and nitrogen requirements of the yeast strain.

FOR RED WINES:

- Aromatic characteristics determined by enzymatic activities are important for wine style.
- Choosing a POF- strain not critical as red wine phenolics inhibit this reaction.
- Use of an enzyme preparation purified from cinnamate esterase activity is key to minimising the amount of precursors available to *B. bruxellensis*; limiting the proliferation of *B. bruxellensis* post alcoholic fermentation will minimise ethyl-phenol production.
- Alcohol, pH and temperature tolerances should be taken into account.
- Starting SO_2 levels and yeast strain production of SO_2. SO_2 binding is even more critical here as there are more SO_2 binding compounds naturally present in red must.
- Understanding YAN and correct supplementation to ensure support for biomass production based on starting YAN, potential alcohol and nitrogen requirements of the yeast strain.

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Q&A

YEAST - *SACCHAROMYCES CEREVISIAE*

Yeasts are responsible for transforming the glucose and fructose in grape juice into wine and creating many of the myriad compounds responsible for flavor, aroma, and texture.

1. Why are there so many different yeast strains?

There are five fundamental characteristics and most fermentation benefits and/or flaws are impacted by one or more of these:

- Alcohol tolerance.
- Optimal temperature range.
- Nitrogen requirements – low, moderate, and high demand.
- Fermentation kinetics – fast, regular, and slow.
- Sensory attributes – the ability to produce mouthfeel and aromatic compounds.

Just as terroir can differentiate the expressions of a grape varietal, the same goes for yeast. *Saccharomyces cerevisiae* strains have mutated and changed their metabolism in response to the diverse environments of grapegrowing. This explains the multitude of strains both in the wild and available commercially.

When a strain of yeast is known to create great wines in a particular area, it is possible to isolate the yeast in a lab and propagate it. This is the source of many terroir-isolate yeasts on the market; **LAFFORT®** has many examples from world-famous cellars in multiple regions such as Bordeaux (**ZYMAFLORE® F15**), Tuscany (**ZYMAFLORE® F83**), and Champagne (**ZYMAFLORE® SPARK**).

There are times when additional characteristics are desired, specificity, higher alcohol, or wider temperature tolerance. This is when crossbreeding is important: a terroir-isolated yeast with great flavor characteristics can be bred with the higher alcohol tolerance from another strain. Look for the "**X**" in **LAFFORT®** yeasts (**ZYMAFLORE® X5, FX10, RX60**, etc.) to find our cross-bred strains.

Whether isolated from cellars from around the world, or crossbred, the **ZYMAFLORE®** range has yeast that express certain qualities to help a wine achieve the goals of the winemaker. The **ACTIFLORE®** range represents workhorse strains that excel at high-volume winemaking to assure fermentation security.

2. Are there any ingredient interactions to avoid when using Saccharomyces cerevisiae?

Only one. Do not add DAP (Diammonium Phosphate) to yeast rehydration water. Ammonia is toxic to yeast cells during rehydration, and the presence of DAP will dramatically affect viability.

3. What happens if I use too little or too much yeast?

Yeast takes time to grow and build up to the levels needed for effective fermentation. Too little yeast allows spoilage microbes to take hold in early stages of fermentation to create off aromas and flavors. Also, if the biomass does not reach sufficient quantity, a cooler fermentation may slow or stop.

SACCHAROMCYES & NON-*SACCHAROMYCES* YEAST APPLICATION.

OBJECTIVE	YEAST	DOSAGE	NOTE
BIOprotection	**ZYMAFLORE ® ÉGIDE**^{TDMP} (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*)	2 - 5 g/hL 20 - 50 ppm	On grapes/must.
Mouthfeel and aromatic development	**ZYMAFLORE ® ALPHA**^{TD} (*Torulaspora delbrueckii*)	30 g/hL 300 ppm	Initiation of cold soak
Primary fermentation	**ZYMAFLORE ®, ACTIFLORE ®** (*Saccharomyces cerevisiae)*	20 - 30 g/hL 200 - 300 ppm	Initiation of alcoholic fermentation

ACTIFLORE® and **ZYMAFLORE®** yeasts work famously together with **SUPERSTART® BLANC** and **SUPERSTART® ROUGE**

Q&A

YEAST - *SACCHAROMYCES CEREVISIAE*

Too much yeast risks excessive biomass production which can spike temperatures early in fermentation, or consume nutrients too quickly. This would provide insufficient nutrition to complete fermentation, causing stuck fermentations.
When it comes to how much yeast inoculum to use, there is a known 'Goldilocks' zone of 150 - 300 ppm, which varies according to potential alcohol. Rates go up to 500 ppm for restarting stuck fermentations.

4. How do I prepare yeast for inoculation?

One of the best ways to ensure a complete fermentation, with clean aroma, and maximum flavor development is to follow a precise yeast preparation protocol. **SUPERSTART® ROUGE** and **SUPERSTART® BLANC** are recommended to strengthen yeast cell walls and improve metabolism, thereby increasing resistance to alcohol, heat, and toxins while improving aromatics and flavors.

- Use a thermometer and start with chlorine-free water (40°C, 104°F), 20 times the weight of the yeast needed.
- Evenly mix in **SUPERSTART® ROUGE** or **SUPERSTART® BLANC** rehydration nutrient at a rate equal to the inoculation dose of yeast.
- When the temperature is at 37°C (99°F) sprinkle yeast over the surface of the water, mix in gently.
- Let stand for 20 minutes.
- Do not add juice or any ammonium-based nutrients.
- Foaming during yeast rehydration varies greatly according to yeast strain, and is NOT indicative of yeast performance.
- Add enough juice from the must to drop the temperature by 10°C/18°F.
- Wait 10 minutes.
- Repeat the juice addition and 10-minute wait intervals until inoculum is within 10°C/18°F of the must.
- Fully homogenize inoculum into the must.
- Total time from yeast rehydration to inoculation should not exceed 45 minutes.

A thermometer is a key tool for working with yeast, not using a thermometer is one of the primary causes of poor yeast implantation.

5. How can I change fermentation kinetics?

Fermentation speed is based on sugar concentration, temperature, yeast strain, nutrition, and yeast dose rate. In general, fermenting either too fast or too slow may produce off flavors/aromas, and may lead to a stuck fermentation.
Higher sugar musts take longer to ferment. Lower temperatures slow fermentation kinetics. All things being equal, a ferment at 60°F will ferment slower than at 70°F. This works to the low temperature limit of the yeast when the biochemical reactions in the yeast cell slow and eventually cease.
The biochemistry of each strain dictates the baseline kinetics and this can vary widely. Knowing the kinetics of a particular strain can be used to improve wine quality. For example, **ZYMAFLORE® X5** has a relatively higher kinetic rating compared to **ZYMAFLORE® VL3** making **ZYMAFLORE® X5** more suitable for lower temperatures.
Nutrition can also influence kinetics. Too much nutrition, like too much yeast inoculum, has potential to produce more biomass and increase fermentaion kinetics.

6. What factors tell me I have a problem with fermentation?

Any signs of slowing fermentation curve, reduction aromas, or off flavors are good indicators of problem ferments. A successful fermentation will have none of these issues Some yeast such as **ZYMAFLORE® FX10** have been bred to be less prone to hostile environmental factors and more reliably finish fermentation cleanly.

7. What is the killer factor?

The Killer phenomenon was thought to play an important role in the balance of the microbial population in winemaking but is now considered of very low impact.
Saccharomyces cerevisiae have 'Killer', 'Sensitive', and 'Neutral' strains. Killer strains secrete a protein toxic to the so-called Sensitive strains. Neutral strains do not secrete the killer protein and are not sensitive to the toxin. It has been also established that a Killer strain may be susceptible to another Killer strain. The best-known toxins are K1 and K2. Toxin K1 is a thermo-sensitive glycoprotein with optimum activity in the pH range of 4.2-4.6, while the similar toxin K2 has a wider pH range of 2.8 to 4.8.
The death of 'Sensitive' yeast is not immediate, but the time varies according to the sensitivity of the strain, environmental conditions, the population ratio of Killer to Sensitive yeast and growth stage of the populations. Yeasts in active growth phase are more susceptible to toxins from the Killer proteins than in the stationary phase.
Physical and chemical factors in the environment can affect the activity of the Killer protein toxin. The thermal stability of the toxin is low (with half-life at 32°C (90°F) of 30 minutes) and its Killer activity is related to temperature. pH also plays an important role, and this in synergy with temperature. At pH below 2.9 there is no longer detected activity. Phenolic compounds from grapes have an inhibiting effect, as do additives or auxiliaries such as bentonite or

YEAST - *SACCHAROMYCES CEREVISIAE*

enological tannins.
In general, a Killer strain implants quickly and a Sensitive strain more slowly. In a situation where a Killer yeast is added to a fermentation with Sensitive yeast, a high percentage of Killer yeast is necessary to eliminate the susceptible population. Spontaneous fermentations are sometimes dominated by Sensitive strains despite significant proportions of Killer strains.
Detailed data from Bordeaux fermentations, as well as industry observations throughout the world, show that Sensitive strains can be properly implanted in the fermentation of wine, despite a strong representation of Killer strains in the indigenous microflora. Indeed, some of the most popular strains of Laffort range (**ZYMAFLORE® VL3, F33, F15**) are 'Sensitive', but for more than twenty years, have never showed a problem of implementation.

8. What is a Bayanus strain?

Saccharomyces bayanus is an old phenotypical characterization of wine yeast that was originally thought to be a distinct species, stronger than *Saccharomyces cerevisiae* and more effective at completing fermentation. The term is still used today but is not genetically correct.
In 1953, Peynault and Domercq, in the work 'Etudes des levures de la Gironde', described of a group of strains often encountered at the end of alcoholic fermentation that were unable to ferment galactose. Due to their presence at completion of fermentation, they were designated as having the best aptitude for fermentation. The name '*Bayanus*' came to represent all strains of yeast that were most efficient at fermentation. After genetic testing became widely available, classically labeled *S. bayanus* strains turned out to be *S. cerevisiae*, with one major difference being the activation of specific gene site, a mutated HXT3 allele, which produces a hexose transport protein. The mutated form (Hxt3p*) is linked with *Saccharomyces* species that are more fructophilic.
Today there is a classified and distinct species of yeast designated *S. bayanus*, which is considered a hybridization of several other yeasts. The true *S. bayanus* is not necessarily fructophilic, and no longer has anything in common with the galactose negative yeasts of the previous era, nor is it used in the wine industry.
The legacy of the old categorization is still evident in the naming of strains like **ACTIFLORE® BO213**, which has excellent fructose metabolization and is a true *S. cerevisiae*.

9. Are dry pitch yeasts as robust as rehydrated yeast?

Active dried wine yeasts require proper rehydration to be fully effective. Loss of viability if dry-pitched means a yeast may not properly implant, struggle to establish, and ultimately lead to loss of quality in the resulting wine. With recent advances in yeast crossbreeding, new strains have been discovered that allow for dry pitching to be done. A **LAFFORT®** technical representative can help determine if a dry pitch yeast is appropriate for any winery.

10. Are there any drawbacks by combining 2 or more different yeast strains at pitch?

Combining different *S. cerevisiae* strains can sometimes create a great outcome. However the greater impact on wine profile will come from whichever yeast had the better implantation in that fermentation, rather than the blend of yeasts.
Variability is generally too high to consistently guarantee reproducibility and thus it is not recommended.

11. How will chitosan react with inoculated yeast?

If used at the maximum legal level of 100 ppm, there is no significant impact on inoculated yeast from Chitosan. There may be a slight reduction in total viable biomass, but not sufficient to influence primary fermentation

Q&A

NON-SACCHAROMYCES YEASTS

Non-*Saccharomyces* strains are multi-purpose tools for winemaking and either offer BIOprotection from microbes that can negatively impact quality and organoleptics, or add complexity and aromatic interest to wines.

1. How do I use non-Saccharomyces with Saccharomyces cerevisiae?

For sensory complexity, use a combination of *Torulaspora delbrueckii* (**ZYMAFLORE® ALPHA**^{TD}) followed by *Saccharomyces cerevisiae*. This is a sequential pitching that closely matches the natural hand-off between organisms in the fermentation process. Use **ZYMAFLORE® ALPHA**^{TD} at 300 ppm, allow fermentation to begin, and then after approximately a 6-9° Brix drop, pitch a **ZYMAFLORE®** yeast of choice. This process is like a 'controlled' native fermentation with lower risk, high mouthfeel, and aromatic complexity.

For BIOprotection on incoming fruit, **ZYMAFLORE® ÉGIDE**^{TDMP} combines *T. delbrueckii and Metschnikowia pulcherrima.* They are perfectly suited to implant quickly, out-compete spoilage organisms, and not start fermentation or uptake nutrients depending on time and temperature of the must. Extremely effective, this product works well when dry pitched on cold fruit and at low doses of only 20 - 50 ppm.

2. What are the main differences between Torulaspora delbrueckii & Metschnikowia pulcherrima?

M. pulcherrima implants favorably as a dry pitch and at very cold temperatures down to near freezing. This allows it to dominate immediately on incoming fruit. *T. delbrueckii* prefers slightly warmer temperatures and rehydration. It will still implant dry, albeit with a little lag time. This allows *T. delbrueckii* to take over right where *M. pulcherrima* leaves off. *T. dulbreuckii* has a greater contribution to aromatic complexity and mouthfeel.

*3. Do **ZYMAFLORE® ÉGIDE**^{TDMP}, **ZYMAFLORE® ALPHA**^{TD} or **ZYMAFLORE® KHIO**^{MP} require rehydration?*

ZYMAFLORE® ALPHA^{TD} requires rehydration at 25-30°C/77 - 86°F. **ZYMAFLORE® ÉGIDE**^{TDMP} and **ZYMAFLORE® KHIO**^{MP} can be simply added dry to incoming fruit or into receiving vessel, or rehydrated with the same protocol as **ZYMAFLORE® ALPHA**^{TD}

*4. Why does **ZYMAFLORE® ÉGIDE**^{TDMP} show little use of YAN and not ferment, while **ZYMAFLORE® ALPHA**^{TD} will utilize YAN and ferment up to 8%?*

ZYMAFLORE® ÉGIDE^{TDMP} has a dose rate only 1/10th of normal yeast, allowing for enough population to out-compete negative organisms at cold temperatures but not kick off fermentation. **ZYMAFLORE® ALPHA**^{TD} is pitched at normal rates to allow for a population large enough that fermentation does begin. When using **ZYMAFLORE® ALPHA**^{TD} for building mouthfeel and aromatics, add an additional 50 ppm YAN to account for the growth of the *Torulaspora delbreuckii.*

5. What does Lachancea thermotolerans do?

L. thermotolerans is a new yeast that can reduce alcohol and increase acidity, great for high brix and ultra-ripe fruit. Ask your **LAFFORT®** Technical Representative for more information.

LAFFORT® YEAST RANGE

	Grape variety	Yeast	*Alcohol Resistance (% v/v)	**Nitrogen Requirements	Optimal Fermentation Temperature °F	Fermentation Kinetics	Sensory Impact
ALL TYPES OF WINES	Whites and reds for complex aromatic profile and increased mouthfeel.	ALPHA	8 -10%	Medium	50 - 79	Slow	Varietal Volume
RED WINE	Cabernet, Merlot, Pinot Noir, Malbec...	F15	16%	Medium	68 - 90	Rapid	Fruit Volume
	Grenache, Syrah, Barbera, Sangiovese...	F83	16.5%	Medium	68 - 86	Regular	Fruit Volume
	Cabernet, Cabernet Franc, Merlot, Tempranillo...	FX10	16%	Low	68 - 95	Regular	Neutral Volume
	Pinot Noir, Merlot, Gamay...	RB2	15%	Low	68 - 90	Regular	Varietal
	Zinfandel, Petite Sirah, Syrah...	RX60	16.5%	High	68 - 86	Regular	Varietal
	Pinot Noir, Syrah, Zinfandel, Tempranillo...	XPURE	16%	Medium	59 - 86	Regular	Fruit Volume
WHITE & ROSÉ WINE	Chardonnay	CX9 / CH9	16%	Low/ Medium	57 - 72	Regular	Varietal Volume
	Grenache Blanc, Riesling, Pinot Gris, Viognier...	DELTA	14.5%	High	57 - 72	Regular	Varietal Thiols
	All sparkling base wines. Tirage bottling.	SPARK	17%	Low	50 - 90	Rapid	Neutral
	Late Harvest, Semillon, Riesling...	ST	15%	High	57 - 68	Regular	Varietal
	Chardonnay, Riesling, Gewurztraminer, Muscat...	VL1	14.5%	High	61 - 68	Regular	Varietal
	Chardonnay, Viognier, Roussanne...	VL2	15.5%	Medium	57 - 68	Regular	Varietal Volume
	Sauvignon Blanc, Vermentino, Gewurztraminer Pinot Gris, Rosé...	VL3	14.5%	High	59 - 70	Regular	Varietal Volume
	Sauvignon Blanc, Albarino, Rosé...	X5	16%	High	55 - 68	Rapid	Varietal Thiols
	Chardonnay, Viognier Pinot Gris, Chenin Blanc, Rosé...	X16	16.5%	Medium	54 - 64	Rapid	Esters
ORGANIC	Organic	011 BIO	16%	Low	57 - 79	Rapid	Neutral

ZYMAFLORE® EGIDE™ and ZYMAFLORE® KHIO™ are not included in this table due to the fact that they are bioprotective agents.

YEAST STORAGE AND PREPARATION

PRODUCT	STORAGE TEMPERATURE AND PLACE	SHELF LIFE UNOPENED AND OPENED	PREPARATION	SPECIAL CONSIDERATIONS
ZYMAFLORE®, ACTIFLORE® (*Saccharomyces cerevisiae*)	Dry area, moderate temperature.	4 years from production date, activity loss is at least 5% per month after opening.	Sprinkle on 10 x volume of chlorine-free water at 37°C (99°F). If using rehydration product, use 20 x volume of water at 40°C (104°F). Wait 15 mins then step down temperature with juice additions until within 10°C (18°F) of must.	Do not exceed 40°C (104°F) water temperature
ZYMAFLORE® ALPHA^{TD} (*Torulaspora delbrueckii*)	Dry area, cool temperature 2 - 10°C (36 - 50°F)..	30 months from production date, do not use open packaging.	Sprinkle on 10 x volume of chlorine-free water at 26-30°C (77-86°F). Wait 15 mins then step down temperature with juice additions until within 10°C (18°F) of must.	Do not exceed 30°C (86°F) water temperature
ZYMAFLORE® ÉGIDE^{TDMP} (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*)	Dry area, cool temperature 2 - 10°C (36 - 50°F)..	2 years from production date, do not use open packaging.	Apply dry or, if rehydrating, use the protocol for **ZYMAFLORE® ALPHA**^{TD}.	Keep must temperature below 8°C (46°F) for best results

INNOVATIVE USE OF NON-*SACCHAROMYCES* IN BIOPROTECTION

T. delbrueckii and *M. pulcherrima* Applied to a Machine Harvester.

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SUMMARY

GOALS:
This study addresses the increasing demand for "natural" and certified organic wines, along with the need for improved worker safety. Winemakers continue to search for alternatives to SO_2 as an antioxidant and antimicrobial agent. This study compares the use of blended non-*Saccharomyces cerevisiae* yeasts - *Torulaspora delbrueckii* (Td) and *Metschnikowia pulcherrima* (Mp) - as antimicrobial agents to a standard addition of SO_2 on Cabernet Sauvignon. This fruit possesses over 10 times the normal microbial flora typically found in California. In conjunction with this comparison study, a proof of concept pro-totype illustrates the use of a novel spray method for the application of these non-*Saccharomyces* yeasts onto a grape machine harvester for BIOprotection.

KEY FINDINGS:

Research Winery:

- Overall, the blended yeasts performed better than a standard addition of SO_2 at controlling wine spoilage organisms on compromised fruit.
- Organisms related to wine spoilage responded differently to *Td/Mp* than to SO_2. The *Td/Mp* treatment exhibited an advantage over the SO_2 treatment. The *Td/Mp* treatment appeared to work best against Zygo*saccharomyces*, *Lactobacillus kunkeei*, *Hanseniaspora uvarum*, and acetic acid bacteria. It was less effective against *Pediococcus* and other *Lactobacillus* species.
- Different stages of the trial fermentations were affected differently by *Td/Mp* and SO_2. The *Td/Mp* populations performed best during prefermentation and the early stages of fermentation.
- *Td/Mp* showed an antagonistic effect on microorganisms responsible for wine spoilage. There were fewer microorganisms related to spoilage growing in the three bioreactors with non-*Saccharomyces* species than in the bioreactors acting as experimental controls with 60 mg/L SO_2 added during processing.
- *Td/Mp* treatment increased the implantation capacity of *S.cerevisiae* compared to the use of SO_2. Using identical inoculation rates of *S.cerevisiae*, we found more *S.cerevisiae* cells growing in the *Td/Mp* bioreactors than in the bioreactors treated with SO_2. Furthermore, we observed greater population reduction and fewer cells/mL of *S.cerevisiae* at the end of fermentation.

Field Trial:

- A reduction in spoilage microorganisms occurred when using *Td/Mp* directly applied to the harvester.
- Applying *Td/Mp* yeasts to the grape harvester reduced aromas related to volatile acidity coming from the machine.

Impact and Significance:
The use of *Td/Mp* yeasts provides an alternative to SO_2 for controlling the growth of organisms related to wine spoilage. Incorporating these yeasts as a bioprotectant by applying them during the harvest and transport processes reduces the risk of detrimental microbial organisms in the harvested fruit, juice, and wine.

Key words:
Metschnikowia pulcherrima, microbial populations, *Torulaspora delbrueckii*, vineyard management, yeast ecology.

OVERVIEW

Winemakers continue to search for alternatives to SO_2 in winemaking. Many producers seek to reduce SO_2 to take advantage of market opportunities in the "natural" wine movement by following organic certification guidelines, to improve worker safety, and to utilize natural products known to have similar efficacy to synthetic products for targeted applications. Winemakers currently use SO_2 to control microbial growth. However, it is also a powerful antioxidant and inhibits browning reactions. Because the properties of SO_2 allow many different wine-making applications,1 reducing or completely removing SO_2 from winemaking requires an investigation of each processing step, beginning with grape harvesting and transport.

BIOprotection is a relatively new term and emerging concept in several food industries[2,3,4].In this study, the term refers to a natural agent that controls the growth of unwanted organisms through ecological processes such as competition. This study examines the efficacy of a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* (*Td/Mp*, 1:1 ratio by cell count) as a BIOprotection agent. This mixed culture is a commercial product currently used by winemakers. The nature and impact of these yeast species on winemaking is a current area of research at universities and developmental laboratories worldwide[2,5,6].

This study investigates *T. delbrueckii* as a co-inoculum for the BIOprotection of grapes and juices. Some data suggest that the effectiveness of *T. delbrueckii* as a bioprotectant depends on the matrix.21 Another study illustrates that *T. delbrueckii* populations show much less severe losses in viability during the early stages of fermentation than other non-*Saccharomyces* species, making it an ideal choice as a competitive species for inoculation during prefermentation[7,8].

In addition to its use for BIOprotection, *T. delbrueckii* is known to enhance the aromatics of wine produced in a cofermentation with *Saccharomyces cerevisiae* and is widely used in industry for this purpose [9].Evidence from the University of Bordeaux found that co-inoculation with *T. delbrueckii* and *S.cerevisiae* produced 54% less volatile acidity and 60% less acetaldehyde than inoculation with *S.cerevisiae* alone[10]. Other studies show a positive sensory impact of cofermentation in both sequential and simultaneous mixed cultures of *T. delbrueckii* and *S.cerevisiae*, most notably an increase in fruity aroma linked to specific esters and tropical aroma profiles releasing volatile thiols (3SH and 4MSP)[9,11].

T. delbrueckii is a well-documented yeast and contributes to mouthfeel sensations along with many other non-*Saccharomyces* species[10,11,12,13,14]. Many of the mechanisms for increased mouthfeel result from an increase in mannoprotein content from the cell[13]. *T. delbrueckii* is also known to metabolize sugar to produce alternative compounds such as glycerol or pyruvic acid via the Crabtree effect[15]. An additional study reveals an impact regarding the modulation of astringency resulting from *T. delbrueckii* fermentation.

The use of *M. pulcherrima* as a biological control agent is possible thanks to its ability to produce the natural antimicrobial compound pulcherrimin. This compound is an insoluble red pigment with antifungal activity[6]. Pulcherrimin has been shown to deplete iron in growth media, which in a fermentation could result in inhibition of organisms requiring iron for growth.[17] This mechanism of iron depletion occurs via the precipitation of iron(III) ions caused by an interaction with pulcherriminic acid, a precursor of pulcherrimin secreted by *M. pulcherrima*[6]. Several microorganisms exhibit inhibitory effects from pulcherrimin, including *Candida tropicalis*, *Candida albicans*, *Brettanomyces/Dekkera*, *Hanseniaspora*, *Pichia*, and *Botrytis cinerea*. *S.cerevisiae* appears unaffected by this antimicrobial activity.[3] In addition, some strains of *M. pulcherrima* produce a killer factor to suppress growth of killer-sensitive organisms. *M. pulcherrima* is also described as a biofungicide capable of reducing *B. cinerea* on postharvest fruits via nutrient competition[18].

Uninoculated non-*S.cerevisiae* yeasts, often called "wild" yeasts, are commonly associated with the production of ethyl acetate and negative sensory characteristics[14]. However, mixed cultures of *T. delbrueckii* and other yeasts are known to produce positive aromas without the negatively associated sensory attributes.[14] *M. pulcherrima* is known to produce high concentrations of esters[19,20,21,22,23] and also possesses ß-glucosidase activity, which catalyzes the release of varietal aromas.[5] An additional study illustrates that co-inoculations of *M. pulcherrima* with *S.cerevisiae* reduce the total amount of acetic acid in the final wine.[4] Wines inoculated initially with *S.cerevisiae* and *M. pulcherrima* show contributions of 2-phenylethanol and several esters in the final product[12].

In this study, we validated the bioprotective nature of the mixed cultures at the University of California Davis Research Winery. The commercially available BIOprotection product is compared to a standard addition of SO_2 on fruit possessing a high number of microbial organisms related to wine spoilage.

In addition, we built a prototype blaster as a novel method for applying the dry mixed culture onto a mechanical grape harvester in the vineyard. The aim was to encourage the growth of specific inoculated yeast rather than oxidative spoilage organisms, after a winemaker complained that the harvester "always smells like VA (volatile acidity, typically acetic acid and ethyl acetate) during harvest." We quantified the impact of BIOprotection in both trials by evaluating the microbiota of fermentation using qPCR Scorpions from ETS Laboratories in Saint Helena, CA and performed a statistical analysis of the resulting ecological population data sets.

MAJOR OBSERVATIONS & INTERPRETATIONS

The *Td/Mp* inoculation was better than the addition of SO_2 at controlling organisms related to wine spoilage from highly compromised fruit. The acetic acid bacteria level found in the fruit was 10 times the average value measured by ETS Laboratories during the 2018 harvest based on microbiota evaluation using qPCR from ETS. Physical examination of the fruit revealed excessive damage resulting in macerated grapes and grape skin wounds consistent with bird damage. Experienced winemakers noted aromas related to a compromised crop and significant insect populations on the fruit.

Sampling occurred on days 1, 4, 6, 8, and 15. We decided before the experiment that we would seek greater resolution of data points during the prefermentation and early fermentation stages. Thus, the majority of sampling occurred during the early stages of fermentation. Furthermore, we had to sample on days when the research winery was open and took extra care not to interfere with student and faculty experimentation.

Our exploratory approach to the data included examining the raw data, searching for outliers, transformations of the data, descriptive statistics, and robust statistical analysis. We seek to provide a narrative for the data while presenting our findings in simple terms. To prove the statistical significance of our work, we also provide some information on our statistical analysis and results.

For the sake of simplicity, we include the data as a raw representation of the sum of cells detected (Figure 1). Note that the cell counts are the sum of all the measurements taken for each organism. We also include the organismal response to the treatments in this figure. In this way, the raw data is presented for each spoilage organism, as well as for the overall number of spoilage organisms. Notice some organisms contribute more to the total amount of organisms than others. This provides the first glimpse of what is known as an outlier.

If we divide the data into subsets, we can see how the phases of fermentation influence the growth of the organisms. The protective *Td/Mp* populations only survive the initial stage of fermentation (Figure 2). Once the alcohol of the system reaches between 3 and 7%, they die. The same is true for some of the spoilage organisms, which possess various levels of ethanol tolerance. In the case of the controlled bioreactors, after day 8, many of the microorganisms related to wine spoilage had died. Using the *Td/Mp* populations as a protective factor against unwanted organisms worked better than SO_2 during the earliest stages of fermentation.

In Figure 1, we split the data into subgroups labelled A, B, and C. These subgroups represent different levels of contribution to the overall total number of spoilage organisms. One population contributed more than any of the other populations. In statistical terms, we call this population an outlier in subgroup A. Interestingly, the addition of

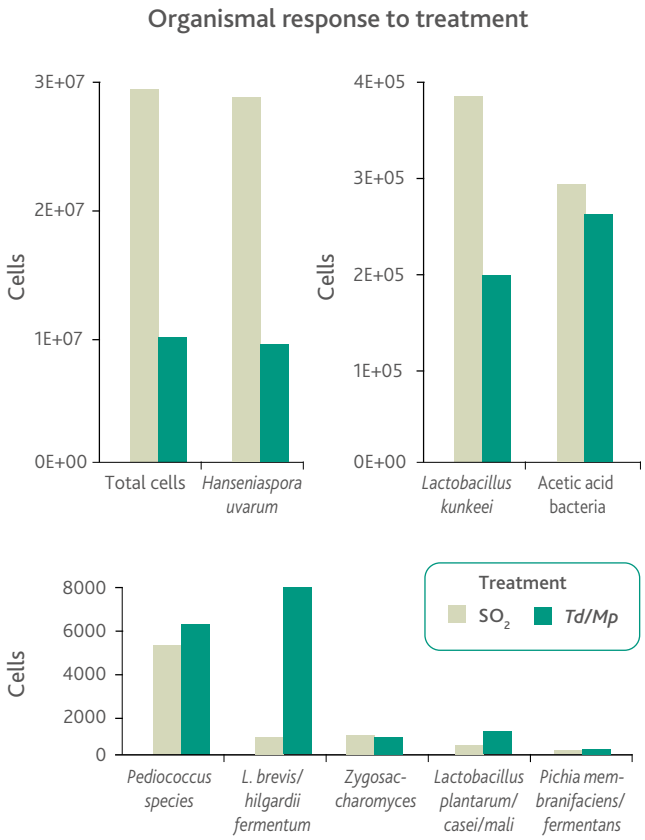

Figure 1. Overall, the addition of Td/Mp (a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) performed better than a standard addition of SO_2 at controlling wine spoilage organisms in the research winery. Note that the cell counts are the sum of all the measurements taken.

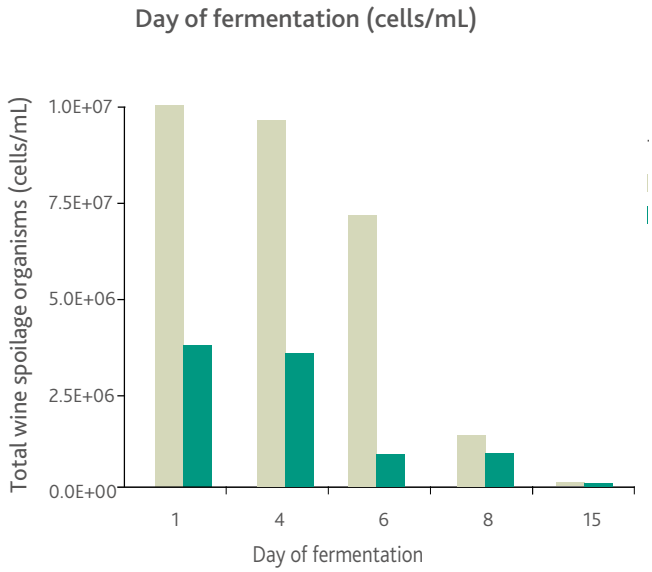

Figure 2. Different stages of the laboratory fermentation are affected differently using Td/Mp (a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) versus SO_2. This graph depicts the sum of all the microorganisms listed by day.

SO_2 resulted in a marked increase in populations of *Hanseniaspora uvarum* compared to *Td/Mp* treatment. *H. uvarum* dominated the control fermentation, reaching ~10 million cells/mL during the initial stages of fermentation. In comparison, *Td/Mp* treatment resulted in much less *H. uvarum*, with cell counts reaching 4 million cells/mL. *H. uvarum* cells appeared to be uninhibited by the addition of SO_2. A record of each organismal population response is available in Table 1. Finding outliers is often done with a Monte Carlo simulation. As we explored the data, we took note of the outlier and instead of focusing on it—contemplating its removal or reason of existence—we simply included it as part of the fermentative system of organisms and moved to a transformative approach to data mining.

TREATMENT	ORGANISM	TOTAL CELLS COUNTED DURING FERMENTATION
SO_2	*Acetic Acid Bacteria*	305,040
Td/Mp	*Acetic Acid Bacteria*	260,620
SO_2	*Hanseniaspora uvarum*	28,309,780
Td/Mp	*H. uvarum*	9,387,320
SO_2	*Lactobacillus brevis/hilgardii/ fermentum*	1101
Td/Mp	*L. brevis/hilgardii/fermentum*	7763
SO_2	*Lactobacillus kunkeei*	388,900
Td/Mp	*L. kunkeei*	214,650
SO_2	*Lactobacillus plantarum/casei/mali*	571
Td/Mp	*L. plantarum/casei/mali*	1931
SO_2	*Pediococcus*	5450
Td/Mp	*Pediococcus*	6290
SO_2	*Pichia membranfaciens*	292
Td/Mp	*P. membranfaciens*	483
SO_2	*Zygosaccharomyces*	1451
Td/Mp	*Zygosaccharomyces*	1291

Table 1. Total cells for each tested organism. Td/Mp, a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*.

Transformations are a key part of data analysis, in much the same way as outlier detection algorithms. Transforming the data allows us to change the dimensionality of the data and examine the data set in more detail. A common technique for understanding the sanitary effects of agents in microbiology is known as log-kill. The FDA uses guidelines based on log-kill or log-change to determine the efficacy of sanitation and sterilization procedures. Transforming the data into log-change allows us to understand the relative rate of growth and death of an organism.

It is essential for the protective effect of the *Td/Mp* not to interfere with the ability of *S.cerevisiae* to complete the alcoholic fermentation. We examined the *S.cerevisiae* populations during the different stages of fermentation and calculated the log-change throughout the fermentation. In doing so, we examined the function of *S. cerevisiae* in terms of biomass development, reproductive rates, and cell death rates. Using log-change, we were able to describe changes in the dynamic system that provide better insight into rate changes than do cell counts alone. We found that *Td/Mp* treatment resulted in better implantation for *S.cerevisiae* compared to SO_2 treatment (Figure 3). Better implantation indicates more substantial rates of growth and more biomass.

We also noted more pronounced cell death in the late stages of fermentation. We suspect that the redox potential of the system may also be affected by the presence of *Td/Mp* populations and intend to study this further in future collaborative endeavors with our research partners. Furthermore, the rapid die-off of the *S. cerevisiae* may inhibit its ability to contribute off-aromas and flavors in the middle and late stages of fermentation.

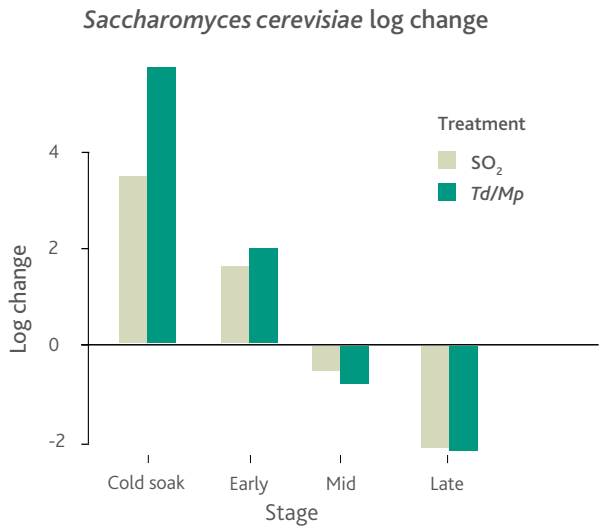

Figure 3. The *Saccharomyces cerevisiae* with Td/Mp (blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) showed better implantation compared to an identical inoculation with SO_2. This graph illustrates the growth (positive values) and death (negative vales) of S. cerevisiae as the logarithmic change between the stages of fermentation.

After exploring the microbiota data with descriptive statistics, searching for outliers, determining the data distribution, and performing various transformations, we sought to determine whether the resulting data from our study is conclusive. To do this, we needed to define statistical significance. We set out to answer the question: "Can we illustrate a significant advantage of using a group of non-*Saccharomyces* yeasts compared to a standard addition of SO_2?"

We had already performed an exploratory dive into the data by examining our assumptions from analysis of variance (ANOVA) Type II, including random sampling, normal distribution, and balanced experimental design. This data is not shown but is provided through a link to Github in the final section of this report. We made a log transformation (*S. cerevisiae*, mentioned above) and examined outliers of the data. We also used several data mining methods commonly found in geometric data analysis or data science. However, due to the scope of this report, we do not discuss the details of the data mining approach in more depth. Instead, we simply provide raw data numbers and validate our results using statistics.

Once significance was determined with ANOVA Type II in the research winery, we performed an additional proof of concept study at a winery where the winemaker had complained: "My harvester smells like VA!" Knowing that the machine harvester might be serving as a vector for spoilage organisms in the vineyard, we constructed an airpowered applicator named Yeast Blaster Prototype One (Figure 4). Yeast Blasters, blaster components, and build-your-own blaster plans are available from LAFFORT® USA in Petaluma, CA. The field trial consisted of daily applications of *Td/Mp* on the harvester at the end of a washdown procedure (Table 2). This trial illustrated a proof of concept, but we need to perform significance testing on the application, including multiple replicates with several different harvesters and iterations of a field trial.

According to the winemaker, application of *Td/Mp* to the grape harvester reduced the smell of VA coming from the machine. We also noted decreases in cells per mL in fruit coming from the harvester (Table 2). However, field trials have many more variables than do validations in a research winery. The second and third weeks of the trial had average low temperatures nearly 2°F colder than those during the first week, which may have affected the results. This preliminary study provides anecdotal evidence as a reason for a continuation of our research with multiple harvesters.

HARVESTER APPLICATION TRIAL (cells/mL)	PRE-TREATMENT Week 1	PRE-TREATMENT Week 2	PRE-TREATMENT Week 3
Bacteria			
Acetic acid bacteria	22,000	7800	4600
Oenococcus oeni	520	700	240
Lactobacillus brevis, L. hilgardii, L. fermentum	40	50	300
Lactobacillus plantarum, L. casei, L. mali	4800	1300	720
Lactobacillus kunkeei	40	<40	230
Pediococcus species	290	120	230
Yeasts			
Hanseniaspora uvarum	26,800	7100	10,900
Zygosaccharomyces species	40	50	50
Pichia membranifaciens, P. fermentans	230	100	0
Brettanomyces bruxellensis	<40	<40	<40
Yeasts and bacteria			
Sum of bacteria	27,690	9970	6320
Sum of yeasts	27,070	7250	10,950
Sum of yeasts and bacteria	54,760	17,220	17,270

Table 2: Bacteria and yeast populations on machine harvester as measured in the first fruit harvested at the commencement of shift every week for three weeks, with the first analysis (Week 1) done before *Td/Mp* (a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) application.

BROADER IMPACT

This study validated product claims that using *Td/Mp* as a bioprotectant can reduce the need for SO_2 in winemaking. The microbiota investigation used qPCR data to quantify a greater reduction of spoilage-related organisms in wine from grapes treated with *Td/Mp* versus grapes treated with SO_2. Specific organismal responses to treatment are found in Figure 1. The study also tested a novel dry application of *Td/Mp* with the Yeast Prototype Blaster One (Figure 4). Early stages of product validation in vineyard trials showed promise in North America. The use of *Td/Mp* to reduce the amount of SO_2 needed during winemaking is thus a validated process that will continue to gain traction as more wineries and vineyards seek out new agricultural processes related to BIOprotection.

This study examined the role of *non-Saccharomyces* yeasts as a bioprotectant but only tested the responses of 10 organisms. Published peer-reviewed data indicate that the process may also work for *B. cinera*.[17] In addition, several winemakers have inquired about the use of *Td/Mp* against powdery mildew. We are currently considering developments into vineyard applications (Figure 5). A continuation of this study would link these topics in a future experiment. The use of *Td/Mp* as a bioprotectant in the vineyard during harvest provides the earliest protection against microbial spoilage.

EXPERIMENTAL DESIGN

Prefermentation.

In this portion of the trial, we tested the efficacy of a mixed inoculation of *Td* and *Mp* packed in a 1:1 ratio by cell count. The trial used Cabernet Sauvignon fruit possessing a high microbial load in an experiment involving a control and two replicates. We used ~1 ton of Cabernet Sauvignon, donated from Elk Grove AVA and processed at the University of California, Davis. Harvest occurred on the night of 22 Sept 2019. The grapes received a 30 mg/L addition of SO_2 during harvest and were then incubated in a chilled room for three days before fruit processing.

The harvested Cabernet Sauvignon fruit was then incrementally transferred to six bioreactors. Each fermenter possessed 132.5 L (35 gallons) of crushed Cabernet Sauvignon after filling. The group of six bioreactors included two sets, one for control and one for the mixed bioprotectant culture. The control tanks received a 30 mg/L SO_2 addition commonly found in a practical winery setting with compromised fruit. Thus, the control tanks received a total of 60 mg/L SO_2. The mixed-culture tanks received no additional sulfur. Instead, they received 50 g/hL of *T. delbrueckii* and *M. pulcherrima* in a 1:1 ratio by cell count. All tanks were set to 56°F for a three-day cold soak. The experimental cellar team adjusted the yeast assimilable nitrogen to 250 mg/L and the titratable acidity to 6.0 g/L. We ran a complete juice panel and SCORPIONS for Yeast and Bacteria at ETS Laboratories. Sampling included equal amounts from each of the bioreactors. The initial juice chemistry is given in Table 3.

ANALYSIS	SO_2	*TD/MP*
Titratable acidity	3.4 g/L	3.5 g/L
pH	3.67	3.63
Volatile acidity (acetic)	0.07 g/L	0.09 g/L
L-malic	0.71 g/L	0.73 g/L
Brix	23.9 degrees	23.7 degrees
Glucose + fructose	249 g/L	242 g/L
Yeast assimilable nitrogen	129 mg/L (as N)	127 mg/L (as N)

Table 3. Initial juice chemistry from bioreactor fermentations at UC Davis Research Winery. Td/Mp, a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*.

Primary fermentation:

The temperature of the jackets was raised to ~65°F at the end of 27 Sept 2019, after the three-day cold soak. The control bioreactors were inoculated on 28 Sept 2019 with a strain of *S. cerevisiae* (ZYMAFLORE® FX10). The *Td/Mp* trial fermenters were inoculated with *S. cerevisiae* on 30 Sept 2019 after a near 3°F drop in Brix. Both inoculations included 20 g/hL of rehydration nutrient (Laffort SUPERSTART ROUGE). The tank jacket temperature setting corresponded to 75°F at inoculation.

Postfermentation:

After alcoholic fermentation was complete, the individual bioreactors had their contents drained and combined into two stainless steel drums separated as trial and control. An addition of *Oenococcus oeni* ensured that malolactic fermentation took place (LACTOENOS® SB3 DIRECT). The research winery laboratory

concluded postfermentation with a verification of complete malic to lactic acid conversion and the addition of 50 mg/L SO_2. The final wine chemistry is listed in Table 4.

Preliminary prototype application:

Following the development of dosing protocols illustrating that the blaster can perform at a safe distance of around 5.2 m (17 feet) and inoculate a harvester in about 5 min, this preliminary field study investigated the application of *Td/Mp* as a dry product onto a wet surface (Figure 6). We were only given access to one harvester for this trial. In this portion of the study, the harvester followed standard operating procedures in the vineyard, followed by spraying of the dry *Td/Mp* onto all surfaces that contact fruit daily. Weekly samples were acquired from the first fruit harvested by the machine at the commencement of a shift, and qPCR was performed for microbial determination and quantification. The first application of *Td/Mp* was performed once the machine possessed cells responsible for wine spoilage at a concentration of 1×10^4 cells/mL. The incorporation of *Td/Mp* into the standard sanitation protocol was measured using qPCR. There are no replicate data for this experiment, as it is anecdotal and preliminary.

Standard operating procedure:

- Run the harvester throughout the night, then wash the harvester down with water at ~0600 hr after finishing.
- The next evening, ~0000 hr, resume harvesting operations after wet down.

Sampling procedure:

- Take samples from the juice and berries of the first three bins picked for the evening once they arrive on the crush pad. Send samples to ETS Laboratories for qPCR.
- Once samples have a verifiable population of 10^4, commence with the experimental operating procedure.

Experimental operating Pprocedure:

Apply *Td/Mp* as a dry product using the blaster prototype and product coverage calculations and protocol. Using the blaster prototype, spray the harvester with the *Td/Mp* product after finishing the standard operating procedure.

ANALYSIS	SO_2	*TD/MP*
Free SO_2	< 2 mg/L	< 2 mg/L
Volatile acidity (acetic)	0.50 g/L	0.50 g/L
L-malic acid	< 0.05 g/L	< 0.05 g/L
Glucose + fructose	< 0.1 g/L	0.1 g/L
Ethanol at 20°C	14.13% vol	14.05% vol
Ethanol at 60°F	14.08% vol	14.00% vol

Table 3. Initial juice chemistry from bioreactor fermentations at UC Davis Research Winery. Td/Mp, a blend of *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*.

Data analysis:

We received our DNA reports from ETS Laboratories. Once we transcribed the data to Microsoft Excel spreadsheets, we imported the data into the R programming environment (R Studio version 1.2.1335 Build 1379). The dependent libraries used to write and construct the models include dplyr, tidyselect, ggplot2, lsmeans, grid, rcompanion, and others. All the code generated during our data-mining expedition is listed as part of an open-source group of projects available for learning and free distribution among those interested in applied wine data science. The code used for this analysis, our statistical findings, and the data set for the project can be found at https://github.com/dandeelyon/NonSac.

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AVOIDING STUCK FERMENTATIONS

Just in time for vintage.Outline some of the key contributing factors in stuck fermentations and how to avoid them.
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Stuck and sluggish fermentations can cause significant economic losses for a winery due to the extended labour requirements and the purchase of additional yeast and nutrients required to restart them. Wine quality is often significantly impacted causing additional financial loss. Extended periods of time with residual sugar and lack of SO2 protection increase the risk of microbial spoilage primarily due to Acetobacter spp and Brettanomyces bruxellensis. The intention of this article is to examine some of the key contributing factors to stuck fermentations and how best to avoid them.

STAGE 1: MUST

Many of the contributing factors to stuck fermentations are found in must before yeast is inoculated. But often stuck and sluggish fermentations are an adaptive process with a number of factors culminating in the arrest of fermentation.

NITROGEN

The portion of nitrogen relevant to fermentation is yeast assimilable nitrogen (YAN) content, which is the nitrogen able to be taken up by *Saccharomyces cerevisiae*. Its starting level is generally vineyard dependant (Boulton et al. 1999). YAN can be found in two forms: mineral or organic. Mineral nitrogen is made up of ammonia (NH_4), and can be added as di-ammonium phosphate in Australia.

Organic nitrogen is made up of free amino acids with the exception of proline and can be added through autolysed yeast. In grapes, mineral nitrogen makes up to one third of the total nitrogen, whilst the organic fraction makes up two thirds to three quarters grape derived of YAN (Ribereau-Gayon 2006). At the beginning of fermentation *Saccharomyces cerevisiae* utilise YAN to build cell biomass corresponding to the amount of sugar present in the must (Table 1). In cases of YAN deficiency, yeast are not able to build to sufficient populations and the fermentation will likely be slower as there are less cells fermenting in the must (Bisson et al. 2005). The level of biomass is also impacted by whether the strain has a high (more biomass produced) or low nitrogen requirement (less biomass produced) (Figure 1).

WATER ADDITIONS, STRESS CONDITIONS, AND JUICE CHEMISTRY

Water additions to grapes at the crusher were legalised for the 2017 Australian vintage, enabling the addition of water to adjust Baume down to 13.5. But a starting Baume of 17 and a resulting Baume of 13.5 can lead to a significant amount of dilution of not only sugar but vitamins, minerals, lipids and amino acids.

Juice chemistry including sugar level, SO_2, pH and volatile acidity may impact alcoholic fermentation. A very high sugar level can cause osmotic stress at the beginning of fermentation and lead to high levels of ethanol at the end of fermentation. *Saccharomyces cerevisiae* are sensitive to SO_2 , ethanol >10% as well as very low pH and volatile acidity above 0.8 g/L of acetic acid. The levels of sensitivity are strain specific.

Vine health aside, heat stress can significantly impact juice chemistry. Baeza et al. (2019) found that there was a positive correlation between sugar content and available water, but also phenolic compound production, mainly in the form of anthocyanins (Downey et al. 2006). Several authors have reported links to increased levels of key aroma compounds such as norisoprenoids, carotenoids and monoterpenes (Reynolds and Wardle 1989, Belancic et al. 1997). Other changes relative to grape maturation are the degradation of malic acid and the accumulation of tartaric acid. Whilst tartaric acid is not affected by heat stress, malic acid above 46°C (115°F) is degraded (Lakso and Kliewer 1975, Drappier et al. 2017). High concentrations of phenolic compounds are inhibitory towards yeast (Pastorkova et al. 2013).
Mould-affected grapes may have toxins, pathogenesis-related enzymes and other toxic compounds produced by the grapes

STRATEGIES TO OVERCOME STUCK FERMENTATION: STAGE 1 MUST

1.UNDERSTANDING STARTING LEVELS OF NUTRITION

Measuring the level of YAN is a simple method to assess the nutrition status of must. Yeast need enough nitrogen to produce sufficient biomass for the specific level of potential alcohol which will differ if the yeast strain is a high or low nitrogen demanding strain. Laffort proposes a nitrogen adjustment calculator specifically for this purpose (https://laffort.com/en/decision-making-tools). The chances of a successful alcoholic fermentation are greatly increased if the starting YAN is adjusted accordingly.

2.FACTOR IN WATER ADDITIONS, STRESS CONDITIONS AND WINE CHEMISTRY

Basic chemistry of must is key to assessing what conditions yeast are being asked to grow in. This will likely change after additions are made at the crusher including acid, water and SO_2. Yeast will not grow under pH 2.8 g/L and above 0.8 g/L acetic acid, so ensuring must chemistry permits the multiplication of yeast cells before fermentation has even begun is critical. Microorganisms including lactic acid bacteria, *Saccharomyces cerevisiae* and *non-Saccharomyces* yeast spp as well as acetic acid bacteria prefer higher pH, which could lead to competition for nutrients if pH is left unchecked at this stage.
Water dilution can impact the fermentation by diluting nitrogen, nutrients, lipids, vitamins and minerals which will need to be replaced should they fall below critical levels.

3.YEAST SELECTION

Yeast strains of *Saccharomyces cerevisiae* have different tolerances to alcohol, optimum fermentation temperature ranges and nitrogen demand. Choosing an optimal strain for the specific conditions will improve the chances of completing alcoholic fermentation.

when presented with a fungal infection (Takemoto et al. 1991). It is possible these factors may not only affect yeast multiplication but also fermentation (Bisson et al. 2005, Smith and Banks 1986). Non-*Saccharomyces*, *Saccharomyces cerevisiae* and lactic acid bacteria are able to produce medium chain fatty acids that are inhibitory towards *Saccharomyces cerevisiae*, markedly at the tail end of alcoholic fermentation at high concentrations of ethanol and temperature extremes.

STAGE 2: FERMENTATION

Fermentation in wine is initiated by the multiplication of yeast species in grape juice, either inoculated or spontaneously developed. Typically, strains of *Saccharomyces cerevisiae* are inoculated into must with desirable characteristics in terms of alcohol tolerance, fermentation kinetics and sensory impact. Their role is to metabolise the sugar present and convert it to alcohol and carbon dioxide. In perfect ripening conditions, the ratio of fermentable sugars glucose and fructose is1:1. As the grapeshead towards over ripeness the ratio can change to favour fructose over glucose (Kliewer 1967, Shiraishi 2000). *Saccharomyces cerevisiae* metabolises glucose more easily than fructose (Guillaume et al. 2007). As a consequence, fructose is often the main sugar left in a stuck or sluggish fermentation. A higher fructose-to-glucose concentration in stuck wines is likely the consequence and not the cause of a stuck fermentation. The limiting factor is the transportation of sugar into the cell (Luyten et al. 2002), and in the presence of ethanol it is even harder for yeast to take up fructose (Berthels et al. 2007). Factors affecting fermentation are discussed below.

	Yan required (mg N/L)	Yan 1st addition (mg N/L)	Yan 2nd addition (mg N/L)
12% vol	180	150 -initial YAN	30
13% vol	190	155 -initial YAN	35
14% vol	200	160 -initial YAN	40
15% vol	220	170 -initial YAN	50

Table 1. Minimum amount of YAN recommended to build enough population for a corresponding potential alcohol. The third and fourth column are recommended rates of YAN to be added within the first 24 hours of yeast inoculation and at one third of the way through alcoholic fermentation, respectively. For low N demanding yeast, add 10 mg N/L (at 2nd addition); for medium N demanding yeast, add 20 mg N/L (at 2nd addition) for high N demanding yeast.

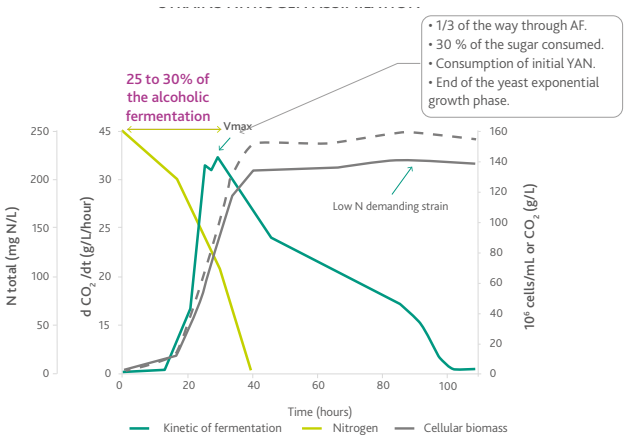

Figure 1. Assimilation of nitrogen and production of biomass for a high and low nitrogen demanding strain during alcoholic fermentation. Source: personal communication, Marina Bely, University of Bordeaux.

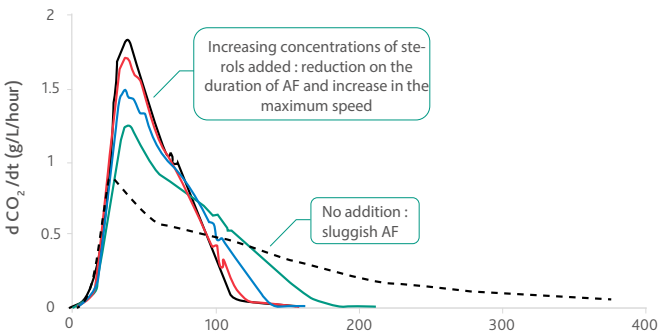

Figure 2. Fermentation kinetics with various concentrations of sterols added. Sourced from (Casalta et al., 2019)

LIPIDS, STEROLS AND OXYGEN

Sterols and unsaturated fatty acids are important for their role in cell wall fluidity and permeability. Synthesis of sterols is conducted in the presence of oxygen in the yeast exponential growth phase, so as the alcohol level increases, the ability of yeast cells to synthesise lipids decreases. This means that as the cells multiply, the amount of lipids present will deplete. Increasing amounts of sterols (Figure 2) can reduce the duration of alcoholic fermentation (Casalta et al. 2019). An absence of sterols leads to a sluggish alcoholic fermentation. Lipids can come from juice lees and solid parts of the must, with concentrations having been shown to vary based on the vigour of a vineyard (Casalta et al. 2019). Inactivated yeast are rich in unsaturated fatty acids and sterols and are able to supplement must if added at the beginning of the yeast rehydration phase. The amount of sterols in the membrane, especially ergosterol, as well as the degree of unsaturation of the membrane phospholipids favour the penetration of glucose in the cell (Ribereau-Gayon 2006).

Oxygen promotes yeast cell multiplication and sterol production making it critical in the exponential yeast growth phase. Conversely, oxygen becomes less critical from a fermentation perspective at the latter stages of fermentation as yeast have finished their multiplication stage and are only fermentative. This exposure to oxygen is critical to membrane fluidity and construction.

NITROGEN CONTENT

There are two stages in fermentation where nitrogen additions are critical. Nitrogen additions within the first 24 hours of yeast inoculation are required to build cell biomass relative to potential alcohol. Excess nitrogen at this stage can lead to a surplus in biomass and volatile acidity production (Mendes Ferreira et al. 2010). An excessive addition at the beginning of alcoholic fermentation has been demonstrated to block fermentation (Sablayrolles et al. 1996). Yeast populations reach their maximum one third of the way through alcoholic fermentation (Figure 1). At this point all nitrogen has been consumed, with two thirds of the sugar in the fermentation still to be metabolised. A second nitrogen addition maintains the population through the remainder of alcoholic fermentation (Table 1), with the amount dependant on how much of the population has been produced in the first third of alcoholic fermentation.

TEMPERATURE

Saccharomyces cerevisiae optimal growth is around 25-28°C (77-82°F), but often white fermentations are conducted at lower temperatures (14-16°C - 57-61°F) and red wine fermentations at higher temperatures (above 20°C - 68°F). The temperature will affect the rate of cell multiplication as well as the rate of fermentation. Abrupt temperature shocks can cause a fermentation to arrest (Suutari et al. 1990). These temperature shocks impact yeast cell membranes, enzyme function and typically produce stress shock proteins in response. Should the cell be deficient in nitrogen and key vitamins it may not be able to cope with specific shocks (Bisson et al. 2005).

STRATEGIES TO OVERCOME STUCK FERMENTATION: STAGE 2

1.COMPLEMENT EXISTING LIPIDS AND STEROLS PRESENT IN GRAPE MUST VIA THE USE OF REHYDRATION FACTORS - CRITICAL FOR HIGH ALCOHOL RED WINES AND LOW VIGOUR VINEYARDS (CASALTA ET AL. 2019)

Yeast rehydration nutrients can supplement the amount of lipids and sterols in musts. It is critical that they are present at the yeast rehydration phase when cell membranes are formed. Red wines high in alcohol require more sterols in the cell membrane as the ethanol can affect membrane fluidity at the end of alcoholic fermentation.

2.ADJUST NITROGEN ONE THIRD OF THE WAY THROUGH FERMENTATION TO ENSURE COMPLETION

At one third of the way through alcoholic fermentation it is expected that all of the nitrogen will have been depleted and yeast populations will be at maximum level. It is important at this stage to supplement enough nitrogen to maintain the populations throughout alcoholic fermentation. If there are vitamin and mineral deficiencies a complex organic nutrient will provide nitrogen as well as key micronutrients.

STAGE 3: MATURITY PHASE

This is the hardest stage to remedy a sluggish fermentation although this is often when an arrest becomes apparent. If yeast cells do not have enough lipids and sterols during multiplication via the addition of yeast rehydration nutrients and oxygen, cell walls can become rigid and likely compromise high alcohol concentrations. Insufficient nitrogen at the beginning of fermentation may also become evident, with not enough nitrogen provided to achieve the optimal yeast population to complete alcoholic fermentation. Yeast that are well constructed and have sufficient nutrients in the growth phase are better equipped to dealing with temperature shocks, the presence of toxins and high alcohol. Whilst must starts out with a glucose fructose ratio of 1:1, by this stage of the fermentation phase it is likely that the majority of residual sugar is fructose. It has been demonstrated that the change in the ratio of glucose to fructose can inhibit a fermentation (Schutz and Gafner 1993). Not only is fructose a less preferred sugar, it is harder for yeast to metabolise in the presence of high alcohol (Berthels et al. 2007). Yeast selection made at the beginning is even more important at this later stage of fermentation, as both the alcohol tolerance of the yeast strain and its ability to metabolise fructose come into play.

STRATEGIES TO OVERCOME STUCK FERMENTATION: STAGE 3

1.KEEP YEAST IN SUSPENSION

At the tail end of alcoholic fermentation yeast cells may drop out of suspension as the rate of fermentation reduces. Keeping yeast in suspension may enable the yeast to access sugar and key nutrients. Options include **BI-ACTIV®** (**LAFFORT®**) which detoxifies must and provides survival factors for yeast via inert elements without the need to add any mineral nitrogen in the form of diammonium phosphate. Alternatively, tank agitation can keep cells in suspension providing the user is mindful of oxygen pick up which can be detrimental at this point.

2.DETOXIFY

Often a combination of factors are involved in a sluggish fermentation, but if the fermentation has stopped it can be beneficial to 'clean up' the wine either by centrifugation (removal) or the addition of yeast hulls (absorption). In this case re-inoculation is necessary to complete alcoholic fermentation.

3.RE-INOCULATE OR CROSS SEED

Re-inoculating is often the last option available to remedy a stuck fermentation. Strain selection here is ultra-critical as yeast will be going into a very hostile environment with high levels of alcohol, poor nutrition and fructose as a carbon source. By inoculating a fresh culture, it is possible to maximise the sterol content using rehydration nutrients high in ergosterol content and incorporating oxygen in the yeast build up stage. **ACTIFLORE® BO213**, from **LAFFORT®,** is an example of a yeast strain that has the best chance of fructose uptake and a high tolerance to alcohol (Marullo et al. 2019). Yeast with a better chance of taking up fructose have been identified as having a particular form of the HXT3 transporter which has a higher affinity for fructose (Guillaume et al. 2007). Not all yeast strains have this hence why it is important to choose a robust strain with both a high tolerance to alcohol and an affinity towards fructose when dealing with high alcohol and/ or stuck fermentations. The yeast build-up process is important to acclimatise the yeast to the harsh environment. Calculators for restarting fermentation are available at www.laffort.com/ en/ protocols-and-itineraries.

Cross-seeding yeast lees from an active fermentation may provide yeast that have the appropriate level of nutrition, vitamins and minerals. However, this culture will face the same adversities found in a sluggish fermentation (high alcohol, high proportion of fructose, presence of inhibitors). The strain present would need to have a high alcohol tolerance and a high affinity towards fructose.

TAKE HOME POINTS

- Measure YAN in must and supplement in both organic and inorganic forms accordingly. Critical points in alcoholic fermentation are:
- Nitrogen supplementation #1 within 24 hours of yeast inoculation (objective build sufficient yeast biomass to complete alcoholic fermentation.
- Nitrogen supplementation #2 at one third of the way through alcoholic fermentation.
- In most cases, a nitrogen content of below 150 mg/L is considered deficient.
- The higher the potential alcohol, the more nitrogen is required to achieve the correct biomass.
- Strain selection appropriate to must.
- Water additions will minimize the amount of alcohol produced by reducing the concentration of sugars present, but will also dilute key nutrients and lipids important for yeast cell membrane structure.
- Yeast rehydration nutrients high in ergosterol and use of oxygen in the yeast exponential growth phase are especially critical in high alcohol red wines to ensure alcoholic fermentation completes.
- Fructose is the predominant sugar in a stuck fermentation. Must detoxification and de-alcoholisation will not change the high proportion of fructose (relative to glucose) remaining in a stuck fermentation.
- Restarting a stuck or sluggish alcoholic fermentation requires a yeast strain possessing at least one if not two copies of the HXT3 transporter that has a higher affinity for fructose.
- The best way to avoid stuck and sluggish fermentations from happening is by addressing the must before fermentation has commenced by rehydrating yeast with rehydration factors and oxygen, providing oxygen in the yeast log phase.
- Assisting yeast to stay in suspension may assist yeast to complete alcoholic fermentation if they have had adequate nutrients and rehydration factors in the yeast log phase.

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NUTRITION

NUTRITION

APPLICATIONS AND USE

PRODUCT	PREPARATION	SPECIAL CONSIDERATIONS	ADDITION RATE RANGE
DYNASTART®	40 - 43°C (104 - 110°F) 20 x weight in water.	Add yeast when temperature is at 37°C (99°F). Use for yeast rehydration.	200 - 300 ppm (20 - 30 g/hL)
SUPERSTART® ROUGE	40 - 43°C (104 - 110°F) 20 x weight in water.	Add yeast when temperature is at 37°C (99°F). Use for yeast rehydration.	200 - 300 ppm (20 - 30 g/hL)
SUPERSTART® BLANC	40 - 43°C (104 - 110°F) 20 x weight in water.	Add yeast when temperature is at 37°C (99°F). Use for yeast rehydration.	200 - 300 ppm (20 - 30 g/hL)
SUPERSTART® SPARK	40 - 43°C (104 - 110°F) 20 x weight in water.	Add yeast when temperature is at 37°C (99°F). Use for yeast rehydration.	200 - 300 ppm (20 - 30 g/hL)
NUTRISTART®	10 x weight in water or must.	Use for fermentation only.	200 - 600 ppm (20 - 60 g/hL)
NUTRISTART® ORG	10 x weight in water or must.	Use for fermentation only.	300 - 600 ppm (30 - 60 g/hL)
THIAZOTE® PH	Add directly in must.	Use for fermentation only.	100 - 500 ppm (10 - 50 g/hL)
NUTRISTART® AROM	10 x weight in water or must.	Use for fermentation only.	200 - 600 ppm (20 - 60 g/hL)
OENOCELL®	10 x weight in water or must.	Use towards end of fermentation or 2 days before restart.	200 - 400 ppm (20 - 40 g/hL)
BI-ACTIV®	Aerate 10 minutes first. Add directly.	Use towards end of fermentation or 2 days before restart.	300 - 600 ppm (30 - 60 g/hL)
MALOBOOST®	10 x weight in water or wine.	Use during MLF.	200 - 400 ppm (20 - 40 g/hL)

NUTRIENT STORAGE

PRODUCT	STORAGE TEMPERATURE AND PLACE	SHELF LIFE UNOPENED AND OPENED
All **LAFFORT®** Nutrients	Cool, dry area, in sealed original package.	Unopened 3 years. Once opened use within 2 months.

Q&A

NUTRIENTS

A stressful primary fermentation can result in unfermented sugar, decreased aromatics, off aromas, and the production of factors inhibiting MLF. Yeast must have complete nutrition for a stress-free environment. Grape nutrition and nutrient additions need to be balanced in both growth and survival factors for the yeast to ensure a clean and strong fermentation to finish.

1. What is the difference between a yeast rehydration product and a yeast nutrient?

A yeast rehydration product such as **SUPERSTART®** is formulated with yeast derived growth and survival factors (ergosterol, long chain fatty acids), with a goal of strengthening elements of the yeast membrane. **SUPERSTART®** is absorbed into the yeast cell membrane during the rehydration process to help with membrane fluidity, alcohol and temperature tolerance, and have high efficiency of sugar and nitrogen transporters. The benefits of yeast rehydration products carry through to the last generation of yeast. Yeast re-hydration products do not contain DAP which is toxic to the yeast at the rehydration phase.

Yeast nutrients contain growth factors, survival factors, and promote yeast multiplication. Complex yeast nutrients are formulated with yeast autolysate, inactivated yeast, DAP, and thiamine. Yeast nutrients are typically added during the fermentation phase to promote a healthy yeast biomass and support yeast when must is deficient in nitrogen.

Using both **SUPERSTART®** (yeast rehydration product) and **NUTRISTART®** (fermentation nutrient) will ensure a healthy fermentation, boost yeast-derived aroma production, and reduce VA and H_2S production.

2. What is the difference between organic and inorganic nitrogen?

Organic nitrogen comes from amino acids, whereas inorganic or mineral nitrogen comes from ammonium ions. The organic nitrogen source comes from inactivated yeasts and yeast autolysate. A common term is "yeast derived" nutrition. Inorganic nitrogen is diammonium phosphate, or commonly referred to as DAP.

For yeast nutrition, a combination of organic and inorganic nitrogen is common practice during alcoholic fermentation. **NUTRISTART®** is a combination of amino acids, DAP, and thiamine for complete nutrition. **NUTRISTART® ORG** is 100% yeast derived organic nutrition, for winemakers that want to use only organic nitrogen. **THIAZOTE® PH** is DAP with added thiamine which helps yeast with nitrogen uptake.

Organic nitrogen has been shown to have a significant increase of aromatic compounds produced by yeast, both esters and thiols, through the metabolism of amino acids.

3. What is the normal range of nutrient composition of grape must?

The range of Yeast Assimilable Nitrogen (YAN) found in grapes varies from 80 - 400 mg N/L, with 150 mg N/L, as desired minimum for biomass health. There is tremendous range and only analysis can tell you the YAN value of your grapes or must.

The typical nutrient composition of grape must is composed of up to 10% NH_4^+, up to 30% amino acids (excluding proline), up to 50% peptides + proteins as well as 10% or more of other components. All these combined are considered YAN. In terms of nitrogen contribution, YAN in grape must can also be categorized as 25 – 33% mineral nitrogen and 67 – 75% organic nitrogen,

4. How do I calculate YAN/nutrient additions?

First: start with the basics.

- Measure the must/juice YAN.
- During the yeast growth phase, the yeast population of a low nitrogen demanding yeast will utilize an average of 150 mg/L of YAN.

Second, account for alcohol.

- Measure initial brix to calculate the potential alcohol of the wine.
- Red wines (warm fermentation): multiply brix by 0.57 = potential alcohol.
- White/Rosé wines (cooler fermentation): multiply brix by 0.60 = potential alcohol.

Third: account for yeast strain variation.

- Yeast strains can differ in the amount of nitrogen supplement needed to maintain a healthy clean fermentation. The chart on page 32 is based off low nitrogen demanding yeast strains.
- Add an additional 10 mg/L YAN for 'average' Nitrogen demanding yeasts.
- Add an additional 20 mg/L YAN for 'high' Nitrogen demanding yeasts.

Fourth: allocate nutrients to the first and second additions.

- Make the first addition within 24 hours of yeast inoculation.
- Make the second addition at one-third fermentation.

POTENTIAL ABV	YAN TOTAL REQUIRED (mg N/L)	YAN 1ST ADDITION (mg N/L)	YAN 2ND ADDITION (mg N/L)
12%	180	150 - Initial YAN	30
13%	190	155 - Initial YAN	35
14%	200	160 - Initial YAN	40
15%	220	170 - Initial YAN	50
16%	240	180 - Initial YAN	60

For the first addition, encourage biomass with both mineral nitrogen **THIAZOTE® PH** and organic nitrogen **NUTRISTART® ORG,** or in a combination product such as **NUTRISTART®** or **NUTRISTART® AROM**. For the second addition, as both sugar and YAN decrease, organic nitrogen is recommended to maintain a healthy biomass and prevent sulfide formation and stuck fermentations.
Organic nitrogen products are an important part of the overall nutrient program however they can be low in overall YAN contribution. For musts/juices that are significantly low in initial YAN, it is important to incorporate DAP into the nutrient additions. **LAFFORT®** have an on-line nutrient calculator to help create the correct nutrient additions for your specific needs.

LAFFORT®
DECISION-MAKING TOOLS

5. Why is it better to do two additions instead of one?

The first nutrient addition is for the yeast growth phase to build biomass. Too little YAN in the growth phase will lead to inadequate yeast cell numbers to complete fermentation. Excessive YAN during the growth phase will signal the yeast to build excess populations, which can create rapid fermentation kinetics, VA production, and rapid YAN metabolization, leading to nutrient deficiency and H_2S production.
The second YAN addition occurs at one-third completion of fermentation when the yeast population is at a maximum. Nitrogen is required for ongoing maintenance of yeast cells to complete fermentation of the remaining 2/3 of the sugar in the must.
Splitting your nutrient additions will keep a healthy level of YAN in the must, keep the yeast population in balance and fermentation kinetics under control.

6 .When is a nutrient addition too late?

Nutrients are added to support the metabolic processes of the yeast during fermentation. When added in the last phase of fermentation (less than 8° Brix), the yeasts are under more stress from an alcoholic environment and are not metabolizing at the same rate as during peak fermentation. Yeast may not utilize all the YAN in the last phase of fermentation and the wine may have increased residual YAN available for spoilage microorganisms during aging.
There is one situation where a YAN addition late in fermentation is appropriate, when there is a manifestation of H_2S. DAP may work well, but the best choice is **NUTRISTART® ORG**. Frequently, when nitrogen is deficient in late fermentation, there is also a shortage of pantothenic acid, which is required for yeasts to produce sulfur containing amino acids rather than H_2S. DAP alone may remove the H_2S, only for it to return the next day. The additional pantothenic acid will complement the added YAN and allow the sulfite reductase pathway to work effectively.
In addition, there are options that do not have YAN but detoxify the medium and contribute survival factors (sterols and long chain fatty acids). For late additions where a fermentation is sluggish and may need help, utilize **OENOCELL®** or **BI-ACTIV®**.

7. Are nutrients needed for high initial YAN levels in must?

High initial YAN levels often are the result of large amounts of nitrogen in the vineyard, through cultural practices, fertilizers, or irrigation water. High YAN can cause yeast to produce a large biomass, which requires a high amount of nitrogen and micronutrients to maintain a healthy fermentation. Musts with high initial YAN can be low in micronutrients such as pantothenate (vitamin B5) an important component in the synthesis of amino acids. A shortage of pantothenate can lead to an increase of sulfides. High YAN's require an equally high pantothenate content for yeasts to prevent H_2S formation.
High YAN musts (> 300 ppm) will benefit from organic nutrients added at the one-third of fermentation completion so that there is sufficient micro nutrients in the must to continue fermentation through the secondary phase. We recommend using **NUTRISTART® ORG** to add essential micronutrients without adding too much nitrogen to the must.

8. How can I predict when my fermentation is sticking?

INFLECTION OF THE FERMENTATION CURVE

During the active growth phase, brix will drop quickly, and then the rate will slow gradually. If the brix drop slows and the ferment curve flattens, this may be a sign that the yeast culture is struggling.

TEMPERATURE

Yeast strains have optimal temperature ranges for fermentation, which are listed in the yeast description charts. It is important to keep fermentation temperatures within the optimal range

for healthy fermentation. High temperature spikes can damage cellular functions, such as enzymatic production that is involved in metabolic pathways. The high temperatures create a stressful environment for yeast and they produce toxins and heat shock proteins in a survival response to the heat. Conversely, cold temperature can slow down cellar metabolic processes and permanently damage the cell structure, lowering cell viability. If your fermentation has encountered rapid temperature spikes (hot or cold), watch for a slowing in brix drop as a warning sign that yeast population has been damaged.

HYDROGEN SULFIDE AND REDUCTION

Off aromas like hydrogen sulfide (rotten egg) indicate that the yeast culture needs more oxygen or nitrogen in some form. This can happen even if you have added the recommended nutrient additions. Fermentations are dynamic and unique, so a general recipe does not always work to keep the fermentation healthy. Sulfur containing compounds, sulfates and sulfites are reduced to H_2S inside the yeast cell by sulfite reductase. Pantothenic acid is required to catalyze the production of sulfur-containing amino acids from the H_2S. Without pantothenic acid, the H_2S is excreted directly into the fermentation.

For red wines, oxygen alone may be sufficient to remove reduction. For white wines, a small addition of either **THIAZOTE® PH** or **NUTRISTART® ORG** can help get the yeast to stop producing hydrogen sulfide during early to mid-fermentation. If the fermentation produces H_2S later in the process, an addition of **NUTRISTART® ORG** can reduce the H_2S production by the yeast.

VOLATILE ACIDITY

It is not unusual to have volatile acidity aromas before yeast fermentation begins, either due to oxidation or *Hanseniaspora* species, and this will blow off or be metabolized during fermentation.

During fermentation, if the yeast culture struggles, there is an opportunity for bacterial growth that can cause volatile acidity (acetone, nail polish, etc.). Many microbes can produce these compounds. Acetic acid bacteria can produce VA in aerobic environments, so it is important to protect the wine from oxygen (air) once the fermentation is no longer producing CO_2. *Lactobacillus* species can cause issues in aerobic and anaerobic environments. The presence of rapidly increasing volatile acidity in an otherwise healthy fermentation implies likely contamination by *Lactobacillus* species and requires immediate attention.

9. Can I control fermentation kinetics through nutrients?

When high amounts of nitrogen are available in the must/juice for the yeast, the fermentation kinetics can be very rapid. By splitting your nutrient additions into two separate additions, you can help slow the rate of the fermentation. Using a product like **NUTRISTART® ORG** (contains no DAP), will also help keep the rate of fermentation at a healthy rate.

10. Is there a different strategy for nutrient additions for wild/natural fermentations?

In wild fermentations there is no need for a yeast rehydration nutrient. However, a wild yeast culture will require the same amount of nitrogen during growth phase and will face the same challenges as commercial yeast strains during fermentation. When working with wild/natural fermentations, it is suggested to keep the same nutrient strategy recommended for commercial yeast fermentations with average Nitrogen demands.

11. Is glutathione a fermentation nutrient?

Glutathione is an antioxidant made up of three amino acids: glutamine, glycine, and cysteine. Glutathione is produced by cells and grape cells can have varying levels of glutathione depending on the growing season and type of farming. Glutathione's role in winemaking is an antioxidant, not a nutrient. Glutathione helps to protect the fermentation esters in white and rosé wines.
Yeast cells can produce more glutathione by metabolizing the amino acids glutamine, glycine, and cysteine. **FRESHAROM®** is a yeast extract product that is rich in sulfur-containing amino acids. When added after the regular nutrient additions, yeast will use the amino acids in **FRESHAROM®** to facilitate fermentation and produce glutathione for added protection of white and rosé wines.

12. Do I need to adjust nutrient additions for co-fermentations, either with multiple yeasts or in conjunction with MLF?

When using a non-*saccharomyces* yeast strain, like **ZYMAFLORE® ALPHA TD**, an additional 50 ppm of YAN is required for the extra yeast population.
When using multiple *Saccharomyces cerevisiae* strains for fermentation, the difference in implantation of each strain will have the biggest impact on the growth phase nitrogen requirements for each yeast strain. It is difficult to predict the outcome of multiple strain inoculations due to this variability in implantation, and this can vary dramatically from ferment to ferment and from year to year. We recommend treating the ferment as a single yeast strain inoculation for the purpose of calculating nutrient additions.
No additional nutrients are required for co-inoculation with malolactic cultures, but we recommend the addition of **MALOBOOST®** at the completion of primary fermentation to provide peptides for malolactic bacteria growth.

ARTICLE

YEAST NUTRITION & ORGANIC SUPPLEMENTATION

YEAST DEMAND FOR NITROGEN

The nitrogen sources that can be used by *Saccharomyces cerevisiae* are ammonium (NH_4^+) and amino acids (organic nitrogen). They both represent assimilable nitrogen and are present in must at varying concentrations, sometimes not in sufficient quantities to meet the requirements of the yeast. The three following factors must be taken into consideration:

- Below 150 mg N/L, must is deficient. It is therefore important to supplement it with nitrogen elements.
- Yeast nitrogen requirements depend on sugar concentration. The higher this concentration, the greater the amount of yeast biomass needed to successfully achieve a thorough breakdown of the sugars during alcoholic fermentation. Although, the yeast biomass must not be too excessive to avoid an induced nitrogen deficiency.
- The nitrogen initially present in must is rapidly assimilated during the first third of the alcoholic fermentation, at the point when the biomass is at its highest density. Consequently, irrespective of the initial nitrogen content, its addition during alcoholic fermentation allows to preserve the biomass formed, which is dependent on the yeast strain and proportional to the initial nitrogen concentration.

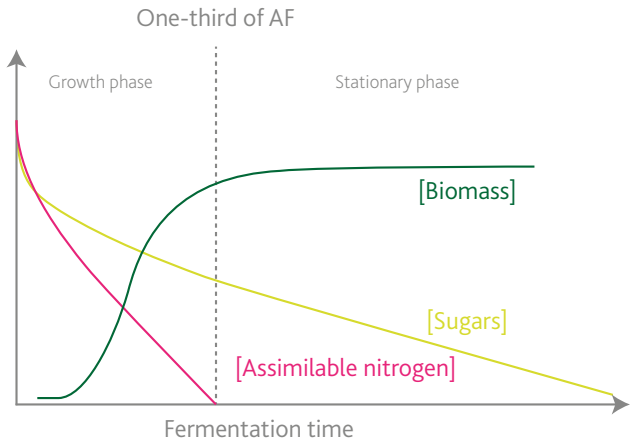


Figure 1. Assimilation of nitrogen and production of biomass during alcoholic fermentation.

WHY ORGANIC NUTRITION?

Organic nitrogen is supplied by adding yeast derivatives (usually autolysed yeast). In addition to amino acids, these yeast derivatives include lipids, vitamins and minerals which also contribute to the efficient performance of the yeast.

Yeast has the ability to simultaneously assimilate organic nitrogen and mineral nitrogen from the beginning of the alcoholic fermentation. Organic nitrogen must be present in order to:

- Limit the production of SO_2 and sulphur compounds (H_2S and mercaptans).
- Produce healthy, but not excessive, biomass.
- Limit the risk of stuck or sluggish fermentation.

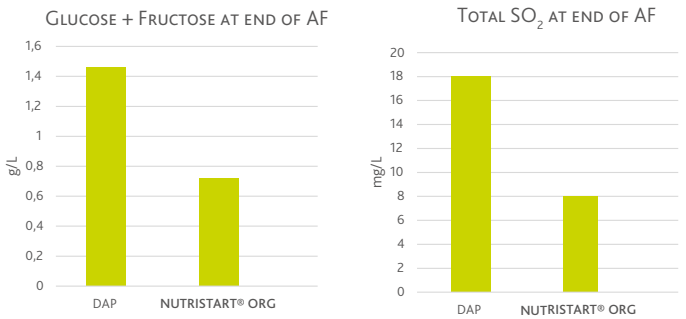


Figure 2. Concentrations of Glucose + Fructose and total SO_2 at the end of alcoholic fermentation. Must derived from sauvignon blanc (TAP vol. 13.9%, initial Nass: 125 mg N/L), 2016. At the one-third point of alcoholic fermentation, 35 mg N/L were added with DAP or **NUTRISTART® ORG**, deliberately making yeast conditions difficult.

WHEN RESEARCH LEADS TO A BETTER UNDERSTANDING OF THE NUTRISTART® ORG PERFORMANCE

By carrying out an extensive study on **NUTRISTART® ORG**, we were able to learn about this product's subtle composition after developing specific assay methods (Figure 3).

LIPIDS

Palmitic acid (C16:0), Stearic acid (C18:0), Palmitoleic acid (C16:1), Oleic acid (C18:1), Squalene, Zymosterol, Lanosterol, Ergosterol

AMINO ACIDS

ASP, GLU, CYS, ASP, SER, GLN, GLY, THR, ARG, ALA,GABA, TYR, ETN, VAL, MET, TRP, PHR, ILE, LEU, ORN, LYS

VITAMINS

Para aminobenzoic acid, Pyridoxine, Riboflavin, Biotin, Pantothenic acid

MINERALS*

Mg, Ca

Figure 3: Elements detected in **NUTRISTART® ORG**.

** Other minerals are in the process of being assayed.*

An experiment design setting up models for 58 trials and omitting various compounds was then carried out to discover the impact of these various nutrients on alcoholic fermentation.

COMPOUNDS INCREASING THE MAXIMUM YEAST POPULATION DURING AF*	COMPOUNDS REDUCING THE LAG PHASE LENGTH OF AF	COMPOUNDS INCREASING THE MAXIMUM RATE OF AF
ASP	CYS	ABA
ARG	GABA	ARG
C18	GLN	ASN
C18:1	GLY	ORN
Calcium	Pyridoxine	Lanosterol
GLU	TRP	
Lanosterol	VAL	
Riboflavin		

Table 1: Effect of the various constituents of **NUTRISTART® ORG** on alcoholic fermentation parameters (Results obtained following a statistical analysis based on a multiple linear regression and a Kruskal-Wallis test – methods performed according to a Hadamard experiment design).

** Nutrition must enable an optimum, but not excessive, population to be attained.*

Our latest research shows that not all of the constituents have the same effect on yeast and alcoholic fermentation. We will continue with this study in order to have a detailed understanding of the role of each constituent.

ORGANOLEPTIC EFFECTS OF ORGANIC NUTRITION

Numerous experiments show that improved outcomes of alcoholic fermentation can be achieved with the use of organic nitrogen. Even in the case of wines considered dry (glucose + fructose < 2 g/L), small amounts of fermentable sugars can be used by degrading microorganisms and can have an adverse effect on the quality of the wines (Figure 2).

Besides its effects on fermentation kinetics, the addition of oragnic nitrogen can increase the fruitiness of wines and limit the aromatic mask linked to the production of sulphur compounds during the alcoholic fermentation.

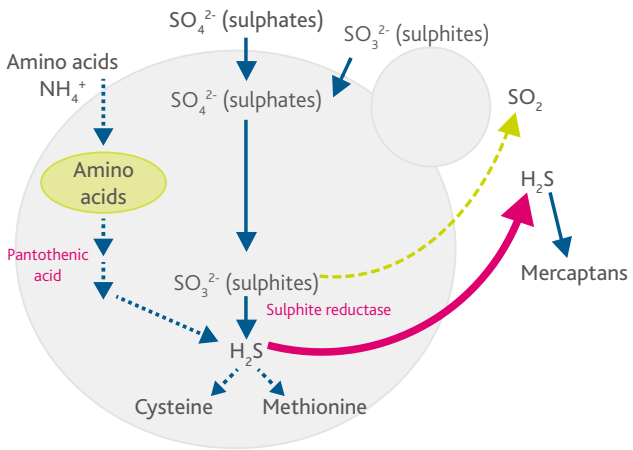
Except for the source of the nitrogen added, a comparison of wines produced under the same conditions reveals significant preferences for wines derived from musts supplemented with NUTRISTART® ORG (Table 2). The wines are considered fruitier, fresher, less vegetal and subject to less reduction than those supplemented with mineral nitrogen alone.

	MINERAL / ORGANIC COMPARISON
Number of tasters	20
Number of correctly detected differences	13
Results	99% significant difference
Preference	Organic: 13/13

Table 2. Triangular tasting tests (ISO 4120-2004) of red wines. Comparison of two vinified Merlot wines with 65 mg N/L nitrogen added in the form of THIAZOTE® or NUTRISTART® ORG.

DID YOU KNOW ?

The key enzyme in the production of H_2S is sulfite reductase. When the H_2S and amino acids pathways meet the sulphur amino acids (cysteine and methionine) are produced. Where there is an imbalance between these two pathways and a nitrogen deficiency, the precursors of these sulphur amino acids are limiting, leading to an accumulation of H_2S.



ASSIMILABLE NITROGEN CONTRIBUTION BY NUTRIENT

	EQUIVALENCE	BALANCE AND COMPOSITION			
PRODUCT	YAN CONTRIBUTION PER 100 ppm	ORGANIC AVAILABLE NITROGEN	MINERAL AVAILABLE NITROGEN	VITAMINS AND MINERALS	NUTRITIONAL BALANCE
NUTRISTART® ORG	10 ppm	••••		••••	•••
NUTRISTART® AROM	14 ppm	•••	•	•••	••••
NUTRISTART®	15 ppm	•	•••	••	••
THIAZOTE®	21 ppm		••••	•	•

LAFFORT®: THE ORGANIC COMMITMENT

Organic Wine

The products and families of products for use in Organic winemaking are supervised by the EU Regulation n ° 203/2012 and NOP (National Organic Program) USDA (United States Department of Agriculture). Many are approved by OMRI and CCOF.

The Certifications

The list of LAFFORT® products used within the regulated framework of Organic wine and / or NOP is available on our website (direct access by scanning the QR code below). We have chosen the following external certification organizations; Ecocert, OMRI and USDA. Our products have their own certification, corresponding to individual specifications defined by each organization. (www.ecocert.fr, www.omri.org, www.usda.gov, www.ccof.org).

To Find our Certificates

Log on www.laffort.com, "Downloads", "Certificates" category.

www.laffort.com/en/certificates

CCOF Approved for use in "Made with Organic Grapes":

TAN'COR®
TAN'COR® GRAND CRU
QUERTANIN® SWEET
TANIN GALALCOOL®
TANIN VR SUPRA®
TANIN VR COLOR®
TANIN VR SUPRA® ELEGANCE
TANIN VR GRAPE
GELAROM®
CASEI PLUS

CCOF Approved for use in "Made with Organic Grapes" and "Organic Wine":

ACTIFLORE® F33*
ACTIFLORE® ROSE*
ACTIFLORE® B0213*
ZYMAFLORE® X5*
ZYMALFORE® X16*
ZYMAFLORE® VL3*
ZYMAFLORE® VL2*
ZYMAFLORE® VL1*
ZYMAFLORE® CH9*
ZYMAFLORE® CX9*
ZYMAFLORE® ST*
ZYMAFLORE® F15*
ZYMAFLORE® FX10*
ZYMAFLORE® F83*
ZYMAFLORE® RB2*
ZYMALOFRE® RX60*
ZYMAFLORE® 011BIO*
LAFAZYM® AROM
LAFAZYM® EXTRACT
LAFAZYM® CL
LAFASE® HE GRAND CRU
DYNASTART®*
SUPERSTART® BLANC*
SUPERSTART® ROUGE*
FRESHAROM®*
MALOSTART®
BI-ACTIV®
OENOLEES®*
AUTOLEES®*
MANNOSTAB®*
GELATINE EXTRA N°1*
MICROCOL® ALPHA*
LACTOENOS® 450 PREAC
LACTOENOS® SB3 DIRECT

**Must demonstrate commercial unavailability for organic equivalent for use in organic wine*

OMRI Certification

DYNASTART®
SUPERSTART® ROUGE
SUPERSTART® BLANC
OENOLEES®

MALOLACTIC FERMENTATION

MALOLACTIC FERMENTATION

APPLICATIONS

OBJECTIVE	INOCULATION TYPE	STAGE	RECOMMENDED LACTOENOS® PREPARATION
Save time and faster completion of MLF. Optimize management of the fermentation processes. Reduce volatile acidity.	Early co-inoculation	24 - 48 hours after the start of alcoholic fermentation	**LACTOENOS® SB3 DIRECT** **LACTOENOS® 450 PREAC**
Monopolistic control of the ecosystem. Safeguard the conventional vinification sequence (AF followed by MLF).	Late co-inoculation	At 0 - 4° Brix	**LACTOENOS® SB3 DIRECT** **LACTOENOS® B7 DIRECT** **LACTOENOS® 450 PREAC**
Red wine MLF post pressing. MLF in barrel. Thermo-vinification. Reduce the potential of SO_2 to combine with wine compounds.	Sequential inoculation	At dryness and post-pressing	**LACTOENOS® SB3 DIRECT** **LACTOENOS® B7 DIRECT** **LACTOENOS® 450 PREAC**
Restart stuck MLF. Spring MLF.	Curative inoculation	To restart a stuck MLF	**LACTOENOS® B7 DIRECT** **LACTOENOS® 450 PREAC** **LACTOENOS® B16 STANDARD**

SPECIFICATIONS

PRODUCT	ALCOHOL	PH	TOTAL SO_2	TEMPERATURE
LACTOENOS® B7 DIRECT	≤ 16% vol	≥ 3.2	≤ 60 mg/L	≥ 15°C (59°F)
LACTOENOS® SB3 DIRECT	≤ 15% vol	≥ 3.3	≤ 40 mg/L	≥ 15°C (59°F)
LACTOENOS® 450 PREAC	≤ 17% vol	≥ 3.3	≤ 60 mg/L	≥ 16°C (61°F)
LACTOENOS® B16 STANDARD	≤ 14% vol	≥ 2.9	≤ 60 mg/L	≥ 15°C (59°F)

STORAGE

PRODUCT	STORAGE TEMPERATURE AND PLACE
All **LAFFORT® LACTOENOS®** bacteria	30 months at -18°C (0°F) 18 months at 4°C (32°F)

Q&A

MALOLACTIC FERMENTATION

A crucial step in the winemaking process, malolactic fermentation, has a huge influence on the stability of a wine. By seeding the wine's microbial ecosystem with a selected, active, and controlled microorganism, you help prevent the development of undesirable yeast and bacteria at the end of the alcoholic fermentation process.

1. Which wine parameters have an effect on MLF success?

Temperature
Lactic acid bacteria prefer temperatures above 15°C (60°F) with an ideal zone between 18°C to 24°C (65°F to 75°F). Temperature acts on membrane fluidity: an excessively high temperature will liquefy the cell membrane; too low it becomes rigid. Ethanol narrows these parameters even further.

Alcohol
Any wine above 13% ABV is considered a difficult environment for lactic acid bacteria, however, some commercial strains are equipped to tolerate up to 17% ABV.

pH
All living cells are dependent on specific pH parameters to function. Lactic acid bacteria used in winemaking require pH levels typically above 3.3, while some strains like Lactoenos B16 can tolerate much lower pH levels.

SO_2 levels
It is important to keep Total SO_2 levels below 60 mg/L so as to not interfere with LAB population survival. Commercial strains are designed to complete MLF quickly, then be eliminated easily with an SO_2 addition.

Malic acid
Below 1 g/L of L-malic acid, it is difficult to trigger MLF as the quantity is not sufficient for the bacteria to activate malic acid enzymes and choose this metabolic pathway. Fermentability is optimal with an L-malic acid content of between 1 and 5 g/L.

Toxins
Besides ethanol, medium chain fatty acids released by yeast in stressful conditions during alcoholic fermentation are known to delay or prevent MLF. The medium chain fatty acids affect membrane fluidity and disrupt normal ML fermentation.

2. What is the dosage rate of an ML culture?

The standard dose is 1 g/hL (10 ppm). Packages are typically sold in hL quantities, for example, 25 hL package weighs 25 grams.
Note that malolactic bacteria are susceptible to oxygen. Once opened, the package needs to be used immediately.

3. What is the difference between direct inoculation, PreAc strains, and standard build-up cultures?

Direct inoculation are those strains that may be added directly to your wine without the need to rehydrate. **LACTOENOS® SB3** or **LACTOENOS® B7** are direct inoculation strains.
PreAc strains require less time compared to standard build-up cultures to increase biomass and acclimate before pitching. Thanks to pre-acclimatization during production, **LACTOENOS® 450 PREAC** only requires an overnight build-up (rehydration and time) to build strength before adding to wine.
Standard build-up cultures such as **LACTOENOS® B16** can take up to 3 days to reach optimum viable cell counts. Direct inoculation cultures save time by eliminating extra steps, whereas build-up cultures tend to be more robust and can handle more extreme conditions.

4. Is a specific ML nutrient required for ML fermentation?

Specific ML nutrients are appropriate in wines that have one or more challenging parameters. During a healthy primary fermentation, yeast provide MLF nutrition through natural autolysis that breaks down yeast cells to release essential vitamins, minerals, and peptide fragments. For challenging primary ferments, and especially for uninoculated MLF, a specific nutrient may be necessary. **MALOBOOST®** is a mixture that is rich in peptide fragments and amino acids.

5. In general, what is the average time an ML fermentation should take to complete?

There are several factors that influence the duration of MLF, studies have shown that commercial strains are the most efficient to complete fermentation, while native strains are much more variable. The biggest impacts come from temperature and inhibitory components in the wine.

- Early co-inoculation – 3 days to 2 weeks.
- Late co-inoculation – 1 to 4 weeks.
- Sequential inoculation – 3 to 6 weeks.
- Indigenous MLF – 3 to 11 weeks.
- Using an ML nutrient such as **MALOBOOST®** may reduce MLF by several weeks.

6. What happens if the ice packs melt in shipping?

Frozen bacteria can tolerate a temperature of 25°C (77°F) for a few days without losing their efficiency. This occasionally occurs during transport and is not to be worried about.
The **LAFFORT®** range of freeze-dried malolactic bacteria can be stored in the refrigerator at 4°C (39°F) for 18 months, and in the freezer at -18°C (0°F) for up to three years from the date of production.
Note that the best option is to always store bacteria in the freezer.

7. Before adding the ML culture, should I detoxify with yeast hulls as a standard step prior to MLF?

In general no, the majority of fermentations create an acceptable environment. If your primary fermentation struggled to finish or stuck, then it is recommended to detoxify the wine with yeast hulls before adding the ML bacteria culture.
Besides ethanol, medium chain fatty acids released by the yeast metabolism are one of the most common inhibitors of lactic bacteria. In case of excessive quantities, their toxic effect can be efficiently eliminated by treating the wine with yeast cell walls (**OENOCELL®**) which can adsorb fatty acids and other inhibiting metabolites. Add **OENOCELL®** at 20 to 40 g/hL (200 - 400 ppm) and during the 48 hours following the addition, mix anaerobically several times to promote the adsorption and inhibition removal. Bacteria must then be inoculated rapidly or activate the native bacteria with the addition of **MALOBOOST®** to maximize the clean environment.

8. What are the benefits and risks of co-inoculation with yeast and bacteria?

Early co-inoculation (24-48 hours after the yeast inoculation) saves time as MLF begins before AF is completed. In late co-inoculation (at around 0-4 Brix), the bacteria adapt to the medium while AF is finishing and MLF begins as AF is completed.
Early co-inoculation should be utilized in healthy fermentation conditions: moderate final alcohol, clean fruit, and vineyard blocks which are known to finish AF strongly. With questionable conditions: high potential alcohol, mold, and blocks with historical stuck fermentations, it is best to avoid early co-inoculation. Once you are confident that the AF curve looks good and the wine will finish AF, add the ML starter at around 0-4 Brix.

9. What should I do if my ML fermentation has not started or has started and did not complete the fermentation?

There are many reasons for slow-to-start, or slow-to-finish, or stuck, malolactic fermentations, generally through either microbial or chemical inhibition. First, analyze the microbial status of your wine to assess the health of the malolactic bacteria and the risk of any competing microorganisms, particularly *Brettanomyces*. Second, determine the status of your wine chemistry, alcohol %, glucose-fructose, pH, L-malic acid, free SO_2, and total SO_2.
Other inhibiting metabolites also exist, such as short- and medium-chain fatty acids produced by yeasts during AF, or afterwards by *Brettanomyces*. C8 (octanoic acid), C10 (decanoic acid) and C12 (dodecanoic acid) fatty acids inhibit MLF bacterial growth and enzyme activity, particularly by disrupting membrane function. An assay of the fatty acids gives an indication of the wine's toxicity for lactic bacteria, and ethanol exacerbates their effect, and these can be removed by detoxification with **OENOCELL®**.
Often it can be an interaction of multiple factors, a single one may not be a problem, but a combination can cause an issue.
And remember that occasionally, simply racking the wine may be sufficient to kick off or finish the MLF. In more difficult cases, correct any deficiencies and inoculate with a robust strain that can handle a wide array of conditions such as **LACTOENOS® B7**, and also see the MLF restart protocol on page 157.

10. What is the easiest way to achieve partial MLF or prevent MLF completely?

Many methods exist to stop or prevent MLF, including SO_2, **LYSOZYM**, and filtration. Each has advantages and disadvantages. SO_2 added and maintained at over 60 ppm can effectively kill the bacteria but will deplete over time and MLF may restart in the bottle. **LYSOZYM** degrades the bacteria cell wall to inactivate the cell and has minimal sensory impact. However, it will add heat-labile proteins that need removal with bentonite or may interact with phenolics and reduce color.
Filtration can be used to remove the bacteria, however, keep in mind that recontamination is always a risk. Always consider the end goal before deciding which method is best. With wines that have residual malic acid, it is recommended to sterile filter at bottling time.

11. For low pH wines, what is the best type of ML culture to use?

Strains that require acclimatization typically can handle a wider range of wine conditions including lower pH. **LACTOENOS® B16 STANDARD** is a 3-day build up strain that can handle wine pH down to 2.9.

12. Can ML bacteria influence the fruit aroma/flavor in a wine?

MLF lactic bacteria are capable of metabolizing sulfur containing amino acids: methionine and cysteine. It is now recognized that sulfanyl-3-methyl propionic acid, a compound derived from this metabolism, positively contributes to red fruit aromas in wine.
It is, in our current state of knowledge, the only compound clearly identified as being implicated in the aromatic impact of lactic bacteria during MLF. Inversely, it is recognized that sluggish MLFs that delay the wine's microbiological stability are detrimental to the fruity aromas derived from alcoholic fermentation. With the indigenous flora, it should be specified that certain compounds such as biogenic amines can mask aromas. Research, led by Professor Gilles de Revel at the University of Bordeaux Oenology Faculty (ISVV), is currently in progress, studying the effect of bacteria and fruitiness in wine. While the initial results show that it is difficult to establish the existence of a bacteria strain effect used during MLF and fruity notes, it appears that certain vinification pathways protect fruity aromas more than others. Co-inoculation is a technique that can shorten the time between end of AF and end of MLF, thereby protecting the fruity aromas.

CITRIC ACID METABOLISM IN LACTIC BACTERIA AND CONTROLLING THE DIACETYL CONTENT IN WINE

LAFFORT info, Vincent RENOUF[1] & Marie-Laure MURAT[2]

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INTRODUCTION

Diacetyl (2,3-butanedione: $C_4H_6O_2$, Figure 1) is an acetoinic molecule responsible for the 'buttery' character perceived in wine during malolactic fermentation (MLF), which is by no means universally appreciated by wine tasters.

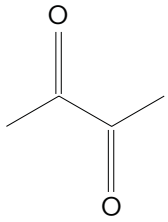

Figure 1. The chemical formula of diacetyl.

In wine, diacetyl is mainly produced by lactic bacteria, particularly the *Œnococcus œni* species, which is responsible for MLF (Renouf et al 2006). Diacetyl and other acetoinic molecules produced by lactic bacteria (acetoin and butanediol) are the degradation by-products of citric acid (Figure 2), one of the organic acids naturally present in grape juice.

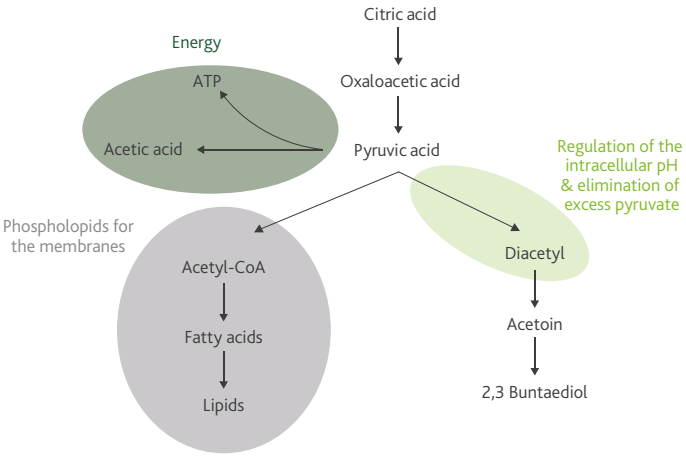

Figure 2. Pathways of citric acid degradation by lactic acid bacteria.

Pyruvate is the key metabolite at the crossroads of these metabolic pathways. It may be either used to synthesise lipids that are used in turn to build membrane phospholipids, which are essential components of the cell membrane, or consumed to produce acetoinic molecules.

The formation of these molecules is therefore considered a detoxification process for cells, which must eliminate excess pyruvate once the phospholipid demand is satisfied. The formation pathway for acetoinic compounds is also essential as it contributes to regulating intracellular pH. Citric acid is also used as an energy source by lactic bacteria. Firstly, the acetyl phosphate molecules produced from pyruvate are converted to acetic acid, releasing phosphate used for adenosine triphosphate (ATP) synthesis (Wagner et al. 2005), and secondly, the decarboxylation and translocation of the citrate molecule (which exists in the ionised form H_2citrate- at wine pH) are the two components of the proton driving force that generates energy (Seiz et al. 1963, Ramos et al. 1995), which is also the case during the malolactic transformation reaction.

Under conditions favourable for bacteria, the metabolic pathway of the pyruvate molecule is oriented towards lipids or diacetyl, depending on the cell's lipid and energy requirements. Conversely, under limiting growth conditions, the bacteria mainly use citric acid to produce acetoinic compounds.

In wine, the organoleptic impact of diacetyl has been debated for many years (Peynaud 1947, Rankine et al. 1995). At the end of MLF, concentrations vary between 1 and 10 mg/L, or sometimes even higher. Wine tasters generally agree that the diacetyl content must not exceed 5-6 mg/L (Davis et al. 1986), although it depends on the characteristics of each wine (Martineau et al. 1995). Below that level it is considered to contribute to the wine's bouquet, while higher concentrations have a negative impact. Chardonnay wines generally have the highest concentrations and, unsurprisingly, the impact of diacetyl is mainly a concern in white wines that undergo MLF (acidic wines and/or those intended for ageing, or base wines for sparkling wine).

MLF is intended to reduce acidity and ensure microbiological stabilisation, but must not leave the wine overwhelmed by heavy buttery aromas.

This article presents a brief summary of the essential knowledge on this subject and the results of recent experiments. It proposes practical solutions for controlling the diacetyl content of wine while ensuring successful completion of MLF.

CITRIC ACID IS AN INDISPENSABLE SUBSTRATE FOR BACTERIA

As previously mentioned, the citric acid degradation pathway provides *O. œni* key elements for its cell viability (population) and vitality (activity). The degradation of citric acid leads to energy production, regeneration of reducing capacity, regulation of intracellular pH, and membrane phospholipid synthesis. The vast majority of indigenous strains and those selected for malolactic starters use this pathway during their development in wine.

Citric acid is present in grapes, and even if concentrations may be modified by yeast activity, they generally range from 0.2-1 g/L at the end of alcoholic fermentation (AF), rarely more. This is not proportionate to the total acidity (TA) of the wine; for example, citric acid concentrations in wines with high TA (12 g/L H_2SO_4) may be lower than those in wines with a TA of 6 g/L H_2SO_4. Furthermore, not all citric acid is consumed during MLF, generally resulting in 20 - 50% consumption of the initial concentration. Figure 3 illustrates the usual variation in citric acid content during MLF.

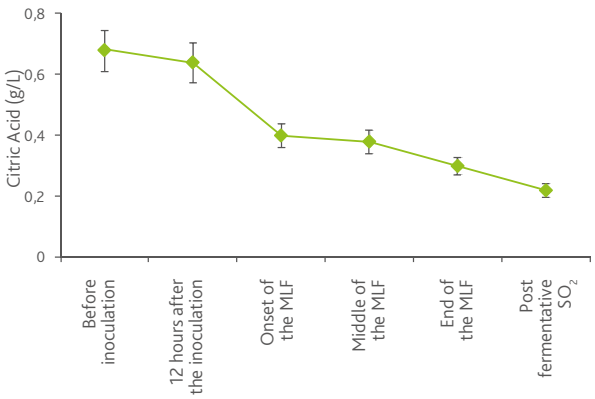

Figure 3. Evolution of the citric acid in a Chardonnay wine inoculated after the end of AF with **LACTOENOS® 350 PREAC**. Wine parameters before the bacterial addition: ETHANOL = 13.2% vol., pH = 3.3, L-MALIC ACID = 2.4 g/L, CITRIC ACID = 0.68 g/L.

As shown in Figure 3, citric acid consumption occurs mainly between inoculation with bacteria and the start of MLF. Apparently the cells need citric acid to survive inoculation in their new environment. This is due to the fact that the bacteria not only need energy at that time but they also have to regulate their intracellular pH (5.5 - 6) to a new acid environment, i.e. wine. Once they have adapted to the medium, the bacteria proliferate and need to produce new membranes, which requires an increase in phospholipid synthesis and therefore a supply of citric acid. Once the population has reached a sufficient level, the bacteria mainly consume malic acid, probably as it is naturally present in much larger quantities in wine than citric acid. It is important to emphasise that the phenomenon is identical when bacteria are co-inoculated with yeast into must with high sugar content. Among the available substrates, lactic bacteria initially degrade a small quantity of citric acid and then turn to malic acid, only using minute quantities of glucose and fructose, which remain the key target of the yeasts that are also active in the must at that time. Careful monitoring of the co-inoculation process demonstrated the kinetics of substrate use (data not shown).

Citric acid is not consumed by the bacteria during the remainder of MLF, with the exception of small amounts degraded right at the end of the process.

Figure 3 shows that a total of 0.46 g/L citric acid was used by the bacteria in that particular wine sample during MLF. This resulted in a final diacetyl content of 2.2 mg/L. This is well below the theoretical yield of the reaction (according to Bartowsky and Henschke (2004), 1 mol citric acid produces 0.5 mol acetoinic compounds). These results therefore indicate that the citric acid degradation products were used by the bacteria for other essential purposes (energy, phospholipids), rather than simple diacetyl production.

The quantity of diacetyl produced also depends on the aptitude of the bacteria and any environmental stresses to which they are subjected. Indeed, as previously described, diversion from the citrate to the pyruvate pathways depends exclusively on physiological requirements. In general, factors such as pH, high temperatures and the general composition of the wine medium, which affect bacterial growth, modify diacetyl production levels.

In view of the negative aromatic impact of diacetyl, microbiologists attempted to identify strains of *O. oeni* that were incapable of degrading citric acid under any environmental conditions. These strains did not possess the genes involved in synthesis of the enzymes responsible for citrate degradation. However, in view of previous observations of the adaptive advantages conferred by the citric acid degradation pathway, it was unsurprising that these strains had difficulty developing in wine under difficult conditions, and that they performed far poorer than other malolactic starters. As illustrated by the comparative test in Figure 4, the latency phase of the malolactic starter incapable of degrading citric acid was twice as long as that of the conventional starter, even under favourable conditions (% alc., pH and temperature). On completion of MLF, the conventional starter had only degraded 0.17 g/L citric acid

and produced less than 2 mg/L diacetyl (1.8 mg/L). Furthermore, although the non-citrate metabolising starter had not degraded any citric acid, volatile acidity was significantly higher than with the conventional malolactic starter (0.36 g/L H_2SO_4 compared with 0.25 g/L H_2SO_4). The longer latency phase certainly contributed to this increase in volatile acidity by giving other microorganisms a chance to develop.

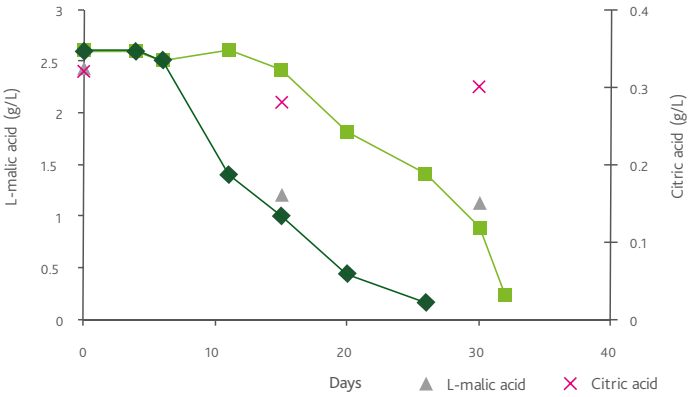

Figure 4. Comparison between a conventional malolactic starter (in green: **LACTOENOS SB3®**, **LAFFORT®**) and a malolactic starter incapable of degrading citric acid (in pink) in a Merlot wine (ACV = 13.4% vol, pH = 3.68, L-MALIC ACID = 2.6 g/L, CITRIC ACID = 0.32 g/L (test carried out in the laboratory at 25°C / 77°F)).

Depriving bacteria of the possibility of using citric acid and its associated benefits for cell development was therefore not the optimum solution as it was detrimental to the starter's efficiency in adapting to the medium, delaying the triggering of malolactic activity and leading to higher acetic acid production. Fortunately, there are other solutions to diacetyl management.

CONDITIONS FOR DIACETYL PRODUCTION BY BACTERIA

As previously mentioned, bacteria require citric acid when phospholipid and energy demands are high and intracellular pH regulation is necessary. Phospholipid demand depends on bacteria development. This factor is unavoidable as it is now well known that a minimum population of 10^6-10^7 cells/mL is required to trigger MLF. On the contrary, energy demand and pH regulation depend directly on the parameters of the bacterial environment. In general, these requirements increase whenever the bacteria are growing under difficult conditions. For example, a 30% increase in diacetyl production was measured in a Chardonnay wine during MLF when pH = 3.1 rather than pH = 3.5, all other conditions being equal. In the same wine at pH = 3.5 diacetyl production increased by 12% when the total sulfur dioxide (SO_2) content was reduced (25/45 mg/L).

It is, however, difficult to draw general conclusions on the basis of these observations as many parameters must be taken into consideration. For example, considering the impact of temperature, diacetyl production is much higher at 25°C (77°F) than 16°C (60°F), which is only significant in wines with a high alcohol content (Table 1). High temperatures probably accentuate the impact of ethanol, obliging the bacteria to metabolise citric acid. It is also known that high temperature and ethanol have a direct impact on cell membranes, probably resulting in a higher phospholipid demand. The situation was reverse in wine with a lower alcohol content – diacetyl production was slightly higher at 16°C (60°F) than 25°C (77°F). Consequently, it would certainly be preferable to maintain the temperature in the vicinity of 20°C (68°F), i.e. the optimum temperature for bacterial growth in wine. Finally, note that the relatively high diacetyl values obtained during this test were probably due to the fact that the wine was supplemented with citric acid, which also confirmed that the risk of diacetyl production increases concurrently with wine citric acid content.

ACV (% VOL)	15		12	
Temperature	16°C (60°F)	25°C (77°F)	16°C (60°F)	25°C (77°F)
Diacetyl mg/L	2.3 ± 0.7	33.5 ± 1.1	7.2 ± 0.9	5.4 ± 0.9

Table 1. Effect of temperature and alcohol content by volume (ACV) on diacetyl production at the end of MLF in a wine with a high initial citric acid content (2.5 g/L) (laboratory test).

It should also be emphasised that the duration of MLF plays a decisive role in diacetyl production. Irrespective of circumstances, the longer it takes to complete MLF, the larger the quantity of citric acid degraded and therefore the greater the risk of diacetyl production. If MLF has started but completion is sluggish, then this is a sign that the bacteria are having problems and will probably start to degrade citric acid.
Finally, note that the final concentration in wine is also moderated by the rate of diacetyl reduction. Indeed, like all ketones, diacetyl is an unstable compound which can be rapidly reduced to the corresponding alcohol – acetoin and then butanediol in this case. These molecules are much less odoriferous than diacetyl, therefore the diacetyl formation rate depends not only on the citric acid degradation rate, but also the acetoin conversion rate. This conversion is carried out by *Saccharomyces* yeasts, in particular when they are in decline at the end of AF, as well as *O. oeni*, also during their decline but at the end of MLF (Martineau and Henick-Kling 1995).

HOW CAN THE DIACETYL CONTENT IN WINE BE CONTROLLED AT THE END OF MLF?

In view of the previous observations, the first essential point is to ensure that the medium is as favourable as possible for the bacteria and that conditions are optimal for MLF, i.e. moderate sulphiting of the grapes to restrict the amount of residual SO_2 when the bacteria are added, and maintaining stable temperatures as close as possible

to 20°C (68°F) during MLF. The choice of yeast strains is also a major factor as, firstly, the quantity of pyruvate varies at the end of AF depending on the yeast strain, and secondly, the yeasts interact with the bacteria in several ways (Alexandre et al. 2004). Different yeasts make the medium more or less favourable to bacterial growth, depending on their production of SO_2 and medium-chain fatty acids (Murat et al. 2007). The yeast autolysis rate at the end of AF also plays a key role as, firstly, it adds nutrients required by the bacteria to the medium, and secondly, as previously described, the yeasts have a strong diacetyl reductase activity during their decline phase.

It is also essential to use a bacterial strain that is suited to the conditions in the wine. Specially selected bacteria are required for wines with a high alcohol content or low pH. The inoculation stage is also a key factor. While in the past, selected bacteria were added once AF had been completed, co-inoculation techniques – where the bacteria are inoculated into fermenting must – are now increasingly widely used. The main objectives are to complete MLF more rapidly and to maintain effective control of the microbial ecosystem in the wine during fermentation by imposing selected strains of yeast and bacteria. This prevents contamination due to microfloral spoilage (Renouf et al. 2008a), while ensuring more economical and ecological control of MLF. For example, when the bacteria are active at the high temperatures of AF, it is unnecessary to heat the wine, as can be required to complete MLF (Renouf et al. 2008b, Laurent et al. 2009). Adding the bacteria to the must at the beginning of AF also provides them with a more favourable medium for their development. Must is a nutrient-rich, warm and low alcohol environment, which facilitates the rapid adaptation of the bacteria, so they need less citric acid after inoculation. More importantly, the diacetyl produced during MLF may be immediately reduced via the diacetyl reductase activity of the yeast in the medium. As a result, the final diacetyl content of the wine may be halved by co-inoculation, as compared with late MLF.
The timing of post-fermentation sulphiting also has a major impact. Close monitoring of the L-malic acid degradation kinetics during MLF (assays on a twice-weekly basis) is recommended when diacetyl content is a major concern for the winemaker. This enables the winemaker to sulphite the wine rapidly as soon as MLF is complete to avoid the second phase of citric acid consumption, which occurs at that time. When the L-malic acid content is equivalent to that of citric acid (about 0.3 g/L, on average), the bacteria are once again just as likely to use either of these substrates. In many cases, they consume a little citric acid and produce diacetyl at that time; therefore, although the bacteria consume a much higher proportion of L-malic acid during the first part of MLF, when there is no more L-malic acid available, or equal substrate availability, they start degrading citric acid again. This is why it is recommended to sulphite the wine as soon as the L-malic acid content drops below the threshold of 0.2 - 0.3 g/L. In this case, the last few milligrams of L-malic acid are degraded by the residual enzyme activity of the bacterial cells inhibited by sulphiting, which then do not survive long enough to start consuming citric acid.

Finally, the way the wine is stored after MLF (i.e. whether it remains in prolonged contact with the lees or is run-off rapidly) has a major impact on the final diacetyl concentration (Nielsen et al. 1999). The yeast lees reduce diacetyl to acetoin and then butanediol, which have perception thresholds over 100 times higher than diacetyl. Sulphiting also has a significant impact, as diacetyl combination is reversible. When SO_2 is present, the concentration of free diacetyl drops and, on the contrary, its aromatic impact increases when SO_2 levels are inadequate. Of course, good management of the diacetyl content upstream in the process also contributes to reducing the amount of SO_2 required.

CONCLUSION

The degradation of citric acid by lactic bacteria during MLF should not be considered a real problem. First of all, the metabolic pathways for degrading citric acid are necessary for lactic bacteria to perform efficiently in the medium, even under stressful conditions. Secondly, it is possible to prevent excessive production of diacetyl by applying simple rules: using a malolactic starter suited to the conditions, developing early co-inoculation in the most sensitive wines, compliance with proper sulphiting and temperature conditions, regular monitoring throughout MLF to ensure that post-fermentation sulphiting is carried out before the shortage of L-malic acid causes the lactic bacteria to consume citrate, and finally, regular monitoring of free SO_2 following post-fermentation sulphiting. These simple measures are much more effective than using lactic bacteria that are incapable of degrading citric acid, as these bacteria are less efficient at malolactic conversion, which raises several other significant quality control issues.

Finally, it should be noted that its ketone functions make diacetyl a highly reactive compound capable of combining with S-based amino acids to produce odoriferous molecules with desirable floral or toasty aromas. A great deal of further work is required to clarify the role of MLF in developing wine aroma and flavour (De Revel et al. 1999, Malherbe et al. 2009), but it is quite clear that preventing the controlled production of diacetyl probably means depriving the wine of certain compounds that contribute to its aromatic complexity at the end of MLF.

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MALOLACTIC NUTRITION

PRODUCT	PURPOSE	PREPARATION	SPECIAL CONSIDERATIONS	ADDITION RATE RANGE
MALOBOOST®	Promote MLF activity and kinetics.	10 x weight in water or wine.	Use during MLF. Can be added 24 hours after beginning of MLF.	200 - 400 ppm (20 - 40 g/hL)
ENERGIZER®	**LACTOENOS® 450 PREAC** preparation only.	For 50 hL. Add with bacteria to 1L of wine + 1L of water at 68°F (20°C). Follow protocol for inoculation.	**LACTOENOS® 450 PREAC** only. Use as described in protocol.	50 ppm (5 g/hL)
REACTIVATER	**LACTOENOS® B16 STANDARD** preparation only.	For 50 hL Add with bacteria to 5L of wine + 5L of water at 68°F (20°C). Follow protocol for build up.	**LACTOENOS® B16 STANDARD** only. Use as described in protocol.	60 ppm (6 g/hL)

YEAST PRODUCTS

YEAST PRODUCTS

Q&A

YEAST PRODUCTS

Through original research and sponsoring multiple PhD theses over two decades, LAFFORT® has created a range of specialty winemaking products rich in naturally occurring compounds derived from yeast cells.

1. Which yeast products can I use during fermentation?

Yeast products are used during fermentation to facilitate fermentation, promote gentle fining action, improve sensory attributes, and contribute beneficial antioxidants to preserve aromatics. Yeast products developed for fermentation generally have insoluble yeast fractions, which will settle out over time.

OENOLEES® and **POWERLEES® ROUGE** are excellent for red wines, rich in insoluble yeast cell wall constituents that provide a gentle fining, add perception of sweetness, and detoxify the wine to help ensure a healthy alcoholic fermentation. **POWERLEES® ROUGE** also contains β-glucanase, to help promote yeast autolysis and produce wines with better sweetness and mouthfeel earlier.

FRESHAROM® is rich in glutathione, an antioxidant, to help protect fermentation esters in white and rosé wines from oxidation during aging and in the bottle. **FRESHAROM®** is added at the second nutrient addition, around one-third of fermentation completion, and will also contribute to sweetness and mouthfeel.

YEAST PRODUCT APPLICATIONS

OBJECTIVE	GRAPE OR MUST TYPE	YEAST PRODUCT	DOSAGE	ADDITION TIMING	NOTE
Provide antioxidant post fermentation.	White & Rosé	**FRESHAROM®**	200 - 300 ppm	First one-third of alcoholic fermentation	Correct any nitrogen deficency prior to addition.
Improve aging potential.	White & Rosé	**FRESHAROM®**	200 - 300 ppm	First one-third of alcoholic fermentation	Correct any nitrogen deficency prior to addition.
Gentle fining activity.	White, Rosé & Red	**OENOLEES®**	200 - 400 ppm	Alcoholic fermentation Aging Final correction	Requires at least 4-6 weeks settling prior to bottling.
Increase mouthfeel, gives a perception of sweetness, and increases fruit aromas.	White, Rosé & Red	**OENOLEES®**	200 - 400 ppm	Alcoholic fermentation Aging Final correction	Requires at least 4-6 weeks settling prior to bottling.
Increase mouthfeel, gives a perception of sweetness, increases fruit aromas.	Red	**POWERLEES® ROUGE**	150 - 400 ppm	Alcoholic fermentation Aging Final correction	Requires at least 4-6 weeks settling prior to bottling.
Make wines more approachable with reduced aging.	Red	**POWERLEES® ROUGE**	150 - 400 ppm	Alcoholic fermentation Aging Final correction	Requires at least 4-6 weeks settling prior to bottling.
Add roundness on palate. Fills in texture gaps.	White, Rosé & Red	**MANNOFEEL®**	25 - 150 mL/hL	Aging Final correction	Add to wine at least 48hrs prior to bottling.
Contribute sweetness sensation.	White, Rosé & Red	**AUTOLEES®**	100 - 300 ppm	Aging Final correction	Add prior to cellar filtration.
Reduce phenolic, green and astingent characters.	Red	**AUTOLEES®**	100 - 300 ppm	Aging Final correction	Add prior to cellar filtration.

2. Do yeast products contribute to YAN during fermentation or aging?

Healthy fermentations require certain levels of assimilable nitrogen to thrive and yeast will use any available food source when those needs are not met. The best way to ensure you are maximizing the benefits of these products is to manage your YAN with proper yeast nutrition using DAP, **THIAZOTE® PH**, **NUTRISTART®** and **NUTRISTART® ORG**. Yeast products are considered supplemental to the nutrition program for your fermentation and do facilitate fermentation.
While small amounts of nitrogen may be present post fermentation, yeast products will not contribute to YAN during aging or cause unwanted microbial growth.

3. Is there an easy way to measure the concentration of glutathione?

Glutathione is a tripeptide made of three amino acids: cysteine, glutamate, and glycine naturally found in grapes which act as a powerful antioxidant. **LAFFORT®**'s **FRESHAROM®** is an inactivated yeast rich with glutathione precursors (*cysteine, N-acetyl cysteine*) that are assimilated and metabolically converted to glutathione. This is released into the wine to preserve freshness and inhibit browning and other oxidative characteristics.
There are relatively quick over-the-counter products available for testing glutathione in the human body, however, for the most reliable results in wine it is recommended to use Ultra High-Performance Liquid Chromatography techniques. ETS Labs in St Helena can perform Glutathione tests, and **LAFFORT®** performs this test and much more at the **EXCELL** Laboratory in Bordeaux, France.

4. What sensory impact can I expect?

FRESHAROM® acts as an antioxidant, protecting the youthful fruit and fermentation aromas and flavors from oxidation in white and rosé wines. Additionally, **FRESHAROM®** can enhance the sweetness perception and build more mouthfeel in the wine.
POWERLEES® ROUGE and **OENOLEES®** are both equipped to reduce astringency and improve aging, although **POWERLEES® ROUGE** has the added benefit of a β-glucanase enzyme to aid autolysis for early-to-market wines. Typical sensory changes can include softening of astringency, improved mouthfeel and mid-palate weight, increased perception of sweetness, reducing green character, improved fruitiness, and freshness as well as aromatic intensity.
MANNOFEEL® and **AUTOLEES®** are two other yeast products in the **LAFFORT®** line-up that are rich in mannoproteins for tartrate stability and offer multiple benefits of mouthfeel and sensory improvement. These products are fully soluble and are typically used during aging and before bottling.

5. Can yeast products help me reduce SO_2 levels?

SO_2 is the Dr. Jekyll and Mr. Hyde of winemaking. It is essential as an anti-microbial agent and as an antioxidant, it is also reviled by the natural wine movement, toxic in extreme doses, an allergen, and tastes disagreeable at high levels. Yeast-derived products can certainly aid in reducing SO_2 but will not completely replace it.
Reducing the need for SO_2 can be accomplished with yeast products that contribute to natural yeast-derived glutathione levels such as **FRESHAROM®**. Glutathione is a powerful antioxidant, substantially more than SO_2. The decrease in need for SO_2 will show during the aging period as the glutathione protects the wine.
Contact your **LAFFORT®** representative for reduced SO_2 winemaking products and protocols, and see page 145-147.

6. How quickly do yeast products react, and how close to bottling can they be added?

Yeast products are divided neatly into those that are 100% soluble and those that are not, depending on the parts of the yeast cell that are used.
Fermentation products that contain yeast cell walls are not fully soluble, including **FRESHAROM®**, **OENOLEES®**, **POWERLEES® ROUGE**, and **POWERLEES® LIFE**.
FRESHAROM® is only used during fermentation, its properties are dependent on the action of yeast on a succession of precursors, and its effects are found through the life of the wine. These reactions happen during fermentation, and the insoluble components are removed by racking.
POWERLEES® ROUGE and **OENOLEES®** offer light fining and sensory improvement within a couple days and can also benefit sur-lie aging over several weeks. They can be added during fermentation and always take some weeks to settle out of wine before racking: it is recommended to add these products at least several weeks before bottling.
MANNOFEEL® and **AUTOLEES®** are fully soluble extracts of yeast cell walls, formulated to react quickly and can be added to the wine right up to the week before bottling.

7. Will Yeast Products interfere with filtration?

For products containing inactivated yeast and yeast cell walls such as **POWERLEES® ROUGE**, **OENOLEES®**, and **FRESHAROM®**, it is recommended to rack off before filtering. Specially formulated products containing mannoproteins, such as **MANNOFEEL®**, generally will not interfere with filtration and may be added post cellar filtration. With **AUTOLEES®**, there is a risk of increasing the clogging index in reaction with other wine colloids, and it is recommended to add **AUTOLEES®** before prefiltration bottling. Always check with your **LAFFORT®** technical representative to confirm the timing of your addition.

8. Are there differences between inactivated yeasts, yeast cell walls, yeast derivatives, and yeast products?

Yes! Yeast-derived products are made of the different parts of the yeast cell, including the entire inactivated cell, the cell wall, and the cell interior. Each product has a unique function for winemaking. For each yeast derived product, there is a different blend of the yeast fractions. These blends are developed through extensive research and trials.

- 'Inactivated yeasts' are where the yeast cell is intact, but not active. This part is used in nutrition, detoxification, and glutathione products.
- 'Yeast autolysates' are the intracellular contents (cytoplasm) used for nutrition, detoxification, and glutathione products.
- 'Yeast cell walls' (a.k.a. yeast hulls) contain sterols, long chain fatty acids, polysaccharides, and mannoproteins. Yeast cell walls may be used intact for detoxification, such as **OENOCELL®** and **BI-ACTIV®** or may be extracted to obtain the soluble fractions which help with mouthfeel, sweetness, and detoxification.

9. What are mannoproteins?

Mannoproteins are a group of proteins found in yeast cell walls linked by β-glucan chains. Mannoproteins were first studied in winemaking for their ability to stabilize tartrates without seeding or refrigeration. They have also been found to provide many beneficial winemaking properties including building mouthfeel. **MANNOFEEL®** and **AUTOLEES®** are two mannoprotein based products from **LAFFORT®** that in addition to tartrate stability also offer sensory improvement through increased freshness, perception of sweetness, and volume. The best way to experience these attributes is to perform bench trials and see the changes for yourself. Ask your **LAFFORT®** Technical Representative for more information on bench trials or acquiring samples.

10. Why are yeast products sometimes blended with enzymes or chitosan?

LAFFORT® offers **MICROCONTROL®**, a combination of yeast derivatives and chitosan. Chitosan has been shown to be effective at disrupting the cell membrane in many microbes, especially *Brettanomyces*, essentially controlling spoilage. The combined effect of yeast products with chitosan can offer gentle fining action to improve microbial control as well as clarity and filterability. **POWERLEES® ROUGE** contains β-glucanase, an enzyme that aids in yeast autolysis, and in combination with yeast products will help improve filterability and build mouthfeel.

STORAGE AND PREPARATION

PRODUCT	STORAGE TEMPERATURE AND PLACE	SHELF LIFE UNOPENED AND OPENED	PREPARATION
FRESHAROM®	Dry area, moderate temperature.	3 years unopened, use quickly once opened.	Add directly to wine.
OENOLEES®	Dry area, moderate temperature.	3 years unopened, once opened use within 1 month.	Mix in 5 - 10 x volume of water.
POWERLEES® ROUGE	Dry area, moderate temperature.	3 years unopened, use quickly once opened.	Mix in 5 - 10 x volume of water.
MANNOFEEL®	Dry area, moderate temperature.	2 years unopened, once opened store in refrigerator and use within 3 months.	Add directly to wine.
AUTOLEES®	Dry area, moderate temperature.	2 years unopened, once opened keep well sealed and use within 1 month.	Mix in 5 - 10 x volume of water.

THE ORIGIN OF SWEETNESS IN DRY WINES

Axel MARCHAL & **Philippe MARULLO**, Institut des Sciences de la Vigne et du Vin, University of Bordeaux, France.

This work has been carried out as part of the theme covering research on identifying wine quality markers. It is derived from a collection of research projects carried out in the 2000s, starting with Anne Humbert's thesis on enzymatic phenomena that intervene during wine ageing. This work showed that the sapid fraction released during maturing on lees is made up of small-sized peptides, between 0.5 and 3 KDa. Subsequently this peptide fraction was purified from autolysis of a yeast and LC-MS-MS analysis made it possible to identify peptides from the membrane protein Hsp (Heat shock protein). This second research project resulted in a patent family (Moine V., 2005) and in the development of **OENOLEES®**. A few years later Axel MARCHAL resumed this work as part of his PhD and is currently carrying out research on sweetness in wines and the role of the Hsp12 protein.

THE ROLE PLAYED BY THE Hsp12 PROTEIN IN THE SWEET FLAVOR OF DRY WINES.

The gustatory balance of a wine comes not only from its tannin structure and its acidity, but also from its sweetness. Yet the molecular origin of the sweet taste in dry wines has long been a mystery, despite the importance of this perception as part of consumer appreciation.

To verify the increase in sweetness associated with the presence of this protein because of yeast autolysis in dry wines, molecular biology and sensorial analysis techniques were jointly implemented (Figure 1). A "mutant zero" Δ° Hsp12 was created using the oenological yeast *Saccharomyces cerevisiae* **ZYMAFLORE® FX10** which thus differed solely from **ZYMAFLORE® FX10**, by the absence of the Hsp12 gene. The yeasts were introduced into a dry wine at identical concentrations (2 x 10^8 cells/mL) and stored at 32°C (90°F), consistent with oenological conditions after primary fermentation. A triangular test was carried out and showed a significant organoleptic difference between the 2 modalities at the 5% threshold. The presence of the Hsp12 protein was associated with an increase in sweetness, confirming the implication of this protein in the association between increased sweetness and autolysis. This research was continued and applied to the effect of several fermentation parameters potentially able to modulate sweet flavor intensity.

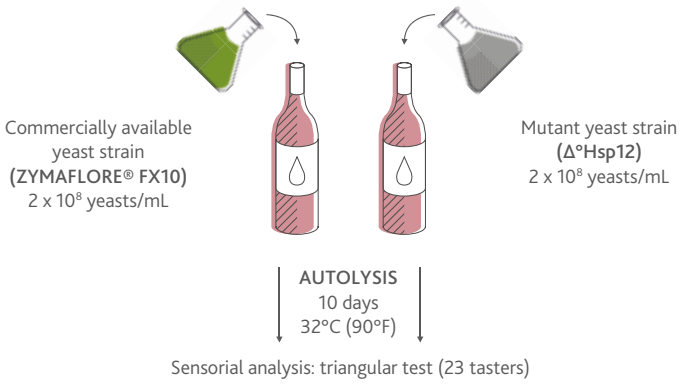

Figure 1. Protocol for identifying the role of the Hsp12 protein in the increase in sweet flavor because of yeast autolysis.

PARAMETERS THAT INFLUENCE THE LEVEL OF EXPRESSION OF THE HSP12 GENE.

Fermentations were carried out in a synthetic medium using two oenological strains **ZYMAFLORE® FX10** and **ZYMAFLORE® VL3**. The level of expression of the Hsp12 gene was measured for each strain, at different stages of alcoholic fermentation (after release of 30, 46, 55, 66 and 76 g/L of CO_2). The results presented in Figure 2 show that the level of expression of Hsp12 gene increased progressively as alcoholic fermentation progressed for both strains. This experiment confirms previous work and suggests that the ethanol produced during alcoholic fermentation could cause stress that lies behind the increased

expression of the Hsp12 gene. While ethanol has a sweet taste when tasted at low concentrations in water, we previously showed that the addition of 1.5% of ethanol to a dry wine does not modify the perception of its sweet flavor. However, expert tasters frequently perceive an intense sweetness in wines with a naturally high degree of alcohol from fermentation. This could be explained by the inducing effect of ethanol on expression of the Hsp12 gene: in wines with a high degree of alcohol, synthesis of the Hsp12 protein was greater at the end of alcoholic fermentation, giving the wine a more intense sweet flavor.

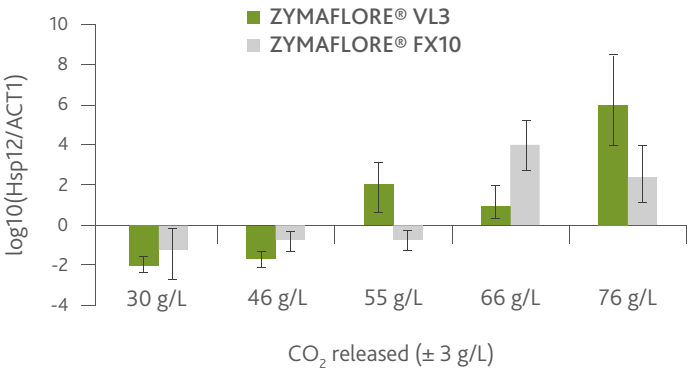

Figure 2. Monitoring Hsp12 gene expression of 2 yeast strains **ZYMAFLORE® FX10** and **ZYMAFLORE® VL3** during alcoholic fermentation at 26°C (79°F).

A wine without the Hsp12 protein was obtained by fermenting a Merlot must derived from thermovinification by the strain Δ° Hsp12 to dryness. Also, the biomass of eight other yeast strains previously studied was collected halfway through their fermentation. This biomass was added to the dry Merlot wine at the completion of alcoholic fermentation, at concentrations like those found at the end of vinification. As the wine was dry, the yeasts could not develop. To encourage their autolysis, the wines were placed at 32°C (90°F) for 10 days, to imitate the conditions of hot post-fermentation maceration. The tasting panel then graded the sweet flavor on a scale of 0 to 7 (Figure 3).

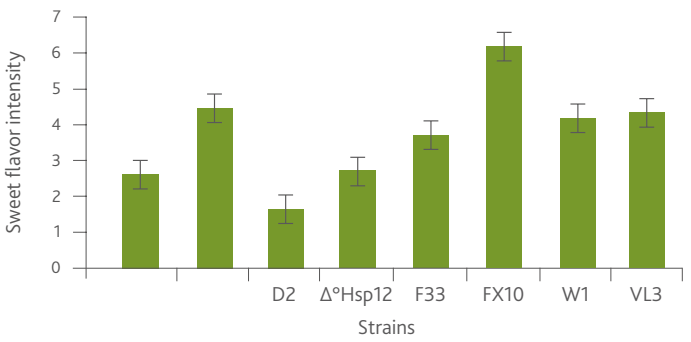

Figure 3. Impact of yeast strains on sweetness perception after autolysis.

The results showed a strong strain effect, i.e., the tasters perceived clear variations in sweetness according to the strain of yeast used for autolysis. It was observed that **ZYMAFLORE® FX10** presented a higher sweetness intensity than that which was suggested by its level of Hsp12 gene expression. This could be explained by the post-fermentation mechanisms involved in the release of the Hsp12 protein and its sweet peptides. In any event, a statistical test indicated a correlation between these sensory data and the expression level of the Hsp12 gene for most of the strains (p-value = 0.06).

CONCLUSION

These results demonstrated for the first time that yeast strain has a significant influence on wine sweetness perception. The differences in sweet flavors are correlated with variations in the coding gene for the Hsp12 protein.

This research opens new perspectives for management of taste during wine production. It needs to be pursued to identify the sweet peptides derived from the Hsp12 protein and to specify the post-fermentation conditions that encourage their release into the wine. Furthermore, the yeast's aptitude to increase sweetness in wines is a new criterion to be taken into consideration for the selection of new enological strains.

ARTICLES PUBLISHED ON THE SUBJECT

Marchal, A., Marullo P., Durand C., Moine V. & Dubourdieu D. 2015. Fermentative conditions modulating sweetness in dry wines: genetics and environmental factors influencing the expression level of the Hsp12 gene. Journal of Agricultural and Food Chemistry. 63 (1): 304–11.

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OUR VALUES

THE LAFFORT® VALUES

At **LAFFORT®** we are committed to sustainable development and we believe that our company should create value, not only for its customers but also for its employees and partners.

Therefore, we should adopt responsible conduct and ensure that our activities address issues relating to the environment, social equity and economic viability.

To do this, we rely on the shared values of the people who work daily for the development of **LAFFORT®**:

AGILE

BETTER SERVING OUR CUSTOMERS

- Responsiveness
- Practicality
- Flexibility
- Proactiveness

INTEGRITY

STRIVING FOR EXCELLENCE

- Performance
- Skill
- Perseverance
- Quality

INNOVATIVE

CREATING VALUE

- Creativity
- Enthusiasm
- Initiative
- Forward-looking

UNITED

SUCCEEDING TOGETHER

- Well-being
- Team spirit
- Goodwill
- Trust

ENZYMES

ENZYMES

APPLICATIONS

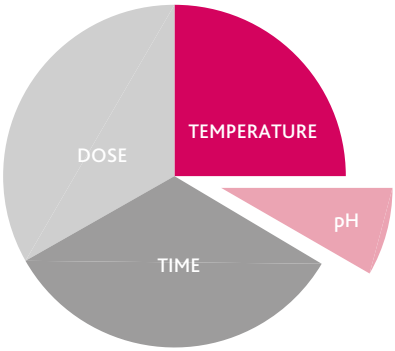
TIMING	OBJECTIVE	GRAPE OR MUST TYPE	ENZYME	FORMAT	DOSE	NOTE
Coat evenly over grapes during loading of press.	Increase press yields.	White & Rosé juice	LAFAZYM® PRESS	Granulate	20 - 50 g/ton	Duration of pressing cycle.
		White & Rosé juice	LAFASE XL® PRESS	Liquid	10 - 40 mL/ton	
	Reduce maceration time.	White & Rosé juice	LAFAZYM® PRESS	Granulate	20 - 50 g/ton	
		White & Rosé juice	LAFASE® XL PRESS	Liquid	10 - 40 mL/ton	
	Accelerate pressing activities in difficult varieties.	White & Rosé juice	LAFASE® BOOST	Liquid	1 - 1.5 mL/hL	2-4 hours contact time. Use with a clarification enzyme.
	Extract flavor from skins.	White & Rosé juice	LAFAZYM® EXTRACT	Granulate	20 - 40 g/ton	During skin contact and during pressing cycle.
	Speed extraction of flavor from skins and improve pressing yield.	White & Rosé juice	LAFASE® XL EXTRACTION ROUGE	Liquid	20 - 40 mL/ton	
Add to juice immediately after pressing.	Fast depectiniztion for flotation.	White & Rosé juice	LAFASE® XL FLOT	Liquid	1 - 4 mL/hL	Up to 24 hours for juice. Minimum 2 hours.
	Accelerate clarification in difficult varieties.	White & Rosé juice	LAFASE® BOOST	Liquid	1 - 1.5 mL/hL	
	Clarification at low temperatures.	White & Rosé juice	LAFAZYM® 600 XL ICE	Liquid	0.5 - 2 mL/hL	
	Clarification and lees compaction.	White & Rosé juice	LAFAZYM® CL	Granulate	0.5 - 2.0 g/hL 5 - 20 ppm	
	Speedy clarification.	White, Rosé, Red & Thermo Vinified juice	LAFASE® XL CLARIFICATION	Liquid	1 -3 mL/hL	
Add during fermentation.	Increase aromatic potential.	White & Rosé	LAFAZYM® THIOLS[+]	Liquid	3 - 6 g/hL 30 - 60 ppm	Can be added up to first 1/3 of fermentation.
Add to grapes during destemming or at first pumpover.	Increase color and tannin extraction.	Red grapes	LAFASE® HE GRAND CRU	Granulate	25 - 45 g/ton	From cold soak through pressing.
		Red grapes	LAFASE® XL EXTRACTION ROUGE	Liquid	20 - 40 mL/ton	
	Extract fruit flavors and aromatics.	Red grapes	LAFASE® FRUIT	Granulate	25 - 45 g/ton	
Add after pressing for whites and rosé, or at destemming for reds. Can be used on finished wine.	Enhance clarification and filtration.	White, Rosé & Red juice or must	EXTRALYSE®	Granulate	5 - 10 g/hL 50 - 100 ppm	24 hours for juice. Up to 6 weeks for wine.

Q&A

ENZYMES

Enzymes occur naturally in grape berries and microorganisms in varying concentrations. Adding enzymes during vinification promotes clarification, extraction of skin compounds of interest, and optimizes pressing. Mastery of the use of enzymes allows for a reduction in the use of other products and eases winemaking downstream.

1. What are the main factors affecting enzyme activity and how do they relate to each other?



Enzyme activity rates are influenced by temperature, contact time, dose rate, and pH. Cooler temperatures require higher dose rates, or more time. When time is short, warmer temperatures and/or higher dose rates are required. To minimize dosage, allow warmer temperatures and/or more time.
Another key factor for enzymes is ethanol. Pectin chains will curl up when alcohol levels increase, making the sites harder to access for enzymes. Thus, enzymes are more effective on juice than on wine.

Temperature
Enzymes are sensitive to temperature but do have a wide range for activity. In general, lower temperatures will slow enzyme activity, and higher temperatures will speed up activity. Enzymes are proteins, and will denature at higher temperatures, hence it is important not to add enzymes directly to must during or right after flash détente, allow the must to cool to 60°C (140°F) first. Certain enzymes are more active at different temperatures, for example, enzymes such as **LAFAZYM® 600 XL**^{ICE} can maintain high activity levels down to 5°C (41°F) while **LAFASE® XL EXTRACTION** can function up to 55°C (130°F), making it a good choice for thermo-vinified musts.

pH
Enzymes work well within normal winemaking pH ranges, 3.0 - 4.0. pH is considered a minor factor for enzyme performance, as most variation occurs at extremes, for example pH over 5.0. Special formulas, such as **LAFAZYM® 600 XL**^{ICE}, retain activity well below pH 3.0.

Time
Use enzymes as early as possible in winemaking as the pectin chain is easier to access while in the aqueous phase. If faster turnaround is needed, use more enzyme or warmer temperatures.

Dosage
Refer to the dosage instructions for the enzyme activity you want to achieve within the appropriate time. For most applications, too much enzyme will simply mean faster performance. Too little enzyme will make the process longer, or not happen at all. Beware of too high of a dosage of red extraction enzymes which could result in grapes turning into a soup-like consistency and/or extract too many phenolics.

2. Are there any interactions to avoid when using enzymes?

There are two ingredient additions that can negatively impact enzyme activity; bentonite and tannins. Both bentonite and tannin can bind with proteins in the juice/wine. Enzymes are proteins, so they have the potential of being removed or inactivated by bentonite and tannin additions. In juice clarification, it is important to allow at least 6 hours for the enzyme to depectinize the juice before the bentonite addition. In red musts, it is recommended to add the fermentation tannin first at the crush pad, then add the enzyme with the first tank mixing.

3. What can I use for difficult to clarify juices, like Muscat?

The ideal method to clarify muscat and other difficult varieties is to use enzymes at two stages. First, use a pressing enzyme directly added to fruit. After press, use a settling enzyme to aid in clarifying. Dosages may need to be increased for especially difficult conditions. **LAFASE® BOOST** can be added at the pressing or clarification stage to add breakdown of complex side chains found in difficult to clarify varieties that inhibit enzyme performance. If only one addition is possible due to production constraints, mix a pressing and clarification enzyme, and add both at clarification.

4. Does bentonite help juice settle with enzyme treatment?

Adding bentonite with an enzyme will deactivate the enzyme, so it is important to delay the bentonite addition to allow time for the

enzyme to work. Bentonite can assist in settling and give excellent juice compaction, especially with the calcium based bentonites. Be sure that enzymatic activity is completed as measured by the pectin test before adding bentonite to juice.

5. How do I apply enzymes directly to white grapes before pressing? How long should I leave the enzyme before pressing?

Enzymes can be diluted even further to be easily sprayed to evenly coat clusters in bins, on destemmed fruit, or while fruit is being loaded into the press. Enzymes can also be applied through a dose pump post destemming when being pumped to the press. The key is to evenly apply the enzymes to all the fruit. We recommend at least one hour of contact time to grape clusters prior to pressing.
Avoid loading the press fully before adding enzyme: the enzymes will form pockets and be less effective. A layering approach would work best.

6. How does the Pectin Test work?

It is important to know that your wines are fully depectinized at juice stage and this can be easily measured with the pectin test. The pectin test simply adds juice to acidified alcohol. After a certain elapsed time, the juice is observed, and the presence of flakes indicates pectins. The **LAFFORT®** Pectin Test Kit contains 10 test tubes, complete instructions, and a stopwatch. Validating that your juice is pectin-negative is critical for effective flotation.

7. What enzymatic characteristics are best for flotation?

The key to effective flotation is complete depectinization of the juice. When pectin is present, juice will not have a clean flotation or separation of solids. Depectinization of white and rosé juice can be problematic at low temperatures, low pH, and with grape varieties containing highly branched pectin chains. These difficult conditions often add up and can be aggravated by winery time constraints. For these reasons, it is ideal to use an enzyme that has high activity at low temperatures and low pH. **LAFASE® XL FLOT** is a liquid enzyme developed for rapid depectinization in low temperatures and can complete activity in less than 2 hours. In further difficult conditions, **LAFASE® BOOST** can be used in addition to aid depectinization.

8. How do enzymes increase press yields or lower the pressure requirements?

By using enzymes such as **LAFAZYM® PRESS** or **LAFASE® XL PRESS** on whole cluster grapes the enzymes can begin breaking down the pectins allowing for increased yields at lower pressures, and/or higher total yields. Pressing enzymes increase the total free-run volume before needing to make a press cut and increase the total pressing volume.

9. What are the benefits of using enzymes in red wines?

Color and tannin extraction are the main benefits of using enzymes in red wines during fermentation. Additional benefits include faster settling and improved filtration later in the wine's life. Pectins can prevent wine clarification during aging, and pectins are much more difficult to breakdown in wine compared to juice. We observe more microbial issues in wines that have settling problems during aging. Review our list of enzymes to find one that fits your wine style. In general, **LAFASE® HE GRAND CRU** is for bigger structured red wines, and will extract more polysaccharides from the grape skin contributing to mouthfeel. **LAFASE® FRUIT** is formulated to focus on extracting anthocyanins, and aroma and flavor compounds for early release red wines. **LAFASE® XL EXTRACTION ROUGE** is a broad-spectrum liquid enzyme to increase yields, increase color and tannin, while also improve clarification.

10. Is there a sensory impact from enzymes, or are they just for processing?

Enzymes have a very wide range of activities beyond simply breaking down pectins. For red wines, **LAFASE® HE GRAND CRU** has hemicellulase & cellulase activities to break down the structural components of the grape cell wall, releasing juice, aroma, and color, and has additional side chain activity rhamnogalacturonase-II, which cleaves polysaccharides, resulting in more mouthfeel and sweetness. **LAFASE® FRUIT** has additional polygalacturonase to enhance cold soak maceration for improved aromas, and **LAFAZYM® THIOLS[+]** has secondary activities formulated for better thiol revelation in fermentation.
The ß-glucanase activity in **EXTRALYSE®** has two key properties. First it breaks down glucan chains in wines, especially those affected by *Botrytis cinerea*, leading to greatly improved filtration. Second, the activity improves the efficiency of autolysis during aging in wines aged on lees, leading to improved mouthfeel.

11. Is it best to add multiple enzymes at the same time or sequentially?

Enzymes typically have different functions and are used at different and specific stages of winemaking. Adding a pressing enzyme directly to grapes such as **LAFAZYM® PRESS** can improve yields at lower pressures. This can be coupled with addition of **LAFAZYM® CL** during settling, which aids in lees compaction improving clean juice yield. At fermentation **LAFAZYM® THIOLS[+]** can enhance thiol revelation and **EXTRALYSE®** can improve filterability. **LAFFORT®** technical representatives can assist in deciding where and when it is most beneficial to add enzymes for your specific stylistic and efficiency goals. For reds, adding an enzyme such as **LAFASE® HE GRAND CRU**, **LAFASE® FRUIT**, or **LAFASE® EXTRACTION ROUGE** can increase color, tannin, flavor extraction, while also reducing cold soak times, depending on which formula is best for your fruit. **EXTRALYSE®** can be used to improve filterability or increase autolysis during aging.

12. If I have botrytis on my grapes, what is the best enzyme treatment strategy?

Botrytis cinerea has two major negatives aspects in winemaking. The mold secretes long chain glucan molecules in grape juice making it highly viscous and difficult to filter, and contains laccase, an SO_2-resistant enzyme that causes browning in juice and wines. One of the first steps in **LAFFORT®**'s *Botrytis Infected Fruit Protocol* on pages 148 - 150 is to determine the level of laccase activity from the percentage of rot in the fruit. The next step is to add tannins based on the corresponding level to bind with laccase and prevent oxidative activity. ß-glucanase enzymes in **EXTRALYSE®** can then break down these glucans into shorter chains making them easier to filter.

13. What is the difference between Polygalacturonase (PG), Pectin Lyase (PL), and Pectin Methyl Esterase (PME)?

Grape juice contains pectin, comprised of a main chain, which may be esterified with methyl groups and multiple side branches and activities. The most important 'pectic' enzymes are Pectin Lyase (PL) and Polygalacturonase (PG). PL will cleave the pectin chain where there are methyl groups attached. PG only cleaves the pectin chain when there is no esterification with methyl groups.
Grape pectin structure changes with ripening towards the non-methylated structure because of the third 'pectic' enzyme, Pectin Methyl Esterase (PME). PME removes methyl groups from the pectin chain, and effectively helps the PG activity.
Each of the three major pectic enzymes also has exo- and endo-activities. The exo-pectinases will act on the ends of the chain, while endo-pectinases will act in the middle of the chain. A blend of exo- and endo-pectinases are important to efficiently break down pectin chains.

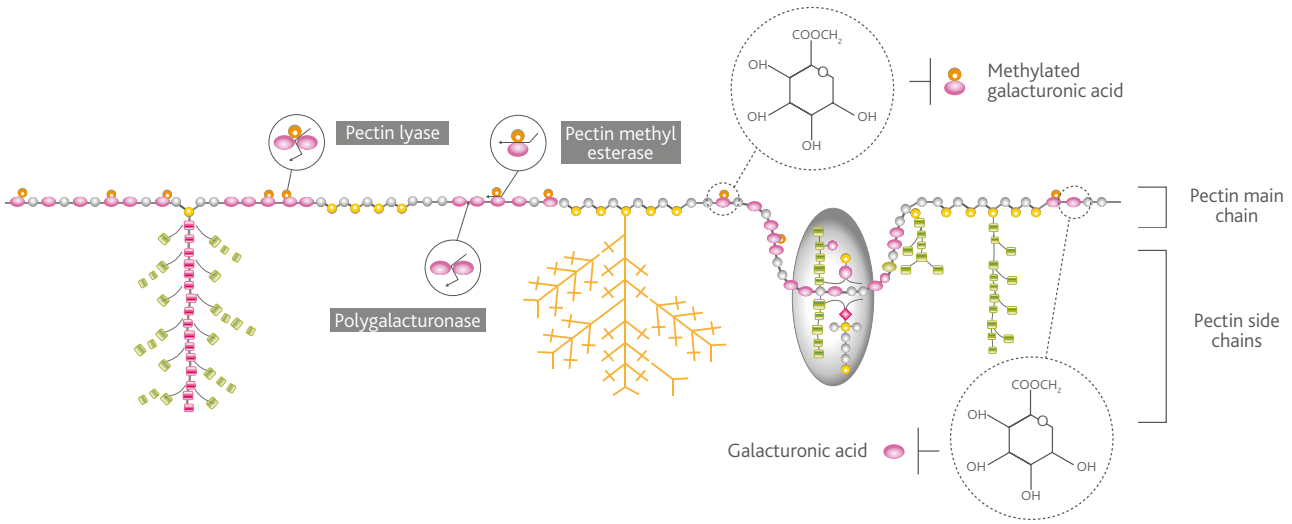
Commercial enzymes are composed of all three of the major pectic enzymes, with varying amounts of endo- and exo activities. The relative proportions of each activity mark its efficacy for application. For example, PL is very important in settling and flotation enzymes. PG is important in grapes with high maturity and in maceration of white and red musts.

14. What is the difference between a primary activity and a secondary activity?

Pectic enzymes are produced primarily by fermentation of *Aspergillus niger* and *Aspergillus aculeatus* under specific conditions. The three primary activities produced include Polygalacturonase (PG), Pectin Lyase (PL), and Pectin Methyl Esterase (PME). Just as varying the temperature and nutrient regime in making wine with *Saccharomyces cerevisiae*, varying conditions of fermentation of *Aspergillus* species will produce different by-products, or side-activities. Some of the more widely found secondary activities include *Rhamnogalacturonase I* (RGI), *Rhamnogalcturonase II* (RGII), *Hemicellulase* (HE), *Cellulase* (CEL), and *Cinnamoyl Esterase* (CE).

As shown in the diagram below, pectin molecules can be highly branched with lots of "hairy" side chains attached, making it difficult for the main enzymes (PL, PG, PME) to break down the main chain. It is the action of the side activities that can remove the side chains so the main enzymes can have access to the pectin main chain.
Also, activities on side chains can have qualitative impacts, including *Rhamnogalacturonase II*, a specific enzymatic activity found in **LAFASE® HE GRAND CRU**, which cleaves off the rhamnogalacturonan II side chains and extracts polysaccharides. These side chains interact with tannins to build mouthfeel and structure.

PECTIN POLYMER SHOWING STRUCTURAL COMPONENTS AND SITES OF ENZYME ACTIVITY.



Pectinase enzyme activity in red wine grape processing is targeted towards degradation of the main backbone of the pectin molecule. The concerted activity of pectin lyase, pectin methyl-esterase and polygalacturonase break the main rhamnose-galacturonic acid chain resulting in enhanced extraction of both tannin and colored anthocyanin molecules. Doco T. et al (1995). Les polysaccharides pectiques de la pulpe et de la pellicule de raisin. Quel devenir pendant la phase préfermentaire? Rev. Fr. Oenol., 153, 16 – 23.

15. How is enzyme performance measured?

Enzyme production creates multiple main and side chain activities, and all **LAFFORT®** enzymes are blends of multiple production runs. Most commercial enzymes will have their activity rated as either Polygalacturonase or Pectin Lyase activity units, and these are generally rated on apple juice although **LAFFORT®** tests all enzyme activities on grape juice and wine.
Be sure to calculate dosage with activity, time, and cost to ensure you are getting the maximum value and benefit for your wines.

16. Are there any negative enzyme activities?

What makes one commercial enzyme better than the other is fitness for purpose, and whether they are sourced appropriately and purified, free of undesirable side activity leading to the production of vinyl-phenols or off-flavors. *Cinnamoyl Esterase* (CE) is produced by some species and strains of *Aspergillus*. CE can convert hydroxy-cinnamoyl esters in juice to hydroxycinnamoyl acid that Phenolic Off Flavor positive POF(+) yeast strains will metabolize into vinyl phenols, giving off aromas of paint, leather, and vinyl. It is important to use enzymes that are purified or have naturally low levels of CE, or use Phenolic Off Flavor negative POF(-) yeast strains. With low quality enzymes, there is a risk of developing negative compounds or faults in your wine.

ENZYME STORAGE AND PREPARATION

PRODUCT	STORAGE TEMPERATURE & PLACE	SHELF LIFE UNOPENED & OPENED	PREPARATION
Granulate enzymes	Cool, Dry, < 25°C (77°F)	Four years unopened, use within two months when opened.	Dilute 10:1 in water
Liquid enzymes	Refrigerated < 10°C (50°F)	Two years unopened, use within two months when opened.	Dilute 10:1 in water

ARTICLE

NEW KNOWLEDGE ON GRAPES CELL WALL STRUCTURE & THE EFFECTS OF MACERATION ENZYMES

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INTRODUCTION

One of the major limitations in understanding the impact of commercial maceration enzyme preparations has been the limited knowledge of the grape cell wall. Whilst many studies have looked at components of the cell wall, the methods for testing the individual components has been limited. Recent work by Gao et al (2016) proposed a new cell wall structure based on a newly developed method to gain a better understanding of the individual components in-situ.

Wine quality is a result of vintage, varietal, grape health, vineyard and winemaking practices. Climatic anomalies can affect the grape development and resultant wine quality. Whilst white wines may be pressed straight away, red grapes are normally crushed to allow for maceration during alcoholic fermentation. The objective of this maceration period is to extract beneficial compounds from the different fractions of the berry to increase the resultant wine quality, including phenolic compounds, organic acids and sugars. Enzyme preparations may be utilised to enhance the extraction of beneficial compounds from the grape skin due to highly specific enzymatic activites.
Understanding of the grape cell wall enables better understanding of the maceration process but greater control over downstream processing in terms of colouring matter stabilisation, tartrate stabilisation and clarification. This article is a summary of recent findings on the impact of commercial enzyme preparations with new information relevant to grape cell wall structure.

GRAPE BERRY

The grape berry is comprised of three main sections: skin, pulp and seeds (Figure 1). A wax layer of soluble lipids covers the grape berry. Aside from providing some protection from pathogens and regulating water loss, it has been suggested that the wax layer prevents the cell degrading enzymes found here from degrading the rest of the berry (Gao et al 2019). It is only after the grapes are crushed that they may commences degrading the berry.

The skin cells form a condensed layer rich in phenolic compounds, aromatic precursors. It also contains a large percentage of neutral polysaccharides as well as acidic pectic components.

The main storage of sugars is the pulp fraction, rich in sugars and organic acids like tartaric acid. This fraction expands significantly during ripening and contains pectins that are more easily hydrolysable.

The seeds are rich in phenolic compounds including tannins, often undesirable in the high concentrations found here as they may confer harsh, bitter notes.

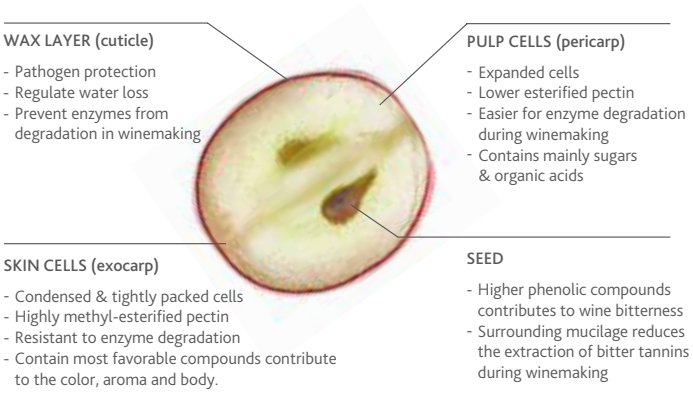

Figure 1. The biological anatomy and biochemical composition of a typical wine grape berry with reference to extractable components taken from Gao and Zietsman et al (2019).

Pectins are a group of polysaccharides derived from plant cell walls have many different forms and properties. Pectins are polyelectrolytes, with gelling behaviour influenced significantly by the presence of Calcium ions (Donald, A.M., 2001). Typically pectins have a backbone that consists primarily of a linear chain of alpha-(1-4)-D-galacturonic acid (GalA) units with rhamnose residues present. They act as a setting agent in fruit jams, but can hinder colouring matter stability and filtration. As the grapes ripen, a large proportion of pectins go from being insoluble to soluble due to specific enzymes in the grapes which are turned on post-veraison. Also, pectins may have a methyl group or an ester group attached making them methyl-esterified. The take-home from this is that many different enzymatic activities are required to break down the cell wall and access specific sugars and phenolics found in the different layers of skin.

INTERACTION OF COMMERCIAL MACERATION ENZYMES WITH GRAPE CELL WALL COMPONENTS – RECENT FINDINGS

Once harvested, maceration of red grapes is influenced by many factors including yeast strain, temperature, SO_2 level, vinification conditions including physical and mechanical interventions (crushing, plunging, pump-overs, header boards). Alternatively, fungal derived commercial enzyme preparations may be used to facilitate maceration process. The research summarised here was carried out by a PhD student Yu Gao in 2016 (South Africa), supervised by John P. Moore in collaboration with **BIOLAFFORT®**.

Finding the perfect balance of cell extraction can be challenging given the localisation of very different compounds in very different sections of the berry, many of which can bring astringency, bitterness, excessive vegetal notes. Variations in ripeness can further pronounce these differences. There is no doubt that best practice maceration techniques can improve the organoleptic quality of the wine by way of extracting key phenolic compounds (anthocyanins and tannins), polysaccharides and aroma compounds which can shape the sensory profile of the resulting wine.

The structure of the grape cell wall is highly complex and has been a limiting factor in thoroughly understanding the mechanisms and impact of commercial enzyme preparations. Commercial enzyme preparations possess a multitude of principal activities targeted at pectin degradation (pectin-lyases, polygalacturonases, pectine-methylesterases) and a vast array of secondary activities. These secondary activities can facilitate the breakdown of the grape berry allowing access for the primary de-pectinisation activities to function. Previous work from Ducasse (2009) has demonstrated that commercial enzyme preparations with specific activities can liberate specific polysaccharides (RGI, RGII, AGP) which have both a significant sensory impact and effect on stabilisation (Vidal et al 2003).

INVESTIGATION THE IMPACT OF COMMERCIAL ENZYMES ON GRAPE BERRY CELL WALLS VARIATION (CABERNET SAUVIGNON)

First developed by Moore et al (2014) a novel method was developed to enable analysis on the grape marc post fermentation as opposed to compounds found in the wine itself. Comprehensive Microarray polymer profiling (CoMPP) involves extracting the components in the grape skin after fermentation and then dissolving this in different medium. These different medium (acidic, alkaline) enable solubilization of different compounds, giving fractions either rich in pectin or hemicellulose. This is then printed onto a nitrocellulose membrane and probed with monoclonal antibodies (mAbs) as well as carbohydrate-binding modules (CBMs) (Moore et al 2014). Gao et al (2015) then conducted work on Cabernet Sauvignon grapes from South Africa to assess changes in polysaccharide composition/ turnover throughout the winemaking process.

The above study provided framework to specifically target key commercial enzyme preparations and their interactions with the grape cell matrix. This second study was carried out so as to represent even distribution in the vineyard using three commercial enzyme preparations from **LAFFORT®** (Table 1) factoring in a variety of grape maturities (Figure 2). The Cabernet Sauvignon grapes were sourced in the 2014 vintage from the experimental vineyard (Stellenbosh Univerisity) and processed to ensure a homogenous sample before being divided into three 5 kg replicates. Chemical analysis at the end of fermentation was conducted to ensure consistency among all replicates. The ferments were inoculated with the same active dry yeast, temperature controlled to 25°C (77°F) and plunged each day. At the end of alcoholic fermentation the must was pressed in a cage press. Malolactic fermentation was not conducted.

PRODUCT	PROPERTIES	APPLICATION
LAFASE® XL EXTRACTION	Liquid enzymatic preparation for red wine maceration and clarification.	Extraction & clarification
LAFASE® HE GRAND CRU	Pectolytic enzyme preparation, purified in CE for the production red wines that are rich in colouring matter and structured tannins, destined for ageing.	Maceration
LAFASE® FRUIT	Purified pectolytic enzyme preparation for the production of fruity, colourful and round red wines.	Maceration

Table 1. Information on the commercial enzymes used in this trial (www.laffort.com).

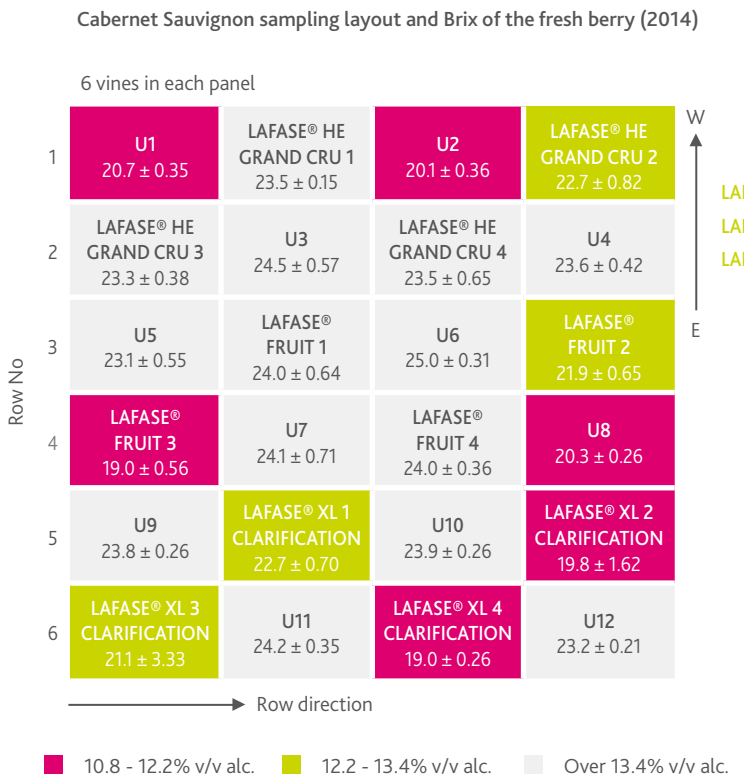

Figure 2. Harvest plan and ripening level variation of Cabernet Sauvignon. Each block represents a panel, which consists of six vines. U refers to untreated fermentations. The level of ripening was categorized into three stages depending on °B value (mean values from three biological repeats). Sourced from Gao et al (2016).

DISCUSSION

There is a clear difference between treated and untreated samples as represented by the two distinct groups in Figure 3. The untreated samples cover a broad area demonstrating a high variability between samples, likely due to the influence of maturity. In contrast the samples treated with enzymes were more consistently grouped, and minimized variation between maturity levels. Depectinisation is significantly facilitated with the addition of enzymes, and there is clustering of the specific enzyme preparations, meaning that each enzyme preparation is able to influence the final wine outcome.

Extraction with NaOH (Figure 4) again distinctly separates enzyme treated from untreated samples. This type of analysis is able to target the different polysaccharides in the hemicellulose rich cell wall fraction in the grape marc post fermentation.
The samples in the bottom right hand corner are predominantly untreated samples which are rich in homogalacturonans (HG), rhamnogalacturonanes I (RGI) and mannanes with a poor degree of esterification. This indicates a poor level of cell wall extraction. The samples which have had enzyme treatment can mostly be found in the top left-hand quadrant of the graph. These samples were found to be higher in xyloglucans with a higher level of esterification, generally indicating a higher level of cell wall degradation. As a consequence, samples treated with LAFASE® XL EXTRACTION (high levels of extraction) vs LAFASE® FRUIT (lower levels of extraction) can be found on either side of this cluster. Differences between preparations can be visualized from Figure 5 which depicts Comprehensive microarray polymer profiling (CoMPP) of the pectin rich fraction of pomace after alcoholic fermentation. This data also suggests that some enzymes might be better at working on the esterified HG (LAFASE® FRUIT) whilst others are more efficient on the de esterified HGs (LAFASE® XL EXTRACTION).

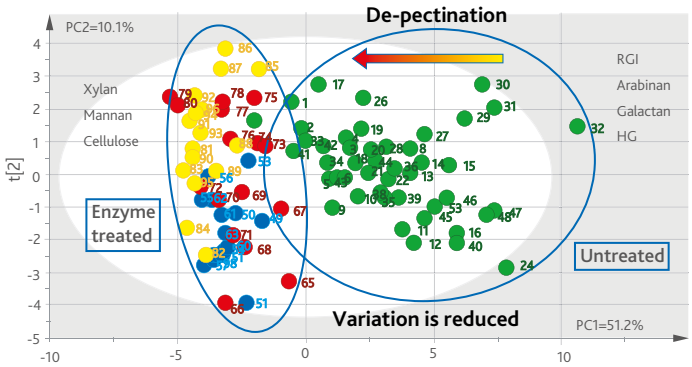

Figure 3. PCA score plot of the pectin-rich extract from alcohol insoluble residues (AIR) sourced from fermented berry pomace. Untreat, untreated fermentation; **LAFASE® FRUIT**; **LAFASE® HE GRAND CRU**; **LAFASE® XL EXTRACTION**. The color is according to the treatment.

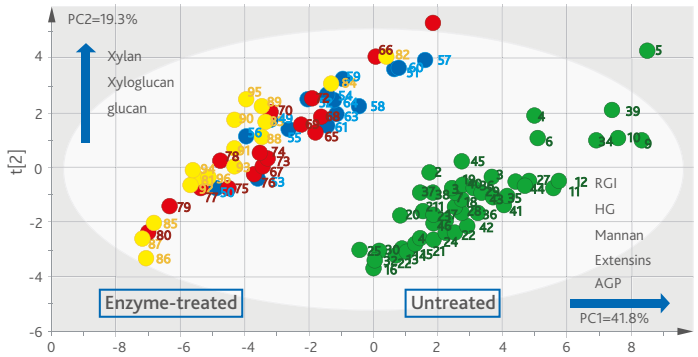

Figure 4. PCA score plot of the NaOH (hemicellulose-rich) extract from AIR sourced from fermented berry pomace. Untreat, untreated fermentation; **LAFASE® FRUIT**; **LAFASE® HE GRAND CRU**; **LAFASE® XL EXTRACTION**. The colour is according to the treatment.

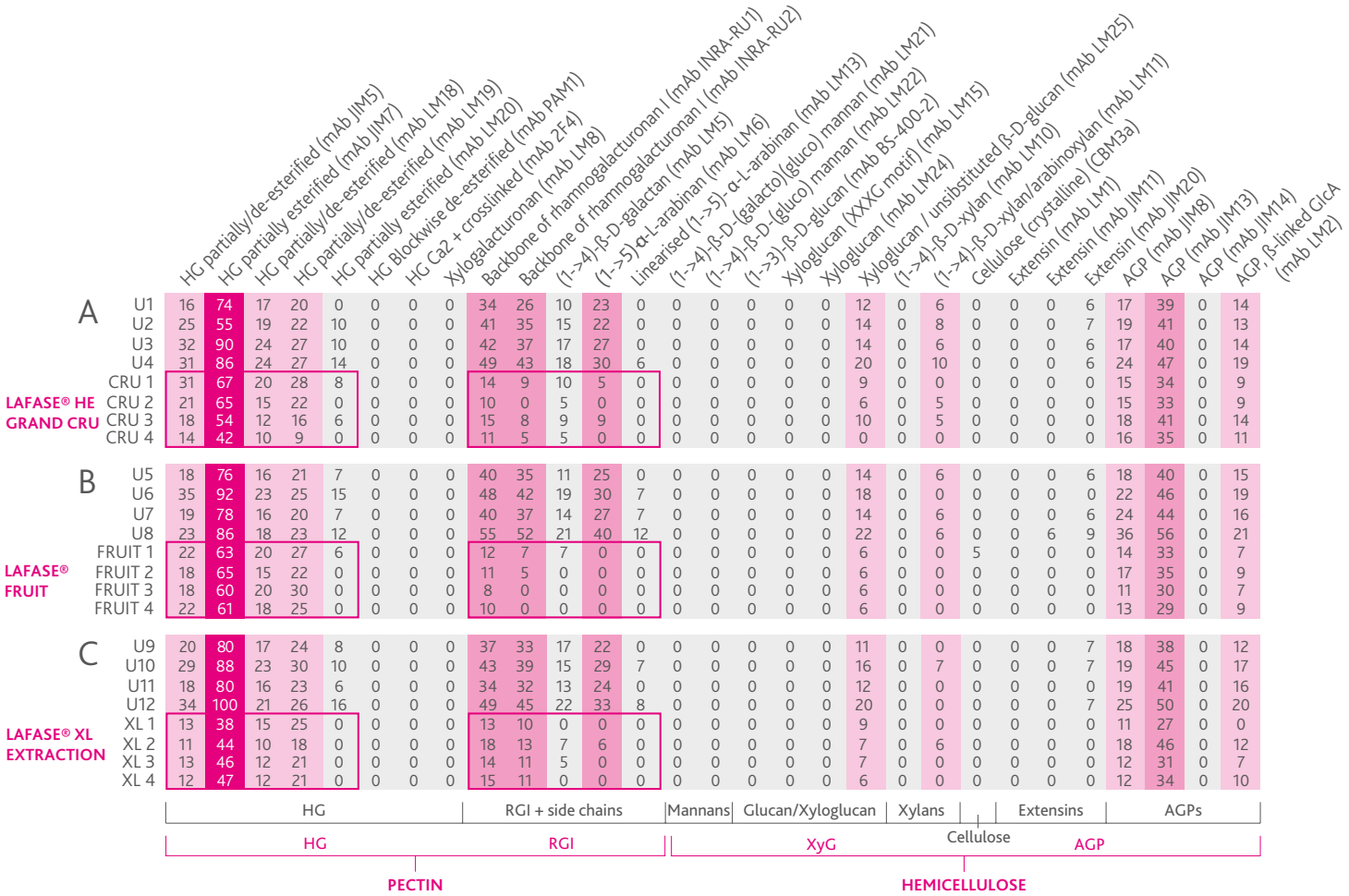

Figure 5. Comprehensive microarray polymer profiling (CoMPP) analysis of the pectin rich fraction(A) U1–U4 and Cru1–Cru4; (B) U5–U6 and Fruit1–Fruit4; (C) U9–U12 and XL1–XL4. HG, Homogalacturonan; RG, Rhamnogalacturonan; AGPs, Arabinogalactan protein. The heatmap shows the relative abundance of plant cell wall glycan associated epitopes present in alcohol insoluble residue (AIR) sourced from fermented berry pomace. The highest signal was set as 100, and others were adjusted accordingly; the color intensity is correlated to the mean spot signal. A cutoff (<5) was applied to all heatmaps. Sourced from Gao et al (2016).

A NEW CELL WALL STRUCTURE AND BETTER UNDERSTANDING OF LAFFORT® ENZYME PREPARATIONS

The results from Gao et al (2016) were able to support the proposal of a new cell wall structure, depicted in a simplified format (Figure 6). These studies were focused on musts and reflect a degree of esterification of polysaccharides, a function of localization in the berry as well as grape maturity. As the grape berry matures, it is likely that the cell wall and pulp start to depolymerise after veraison, increasing cell size and decreasing cell wall size (thinning) as a consequence. During vinification, the grape berries are crushed and the cell walls of the pulp are easily degraded/solubilized in the wine. As proposed by previous studies, it is likely that this generates a must rich in polymers of homogalacturonanes (HG) and rhamnogalacturonan I (RGI), de-esterified, with the presence of arabinogalactan-proteins (AGP) and in a smaller capacity xyloglucans (XyG). The process of de-esterification is likely to start in the pulp and progress outwards versus the wax cuticle.

The structure of the pectins in the pulp is relatively simple in comparison, requiring enzymatic degradation through the action of pectin-lyases, facilitated throught the use of mechanical intervention (crushing or thermovinification) prefermentation. On the other hand, the structure of the pectins in the cell wall is far more complex. In order for enzymes to access the main pectin chain, secondary activities are required to lyse the side pectin chains. These secondary activities are also of interest as they affect the liberation of anthocyainins, tannins and important aroma precursors.

TAKE HOME POINTS

Two main fractions were proposed to form the berry cell wall:

- Pectin-rich (HG high degree of esterification dominant) layer.
- Hemicellulose-rich layer, coated with highly esterified pectin (RGI dominant).

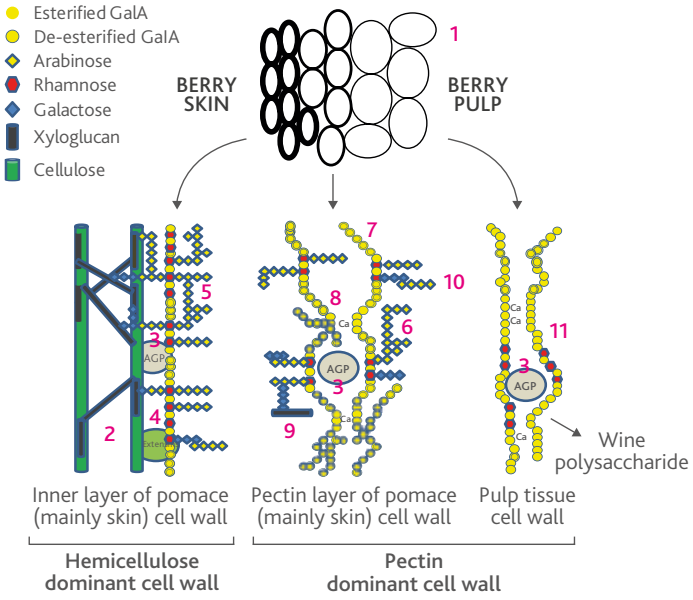

Figure 6. Proposed model of the grape berry cell wall (taken from Gao et al 2016).

Number legend:

1. Tissue differences in grape berry.
2. Tethering model of hemicellulose-dominant fraction.
3. Parallel form of cellulose and associated xyloglucan.
4. AGPs are associated with all fraction of cell wall.
5. Extensins are mainly associated with pomace cell walls.
6. RGI-dominant pectin layer coating hemicellulose fraction.
7. Branched arabinan dominant side chain of RGI.
8. High esterification levels of HG in pectin-rich fraction.
9. Ca^{2+} cross-linking in pectin-rich fraction, masked by esterified HG.
10. Low amounts of xyloglucan are associated with pectin.
11. Unusual linkage "Ara-Gal-Rha" in pectin rich fraction.
12. De-esterified HG, RGI and AGP in pulp cell walls and wine polysaccharides.

Accurate knowledge of grape cell wall structure and its evolution during grape maturity.

- Grape cell wall layers become thinner containing low esterified pectin.
- The hypothetical cell wall model generated helps to explain the differences observed when analyzing berry tissues from previous studies: new model.
- Enzymes have significant impact on pectin.
- The quality of the extracted compounds allows to characterize the extraction intensity.

Commercial maceration enzymes in this study

- Enzyme treatments greatly reduced variation due to different levels of grape maturity.
- The 3 tested enzyme preparations lead to distinctly different wines.
- Residual pectin (RGI rich pectin) on the hemicellulose (inner layer) of pomace cell wall was attacked by the commercial enzymes:
  - **LAFASE® HE GRAND CRU** shows wide action activities (Greatest RGII extraction → increased mouthfeel) : Allows optimal extraction of cellular compounds despite variable RIPENING.
  - **LAFASE® FRUIT** extracts softly → Fruit expression: Oposite end of the spectrum in terms of phenolic extraction to **LAFASE® XL EXTRACTION**.
  - **LAFASE® XL EXTRACTION** has a very high extraction ability in a short amount of time: High phenolic extraction – less requirement for mechanical intervention.

CONCLUSIONS

These studies have enabled a better understanding of the grape cell and how commercial enzyme preparations can interact with the individual components. It highlights the complexity of the enzyme preparation required for depectinisation, maceration for particular wine styles. The use of enzyme preparations conclusively reduced variation between different levels of maturity (up to 3% v/v alcohol difference). The data also demonstrated that all of the enzymes were able to open up the hemicellulose component of the cell wall, exposing components from inner layers. Those of particular interest polisaccharides with known sensory impact such as RG II.

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TANNINS

TANNINS

Q&A

TANNINS

Tannins are highly versatile compounds that can be used for building structure, stabilizing color, deactivating oxidative enzymes, intercepting proteins, and developing mouthfeel. The phenolic family are some of the most versatile compounds in winemaking.

1. What are the differences between fermentation, aging, and finishing tannins?

Tannins are differentiated by the source material and reactivity (polymerization) with other molecules like proteins, oxygen, and phenols. The more reactive a tannin is, the more integration time is required in the wine. Dosage rate of tannin also plays a role in the timing of addition; higher dosage rates at fermentation compared to very low dosage rates for wine finishing.

Fermentation tannins

These include processing tannins like **TANIN VR SUPRA®**, and may use multiple tannin sources, from the seeds and skins of grapes to ellagic sources of oak and oak galls, to others like the chestnut and quebracho. Reactivity is rapid when in contact with freshly crushed grapes, providing a sacrificial role to bind with proteins, react with oxygen, and inhibit laccase (from *Botrytis*). Fermentation tannins can also stabilize color during fermentation (**TANIN VR COLOR®**), reduce the perception of underripe 'green' qualities, and/or increase the structure of the resulting wine.

Aging Tannins

Aging tannins are almost exclusively grape and/or oak tannin blends to bolster the structure and mouthfeel of the wine and can add to the oak tannins imparted by barrels or staves. These tannins require at least a couple weeks (e.g., **TAN'COR GRAND CRU®**) to polymerize, with full integration seen a month or two later.

Finishing Tannins

Finishing tannins are primarily tannins extracted from oak staves that are toasted similarly to barrel classifications such as light, medium, or heavy toast. These tannins help reduce oxidation, boost aromatics, improve astringency and mouthfeel, and can reduce 'green' characteristics of wine made from underripe fruit. Some finishing tannins have slower polymerization rates in line with aging tannins while others, such as the **QUERTANIN®** range, may fully integrate with only two days before bottling. Some aging tannins can cross over to finishing tannins, like **TANFRESH®** with its ability to protect a wine from oxidation over time during aging, and its ability to instantly refresh an oxidized wine when used as a finisher.

2. Are there any interactions to avoid when using tannins?

Avoid adding tannins with enzymes as they will deactivate each other and precipitate. Allow sufficient time (six hours) between additions to benefit from the effects of both. Also avoid adding tannins after bentonite is added for protein stabilization, and too close to tartaric stabilization.

3. How do I know how much tannin to add?

Bench trials are the best way to know how much tannin to add, however there is no time for bench trials when using fermentation tannins. Fermentation tannins are highly reactive and the process of fermentation will integrate the added tannin into the wine. There is a wide dosage range with each fermentation tannin, consult the tannin chart for each product. In general, if you know the fruit you are working with is low in tannin, then use a moderate to high dosage level. If the fruit is higher in tannin, and you desire the sacrificial effect and antioxidant protection, then use a low to moderate dosage. In the case of mold infected fruit, use a moderate to high dosage of fermentation tannin, depending on the level of rot in the fruit.

It is always important to conduct bench trials prior to making a tannin addition to wines after fermentation. Practice and experience with tannins, as well as contacting your **LAFFORT®** technical representative will also help greatly. Tannin additions may be necessary when natural phenolics are too low for structure and color stability, when incoming fruit has been compromised by *Botrytis*, when there is phenolic imbalance arising during élevage, and/or when slight adjustments are required during final blending of a wine. Knowing the right tannin(s) to use at the right time will preserve quality and avoid downstream issues. Gathering the right information is the first step to determining the necessity of a tannin addition. Sources of information may include:

Lab testing

- Grape Phenolic Panel (catechin, tannin, and polymeric anthocyanin levels plus their ratios).
- Wine Phenolic Panel (phenolics, non-flavonoid acids and

oxidation products).
• *Botrytis* Panel.

Traditional sensory methods
• Chewing of seeds and skins in the vineyard.
• Tasting must, ferments, and wine regularly.
• Visual estimation of *Botrytis* infection load.

Vintage history
• Vineyards that have produced wines with low tannin or color intensity in the past can benefit from early tannin additions.

Once phenolics are known or estimated, use the appropriate tannin(s) to address the issue.

FERMENTATION TANNINS

4. What are sacrificial tannins?

Sacrificial tannins react with proteins and enzymes (including laccase from *Botrytis*) in grapes that would otherwise bind with natural grape tannins. Sacrificial tannins preserve the natural grape tannins in the wine.
Tannins have high antioxidant power and can protect juice from oxidation when added during fermentation. In reds, loss of natural tannins can cause loss of color, structure, mouthfeel, and age-worthiness. Sacrificial tannins such as **TANIN VR SUPRA®** are typically a blend of proanthocyanidic (grape seeds and skins, chestnut, quebracho, etc.) tannins.
In white wines, sacrificial tannins are added to bind with protein in the juice for wine stability or to protect the juice from oxidation when fruit is compromised by rot. Gall nut tannins have high reactivity for protein binding. Products like **TANIN GALALCOOL®** are highly effective and should be added during destemming for maximum effectiveness.

5. How do I use tannins to stabilize color?

Color stabilizing tannins are added at the one-third mark of fermentation. These tannins, such as **TANIN VR COLOR®** or **TANIN VR GRAPE®** are high in catechins that polymerize anthocyanins during pigment extraction from the grapes. Polymerization prevents excessive precipitation of the color during aging, thus maintaining more stable color over the life of a wine.
There are two things to remember with color stabilizing tannins. First, these tannins do not add color that is not there – they simply protect the color the grapes naturally have in the skins.

FERMENTATION TANNINS

OBJECTIVE	GRAPE OR MUST TYPE	TANNIN	DOSE	NOTE
Botrytized grapes, anti-oxidant action, laccase inhibition.	Red	**TANIN VR SUPRA®** **TANIN VR SUPRA® ÉLÉGANCE**	100 - 800 ppm, according to the health of the grapes.	Add as soon as possible to grapes, even before arrival in the winery.
	White and Rosé	**TANIN GALALCOOL®**	50 - 200 ppm, according to the health of the grapes.	Perform laccase test in case of *Botrytis*.
Protein precipitation and skin tannin preservation.	Red	**TANIN VR SUPRA®**	100 - 500 ppm	Sacrificial effect.
		TANIN VR SUPRA® ÉLÉGANCE	100 - 500 ppm	Add as soon as possible to grapes.
Protein precipitation.	White & Rosé	**TANIN GALALCOOL®**	50 - 200 ppm	
Color stabilization.	Red	**TANIN VR COLOR®** **TANIN VR GRAPE®** **TANIN VR SKIN®**	150 - 800 ppm	Add during the first third of fermentation.
Structure contribution. Compensation for tannin deficiency.	Red	**TANIN VR GRAPE®**	100 - 400 ppm	Add as soon as possible to grapes.
	Red	**TANIN VR SUPRA®** **TANIN VR SUPRA® ÉLÉGANCE**	100 - 800 ppm	
	Red	**TANIN VR SKIN®**	100 - 300 ppm	

Second, the tannins must be added at the right time, one-third of the way into fermentation. At this point, they can interact with free acetaldehydes to form bridges to stabilize anthocyanins.

6. What are the best tannins to use when the fruit has mold or Botrytis?

The best tannins to use for mold infected fruit are high reactivity tannins for binding and inactivating browning enzymes, and for antioxidant protection of the must. For white grapes, the main concern is oxidative browning from laccase. Gall tannins react quickly with laccase, inactivating enzymatic action. Products like **TANIN GALALCOOL®** are highly effective and should be added during destemming or juice collection for maximum effectiveness. They may also be used post-fermentation if any laccase is detected in the wine. For red grapes, the multiple concerns are preventing the browning enzymes from causing oxidation, lack of tannin for structure, loss of color, and negative flavors from the mold. A broad spectrum fermentation tannin like **TANIN VR SUPRA®** is perfect for addressing these multiple factors. No matter what the varietal, adding tannin during initial grape processing is vital to mitigating the negative character of the compromised fruit. See our *Botrytis* protocol on pages 148 - 150 to decide on the dose rate of tannins.

7. Will tannins used on white or rosé wines affect astringency?

Use of **TANIN GALALCOOL®** and **TANIN GALALCOOL® SP** at low doses will not greatly affect astringency in whites and roses.
Higher doses depend very much on the specific wine and we highly recommend bench trials. Lighter white or rosé wines may need a much lower dosage to avoid any astringency, such as when refreshing those that are tired or oxidized. With heavier, full-bodied whites made with oxidative fermentation methods and aged in oak barrels, there may be more room to use tannins without affecting astringency. Lastly, sweet whites or rosé wine styles can still benefit from tannin additions without astringency due to the mouth-coating effects of the sugar.

8. How do tannin additions affect color stability and copigmentation?

Hydrolyzed oak ellagic tannins play a key role in color stability and co-pigmentation by both protecting anthocyanins from oxidation and encouraging the formation of vitisins, anthocyanin-derived pigment compounds [ADPCs] that can lend to changes in hue. In a solution such as fermenting wine containing ethanol, anthocyanins, and hydrolyzed tannins, anthocyanin levels drop over time as they create anthocyanin-derived pigment compounds. The kind of ADPCs that are created depends on whether an anthocyanin binds with a flavanol (directly or via acetaldehyde bridge) or with a compound such as pyruvate that leads to the addition of a pyrane ring on the ultimate structure.

In the case of an anthocyanin and flavanol bonding (a pigmented compound), the presence of ellagitannin (a non-pigmented molecule) allows for the oxidation of anthocyanins to become vitisins, an ADPC-tannin complex which contributes a hue change that would not occur in the presence of ethanol and anthocyanin alone. This change in hue by the interaction of pigmented and non-pigmented molecules is co-pigmentation and it increases with the amount of ellagitannin within a wine, thus additions of such tannin produced commercially will increase co-pigmentation. **TANIN VR SUPRA®** added during fermentation is an excellent choice for supplementing this process.

9. What is the best tannin strategy when working with Flash Détente?

Due to flash détente's ability to extract maximum compounds from the skin and pulp of grapes, it is advised to first measure total tannins in the grapes as close to the process as possible with a phenolics panel, then add any deficient tannins to the post-flash must once it has been clarified through flotation or centrifuge and cooled a few hours. Elevated amounts of color-stabilizing tannin like **TANIN VR COLOR®** may be needed due to the more complete extraction of anthocyanins during the flash détente process.

10. Are both oak chips and fermentation tannin additions needed?

Not necessarily, although they can be used in a complementary fashion. Oak chips only provide ellagitannins, while a fermentation tannin can contribute proanthocyanidic, gallic, and alternative source tannins thereby providing a more complete tannin profile. If using oak chips and no additional ellagitannins are required, consider using specific pure grape tannin products like **TANIN VR GRAPE®** and **TANIN VR SKIN®** to target proanthocyanidic tannins, such as catechin and epicatechin.

11. Will adding tannin to my white or rosé wine help with protein stability?

Fermentation and aging tannins can certainly help with protein stability, but generally will not make a wine protein stable without adding unpalatable astringency.
At wine pH, tannins are negatively charged and thus have an affinity for positively charged proteins, not only heat-labile proteins, but also enzymes such as laccase, and the reaction will precipitate these out of solution. Without enzymes present, the tannins will first react with those proteins that cause heat instabilities and make the wine slightly more stable.
Finishing (ellagic) tannins may improve protein/heat stability and should be done prior to the addition of bentonite to improve the effectiveness or reduce the amount needed of the bentonite.
Bench trials should be conducted if using finishing tannins after all

stabilities are achieved due to the risk of making the wine unstable again through alteration of the wine chemistry matrix.

AGING TANNINS

12. When is the best time to add tannins for structure?

Structural tannin additions are best addressed in red wines after malolactic fermentation is complete. When wines need a bigger tannin addition, the earlier the better because tannin helps protect wine during aging and there is more time for the added tannin to integrate into the wine. Every time the wine is racked, is another opportunity to make a structural tannin addition. Combination tannin products are great, while specialized products can transform a wine in specific ways. Consult the aging tannin chart in this section for details on the different aging tannin products.

13. How do tannins increase the aging ability of red wines?

Different tannins improve the aging ability of wines in different ways, varying from building big, structured reds to protecting oxidation at all phases of winemaking.

Red wine fermentation tannins.

- Sacrificial tannins during grape processing bind with proteins, enzymes, and oxygen that would otherwise reduce the concentrations of innate tannins extracted from the skins and seeds or degrade aromas and flavors. Use **TANIN VR SUPRA®**.
- Color-stabilizing catechin tannins polymerize anthocyanins, thus creating more stable color that lasts longer during bottle aging. Use **TANIN VR COLOR®** or **TANIN VR GRAPE®**.
- Structural ellagic and proanthocyanidic grape tannins build mouthfeel and function as antioxidants. Use **TANIN VR GRAPE®** or **TANIN VR SKIN®**.

Aging Tannins

- Ellagic and proanthocyanidic grape tannins add structure and function as antioxidants. Use **TAN'COR®**, **TAN'COR GRAND CRU®.**

Finishing Tannins

- Structural ellagic tannins quickly reduce oxidation, increase fruit, improve mouthfeel and function as antioxidants. Use the **QUERTANIN®** range.

14. Can the need for SO_2 in wines be reduced by using tannins during aging?

Tannins play a vital role as an effective antioxidant in red wines, making them a great tool for lowering the need for SO_2 as part of a comprehensive strategy.

AGING TANNINS

OBJECTIVE	GRAPE OR MUST TYPE	TANNIN	DOSE
Balance or wine structure improvement	White & Rosé	TANFRESH®	5 - 60 ppm
		TANIN GALALCOOL® SP	20 - 50 ppm
		TANIN VR SKIN®	50 - 300 ppm
Balance or wine structure improvement	Red	TANIN VR SKIN®	50 - 300 ppm
		TAN'COR®	100 - 300 ppm
		TAN'COR GRAND CRU®	50 - 300 ppm
		QUERTANIN® RANGE	5 - 200 ppm
		TANFRESH®	5 - 60 ppm
Regulation of oxidation reduction phenomena	White & Rosé	TANIN GALALCOOL® SP	20 - 50 ppm
		QUERTANIN® RANGE	5 - 200 ppm
		TAN'COR GRAND CRU®	100 - 200 ppm
Stabilization of color	Red	TANIN VR SKIN®	200 - 400 ppm
		TAN'COR GRAND CRU®	50 - 300 ppm
		QUERTANIN® RANGE	20 - 200 ppm

However, SO_2 is a powerful anti-microbial agent as well as an antioxidant, and tannins are primarily antioxidants.
Simply replacing SO_2 with tannin is not an effective strategy. Reducing SO_2 during winemaking is a complex process. Precautions must also be taken to prevent excessive oxygen uptake during time stored in vessels, wine movements, as well as a comprehensive anti-microbial strategy. Please consult with a **LAFFORT®** technical representative for all the factors to consider, as well as the **LAFFORT®** reducing SO_2 protocol on pages 145-147.

FINISHING TANNINS

15. When is the best time to add tannins to reduce green character?

Reducing 'green' characteristics from a wine is done almost parallel to adding structure because many of the same ellagic tannins correct both issues concurrently. Certain finishing tannins are more suitable for reducing 'green' qualities by promoting more fruit to show in a wine and masking with oak. **QUERTANIN® SWEET** is an excellent example of this kind of tannin.

16. How late before bottling and filtration can I add finishing tannins?

Finishing tannins should be integrated prior to bottling filtration before the polishing crossflow or pad filtration. In the case of the **QUERTANIN®** range, final sterile filtration on the bottling line is recommended at least one week after addition.

17. Can tannins refresh a tired or oxidized wine?

TANFRESH® and **QUERTANIN®** are excellent tools for helping bring a tired wine back to its full potential. They can even be used at low doses in white and rosé wines. These are specific aging and/or finishing tannins that will refresh a wine that has lost aromatics through oxidation, either in barrels, tanks, flex cubes, kegs, and other containers if not sealed or topped properly.

18. Can tannins replace oak aging?

Tannins cannot completely replace the sensory impact of aging wine in barrels or with oak alternatives. The **QUERTANIN®** range can help enhance the oak aging flavors in a wine. If you are looking to bring more toasty oak characters such as vanilla, coconut, or mocha, the Quertanin range has options from light toast to heavy toast in both French and American Oak. When working with neutral oak barrels, a product like the aging/finishing tannin **QUERTANIN®** can fill the role of adding ellagic tannins to the wine. Used barrels impart less ellagic tannin than a new barrel (~25-33%), thus providing less antioxidant protection. A lightly toasted oak such as **QUERTANIN®** with its higher concentration

FINISHING TANNINS

PRODUCT	TYPE	APPLICATION	DOSE
QUERTANIN®	Light toast French oak	Antioxidant properties to protect wine during aging. Eliminates reductive character. Lifts floral and fruit aromatics. Traditional and elegant profile.	10 - 75 ppm
QUERTANIN® SWEET	Medium toast French oak	Rich vanilla aromatics with perception of sweetness. Lifts red fruit such as cherry, redcurrant and strawberry. Masks green character.	20 - 100 ppm
QUERTANIN® CHOC	Medium Plus toast French oak	Perception of aging in new Medium Plus toast barrels. Lifts flavors of blackberry, plum, and blueberry. Hints of chocolate with a sensation of sweetness.	20 - 100 ppm
QUERTANIN® PLUS	Medium Plus toast American oak	Lifts red fruit flavors while adding warm spice aromas. Masks green character and adds the perception of sweetness. Builds mid-palate length without adding astringency.	20 - 100 ppm
QUERTANIN® Q1	Medium Plus toast. French and American oak	High aromatic intensity of toasted almond, vanilla and coconut, giving a perception of sweetness. Brings out dark fruit profile and enhances midpalate weight.	20 - 75 ppm
QUERTANIN® INTENSE	Heavy toast French oak	Perception of aging in new Heavy toast barrels. Increases flavor profile of coffee, toasted almonds, spice and clove. Masks 'off' aromas.	10 - 75 ppm

of hydrolysable tannins versus those extracted from toasted oaks such as **QUERTANIN® CHOC**, yields much higher amounts of ellagic tannins, replacing those that are naturally found in oak.

19. Can I add multiple finishing tannins at the same time?

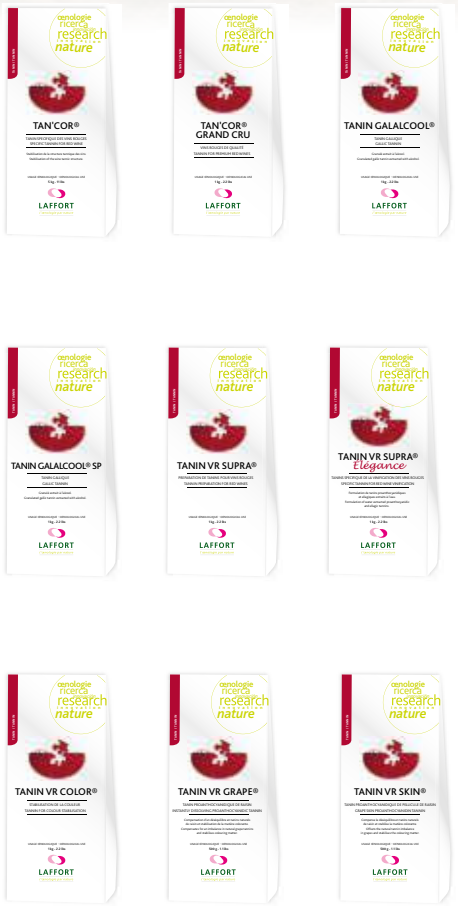
Multiple finishing tannins may certainly be used at the same time. Like having a cellar with a diversity of barrels, running bench trials with different combinations of finishing tannins may lead to a more preferred result than using one tannin alone. Sometimes, a single ultra-premium tannin may reduce the need for multiple tannins. **QUERTANIN® Q1** combines the best qualities of French and American oak finishing tannins for unmatched versatility on a wide variety of wines.

20. Will finishing tannins precipitate out after bottling?

Finishing tannins with sufficient quality should stay in solution long into the life of a bottle if the wine is:

- Heat stable. Any possibility of proteins in or forming in the wine may cause precipitation of tannins.
- Microbiologically stable.
- Dosage range guidelines are followed.
- Tannins are added early enough for full integration ahead of bottling.
- Tannins are homogenized completely.

Note that with **LAFFORT®**'s patented Instant Dissolving Process (IDP) you can add dry tannin direct to the wine and take the mess out of hot water and/or liquid tannins.



TANNIN STORAGE AND PREPARATION

PRODUCT	STORAGE TEMPERATURE AND PLACE	SHELF LIFE UNOPENED AND OPENED	PREPARATION
All **LAFFORT®** granulate tannins	Dry area, cool temperature, away from odors.	4 years from production date, use quickly when opened.	**IDP®** process allows direct dry sprinkling into grapes and wine.
TANSPARK® Liquid tannin	Dry area, cool temperature.	1 year from production date, use immediately.	Liquid formula can be added directly to sparkling base wines.

ARTICLE

ELLAGIC MAGIC

When wine ages in an oak barrel, a new barrel not only adds aromatic complexity to the wine, but also protects the wine against oxidation. This is done by regulating the oxidation-reduction phenomenon during maturation and/or micro-oxygenation. The component which is primarily responsible for this phenomenon is ellagic tannins. These are tannins that are hydrolised to ellagic acid, which in turn is a natural phenol antioxidant. The amount of ellagic tannins in a barrel decreases significantly every time it's used. More than 50% is lost during the first year of use and there's hardly any ellagic tannins left in a barrel after three years of use.

Ernst Kleynhans, LAFFORT® South Africa

GENERAL OVERVIEW & MATURATION OF WINE

Barrel inserts were used to introduce integrated oaky notes and enhance the flavour and aromatic profile of wine. Tannins were used to restore the levels of ellagic tannins that are lacking in older barrels and necessary to protect a wine from oxidation, and becoming brown and oxidative during the maturation process.

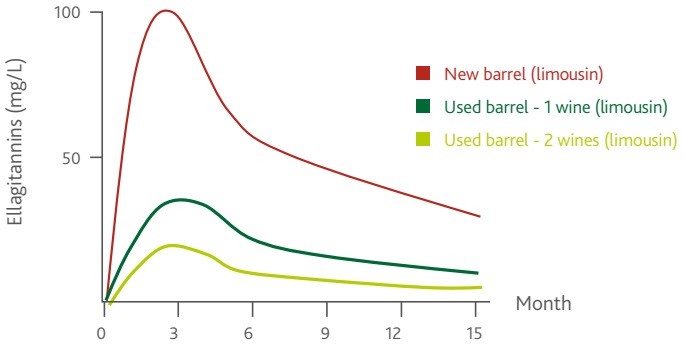
The cost to treat these wines is about R1.70 a litre ($USD0.48 per gallon). The trial was conducted during the 2014 vintage in the Robertson Valley. Maturation of the wine took place in barrels.

For this trial 10 barrels from the same cooperage were selected to reduce variability. Four of these barrels were new 2014 barrels. The other six barrels were older, from the 2004 vintage, thus 10th fill barrels. NOBILE® BARREL REFRESH (barrel inserts) was used to simulate the new wood component and QUERTANIN® (stave wood-quality ellagic tannins was used to adjust the level of ellagic tannins lacking in the older oak barrels. See Table 1 for a complete summary of all the treatments.

WINE	BARRELS			BARREL REFRESH		ADDITIONNAL TREATMENT			TRIAL DURATION (months)
	BARREL AGE	TOAST	FILL	PROFILE	DOSAGE	TANNIN	DOSAGE (at racking)	DOSAGE (3 months intervals)	
1	2004		10th			QUERTANIN®			12
2	2004		10th	FRESH	20% new wood	QUERTANIN®			12
3	2004		10th	SENSATION	20% new wood	QUERTANIN®	1 g/hL (10 ppm)	1 g/hL (10 ppm)	12
4	2004		10th	INTENSE	20% new wood	QUERTANIN®			12
5	2004		10th	REVELATION	20% new wood	QUERTANIN®			12
6	2004		10th	AMERICAN REVELATION	20% new wood	QUERTANIN®			12
7	2014	L/M, FG	1st						12
8	2014	M, FG	1st						12
9	2014	M+, OG	1st						12
10	2014	M+, FG	1st						12

The 2014 barrels were filled with wine and weren't given any additional treatment. These barrels however did have different toasting levels (L/M, Light/Medium; M, Medium; M+, Medium Plus) (FG, Fine Grain; OG, Open Grain). As the wine was drained off the skins and racked into the respective 2004 barrels, each was given a **QUERTANIN®** dose of 1 g/hL (10 ppm). These six barrels continued to be given 1 g/hL (10 ppm) of **QUERTANIN®** every three months for the duration of the 12-month ageing period. In addition, five of these barrels had one **NOBILE®** barrel insert. Each **NOBILE®** barrel insert resembles 20% of a new 225 L barrel. The barrel that was not given a barrel insert served as a control. The maturing red wine barrels were checked at regular intervals and topped up as necessary.

ELLAGITANNIN CONTENT IN BARRELS



SENSORY EVALUATION

After 12 months the SO_2 levels were adjusted and the respective wines were hand-bottled. They were then stored under cellar conditions until they were evaluated in four tasting sessions by winemakers from various wine regions. The wines were evaluated and scored out of a total of 20 points.

Looking at the top five wines, wine 10 was the most preferred wine and scored 16.3. It was from one of the new 2014 barrels with a fine grain and a medium plus toasting.

The wine with the second highest score of 15.7 was also from a new 2014 barrel. The oak had a fine grain and medium toasting. Hot on the heels of this wine was a wine from one of the older 2004 barrels. It scored 15.6 and contained one **NOBILE® BARREL REFRESH REVELATION** insert and had been given a **QUERTANIN®** dose every three months.

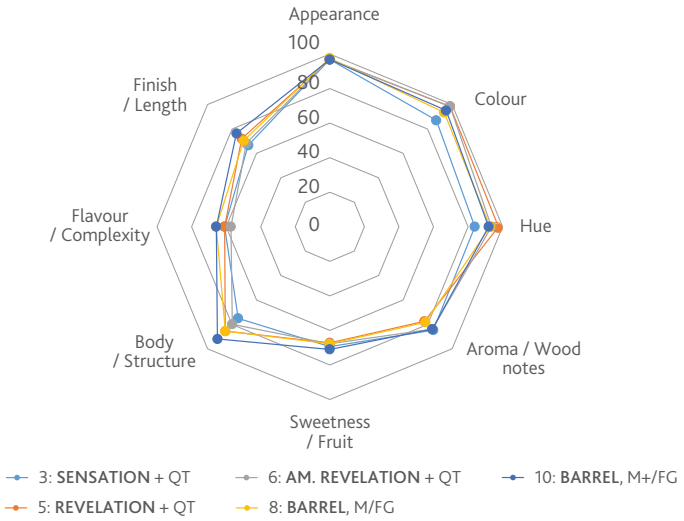
The wine that came fourth with a score of 15.4 also came from a 2004 barrel, but it contained one **NOBILE® BARREL REFRESH AMERICAN REVELATION** insert and had also been given **QUERTANIN®** dosages.

The fifth-placed wine was wine 3 with a score of 15.2. This wine also contained the combination of **QUERTANIN®** and barrel insert, namely **NOBILE® BARREL REFRESH SENSATION**. This barrel insert is convection-oven toasted. The convection-oven toasting process creates a uniform toast throughout the oak which drastically reduces the ellagic tannin concentration. Therefore this barrel insert contributes mostly to the flavour and aroma of the wine, but is not as effective in preventing oxidation.

On the other hand wine 2 (**NOBILE® BARREL REFRESH FRESH**), wine 5 (**NOBILE® BARREL REFRESH REVELATION**) and wine 6 (**NOBILE® BARREL REFRESH AMERICAN REVELATION**) all had barrel inserts that were either untoasted (Fresh) or received a toasting similar to fire toasting (Revelation and American Revelation).

Toasting takes place on the surface of the wood and therefore doesn't affect the concentration of the ellagic tannin as much compared with the convectionoven toasted oak products.

A COMPARISON OF THE 5 MOST PREFERRED WINES



CONCLUSION

All wines were well received by the tasters who evaluated them. All the wines from the 10-yearold barrels did exceptionally well compared with the wines aged in new oak barrels. Although the wines from the barrels with **NOBILE® BARREL REFRESH** and **QUERTANIN®** combinations didn't have the highest score and complexity and structure of wine from a new barrel, they clearly demonstrate that **NOBILE® BARREL** alternatives used in conjunction with the **QUERTANIN® RANGE** can be used to extend the life of older barrels, while protecting the wine.

FINING

FINING

Q&A

FINING

From the removal of oxidized and oxidizable phenolics in white and rosé juice, to the softening of astringency in red wines, to the removal of excess color from rosé, to the improved clarity of all wines, the sheer diversity of raw materials used enables us to adapt fining to the type of wine must and to the desired flavor and aroma profiles.

1. How long do fining agents take to settle?

Fining agents added to wine or juice generally complete their reaction within 15 minutes to one hour after addition and mixing. Products such as bentonite settle and compact well within one week, whereas products such as yeast hulls may take up to six weeks to fully settle.

Settling time depends on the product and application, and depends on the product particle size, surface area, temperature, the volume, height, and shape of the tank, as well as the use of co-fining agents such as colloidal silica, etc. Additional settling time results in more compacted lees. The efficiency of settling also depends on the quality of the fining agent preparation, emphasizing the need to follow the instructions clearly on all fining agent product data sheets.

2. How long can I keep the wine on fining lees?

After settling has occurred, it is recommended to rack off the sediment as soon as possible and avoid potentially redispersing compounds you wish to remove. Routine sulfur additions will stir up the lees and the negative compounds can be released into the wine again.

How long the wine can remain on lees depends on the strength of the bond between the fining agent and the wine compound removed, whether chemical bond, absorption and adsorption, or electrostatic interaction. The bond may not be permanent, and if the lees are disturbed the bound compound may be released into the wine again.

3. How can I speed up settling and compaction after fining?

Proper preparation and implementation of the fining agent is the best way to aid fast and compact sedimentation. The addition of enzymes beforehand for removal of pectins and ß-glucans, as well as the addition of a co-fining agent such as colloidal silica or bentonite may help as well.

SILIGEL is a colloidal suspension of silica used as a co-fining agent. The silica particles carry a negative charge, so in the presence of positively charged proteins (like gelatin) flocculation occurs, followed by precipitation. **SILIGEL** is added prior to the use of gelatin when only seeking clarification, and added after the gelatin when removing phenolics.

4. Can I use pea protein?

Pea protein is another example of a fining material, similar in basis to other protein fining agents, gelatin, potato, etc. At time of writing, the pea protein is not authorized for use by TTB in the USA, but is authorized by the OIV for use in many other countries. **LAFFORT®** offer a range of pea proteins, including **POLYMUST® NATURE**, **POLYMUST® V** (now **POLYMUST® BLANC**), and **VEGEFLOT®**. Contact your **LAFFORT®** technical representative for information on how to assist in getting these products TTB approved.

5. What precautions are needed to prevent over-fining or stripping my wine?

The term 'over-fining' has two main definitions used in enological language. First, over-fining can be used to describe a situation when a fining agent is not fully dispersed in the wine, leading to over treatment of part of the wine and under treatment of the rest of the wine. However, the term 'over-fining' most often refers to the situation where too much fining agent is used, and the wine is 'stripped' of flavor.

In the first definition, a fraction of the protein added in the fining agent does not flocculate and stays in the wine. This phenomenon is more likely to happen with gelatin, but it might occur with different agents if the preparation is not properly homogenized in the wine. Poor flocculation, fast additions, high temperatures or the presence of a haze can also produce over-fining in red wines. The use of **SILIGEL** before the fining agent can help prevent over-fining. If your wine has been over-fined, perform lab trials first, and add bentonite for whites or tannins for reds. Also note that larger tanks with inadequate mixing are more likely to have this issue.

The second definition, stripping wine flavors by adding too much fining agent, can be remedied by performing fining trials and adding the minimum amount required to achieve the goal of the fining agent.

6. How do I rebuild mouthfeel when a wine is stripped?

Many yeast derived products can help to build mouthfeel in a wine after a significant fining treatment. **OENOLEES®** and **AUTOLEES®** have a high content of a specific peptide fraction that is released naturally by yeasts during autolysis and has an excessively low perception threshold (only 16 ppm compared to 3000 ppm for sucrose) and can and can build back mouthfeel that was stripped out. The high sapid peptide content identified as Hsp12 was discovered through years of research by **LAFFORT®** (Patent EP 1850682).
Mannoprotein products such as **MANNOFEEL®** significantly increase the volume, roundness, and length on the palate. Yeast cell walls have a refining action that promotes elimination of polyphenols responsible for bitterness and astringency.

7. Which fining agent will work in my wine?

Each wine is different and will respond differently to each fining agent at a specific dose. There is no substitute for performing fining trials on a lab scale before adding fining agents to wine in the cellar. Determine the goal of your fining and choose 2-3 different products to trial. Keep in mind your constraints of time, volume, labeling, and legislation regarding allergens. Use two different dosages for each agent trialed, and make sure there is an untreated control.
For improved success in your lab trial:

- Correct the free SO_2 to 30 mg/L to prevent oxidation in the trials.
- Use 375 mL bottles as a minimum volume.
- Include a control in the series of wines.
- Keep the wines at the same temperature that the wine will experience in the cellar.
- Allow 2 to 3 days before tasting.
- Taste blind and measure the turbidity of all including the control.
- Choose the fining agent and dosage according to your tasting preferences and constraints.

8. When is the best time to use fining agents for white and rosé wines?

Fining before or during fermentation is the ideal time to remove oxidized and oxidizable phenolics from white and rosé wines. Juice fining reduces solids and improves clarity early in the winemaking process. Fining during fermentation allows the fining agent to be kept in suspension due to the agitation caused by the fermentation and be thoroughly distributed throughout the must.
When fining pre-fermentation, also consider the cooling capacity of the winery. After depectinization, allow 48 hours for static settling of fining products. It is necessary to have sufficient cooling capacity to prevent fermentation at this time. With no cooling capacity, fining during fermentation is the better option.

9. What are the best fining treatments for removing astringent tannin in red wine?

Some of the best options for astringency in red wine are those that effectively tackle larger structured phenolics. Each wine has a unique colloidal and phenolic make up, which can affect the action of the fining agent.

- **GECOLL® SUPRA** and **GELAROM®** (gelatins) are derived from the hydrolysis of collagen, resulting in a distribution of protein sizes in the gelatin, which in turn affects the effectiveness of the fining action and explains its broad activity towards tannins of various sizes. Gelatin can be used on juice or wine.
- **VEGEFINE®** and **VEGECOLL®** (patatins) are a medium weight protein that targets phenolic compounds reducing astringency and bitterness in red wines. **VEGEFINE®** is purified and highly reactive so the dosage range is lower than other fining agents.
- **POLYMUST® PRESS** (PVPP, bentonite, patatin), a combination product developed as a broad-spectrum fining agent for red wine hard press fractions.
- Egg white protein is a medium weight protein and is classically associated with the fining of red wines, due to its lack of reactivity towards smaller anthocyanin-tannin complexes and lower color removal.

10. Are combinations of fining products more effective than using them individually?

In the case of proactive fining on white or rosé at the juice or fermentation phase where there is not much time for trials, we recommend using a combination of fining treatments. Combinations of fining agents are more effective over a broad spectrum of oxidizable phenolics in white and rosé wines, or a range of astringency producing compounds in red wines, than a single fining agent alone.
Examples of combination fining products in the **LAFFORT®** range: **POLYMUST® PRESS** (PVPP, bentonite, patatin), **POLYLACT®** (PVPP, casein), **POLYMUST® ROSÉ** (PVPP, patatin), and **ARGILACT®** (Casein, bentonite).

11. Do protein based fining agents work differently than non-protein based fining agents?

Fining agents can be divided into two categories: proteinaceous and non-proteinaceous. Their interaction with wine compounds can be in the form of a chemical bond, absorption and adsorption, or electrostatic interaction. A chemical bond formation will bind to the compound in question and normally precipitate. Absorption and adsorption carry no electric charge and captures the compounds upon its structure. Electrostatic interactions involve the fining agent and the compound having opposite charges, attracting the larger molecules which combine with the fining agent and settle out.

For proteinaceous agents, the interaction of tannins and proteins initially involves a two-stage process. Firstly, hydrophobic regions on the tannin and protein move into proximity to exclude water and lower the energy of the system. Secondly, hydrogen bonds are formed, which serve to lock the two structures together. Once the protein-tannin association is complete, flocculation follows. The associated compounds aggregate and precipitate out of solution. This process is, in part, governed by the concentration of the added protein. When this concentration is low, simple association occurs. When the protein concentration is high, cross-linking occurs between sites of association, affecting the overall reactivity and function of the fining agent.

A non-protein fining agent such as bentonite has a lattice configuration with inter-laminar cations that take on a net negative charge to react with positively charged proteins in wine in an ion exchange process. Carbon, another non-protein fining agent, has a very high surface area with very small pores and operates on an adsorptive mechanism. Carbon works as a hydrophobic species, in a similar manner to the first stage of protein-tannin interaction.

FINING PRODUCTS STORAGE

PRODUCT	STORAGE TEMPERATURE & PLACE
All **LAFFORT®** fining products	Dry area, moderate temperature. Use quickly once opened. Liquids may be refrigerated for up to three months once opened.

JUICE FINING APPLICATIONS

PRODUCT	DESCRIPTION	DOSAGE	PACKAGE
ARGILACT®	Preparation of selected bentonites and soluble casein. Treatment of white wine and settling of juice.	600 - 1000 ppm for white wine. 400 - 600 ppm for red wine.	1 kg 25 kg
CASEI PLUS	Potassium caseinate developed for treatment of oxidation phenomena and maderization in juice (white and rosé).	50 - 200 ppm for clarification. 200 - 600 ppm for maderization treatment and color correction.	1 kg 5kg
CHARBON ACTIF PLUS GR	Granulated activated carbon for decolorization.	200 - 1000 ppm.	5 kg
GECOLL® SUPRA	Liquid gelatine produced from a selection of exceptionally pure raw materials, exclusively of porcine origin.	40 - 100 mL/hL.	1 L 5L 20L
GEOSORB® GR	A carbon decontaminant for fermenting musts and new wines for reducing geosmin and octenone content, as well as reducing the effects of smoke exposure.	Action on geosmin: 150 - 250 ppm. Action on octenone: 350 - 450 ppm. Activity on smoke exposure: 500 - 1000 ppm.	5 kg 15 kg
MICROCOL® FT	Natural calcium sodium bentonite for protein stabilization and high compaction of lees.	300 to 800 ppm.	15 kg
POLYLACT®	Combination of PVPP and casein for preventing and treating oxidation in juice (white and rosé).	Preventative treatment: 200 - 400 ppm. Curative treatment: 400 - 1000 ppm.	1 kg 10 kg
POLYMUST® PRESS	Association of PVPP, calcium bentonite and vegetal protein (patatin, potato protein isolate) for the preventive fining of press wines and the reduction of oxidized character.	150 - 500 ppm on red press wine. 400 - 1000 ppm on white and rosé press wines.	1 kg 10 kg
POLYMUST® ROSÉ	Association of PVPP with vegetal protein (patatin) for the fining of white and rosé musts and wines.	300 - 800 ppm.	1 kg 10 kg
VEGECOLL®	Vegetal protein (patatin) for juice clarification and flotation.	30 - 100 ppm.	500 g 5 kg
VEGEFINE®	Vegetal protein (patatins) for the static clarification of juice.	Preventive and curative treatment of oxidation: 50 - 300 ppm. To eliminate oxidized phenolic compounds: 100 - 500 ppm.	1 kg 10 kg
VINICLAR® P	Microgranulated preparation of PVPP for preventive and curative treatment of the oxidation of juice.	150 - 300 ppm for preventive use. 300 - 800 ppm for curative use in oxidized wine or must.	1 kg

ARTICLE

FINING DURING FERMENTATION: FOCUS ON WHITE AND ROSÉ

Advantages of fining in must rather than wine on aroma and colour.

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TAKE HOME POINTS

- Oxidisable phenolics (mainly Flavanoids and phenolic acids) can affect colour by turning brown. These brown oxidised phenolics can scavenge important aroma compounds.
- Fining during fermentation may prevent aroma and colour modification by fining out oxidisable phenolics early on.
- Each wine is unique and will require a tailored combination of fining agents suitable for the desired wine style.
- CO_2 from the fermentation will not protect phenolics from oxidising.
- Time from grape to bottle – there is often less time to stabilise colour, greater need to fine.

The most common timing for fining is in the juice stage or in wine, but a multitude of research supports fining during fermentation as a beneficial practice. Fining is a generic term for removal of a particular set of compounds, and there are various methods to accomplish this. Combinations of fining agents (Figure 1) can have a targeted effect on undesirable compounds without affecting nitrogen levels. Both micronutrients and nitrogen can be replaced through the addition of organic nitrogen sources, but key aroma compounds like thiols are lost indefinitely if they are not protected from oxidisable phenolics early on in the juice stage (Coetzee et al., 2013). Wine is a complex matrix of proteins, colloidal matter, sugars, acids, phenolic compounds. Fining removes unwanted components from the juice or wine. This may be for the purpose of clarification, removal of oxidisable and non-oxidisable compounds which can affect colour, bitterness and other off flavours. Fining can improve the wine stability in a number of ways and improve the wine from an organoleptic point of view. Besides having a clarifying effect, fining leads to changes in the polyphenolic structure of wines and in red wines improves the stability of colouring matter by eliminating particles likely to precipitate later in bottle (Lagune-Ammirati and Glories, 1996). Fining also helps reducing the microbial load of the wine (Murat and Dumeau, 2003).

Figure 1. Different applications of fining combinations.

WHAT ARE THE KEY AROMA COMPOUNDS THAT NEED PROTECTION?

Thiols are a key part of expression in many varietals, the most notable being Sauvignon Blanc. Thiols are relatively unexpressed in grape juice, but develop via yeast metabolism during alcoholic fermentation (Dubourdieu et al., 2006). The yeast strain plays a critical role in the formation of the thiols from precursors found in grapes. Cysteinylated and glutathionylated precursors have a high chemical stability against oxidation (Roland et al., 2010). 3-sulfanylhexanol (3SH), 3-sulfanylhexyl acetate (3SHA) and 4-sulfanyl-4-methylpentan-2-one (4MSP) in Sauvignon blanc are elemental, but have also been linked to the black currant aroma of red wine (Rigou et al., 2014). 3SHA is formed from the acetylation of 3SH by the yeast during fermentation (Swiegers et al., 2007).

Methoxypyrazines are grape derived and important contributors to "green pepper", "asparagus", "grassy", "herbaceous" and "vegetative". Three main methoxypyrazines occur in wines, namely 3-isobutyl-2methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine and 3-sec-butyl-2-methoxypyrazine (Marais, 1994). These aromas are not modified by the fermentation, and were shown to be present even after oxidative handling in the absence of SO_2 (Coetzee et al., 2013).

Monoterpenes are plant derived, have characteristic floral, fruit, citrus odors in the form of geranoiol, linalool, nerol and alpha-terpineol, and are present in aromatic muscat varieties (Mateo JJ, Jiménez M., 2000). Terpenes are normally glycosylated and non-volatile in their glycoslylated form. These may be released over the course of the fermentation or with commercial enzymes (Rusjan et al., 2016). Esters, higher alcohols and volatile acids are produced exclusively by microbial intervention (this level is subject to genera, species and strain variation) (Sumby et al., 2010). Esters are much less prone to oxidation than thiols and can contribute fruit aromas.

TYPES OF FINING AGENTS

Fining agents can be divided into two categories: proteinaceous and non-proteinaceous (Table 1). Their interaction with wine compounds can be in the form of a chemical bond, absorption and adsorption or electrostatic interaction. A chemical bond formation will bind to the compound in question and normally precipitate. Absorption and adsorbtion carries no electric charge and captures the compounds upon its structure. Electrostatic interactions involve the fining agent and the compound having opposite charges and attracting, the larger molecules which combine the fining agent will settle out.

	FINING AGENT	CHARGE
Proteinaceous	Gelatine	Positive
	Isinglass	Positive
	Casein	Positive
	Egg white	Positive
	Pea	Positive
	Potato	Positive
Non-proteinaceous	Bentonite	Negative
	Tannins	Negative
	Silica	Negative
	PVPP	No charge
	Carbon	No charge

Table 1. Types of fining agents and their respective properties.

WHAT ARE THE BENEFITS OF FINING IN JUICE RATHER THAN FINING IN WINE?

The removal of oxidisable phenolics are key to preserving aromas and importantly preventing the wine from oxidising and turning brown. Elimination of the phenol acids and flavonoids prevent the formation of o-quinones (brown compounds; Figure 2). If the majority of compounds that can be oxidised are taken out then there is much less to oxidise. Apart from changing the colour, o-quinones can then react with thiols, rendering the bound thiol inodorous thereby removing important aroma (Singleton V.L., 1987). When the must is fined rather than later on in the wine, the oxidisable phenolics can be removed before any impact aroma and colour is caused. When fining wine, the oxidisable phenolics present may already have had an impact on aroma and colour and the rate used has to be much lower, as the fining at this point may have a much harsher effect. Glutathione is found in yeast and juice and can play a role in aroma

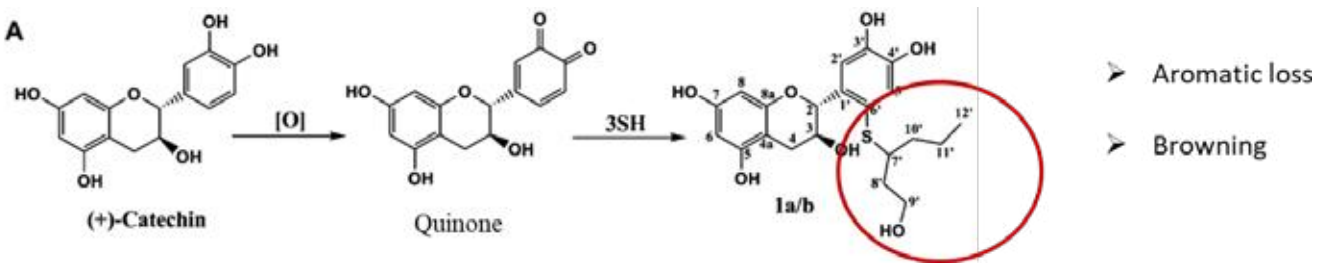

Figure 2. Prevention of the formation of quinones which can trap volatiles aroma.

protection via means scavenging oxygen and can bind to the oxygen in o-quinones forming a stable compound (Cheynier et al., 1993).

During fermentation a fining agent is able to be kept in suspension due to the agitation caused by the fermentation, and as a consequence be thoroughly distributed throughout the must. The desired aromatic outcome can be optimised by managing the right level of NTU for the yeast and fining action. Figure 3 is a trial that was conducted 2014 on the Sauvignon Blanc free run fraction demonstrating an increase in thiols with the turbidity regulation and addition of a fining agent. The purpose of adding the fining agent was to eliminate oxidisable phenolics in the juice which could subsequently bind to volatile thiols in their oxidised form. The yeast strain **ZYMAFLORE® X5** (*Saccharomyces cerevisiae*) was inoculated into the same must with incrementing turbidities 150; 200 and 250 NTU (Fluffy lees were added back to adjust the NTU). Each of these fermentations were conducted with and without the addition of an extract of vegetable protein (patatin) (**VEGECOLL®**) at 30 ppm. Both YAN and lipid content were adjusted to the same level. When conducted with a second strain of *Saccharomyces cerevisiae* (Figure 3b) the levels of thiols released in the same must were much higher, ranging from 46 – 49% increase in thiols, rather than 14 – 24% for the first yeast strain.

The thiol fraction was measured 3 months after the end of AF (Figure 4) for both **ZYMAFLORE® X5** and **ZYMAFLORE® DELTA**. In all cases there was a better preservation in thiols at the end of the three month period where **VEGECOLL®** was used.

With the introduction of cross flow filtration, it can be tempting to skip the fining stage. Cross flows can make the wine clear, but not stable. When floating with a fining agent, there is the added benefit of fining at the same time.

Grapes naturally have glutathione present, but it can also be released from the yeast. Glutathione is important because it can also bind to phenolic acids and prevent them from oxidising. Glutathione can be taken up by yeast so any nitrogen deficiency will incur the loss of glutathione. Fining agents do not have an impact on glutathione levels making fermentation an ideal time to fine.

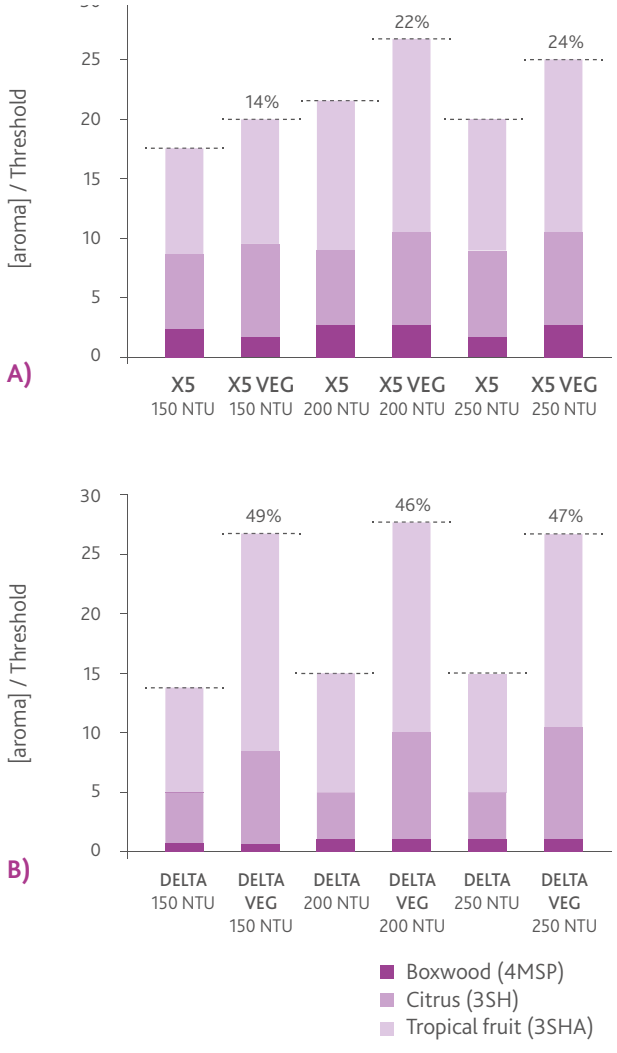

Figure 3. Thiols (concentration/threshold) incrementing must turbidities and with/without **VEGECOLL®**, fermented with A) **ZYMAFLORE® X5** yeast and B) **ZYMAFLORE® DELTA**

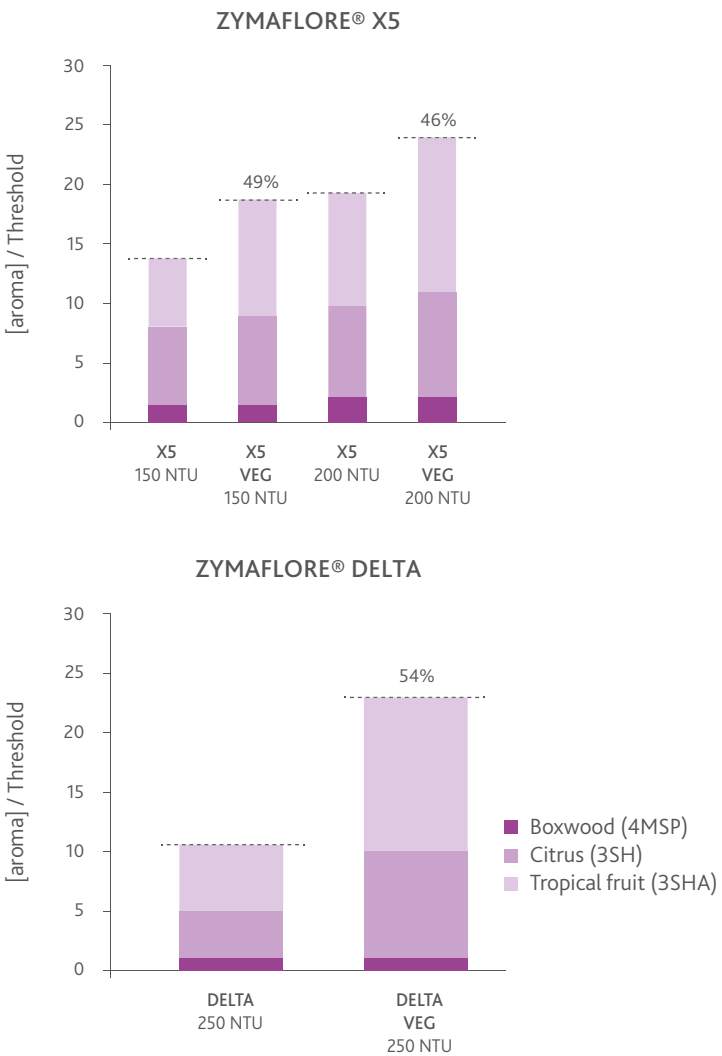

Figure 4. Thiols (concentration/threshold) measured at the end of AF and 3 months after AF.

MULTIPLE ADDITIONS: FINING IN JUICE AND DURING AF

Figure 5 was a trial conducted in France in 2015 on Sauvignon Blanc pressings. The control had controlled oxygenation and settling without any fining agent (A). Overall this variant showed the lowest levels of 3SH and 3SHA. The second variant (B) had a one-off addition of 200 ppm of **VEGECOLL®** added on the juice prior to settling, and had the highest level of 3SH after aging. 3MHA on the other hand was higher in variant D, whereby there was a preliminary addition of **VEGECOLL®** at 150 ppm on the juice prior to settling plus 30 ppm added in alcoholic fermentation. The addition of **VEGECOLL®** at 50 ppm during AF alone produces levels of 3SH and 3SHA on slightly above the control (A), suggesting the importance of fining in the juice stage. A higher preliminary addition with a subsequent fining had the highest levels of the 3SHA, an acetylated thiol reminiscent of passionfruit. Depending on the style of wine desired, subsequent additions can be beneficial and important for aroma.

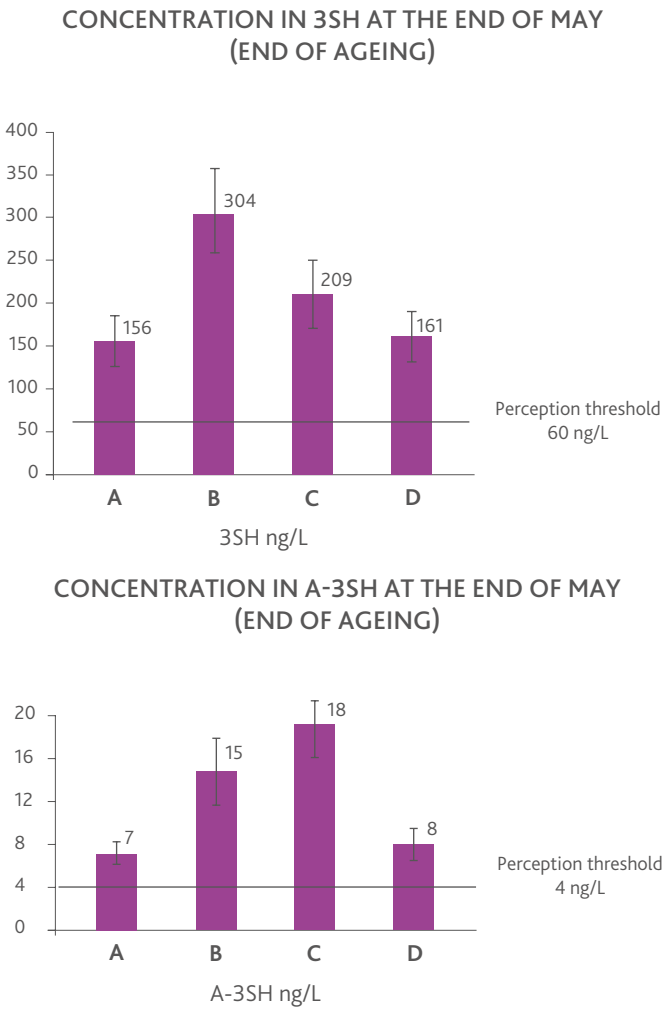

Figure 5. Trial variations: A: Control, juice with controlled oxygenation and settling; B: **VEGECOLL®** at 200 ppm on the juice prior to settling, protection from O_2; D: **VEGECOLL®** at 150 ppm on the juice prior to settling + 30 ppm in Alcoholic Fermentation, protection from O_2; E: **VEGECOLL®** at 50 ppm in Alcoholic Fermentation, protection from O_2. Press juice 2015, Sauvignon Blanc, winemaking in used barrels.

ROSÉ

Fining in rosé is crucial as any effect on browning or aroma will be evident. Trials conducted with a specific combination of PVPP and potato protein (**POLYMUST® ROSÉ**), which stabilises hue and reduces phenolic acids. Synergic effect of PVPP and extract of potato protein can tackle the larger phenolic compounds that form o-quinones making it ideal for most rosé. Figure 6 demonstrates that different combinations of both pea protein with PVPP (**POLYMUST® V**) and potato protein with PVPP (**POLYMUST® ROSÉ**) at incrementing levels had a significant effect on colour measured at 420 nm, 520 and 620 nm, thereby decreasing the amount that can subsequently oxidise, change colour and neutralise thiols.

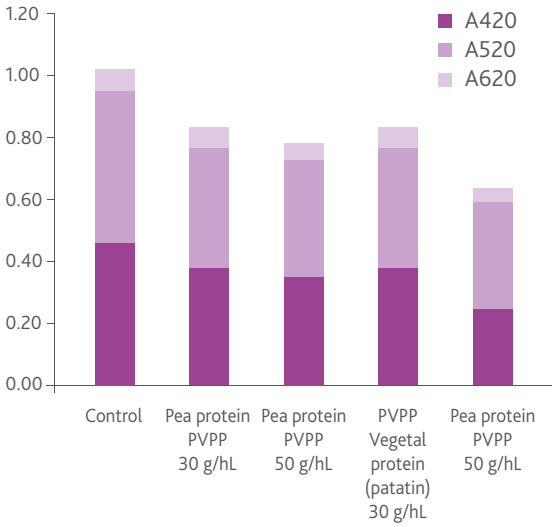

Figure 6. Trials conducted at Cave de Landerrouat 2015, fining on rosé juice.

WHAT HAPPENS WHEN WINES AREN'T FINED?

With the tendency towards producing a more 'natural' product with minimal intervention, some producers are skipping the fining stage. Aside from possible ramifications with colour and aroma, fining takes away harsh, astringent phenolics, often giving the wine a 'phenolic' palate. As discussed previously, fining in the wine is often too late, as the fining agent will have a much harsher impact on desirable compounds. Rosé colour will drop out if there are oxidisable phenolics present which aren't taken out via fining, which leads to colour instability in tank and bottle.

CONCLUSIONS

Wine aroma is made up of thiols produced by yeast from precursors in the grapes, esters produces by microbial interaction, grape derived methoxypyrazines and terpenes which are liberated/volatilised via microbial interaction, chemical or enzymatic hydrolysis. Thiols are highly susceptible to oxidation and important in many white and rosé wines. Fining during fermentation is critical to remove oxidisable phenolics which can bind to thiols produced by yeast to irreversibly remove them. Fining agents and combinations thereof can have a targeted effect on undesirable compounds can be tailored to increase certain volatiles depending on the desired wine style. Glutathione, will scavange oxygen in general, and the oxygen on the ortho quinone to form a stable compound. It is important to note that if the nutrition in the ferment is not sufficient, glutathione levels will drop and take away protection from oxidation. Removal of oxidisable phenolics in the juice stage will prevent negative impacts on colour and aroma – when done in the juice stage, higher doses are less likely to strip the wine.

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AGING AND FINISHING FINING APPLICATIONS

PRODUCT	DESCRIPTION	DOSAGE	PACKAGE
ARGILACT®	Combination of casein and bentonite for treating wines (white and rosé) against oxidation.	400 - 1000 ppm.	1 kg 25 kg
CASEI PLUS	Potassium caseinate developed for treatment of oxidation phenomena and maderization in wines.	200 - 600 ppm for maderization treatment and color correction.	1 kg 5 kg
CHARBON ACTIF PLUS GR	Granulated activated carbon for decolorization.	200 - 1000 ppm.	5 kg
GECOLL® SUPRA	Liquid gelatin produced from a selection of exceptionally pure raw materials, exclusively of porcine origin. Eliminates the tannins responsible for aggressive or astringent characters.	40 - 100 mL/hL.	1 L 5 L 20 L
GELAROM®	Liquid gelatin produced from a selection of exceptionally pure raw materials, exclusively of porcine origin. Intended to bring out the organoleptic potential of the wine.	30 - 60 mL/hL.	1 L 5 L 20 L
GELATINE EXTRA N°1	Highly purified heat soluble gelatin. Fining of red wine for aging.	60 - 100 ppm.	1 kg
GEOSORB® GR	A carbon decontaminant for fermenting musts and new wines for reducing geosmin and octenone content. Also effective on smoke exposure compounds and sensory.	Action on geosmin: 150 - 250 ppm. Action on octenone: 350 - 450 ppm. Activity on smoke exposure: 500 - 1000 ppm.	5 kg 15 kg
ICHTYOCOLLE	Fish-based (Isinglass) fining agent adapted to high-grade white and rosé wine fining and clarification. **ICHTYOCOLLE** restores high sensory clarity and remarkable brilliance to treated wines.	5 - 15 ppm.	250 g
MICROCOL® ALPHA	High quality natural sodium microgranular bentonite with a high adsorption capacity. Intended for protein stabilization in wine over a large pH.	100 - 800 ppm.	1 kg 5 kg 25 kg
POLYLACT®	Combination of PVPP and casein for preventing and treating oxidation in wine (white and rosé).	150 - 900 ppm.	1 kg 10 kg
SILIGEL®	Colloidal silica solution that may be used in combination with all organic fining agents for improved settling speed or clarification.	20 - 100 mL/hL - use 0.5 to 1 mL of **SILIGEL®** for 1 mL of gelatin. Add **SILIGEL®** prior to gelatin.	1 L 5 L 20 L
VEGECOLL®	Vegetable protein (patatin) for red fine phenolic fining during aging or pre-bottling.	30 - 100 ppm.	500 g 5 kg
VEGEFINE®	Vegetable protein (patatins) for the clarification and removal of oxidized phenolics from wine.	To eliminate oxidized phenolic compounds: 100 - 500 ppm.	1 kg 10 kg
VINICLAR® P	Granulated preparation of PVPP.	150 - 300 ppm for preventive use. 300 - 800 ppm for curative use in oxidized wine or must.	1 kg

Using a Venturi or OENODOSEUR is always recommended.

FINING PRODUCT STORAGE AND PREPARATION

PRODUCT	PREPARATION	SHELF LIFE UNOPENED & OPENED	SPECIAL CONSIDERATIONS
ARGILACT®	Dissolve in 10 x its weight in water. Allow to swell three hours.	2 years, do not use open packaging.	
CASEI PLUS	Dissolve in 10 x its weight in water.	2 years, do not use open packaging.	
CHARBON ACTIF PLUS GR	Dissolve in 10 x its weight in water. Allow to swell one hour.	4 years, do not use open packaging.	Allow 48 hours to settle then rack wine or juice. Clarification enzymes will further optimize the action and aid settling in juice.
GECOLL® SUPRA & GELAROM®	Add directly to wine. For barrel additions, dilute in 1/4 its weight of water.	2 years in original, unopened packaging. Opened bottles may last 3 months when refrigerated.	Add after tannins. See below for silicon products. Add before bentonite.
GELATINE EXTRA N°1	Dissolve in 20 x its weight in hot water (35-40°C / 95–104°F), stirring continuously.	5 years in original, unopened packaging.	Maintain the temperature of the gelatin solution throughout the fining operation to avoid gelling.
GEOSORB®	Dissolve in a small quantity of water. Allow to swell 2 - 4 hours.	4 years, do not use open packaging.	Mix thoroughly, then do another mixing 5 - 8 hours later. Allow 48 hours to settle then rack wine or juice. After racking, fine with **GECOLL® SUPRA** and **SILIGEL®** or a filter with diatomaceous earth to ensure efficient removal.
ICHTYOCOLLE	Dissolve in 100 x water by stirring with the help of a blender. Allow to swell two hours.	2 years in original, unopened packaging.	If gelling appears, dilute the solution with additional water (around 10%).
MICROCOL® ALPHA	Dissolve in 10 x its weight in hot water (50°C / 122°F), stirring continuously and vigorously for 2 hours. Allow to swell 12 - 24 hours.	4 years, do not use open packaging.	
MICROCOL® FT	Dissolve in 10 x its weight in hot water (50°C / 122°F), stirring continuously and vigorously for 2 hours. Allow to swell 12 - 24 hours.	4 years, do not use open packaging.	Mix vigorously in order to obtain a homogenous preparation immediately before incorporating into the tank. May be added via an inline dosing pump just prior to the crossflow filter.
POLYLACT®	Dissolve in 10 x its weight in water. Allow to swell one hour.	2 years in original, unopened packaging.	Can be incorporated before or during fermentation settling.
POLYMUST® PRESS	Dissolve in 5-10 x its weight in water. Allow to swell one hour.	3 years in original, unopened packaging.	Maintain agitation for 15 to 30 minutes to optimize effect.
POLYMUST® ROSÉ	Dissolve in 10 x its weight in water. Allow to swell one hour.	3 years in original, unopened packaging.	
SILIGEL	Mix in 10 x volume of water.	18 months in original, unopened packaging. Opened bottles may last 3 months when refrigerated.	**SILIGEL®** is always associated with a proteinaceous fining agent. Follow the protein fining agent usage conditions. Add before other fining products when the objective is to improve clarification conditions. Add after other fining products when the objective is to correct phenolic structure.
VEGECOLL® VEGEFINE®	Mix in 10 x volume of water.	3 years in original, unopened packaging.	Strong agitation may induce foam formation. Do not prepare the solution directly in wine as it will flocculate with wine compounds.
VINICLAR® P	Dissolve in 4 x its weight in water. Allow to swell one hour.	4 years in original, unopened packaging.	

AN INNOVATIVE AND COMPLETE RANGE OF **VEGETABLE PROTEIN** PREPARATIONS FOR THE TREATMENT OF WINES AND JUICES

VEGETABLE ORIGIN ALLERGEN FREE

VEGAN

NEW

VEGECOLL®

Original formulation based on 100% vegetable protein (patatin). **VEGECOLL®** has a very high Zêta potential that makes it one of the **most reactive proteins** in juice and wine. **VEGECOLL®** is particularly suitable for flotation of juice, and premium red wine fining post fermentation or pre-bottling.

VEGEFINE®

With its formulation based on vegetable protein (patatins), **VEGEFINE®** is part of the new line of **LAFFORT®** fining agents. **VEGEFINE®** combines **effective fining** of juices and wines, with an **exceptional compaction of solids and lees.**

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STABILITY

STABILIZATION

Q&A

STABILITY

Consumers buy wine that is clear and appropriately colored, and they like the wine to stay that way. Instabilities, amorphous deposits, cloudiness, and crystals are all negatively perceived, and can be prevented with appropriate practices.

1. What are the most common instabilities?

The three most common instabilities are Protein (Heat), Tartrate (Cold), and Microbial. Often overlooked yet still important is Color instability, and there are also the less common issues such as quercetin instability, ellagic acid instabilities in wines with late tannin additions, phenolic instabilities causing pinking in white wines, and premature aging.

Failure to address potential instability can cause hazes, precipitates, gasses, and even exploding bottles! Consumers see these flaws as reasons to not repurchase, thus making stabilization a critical winemaking process.

2. How do I know which instability my wine has?

To find the precise cause of the instability, laboratory analyses is required, including filtration, microscopy, and chemical assays. Wine labs, both in-house and commercial, can be set up to test for all instabilities. Protein and Tartrate instability testing are easiest to do in-house. Microbial testing ranges from easy (microscopy) to medium (plating) to expensively complex genetic testing (Polymerase Chain Reaction, or qPCR).

3. What would make a stable wine become unstable?

Stable wines may become unstable if subjected to conditions that alter the chemical make-up of the wine or re-introduce microbes. The most common way to change the wine chemistry which may cause instability is to add an acid, a carbonate, sugar, or tannins. Any one of these can cause cold, heat, and/or color instabilities to return.

Cellar cleanliness is paramount to preventing microbially stable wines from being re-contaminated, including thorough cleaning and sterilization of all vessels and equipment, cross-contamination vigilance, regular checks of topping material, regular vessel integrity inspections, and elimination of materials that may harbor substrates useful to microbes (e.g., Chlorine treated wood that leads to TCA).

4. Will blending two stable wines make one stable wine?

No. A common way of making wine(s) unstable is by blending different lots. Even if both lots are independently stable, this does not guarantee the resulting blend will be stable.

The unpredictable nature of how wine chemistry shifts is due to the myriad ways compounds interact in different wines. For this reason, it is advised to make a final blend before embarking on the stabilization of the wine.

5. Can filtration increase or decrease stability?

Filtration will not stabilize a wine for most instabilities. For example, filtration can remove tartrate crystals from stable wine that is cold filtered, but in this case only maintains the stability, rather than creating the stability.

Filtration can certainly stabilize wines from a microbial sense, although even then, only provided the wine is never re-exposed to recontamination in the cellar. When bottling, filters that have been breached can allow microbes into the wine, which then take advantage of a wide-open medium in which to colonize and grow. As few as 1 cell per 750 mL has the potential of growing over time and creating spoilage.

Clogging filters on the bottling line may make a stable wine unstable; they can strip protective colloidal compounds from the wine that passes through, for example, Celstab colloids may be stripped from their protective role around the nuclei of a tartrate crystal. The Celstab can remain upstream of the filter and the unstable wine pass downstream.

Exacting protocols and observations during bottling are critical to maintaining stability into the bottle.

6. Does barrel aging or lees aging improve my stability as a whole?

Aging wine on lees can certainly improve protein stability and it is often found that the amount of bentonite needed to stabilize for proteins is reduced after aging when coupled with stirring on yeast lees. Aging alone will not fully stabilize a wine.

7. What causes pinking and how do I prevent it?

In certain circumstances with white wines, highly reductive protocols combined with fruit damaged by frost (although not always necessarily) can cause a phenomenon called "pinking" in which latent precursors transform to a salmon pink hue upon sudden exposure to oxygen when the bottle is opened. Removal of these precursors is possible with PVPP (**VINICLAR® P**) or careful additions of ascorbic acid may help mitigate the reaction by intercepting oxygen before it can react with the precursors.

8. Which heavy metals in wine can cause instability?

Iron, copper, and other heavy metals exist in trace amounts in wine, sometimes coming naturally from the grapes and other times from exposure to equipment or winemaking processes. These metals can combine with proteins and precipitate, called a casse. The most common incidence involves copper since extremely low amounts (sometimes no more than 0.5 ppm) have been observed to cause instability combined with many known chances for introduction such as foliar sprays, winery equipment, and direct additions to remove sulfur-based aromas. Most other metals have lower introduction rates and consequently lower chances of forming casses. Iron and Copper instabilities can form a white haze, and copper can also produce a reddish-brown amorphous deposit.

Care should be taken to minimize the introduction of metals into wine by examining protocols and equipment. Additionally, using wine lees or a purified lees product like **OENOLEES®** can help by fining out the metal ions prior to filtrations and bottling.

STABILITY PRODUCT APPLICATIONS

OBJECTIVE	JUICE OR WINE TYPE	STABILITY PRODUCT	DOSE
Cold stabilization	Rosé - Heat and Color Stable	**CELSTAB®**	1 mL/L
Cold stabilization	White - Heat Stable	**CELSTAB®**	1 mL/L
Cold stabilization	Heat unstable White and Rosé as well as color-stable Red wines.	**MANNOSTAB®LIQUIDE 200**	50 - 150 mL/hL
Cold and Color stabilization	Red	**CELSTAB® + STABIMAX®**	1 mL/L + 100 mL/hL
Color stabilization	Rosé and Red	**STABIVIN®**	70 - 150 mL/hL
Colloidal stabilization, softer mouthfeel	White, Rosé, and Red	**STABIVIN® SP**	100 - 300 mL/hL
Protein (heat) stabilization	White and Rosé - Juice	**MICROCOL® FT**	30 - 80 g/hL (300 - 800 ppm)
Protein (heat) stabilization	White and Rosé - Finished Wines	**MICROCOL® ALPHA**	10 - 80 g/hL (100 - 800 ppm)
Yeast elimination and inhibition	White, Rosé, and Red	**SORBISOL K**	10 - 25 g/hL
Lactic acid bacteria elimination and inhibition	White, Rosé, and Red	**LYSOZYM®**	10 - 50 g/hL (100 - 500 ppm)
Brettanomyces elimination and inhibition	White, Rosé, and Red	**OENOBRETT®, OENOBRETT® ORG**	4 - 10 g/hL (40 - 100 ppm)
General microbial protection during aging, SO_2 reduction strategy	White, Rosé, and Red	**MICROCONTROL®**	5 g/hL (50 ppm)

Q&A

MICROBIAL STABILITY

1. What causes microbial instabilities?

Microbial stability covers a broad range of microbial contamination, including *Saccharomyces*, *Acetobacter*, *Lactobaccilus*, *Pediococcus*, and *Brettanomyces*, to name a few, resulting in hazes, spoilage, and/or CO_2 build-up. Microbial growth will generally leave residual cell deposits, but more frequently, microbial instabilities will be accompanied by off aromas and flavors and are identified as faults.

2. How can I rescue a fermentation that is sticking, VA is increasing and LAB are taking over?

When lactic acid bacteria feed on available glucose and fructose, it allows volatile acidity to increase, leading to a toxic environment for yeast. Using an enzymatic treatment such as **LYSOZYM®** will arrest the LAB and either there will be enough yeast cell viability left to finish the fermentation or a restart protocol will be required. To restart a malolactic fermentation after a **LYSOZYM®** treatment, add **MICROCOL® ALPHA** to deactivate the enzymes, then adjust the wine chemistry if required to ensure it has the correct parameters for the new MLF culture. In a bad case, reverse osmosis may be required to remove excess volatile acidity that is toxic to yeast.
OENOBRETT® or **MICROCONTROL®** are good options against LAB as well as targeting *Brettanomyces* and can also aid in limiting gram-negative bacteria spoilage. However, bear in mind that they can prolong the lag phase during the restart protocol.

3. How can I stop MLF or unwanted LAB growth?

LYSOZYM® is a microbial stabilization agent that is derived from egg whites with specific enzymatic activity against gram-positive bacteria such as *Oenococcus*. to stop MLF in red and white wines or with *Lactobacillus*. in sluggish fermentations or unintended blooms. In red wines, **LYSOZYM®** can be used to temporarily delay MLF – it is only effective for short periods of time and can be used to knock out native ML strains or spoilage organisms, then the wine can be reinoculated with the strain of choice.

*4. Why does **LYSOZYM®** have a bad reputation?*

LYSOZYM® is certainly effective in its intended use and, as with all products when used correctly it can do a specific task that can save a wine. There are two issues that should always be considered due to the protein nature of the product. The product is a heat-labile protein and will increase the level of heat (protein) instability in the wine. If the wine has been heat-stabilized prior to addition, the wine will need to be tested again and potentially re-stabilized. Secondly, the protein can interact with phenolic compounds and higher dosage rates can decrease color in red wines, especially low color varieties such as Pinot Noir.
Note that as with all heat-labile proteins, **LYSOZYM®** will interact with **CELSTAB®**. As such, use a bentonite such as **MICROCOL® ALPHA** to deactivate the enzyme and stabilize the proteins.

*5. How long does it take for **LYSOZYM®** to work?*

LYSOZYM® begins activity immediately but can take 1-3 days to complete activity. It remains viable in red wines for short periods of time but may offer continuing protection in whites for up to six months. This can be used for advantage in red wines where a short-term delay to malolactic fermentation is desired.

*6. What is the difference between **LAFFORT®** chitosan products?*

The chitosan used in **MICROCONTROL®** and **OENOBRETT®** is is of fungal origin derived from *Aspergillus niger* that is effective against *Brettanomyces* species, with some effect against bacteria by fining.
The new formulation of **MICROCONTROL®** is a blend of chitosan and yeast derivatives designed to soften a wine as well as kill unwanted microbes. **OENOBRETT®** is a blend of chitosan and ß-glucanase enzymes to both kill *Brettanomyces* and aid in efficient settling before racking. **OENOBRETT® ORG** is pure Chitosan.

7. Do I have to rack off Chitosan products?

These products can be left on the lees to continue activity against any wild yeast or bacteria that may be introduced to the wine with minimal impact to the organoleptic qualities.

8. Does Chitosan remove 4-ethyl phenol (4-EP) and 4-Ethyl guaiacol (4-EG) aromas or otherwise impact the sensory character of the wine?

Chitosan does not remove 4-EP / 4-EG or other sensory faults resulting from *Brettanomyces* but will stop the bloom so that faults do not get any worse.

*9. Does **OENOBRETT®** impact/interfere with Saccharomyces cerevisiae and fermentation?*

OENOBRETT® does interfere with the lag phase of *Saccharomyces cerevisiae*, or in the beginning of a fermentation or restart fermentation. Fortunately, *Saccharomyces cerevisiae* is a very hardy yeast and the lag phase will soon build up enough biomass for a regular fermentation.

*10. Can **OENOBRETT®** be used prophylactically?*

OENOBRETT® can be used prophylactically, especially for lots that are naturally susceptible to *Brettanomyces*, such as those with high pH numbers. An example of a prophylactic use would be to add to barrels just before you put the vintage to bed before the following harvest.

11. Do any anti-microbial products penetrate wood staves?

Products do not penetrate the pores of wood staves. Use steam first, or even better, replace contaminated barrels.

12. What is the easiest way to stop fermentation or prevent refermentation in the bottle?

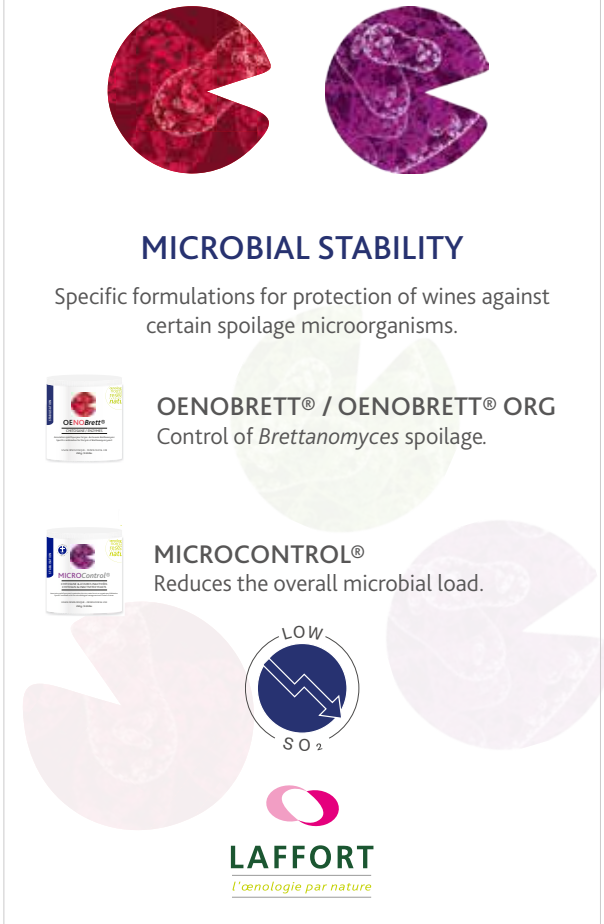
SORBISOL K is a preparation of Potassium Sorbate that is designed as a yeast inhibitor to produce sweet wines as well as an inhibitor of surface spoilage yeasts such as mycoderma (the 'flowers of wine') and must be used in conjunction with sulfur dioxide. To stop alcoholic fermentation, **SORBISOL K** is an effective tool to reduce time and energy spent, as it can be used in place of chilling, filtering or adding high amounts of SO_2 to the wine.

13. What provides residual anti-microbial protection after filtering and bottling?

Filtering below 0.65 µm will remove most yeast cells, and below 0.45 µm will remove most bacteria. Crossflow and cartridge filters of that size rating are a nominal filter size, so if these are the only filtration, there remains a possibility for a microbial bloom to develop in bottle. For the best anti-microbial protection in bottle, use an absolute 0.45 micron membrane filter and a preservative such as **SORBISOL K**.

*14. Will **OENOBRETT®** or **MICROCONTROL®** inhibit MLF?*

Yes. If primary fermentation has completed and a chitosan-based product has been used, it is recommended to have at least 8 days of settling and a clean rack off the chitosan lees prior to inoculation with ML bacteria. If the wine meets the bacteria parameters to complete MLF, the clean rack off the chitosan lees should be sufficient to start MLF without chitosan interaction.



ARTICLE

LOW SO_2 WINEMAKING — MICROBIAL CONTROL POST-FERMENTATION

By JOANA **COULON**[1] and ALANA **SEABROOK**[2]

In a recent article ('Low SO_2 winemaking – bioprotection for microbial control pre-fermentation', published in the Autumn 2019 issue of the Wine & Viticulture Journal) pre-fermentation conditions affecting fermentation kinetics, volatile acidity and ethyl acetate production were discussed. The following article is Part 2 with specific application to using less SO_2 post fermentation whilst maintaining the same high quality.

WINE & VITICULTURE JOURNAL - Summer 2020, V35N1. www.winetitles.com.au

CONTROLLING POPULATIONS DURING FERMENTATION

By controlling microbial populations during alcoholic fermentation and malolactic fermentation via inoculating quickly with desired strains of both *Saccharomyces cerevisiae* and lactic acid bacteria, there is minimal opportunity for spoilage microorganisms to grow. This ensures that the optimal species and strains are able to quickly and efficiently metabolise sugar and produce alcohol (*Saccharomyces cerervisiae*), and convert L-malic acid to L-Lactic acid (lactic acid bacteria), thus minimising the growth of potential spoilage microorganisms (Berbegal et al. 2017, Berbegal et al. 2018).

MICROBIAL POPULATIONS POST FERMENTATION AND DISCUSSION AROUND INFLUENCING FACTORS

Post fermentation, the two species of principal concern are *Brettanomyces bruxellensis* (yeast) and *Acetobacter spp* (bacteria).

B. bruxellensis is literally the dark horse of the wine industry. We know a lot more than we did a couple of decades ago, but its mechanisms still present new learnings. Recent studies have demonstrated the now increased SO_2 tolerance that *B. bruxellensis* presents in modern winemaking (Barata et al. 2008, Curtin et al. 2012, Agnolucci et al. 2014). Where once the presence of volatile phenols due to the presence of *B. bruxellensis* was considered 'terroir' and 'funk', purchasers are becoming savvy at understanding what the problem is and how it might affect the longevity of the wines.

B. bruxellensis is able to tolerate high levels of alcohol and increasing levels of SO_2 (Barata et al. 2008, Curtin et al. 2012, Agnolucci et al. 2014). Wines that have a higher pH will have less molecular SO_2 (the state of SO_2 that has anti-microbial function) (Ribéreau - Gayon et al. 2006). *B. bruxellensis* is suited to wine pH, is able to grow in both anaerobic and aerobic conditions and can adapt to very low levels of glucose and fructose by using alternative carbon sources (Curtin and Pretorius 2014, Crauwels et al. 2015). It is of principal concern due to its role in the production of phenolic off flavours 4-ethyl phenol, 4 ethyl-guaicol and 4 ethyl-catecol. These were described as 'barnyard', 'horse sweat', 'sweaty', 'bandaid' and 'iodine' aromas (Chatonnet et al. 1992).

Species of acetic acid bacteria (AAB) including *A. aceti* and *A. pasteurianus* are present in most wine that has not been sterile filtered at levels of up to 1000 cells/mL. These species grow much quicker than *Saccharomyces Cerevisiae* and much quicker than *B. bruxellensis*. They can metabolise alcohol and convert it to acetic acid which causes an increase in VA (Drysdale and Fleet 1988). They can proliferate when tanks are left on ullage or without SO_2 post fermentation. Practices such as high pH, lower SO_2 regimes and the absence of sterile filtration may promote their proliferation both in tank and bottle (Bartowsky et al. 2003).

CHITIN VS CHITOSAN

Chitosan is a non-allergenic polysaccharide derived from the *Aspergillus spp*. for winemaking applications. It is also a major component of the skeletal structure in crustaceans. Chitin is a major component of yeast cell

walls that is responsible for cell wall rigidity. The primary difference between chitin and chitosan is the acetylation/deacetylation level (an acetyl group is removed to chitin thus becoming chitosan compound), but there are other factors including molecular weight (hence polymerisation level) and deacetylation function distribution. Figure 1 demonstrates the change in molecular structure when going from chitin to chitosan (Rabea et al. 2003).

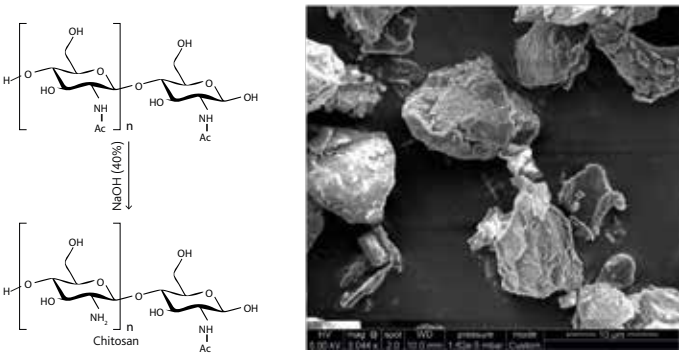


Figure 1. Preparation of chitosan from chitin (a). Sourced from Rabea et al. (2003) (b) chitosan in aqueous solution observed by SEM (microscopic studies conducted by **LAFFORT**® with the Bordeaux Imaging Centre).

Chitosan has been demonstrated to have an effect on membrane potential (the difference between the inside and outside of the cell) (Figure 2). However, other effects may also cause the death of *B. bruxellensis* due to its association with surface lipids, membrane permeability change and chelation of metal ions. At wine pH, chitosan is a positively charged molecule hence very reactive given its polycationic state (a molecule or a compound with multiple positive charges). Figure 2 demonstrates the effect of chitosan/ß-glucanase combinations on cell wall structure.

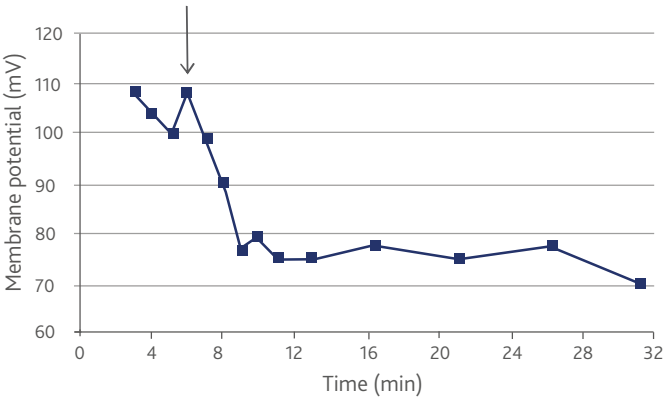


Figure 2. Difference in membrane potential Raafat et al. (2008) with an addition of chitosan of 10 ug/mL.

ADDING IN ENZYMES — THE KEY TO SUCCESS

Chitosan by itself can have an impact on cell viability (Figure 3). LAFFORT® has taken the concept of chitosan further and added ß-glucanase and pectinase activity, mainly promoting sedimentation and acting on the colloidal structure of the wine (ß-glucanase and pectinase activity). Trials conducted between LAFFORT®, BIOLAFFORT® and the Bordeaux Imaging Center investigated the impact of combined ß-glucanase and chitosan on the viability of *B. bruxellensis* cells, both lab cultured (Figure 3a) and spontaneously formed (Figure 3b) in wine (Nazaris et al. 2016). The combined effect is much greater on cell death than chitosan by itself (Figure 4).

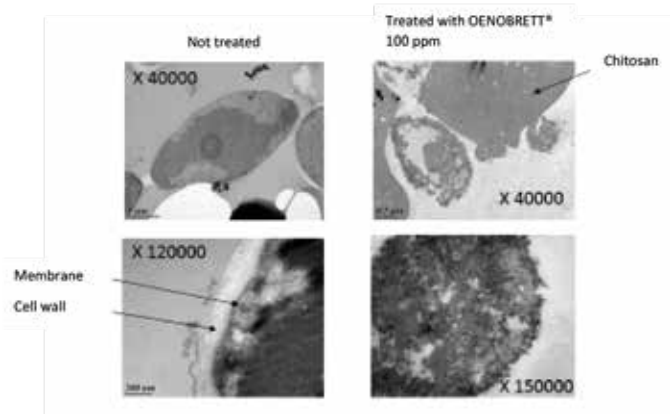


Figure 3a. Effect of **OENOBRETT**® on *Brettanomyces bruxellensis* cultivated on YPD media not treated (top and bottom left), treated with 100 ppm of **OENOBRETT**® after 8 days (top and bottom right).

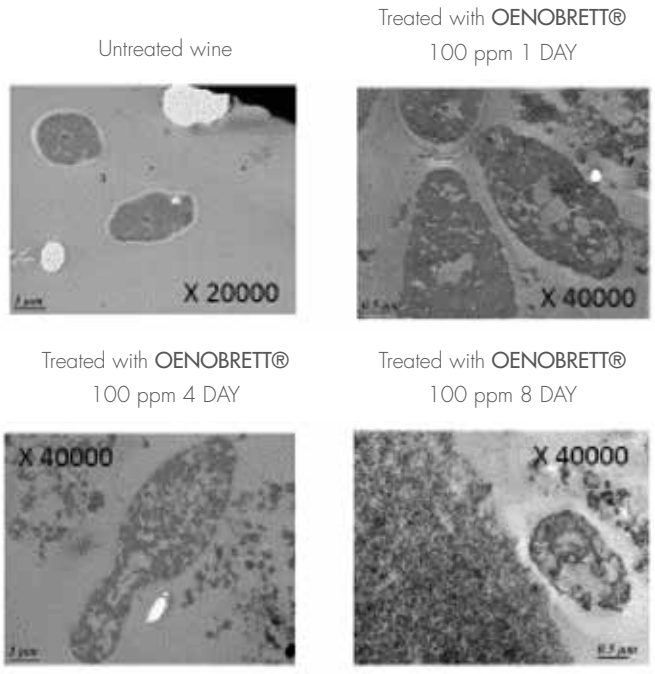


Figure 3b. *B. bruxellensis* cells in a wine naturally contaminated, with and without **OENOBRETT**® treatment 100ppm and observed after 1, 4 and 8 days of treatment.

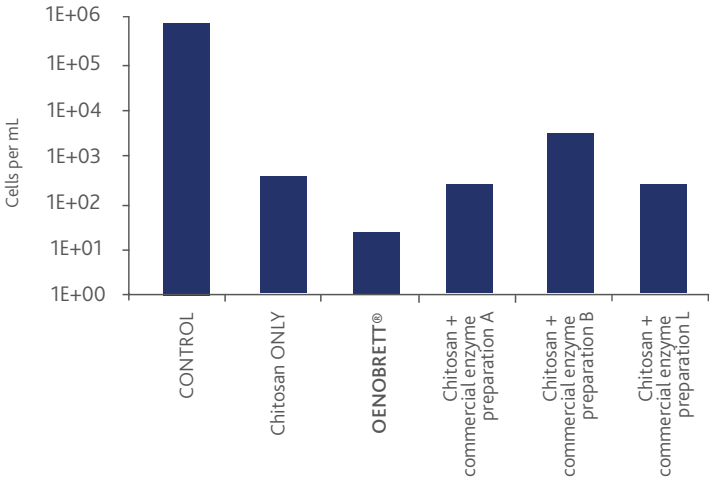


Figure 4. Effect of chitosan with and without enzymatic activities on viable *B. bruxellensis* cells.

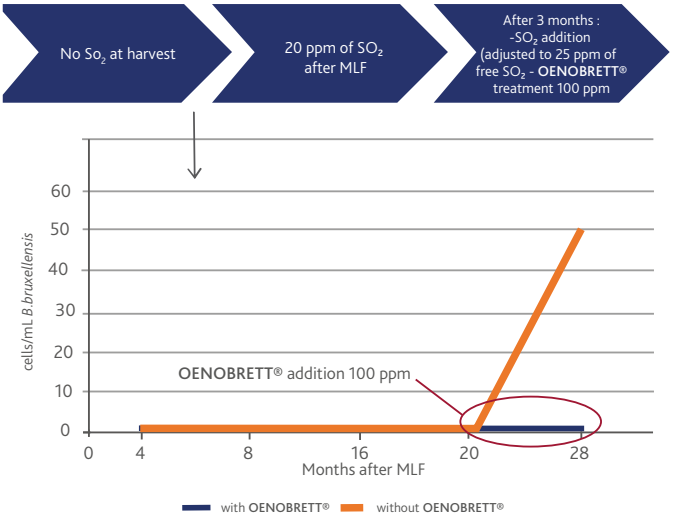


Figure 5. A red wine ex Margaux region with minimal SO_2, monitored for the growth of B. *bruxellensis* up to 5 months post MLF. (**LAFFORT®** and **EXCELL** laboratories, Bordeaux France).

PREVENTATIVE APPLICATION

Chitosan/ß-glucanase combinations may be used successfully as a preventative form of treatment before *B. bruxellensis* has the opportunity to proliferate. Because the combination affects cell viability, it will inhibit any growth before the cell mass is able to grow. Often the presence of *B. bruxellensis* is only determined by the winemaker when taint is detected sensorially. By this stage, the *B. bruxellensis* population has reached high cell numbers, in the order of 50 - 100 cells/mL. By preventing the growth in the first place, a winemaker is able to maintain a higher proportion of molecular SO_2 by ensuring there is a growth prevention strategy such as **OENOBRETT®** in place. This method is also much less dependent on personnel to monitor and detect a problem and becomes part of a standard operating procedure. Trials in 2013 on a red wine from Margaux was able to limit the growth of *B. bruxellensis* by using chitosan as a preventative treatment (Figure 5). Growth of this species in the control was recorded after four months of storage following primary and secondary fermentation. This preventative treatment can be used:

- On topping wine in case of barrel ageing.
- On wine lees.
- On red pressings (these contain a higher microbial load than the free run fraction).
- On wine with high pH (less available molecular SO_2).
- On wine with high micro-organisms population.

CURATIVE APPLICATION – OENOBRETT®

Application of chitosan/ß-glucanase based treatments is often more common once a problem has been detected. As we have seen, its application is able to effectively kill the yeast cell, literally destroying the cell structure. Trials conducted in 2013 demonstrated a complete reduction of population from $3x10^3$ cells/mL to not detected after the addition of 100 ppm of **OENOBRETT®** (Figure 6). The population required to commence producing 4-EP and 4-EG will vary depending on the wine in question, but often produces detectable levels in the $1x10^2$ to $1x10^3$ cells/mL range (Chatonnet et al. 1992). At this population level, SO_2 will be consumed very quickly — both free and total SO_2 will be greatly reduced here and unavailable from an antimicrobial point of view if added. At this cell level it is ultra-critical to reduce the microbial load in order to ensure a portion of molecular SO_2 to prevent further growth.

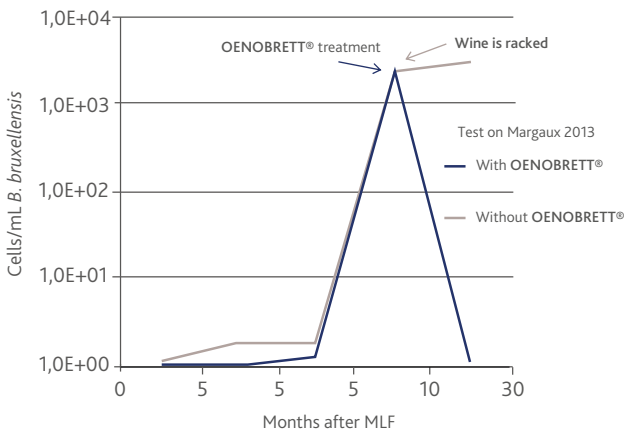


Figure 6. Use of **OENOBRETT®** on a red wine from Margaux after the detection of ***B. bruxellensis*** whereby viable cells are no longer detectable after treatment (**EXCELL** laboratories).

CONTROLLING BACTERIA

The control of both lactic acid bacteria and acetic acid bacteria also comes into question primarily post-alcoholic fermentation, but high pH can induce the proliferation of *Pediococcus spp.* and *Acetobacter spp.* pre-fermentation if the must is left unprotected. The primary role of lactic acid bacteria is to conduct malolactic fermentation (MLF) which is not always a desired outcome. Lysozyme alone may not be sufficient to kill the entire population of lactic acid bacteria, which can reach up to $1x10^8$ cells/mL during MLF, making it quite challenging to stop. The combination of lysozyme, chitosan and ß-glucanase has an effect not only on *Oenococcus oeni* cell membrane (principal bacteria responsible for MLF) but also *Pediococcus spp.* which produces a lot of ß-glucans, making it difficult to lyse with lysozyme alone. Figure 7 demonstrates the arrest in MLF in 2017 trials on base wine for cognac distillation when there is a high population of bacteria that has already started to conduct MLF with the use of a chitosan, ß-glucanase and lysozyme mix used at 200 ppm.
Acetic acid bacteria, on the other hand, is not affected by lysozyme and has limited impact from chitosan (Valera et al. 2017). These species are responsible for spoilage in the form of acetic acid production and are commonly found in levels of 10^3-10^4 cells/mL in unfiltered wines. Combinations of chitosan, ß-glucanase and potato protein may be used in wines where there is an unidentified microbial problem as a blanket strategy. Whilst the *Acetobacter spp.* may not damage the cell wall, **MICROCONTROL®** can drop out by means of fining/sedimentation a portion of the microbial load. Understanding that these populations are present and the risk factors associated with their proliferation after reaching critical mass is imperative to providing protection. Factors such as ullage, low molecular SO_2, high percentages of whole bunches (which can cause high levels of acetic acid bacteria) and unmanaged caps on red wine fermentations may cause proliferations of acetic acid bacteria.

OXIDATIVE PROTECTION

During alcoholic fermentation, the space is often protected due to complete saturation of CO_2 produced by fermentation. As fermentation slows down or goes through MLF, the level of CO_2 may drop and enable oxygen to contact the wine. Strategies to control microbial populations will not protect the wine from oxidation, but limiting the proliferation of spoilage microorganisms will enable the wine to retain more SO_2 in molecular form.

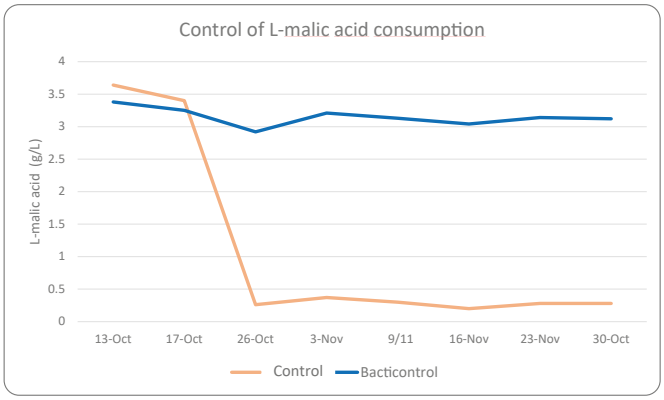


Figure 7. 2017 trials on Cognac base wine (EXCELL laboratories, Bordeaux).

CONCLUSIONS

Using lower rates of SO_2 post fermentation poses the risk of spoilage, primarily due to the proliferation of *B. bruxellensis* and *Acetobacter spp.* after primary and secondary fermentation. *B. bruxellensis* may cause taint due to the production of taint compounds 4-EP and 4-EG and *Acetobacter spp.* are able to metabolise ethanol to form acetic acid. By understanding which microorganisms pose a risk at this stage in production, it is possible to target their growth directly via the use of chitosan and ß-glucanase combinations that disrupt structural components in the cell membrane (chitin and glucans). The chitosan by itself does not display the same efficacy as chitosan and ß-glucanase together on *B. bruxellensis* cell death.

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Q&A

COLOR STABILITY

1. Why is coloring matter instability an issue?

Reductions in the use of fining treatments, earlier bottling, more consistent cellar temperature regulation (instead of cold crashes during winter), and high-extraction vinification processes such as flash détente or thermovinification have all contributed to rising color instability in wines on the market. Along with this, a broader cross section of consumers and decreased acceptance of perceived faults in wine, including precipitates, has led to consumer desire for color-stable wines. While these new processes may improve the sensory quality of wines, they often lead to color instability.

2. What causes color instabilities?

Color instability happens when the concentration of color-unstable anthocyanin molecules causes them to bind with certain fractions of proteins or polysaccharides and precipitate out of solution. It can occur in colder wine conditions just like tartaric instability and sometimes at the same time to produce purple-colored tartaric crystals. Color stability is most important with rosé wines and is gaining attention in red wines, despite general consumer acceptance that red wines precipitate color over time.
Color instabilities can be either crystalline when associated with large amounts of tartrates, or more amorphous, even appearing gelatinous and deeply colored, and are generally found as a fine film coating the inside of the bottle after long term aging.
Color stability in red wines is typically achieved when catechins bind with free anthocyanin to form stable color compounds. In rosés or red wines with low tannins and higher extraction techniques (thermovinification, flash détente), the insufficiency of tannins creates a higher likelihood of free anthocyanins to remain unstable. In case of an unstable wine color, treat with fining agent (**GECOLL® SUPRA** or **OENOCELL®**), gum arabic (**STABIVIN®**) or mannoprotein (**MANNOSTAB® LIQUIDE 200**).

3. How do I determine the dosage rate for color stability?

For stabilization, run color stability checks at different rates and use the minimum amount required to achieve the stability target.
COLD TEST:
Stability is **estimated** by measuring the turbidity before and after cold storage in the following conditions:

√ Filter 30 mL of wine on a 0.65 µm membrane (+ prefilter).
√ Measure the turbidity of the sample: NTU before cold.
√ Place the sample at 4°C for 48 hours.
√ Take out of the cold and, after 15 min at room temperature, measure the turbidity NTU after cold.

ΔNTU = NTU after cold – NTU before cold

Δ turb (NTU)	< 5 NTU	Stable
Δ turb (NTU)	5 - 20 NTU	Moderately Unstable
Δ turb (NTU)	> 20 NTU	Unstable

*In case of an unstable wine, treat with fining agent (**GECOLL® SUPRA, OENOCELL®**) or **STABIVIN®** until the test is positive.*

4. How do I tell which arabic gum to use?

An additional benefit of gum arabic is that it can increase the roundness of wine on the palate. Gum arabic can smooth out tannins and change viscosity which improves mouthfeel. Gums can also reduce perception of acidity, astringency, alcohol and bitterness. Bench trials are recommended to find the right dosage rate to achieve desired effect.
STABIVIN® (gum arabic derived from *Acacia senegal verek*) is most efficient at stabilizing colloids and color pigments in rosé and red wines. While technically still a stabilizer, the gum from the *Acacia seyal* tree is especially prized for its superior mouthfeel qualities which is what makes **STABIVIN® SP** a great option for last minute finishing touches before bottling.

*5. Which arabic gum is used along with **CELSTAB®** to stabilize red wines.*

STABIMAX® is the best option to stabilize color for red wines when using **CELSTAB®** to stabilize tartrates. However, for a stand-alone color stabilization effect, **STABIVIN®** is still the best choice. The two products are blends of gums with different properties, each best suited to its application.

ARTICLE

CHEMICAL CHARACTERIZATION OF COLORING MATTER PRECIPITATES

Chemical characterization of coloring matter precipitates Shipra Prakash under the direction of ERICK **DUFOURC** and AXELLE **GRÉLARD** from the Institut de Chimie et Biologie de membranes et nano-objets (CBMN), UMR5248, CNRS, University of Bordeaux, France.

Coloring matter is defined as a precipitate that appears when the wine is exposed to low temperatures. This precipitate can be formed during maturation depending on storage conditions. Its appearance is not appreciated when it occurs too early in the bottle.
Knowledge on this subject is very limited. Oenologists can decide to prevent this risk of precipitation by fining or cold stabilization. Today a cold test exists to predict wine instability. However a new coloring matter precipitate can reform in the wine during ageing.
Previous studies argued that coloring matter was formed of a complex between phenolic compounds and a polysaccharide or protein-type colloid. This coloring matter complex is described as having a low molecular mass as it can be eliminated by dialysis, but it can be recreated thereafter more or less rapidly according to storage conditions.

APPLYING AN INNOVATIVE TECHNOLOGY IN THE FIELD OF OENOLOGICAL RESEARCH.

Understanding stabilization mechanisms involves identifying the molecules responsible for the instability; for this reason, our team decided to reopen the subject in collaboration with the European Institute for Biology and Chemistry platform at the University of Bordeaux. For this project, ultra-high field Nuclear Magnetic Resonance technology is used (RMN 800 MHz. This non-destructive technique makes it possible to identify and quantify the compounds that constitute coloring matter precipitates.

CHEMICAL COMPOSITION OF COLORING MATTER PRECIPITATES.

Analysis of the precipitates was carried out by carbon NMR during maturation in barrels. The presence of potassium bitartrate was observed in the first months but this disappeared rapidly by natural precipitation over the course of the winter (Figure 2). The duration of maturation in barrels did not modify the other families of substances implicated in coloring matter precipitates, which were dominated by the presence of phenolic compounds and polysaccharides.

The precipitate also contained amino acids and soluble compounds of the wine, such as glycerol and lactic and succinic acids (Figure 3).

PRINCIPAL DIFFERENCES BETWEEN COLOURING MATTER PRECIPITATES IN CABERNET SAUVIGNON AND MERLOT.

The profiles of the precipitates by NMR of the solid matter obtained from Cabernet Sauvignon and Merlot presented the same families of compounds. Only the quantity of polyphenols differed. It was greater in the Cabernet Sauvignon precipitate than in that of the Merlot.
When the precipitates were solubilized in water, NMR analyses of the liquid (Figure 4) indicated that the polyphenols present in the coloring matter from the Merlot were more soluble than those of the Cabernet Sauvignon. This observation indicated differences of proportion within the phenolic compounds of these two grape varieties and presumably in terms of their hydrophobicity.

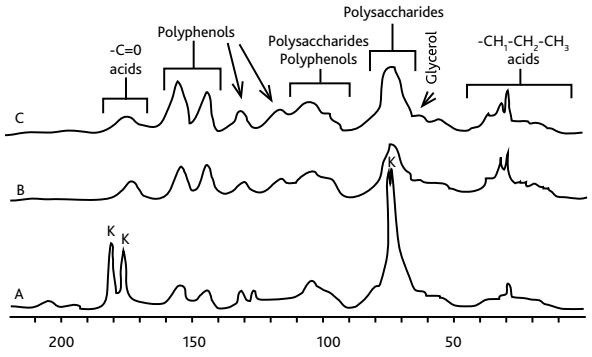


Figure 2. Analysis of coloring matter precipitates (6 days, -4°C/ 24.8°F) derived from Cabernet Sauvignon and Merlot wines (2013) during maturation using NMR 13C. A: Merlot after 1 month maturation; B: Merlot after 4 months maturation and C: Cabernet Sauvignon after 4 months maturation. K indicates potassium bitartrate peaks (from Food Chemistry (2016) 199, 229 - 237).

CONCLUSION.

Thanks to this work, 81% of the molecules that constitute the coloring matter precipitate were identified and quantified;

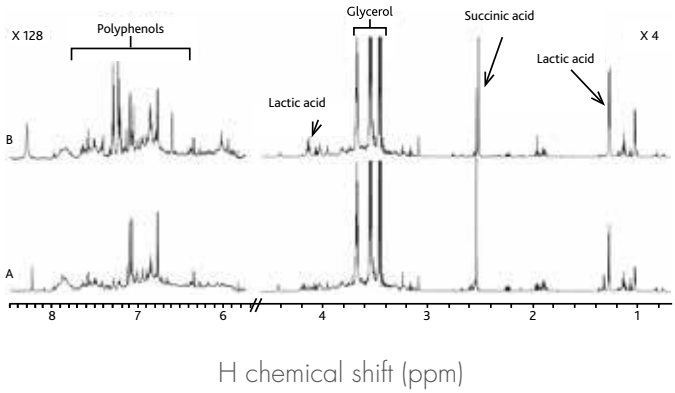


Figure 3 Analysis of the fraction of colouring matter precipitate that was soluble in water (6 days, +4°C) from Cabernet Sauvignon and Merlot wine (2013) after 1 month maturation by NMR 1H (from Food Chemistry (2016) 199, 229 - 237).

51% were polyphenols. These results open new perspectives for stabilization mechanisms and fining in red wines. The pertinence of the cold test has been demonstrated: today it is an efficient method for measuring instability in terms of coloring matter.

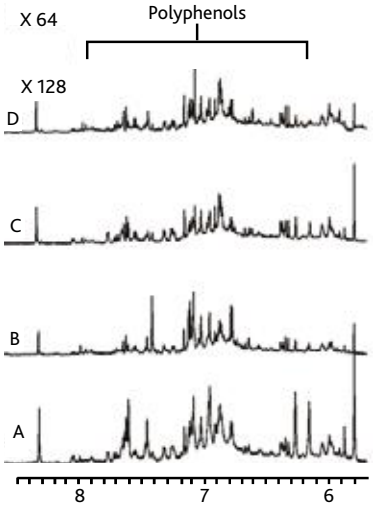


Figure 4 Analysis of the fraction of Merlot colouring matter precipitates that is soluble in water at -4°C/24.8°F, 6 days (A) and at +4°C/ 39.2°F, 6 days (B) and Cabernet Sauvignon at -4°C/ 24.8°F, 6 days (C) and at +4 °C/ 39.2°F, 6 days (D) after 1 month maturing by NMR1H.

ARTICLE PUBLISHED ON THE SUBJECT:

Prakash S., Iturmendi N., Grelard A., Moine V. & Dufourc E. 2016. Quantitative analysis of Bordeaux red wine precipitates by solid - state NMR: Role of tartrates and polyphenols. Food Chemistry. 199: 229 - 237.

Q&A

PROTEIN STABILITY

1. What is protein instability?

Protein hazes are formed either slowly during storage or quickly on exposure to heat; they have a haze-inducing, light-dispersing effect, without sediment, or may form an amorphous deposit at the bottom of the bottle.

This instability occurs when proteins within a wine, upon exposure to heat, unfold and begin to flocculate in suspension, thereby creating a haze in wine. This is most important in white and rosé wines due to their transparent nature. Even red wines with high amounts of proteins and low concentrations of grape and oak tannins may exhibit protein instability.

Protein (heat) stabilization currently has only one solution – bentonite clay. This is negatively charged and pulls out the positively charged proteins. Since bentonite is a naturally occurring material, quality varies greatly from one site to another. **MICROCOL® ALPHA** is sourced from the highest-quality sites making it extremely effective in binding with proteins while minimally affecting aromatics and flavors.

2. How do I test for protein stability?

There are multiple effective tests available based on combinations of temperature and time. Most common in the U.S. wine industry are heating the wine to 80°C / 176°F for 2 hours or heating the wine to 60°C / 140°F for 24 hours. **LAFFORT®** recommends 80°C / 176°F for 30 minutes. Holding for 2 hours at 80°C / 176°F gives very similar results.

Measuring Heat Stability:

- Measure the wine turbidity.
- If > 2 NTU, filter the wine (cellulose ester membrane, 0.65 µm)

#turb1

- Heat 40 mL of wine for 30 minutes at 80°C / 176°F.
- Let it cool for 45 minutes at room temperature.
- Measure the wine turbidity again.

#turb2

- If turb2 – turb1 > 2, the wine is not stable.

Testing different levels of bentonite additions will allow you to decide on the minimum dose rate required to achieve protein stability.

3. What influences how much bentonite I need for protein stability?

The amount of bentonite needed to achieve protein stability (defined as ≤ 2 NTU after 30 minutes at 80°C / 176°F) depends on the pH of the wine, the concentration of heat-unstable proteins within the wine, the type of bentonite being used, and the quality of the bentonite. These parameters are affected by varying factors in the vineyard, in the production of bentonite, and cellar practices.

In the vineyard, heat-unstable proteins increase with grape maturity as does pH. The rise in pH makes it more difficult to remove heat unstable proteins with bentonite because the proteins will have only a slight positive charge or none at all in high pH environments. This change from positive to neutral or negative charge is due to the isoelectric point of the heat unstable proteins. In high pH wines, more bentonite may be needed to achieve protein stability. **MICROCOL® ALPHA** performs well even in high-pH wines.

Factors like vintage variation, varietal (e.g., high-protein Sauvignon Blanc and Gewürztraminer), and fruit soundness can also affect the protein-pH relationship.

Once fruit has arrived at the cellar, exposure to solvents like SO_2 can extract more proteins from the skins and pulp. Reducing SO_2 during processing through bio-protection (**ZYMAFLORE® ÉGIDE**^{TDMP}) and/or sacrificial tannins (**TANIN VR SUPRA®**, **TANIN GALACOOL®**) will lower protein instability downstream. Minimizing skin contact will also reduce protein extraction – if skin contact is important for wine style, expect to need more bentonite for stability.

4. Are there any interactions to avoid when using bentonite?

Only one. Do not add enzymes with bentonite. Bentonite inactivates proteins. Enzymes are proteins. If you add enzymes with bentonite, the enzymes will be deactivated.

5. When is the best time to add bentonite?

Bentonite can be added to juice and/or finished wine. Calcium bentonite, such as MICROCOL® FT is ideal for settling juice. The plate structure does not swell nearly as much as sodium bentonite, so a higher dose is required, typically twice as much. However, calcium bentonite settles more compactly than sodium bentonite, and as a rule, adding twice the amount of calcium bentonite will result in one-quarter of the volume of lees, a big improvement in processing.

Sodium bentonites like **MICROCOL® ALPHA** are gentler in wine while remaining highly effective and require lower dose rates compared to calcium bentonite. Sodium bentonite, such as **MICROCOL® ALPHA**, will always require less to achieve protein stability compared to calcium bentonite and is best used after fermentation.

6. Are there alternatives to bentonite for heat stability?

Tannins can contribute to heat stability by binding with proteins and forming a precipitate, as is normally the case in red wines with higher amounts of grape and barrel or oak alternative tannins. With white and rosé wines, small (50-100 ppm) additions of **TANIN GALALCOOL® SP** can be beneficial in reducing bentonite needs, however tannins are not desirable in whites and thus bentonite is still the only way to achieve complete heat stability.

7. What happens if the bentonite is not properly prepared?

If not properly prepared and swelled, bentonite may clump or not be fully expanded, reducing the surface area available and the effectiveness of the bentonite-protein binding. If the preparation in the cellar is not as efficient as the preparation in the lab, this may lead to under dosing and potential instability in the wine. It is important to have a protocol and train cellar staff on thorough bentonite hydration.

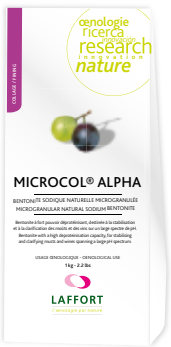
8. Won't bentonite strip my wine?

Bentonite can certainly remove positive aromatic compounds from a wine. This depends on the quality of the bentonite and the aromatic levels of the wine being treated. A highly aromatic wine treated with a premium bentonite is less likely to become stripped. A lower aromatic wine also benefits from premium bentonite as it needs to retain as much aroma as possible. The MICROCOL® series of bentonites are sourced from top quality sites and are known for being highly effective at protein stabilization yet gentle on aromatics.
Bench trials are highly recommended before any bentonite addition to determine the best balance of stability and aromatics.

9. What is the difference between sodium and calcium bentonite?

Bentonite, a.k.a. montmorillonite, is a hydrated aluminum silicate member of the smectite class of clays, comprised mainly of oxides of aluminum and silicon. In the substructure, occasionally aluminum is replaced with a different metal such as iron, manganese or magnesium, generating a deficiency in positive charge and the lattice takes on a net negative charge. This negative charge allows the bentonite to react with positively charged proteins in wine in an ion exchange process with the inter-laminar cations.
In many bentonites used for wine fining, the dominant cation is sodium, producing a high-swelling and high-exchange capacity, at the expense of slowly formed and diffuse lees. Other bentonites have calcium as the dominant inter-laminar cation, giving reduced swelling and exchange capacity, but allowing faster settling and vastly superior lees compaction.

With calcium bentonite, settling can be so rapid that the solution used for fining must be resuspended immediately prior to and during the addition process to ensure complete dispersion. Settling in tank can also be rapid, so that without continued and adequate mixing after addition, a below-optimal protein removal result will be obtained.



Q&A

TARTRATE STABILITY

1. What does tartrate instability look like?

Tartrate instabilities are almost always clear or translucent, or occasionally white crystalline deposits, a mix of potassium bitartrate and tartaric acid, and sometimes also calcium tartrates. They may also be found bound to other compounds, for example phenolics when color instability is also present.
Tartrate instability occurs because of super-saturation of Potassium Bitartrate (KHT) within a wine that precipitates out in the form of crystals as the temperature drops, reducing the wine's ability to retain the salt in solution.

2. What are the options for stabilizing tartrates?

Tartrates can be stabilized by either traditional subtractive or newer inhibitive methods. Subtractive methods force tartrates to fall out of solution from the wine. Inhibitive methods prevent tartrates from falling out of solution by the addition of a protective colloid that blocks the crystal nucleation sites.
Using reduced temperatures of 25 to 32°F (-4 to 0°C) for days or even weeks, the subtractive technique shifts the saturation curve, forcing the potassium bitartrate to be super-saturated, allowing precipitation to occur and the resulting precipitate to be removed. Seeding with KHT powder is optional and helps speed up the process of crystal formation as the powder provides ample nucleation sites. Still, this method can take weeks, require large amounts of energy, be expensive, oxidize the wine, reduce the wine's natural acidity and may be a hurdle for wineries reducing their carbon footprint or applying for Green certifications.
Inhibitors are substances that prevent crystallization. Carboxymethylcellulose (**CELSTAB®**) and mannoprotein (**MANNOSTAB® LIQUIDE 200**) are the two most common and trusted non-subtractive inhibitors. They result in a faster, less expensive, more energy-friendly process that has the additional benefit of not altering the acid levels or pH of the wine.

3. Is color instability the same as tartrate instability?

No, they are not the same although they do form under similar circumstances of cold temperatures. If they present themselves concurrently, the tartrate crystals may be colored. Color instabilities are often found with tartrate instabilities and one may increase the likelihood of the other causing problems.

4. Which laboratory tests are recommended for cold stability?

Contact your **LAFFORT®** Technical Representative to discuss the simple and efficient options of lab testing for tartrate and all other instabilities.
One important consideration for laboratory testing when using **CELSTAB®** is the need for the wines to be protein stable and note that the 60°C / 140°F test is not consistent. It may give correct results for the tendency of the wine to throw a haze from protein but is not sufficiently robust to make sure residual protein does not interact with **CELSTAB®**. When testing for protein stability to validate the use of **CELSTAB®**, the 80°C / 176°F test is required. See the Protein Stability section on page 106 for more information.

*5. **MANNOSTAB®** is a dark brown liquid. What will happen to the color of my wine?*

MANNOSTAB® LIQUIDE 200 will not darken a white wine or rosé. Its purity allows it to remain neutral when added to a wine at the low doses necessary to stabilize.

6. Should I still 'seed' with potassium bitartrate when using an inhibitor?

No. If using an inhibitor, then seeding with KHT is not necessary.

*7. Should I use **CELSTAB®** or **MANNOSTAB®**?*

CELSTAB® and **MANNOSTAB® LIQUIDE 200** are differentiated depending on the goals of the winemaker. Additionally, they both have limitations that can dictate which one should be used.
CELSTAB® is great for whites that are heat-stable and rosés that are both heat-stable and color-stable (see the test on page 103). **CELSTAB®** is not recommended for use with red wines unless supplemented with **STABIMAX®**, see below.
MANNOSTAB® LIQUIDE 200 allows for cold stabilization on whites and rosés that have not been heat-stabilized. **MANNOSTAB®** can also be used in color stable red and rosé wines.

*8. Do I add **CELSTAB®** or **MANNOSTAB® LIQUIDE 200** before or after crossflow filtration?*

Addition of **CELSTAB®** or **MANNOSTAB® LIQUIDE 200** is made after clarification filtration, including crossflow and pad filters.
It should be the last addition made to a wine, except for **STABIVIN® SP** (arabic gum), SO_2, gas adjustments, and ascorbic acid. When added to a wine with an acceptable filtration Clogging Index (<20 for **CELSTAB®** and <50 for **MANNOSTAB® LIQUIDE 200**) that is within all parameters for addition, both products do not raise the Clogging Index significantly and will fully integrate. It should be noted that if sterile filtration differential pressure goes above 0.8 bar, the filtration should be stopped and the membrane changed as colloidal stripping may occur; the protective colloids may be retained on the filter media, allowing the unstable tartrates to pass through the filter and decrease stability.

*9. Can **CELSTAB®** and **MANNOSTAB®** be used in sparkling wines?*

Both **CELSTAB®** and **MANNOSTAB® LIQUIDE 200** can be added prior to tirage bottling.

*10. I added **CELSTAB®** without following the protocol and now I have a haze; can I start over and add more?*

Do not add more **CELSTAB®**. Contact your **LAFFORT®** Technical Representative to receive guidance on how to proceed to recover the wine.

11. Is there a sensory impact from these products?

Both products are neutral regarding mouthfeel, flavor, aroma, pH, and color. Some winemakers report an improvement in mouthfeel, although the difference is slight and should not drastically change a wine.

*12. What is the interaction between **CELSTAB®** and tartrates?*

CELSTAB® is a negatively charged molecule that binds with the positively charged surface layer of potassium bitartrate crystal nuclei, preventing growth beyond the microscopic. Specifically, **CELSTAB®** binds to the 010 face of the crystal and prevents further attachment of potassium bitartrate crystals.

*13. Can **CELSTAB®** be used on red wines?*

CELSTAB® is not advised for use on any red wines without additional treatment due to its affinity for non-polymerized color compounds. A specific arabic gum, **STABIMAX®**, will allow use of **CELSTAB®** on more reds, including younger ones. Contact your **LAFFORT®** Technical Representative for more details. Alternatively, a mannoprotein stabilizer such as **MANNOSTAB® LIQUIDE 200** may be used on red wine, although, again, color stabilization may still be required prior to addition.

*14. Why has my NTU increased after adding **CELSTAB®**?*

There may be a slight change in turbidity after the addition of **CELSTAB®** but remember that turbidity does not equal filterability. The change in turbidity, if it occurs, will not directly affect filterability.

15. Are all mannoprotein stability products the same?

Certain mannoproteins can interfere with the nucleation and crystallization of super-saturated KHT. Of these mannoproteins, those with molecular weight of around 40 kDa were determined by **LAFFORT®** research in France to be the best at stabilization, known as MP40. Found in yeast walls, this stabilizing compound is isolated by a **LAFFORT®** patented enzymatic and filtration process to purify and concentrate it. **MANNOSTAB® LIQUIDE 200** is a homogenous solution of MP40 and will cold stabilize the wine for long periods of aging.

16. What are the overall costs of using these products?

CELSTAB® and **MANNOSTAB® LIQUIDE 200** both offer substantial cost savings per bottle versus traditional cold stabilization. **CELSTAB®** is far less expensive, generally with a treatment cost of 1/3 to 1/10 of the cost of **MANNOSTAB® LIQUIDE 200**.
The greatest monetary savings are the lowered requirements in terms of energy, time, labor, and water. Using Celstab or Mannostab can help wineries reduce carbon footprints and work towards 'Green' certificates.

17. Are these products utilized in an organic, biodynamic, or natural winemaking program?

MANNOSTAB® LIQUIDE 200 is authorized for use by the European Organic legislation N°. 889/2008 and the National Organic Program of the USDA. For any other programs, it is the winery's duty to ensure compliance with and authorization by each program prior to product usage.

18. What about calcium instability?

While **CELSTAB®** and **MANNOSTAB® LIQUIDE 200** are excellent stabilizers for tartrates, they will not correct for calcium instability. For this reason, it is important to ensure wines treated with these products are below 60 mg/L calcium, otherwise calcium tartrates may occur.

19. Is metatartaric acid legal in the USA?

The U.S. wine industry has never expressed an interest in metatartaric acid. It is widely available in many other countries. Contact your **LAFFORT®** Technical Rep if you are interested in working with this.

ARTICLE

ALTERNATIVES TO REFRIGERATION. PRACTICAL COMMENTARY AROUND THE USE OF CMC.

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TARTRATE INSTABILITY

Tartrate instability is the phenomenon that occurs at a specific temperature, when tartaric acid salts become super-saturated: their concentration is higher than the quantity theoretically soluble. Under cooler conditions this state leads to the formation of crystals. All types of wine can incur tartrate instability including white, red and rose wines.

Precipitation of these crystals can be favoured by exposure to cold, colouring matter instability (blending vintages with younger wines), and deacidification treatments before bottling. Wines which were tartrate stable may become unstable after filtration due to the clogging of protective colloids that prevent tartrate formation in filtration.

Tartaric acid itself is not found very commonly in nature and concentrations range from less than 6 g/L in cooler climate grapes and 2 - 3 g/L in warmer climate grapes (Ribéreau - Gayon et al 2006). Tartaric acid can form up to five different salts which all vary in their solubility in alcohol-based solutions (listed below). Between tartaric acid, potassium bitartrate and neutral potassium tartrate, each of these compounds will exist in different levels at different pH (Figure 1). The pH which the wine is at will determine whether the pH will increase or decrease should KHT precipitate. Alcohol levels depending, pH 3.6 KHT precipitation will lower the pH. Below pH 3.6 KHT precipitation will increase the pH (Ribéreau - Gayon et al 2006). At pH 3.6 KHT precipitation is fast and the pH should remain the same.

- Potassium hydrogen tartrate (KHT) or Potassium bitartrate.
- Neutral potassium tartrate (K2T).
- Neutral calcium tartrate (CaT).
- Potassium and calcium tartrate double salt.
- Mixed salt potassium and calcium tartromalate.

There are various methods which are employed to test the cold stability of a wine. The treatment options for these wines can broadly categorised into two categories: Subtractory (Physical removal of constituents responsible for precipitation) or Inhibitory (Inhibition of KHT crystal nucleation and/or growth phase).

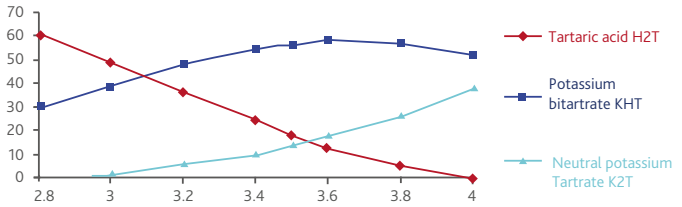

Figure 1. Tartaric acid, potassium bitartrate and neutral potassium tartrate over a pH range of 2.8 – 4.0.

SUBTRACTORY	INHIBITORY
Cold treatment (with or without seeding cream of tartar)	Metatartaric acid: POLYTARTRYL® (Inhibition of crystal nucleation and growth)
Electrodialysis to remove K+ ions	Sodium Carboxymethylcellulose (CMC): **CELSTAB®** (Inhibition of crystal nucleation and growth)
	Naturally occurring Yeast mannoproteins (MANNOSTAB®) (Inhibition of crystal growth)

Table 1. Subtractory and inhibitory methods of preventing tartrate precipitation in wine

E466 CARBOXYMETHYLCELLULOSE (CMC): CELSTAB®

Sodium Carboxymethylcellulose (CMC) was approved for winemaking in Australia in 2011 as a crystalisation inhibitor. There are many variations between commercially available CMC's, with degree of substitution and length of the polymer chain (molecular weight) being very different (Bowyer et al 2010). OIV regulations (OIV resolution 366/2009) outline the molecular weight (indicating the length of the polymer) has to be in between 17 and 300 kiloDaltons, correlating directly

to the viscosity of the solution and therefore ease of use. The degree of substitution of glucose units within the CMC chain is again outlined by the OIV (must be between 0.60 and 0.95). This directly affects the solubililty of the CMC which will in turn impact the effectiveness of the treatment.

The **LAFFORT®** CMC (**CELSTAB®**) is produced to comply with OIV specifications (referenced by FZANZ standard 4.5.1) and selected to have optimal characteristics in terms of molecular weight and degree of substitution. The recommended rate 1 mL/L is based on these properties. **CELSTAB®** is not recommended in red wines for tartrate instability as it can interact with colouring matter and prevent tartrate inhibition. **CELSTAB®** may be used for rose if it is colouring matter stable, however adding **CELSTAB®** to 100 mL of wine and left at 4°C/39.2°F for 48 hours is recommended to ensure there is no residual colouring matter that it can bind to and form a haze (Figure 3).

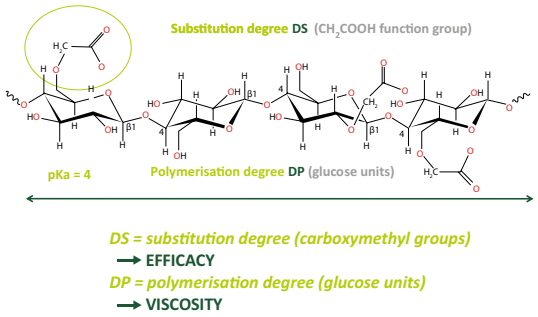

Figure 2. Sodium Carboxymethylcellulose molecule.

In white wine, **CELSTAB®** is recommended as a cost effective and efficient potassium tartrate stabilisation treatment, removing the requirement for refrigeration to tartrate stabilise a wine. **CELSTAB®** should be the last treatment to wine before final filtration and bottling, excluding polysaccharide additions for mouthfeel (ie. **STABIVIN® SP**), SO_2, CO_2 and ascorbic acid.

CALCIUM SALT PRECIPITATION?

Calcium concentration should be below 60 mg/L. It is recommend testing juice as early as possible, during fermentation if necessary. Wine de - acidification with calcium carbonate, treatment with calcium - based products in the vineyard, soil type, poor quality calcium bentonite and skin maceration can elevate calcium levels above 60 mg/L ppm, test treated wines. Elevated calcium levels can cause calcium tartrate precipitation, and **CELSTAB®** is not efficient in stabilisation this type of precipitate.

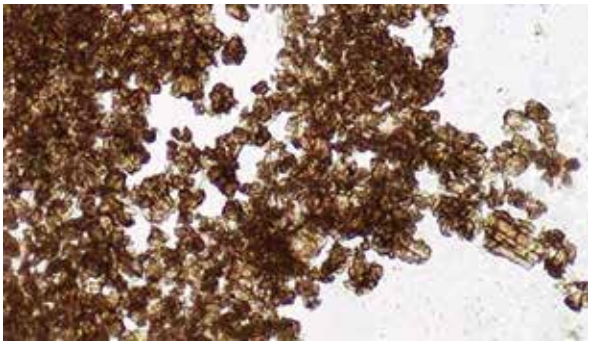

Figure 3. Effect of **CELSTAB®** on a colour matter unstable rose (Picture sourced from EXCELL laboratories, France)

HEAT STABILITY

Wine must be protein stable (heat stable) as measured on filtered wine. CMC can interact with proteins and form a haze (McManus et al 1981) (Figure 4). Wines that have been treated with Lysozyme which is a heat-unstable protein should be bench trialled for **CELSTAB®** compatibility. Additional bentonite may be necessary since elevated protein levels can cause a haze with **CELSTAB®**.

LATE TANNIN ADDITIONS

A protein stability test is necessary in the case of addition of tannins in the last stages of wine preparation before bottling (following barrel ageing or a late addition of finishing tannins). The addition of tannins can cause the formation of a thermo unstable complex with any remaining proteins (McManus et al 1981), thus creating protein haze that the CMC can bind to. As a consequence, the tannin addition can also render the CMC less efficient and render the wine more susceptible to tartrate instability.

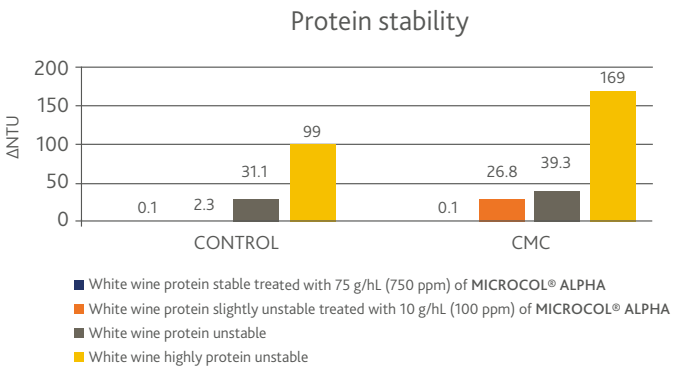

Figure 4. Level of protein stability after the addition of CMC (**EXCELL** laboratories, France).

FACTORS AROUND FILTRABILITY, CLOGGING AND COLLOIDAL LOAD

There is no correlation between filterability and wine turbidity; a clear wine can clog filters. It is essential to assess several parameters when preparing the wine for filtration. Colloidal load refers to unstable colloids are partly composed of anthocyanins in the form of the flavylium (charged +) ion, tannins, polysaccharides (including pectins) and proteins (+). The colloidal load of the wine will affect the filtrability/ clogging index of the wine.

	Control	Tannins 4 g/hL + CMC 100 mL/hL
	0,9	**10**
Turbidity after 48 hours		

Table 2. shows the interaction of a protein stable wine after the addition of 0.4g per 1 L of tannins and CMC.

Products added to the wine may affect the filtrability/ clogging index of the wine. Depending on the nature of the products, CMCs, and mouthfeel enhancing and stabilising polysaccharides may add to the colloidal load of the wine and increase the clogging index. However if this colloidal load is low to start with due to the use of enzymes, fining, bentonite and appropriate temperatures at filtration the addition of additional colloids should not impact the clogging index (Figure 5).

HOW CAN I DECREASE THE COLLOIDAL LOAD OF MY WINE?

1. ENZYME Addition - action on filterability. Ensures pectin and/or glucan chains breakdown, to improve settling (racking).
   a. PECTINASES
   b. ß. GLUCANASES
2. FINING - decreases the load. Ensures settling of particles in suspension (colloids) present in the wine. The addition of negatively charged bentonite allows for the stabilisation of particularly unstable compounds and makes them precipitate.
3. RACKING - decreases the load. Lees removal

Other factors that affect filterability

- TEMPERATURE – the colder the wine the harder it can be to start with.
- DEGASSING - Reduction of the CO_2 load ensures minimal degradation of the cake during DE filtration.

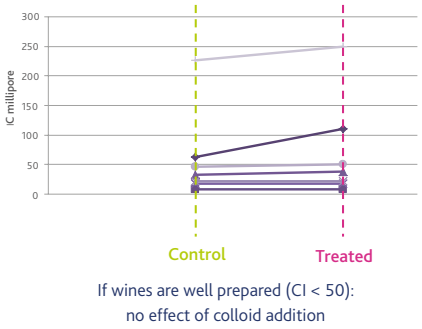

Figure 5. Effect of protective colloids on filterability (BIOLAFFORT, France).

APPLICATION

The initial tartrate instability can be measured by refrigeration test (- 4°C/25°F for 6 days). The clarity of the wine must be < 3.0 NTU. Pad or cross - flow filtration is highly recommended. Add **CELSTAB®** at 1mL per L after pad or cross - flow filtration and at least 48 hours before membrane filtration and bottling. Dilute **CELSTAB®** in 2 times the dosage volume with wine and mix into wine thoroughly.

COST

Lasanta and Gómez (2012) summarised the costs of tartrate stabilisation using various methods factoring in direct costs (energy, chemicals, labour, consumables, water, wine losses) and indirect costs (ammortization over 10 years). In all studies considered CMC was lower priced than the cold treatment with or without seeding. Low et al 2008 only looked at physical processes but the study was conducted in Australian wineries indicating that the energy and labour costs are likely higher than in 2008 but none the less realstic in an Australian context. Figure 5 compares the costs for cold stabilisation with and without seeding in 2008 (Low et al 2008) directly in AUD adjusted to cost per L in comparison to the current cost of **CELSTAB®** in 2019. The figure of 46.9 and 77.5 were derived from the batch cost (Low et al 2008) of $12900 and $21300 for 275 KL batches. The data is then expressed in $/1000L of wine. It is expected that the energy and labour costs from 2008 to 2019 would have increased significantly, making the cost saving of CMC in comparison to traditional cold stabilisation methods even more impactful. Whilst large businesses may be set up for cold stabilisation, many smaller wineries may not have the resources to achieve the cold temperatures for the amount of time required making CMC a practical solution.

	METHOD	DIRECT COSTS (€/HL)	AMORTIZATION (€/HL)	TOTAL COSTS (€/HL)	RATE[e] (%)
Gómez et al., 2002[a]	Cold tratment	0.76	0.19	0.95	100
	Ion exchange	0.07	0.04	0.11	11.58
	Electrodialysis	0.56	0.58	1.14	120
Low et al., 2008[b,c]	Cold treatment	1.38	0.67	2.05	100
	Cold treatment with seeding	3.74	0.69	4.43	216.10
	Semicontinue cold treatment	1.99	0.72	2.71	132.20
	Continue cold treatment	2.60	0.66	3.26	159.02
	Electrodialysis	3.1	1.57	4.68	228.29
Rondeau, 2011[d]	MTA	0.07	-	0.07	7.40
	CMC	0.7	-	0.7	73.68
	MP	3.0	-	3.0	315.78

Table 3. Cost of tartrate stabilisation Lasanta and Gómez (2012)

[a] Adapted from *Gómez et al.(2002).*
[b] Adapted from *Low et al. (2008)*
[c] Currency at April 7;2012 : 0.787€/AUD
[d] Extracted from *Rondeau (2011*)
[e] Rate considering cold treatment as 100

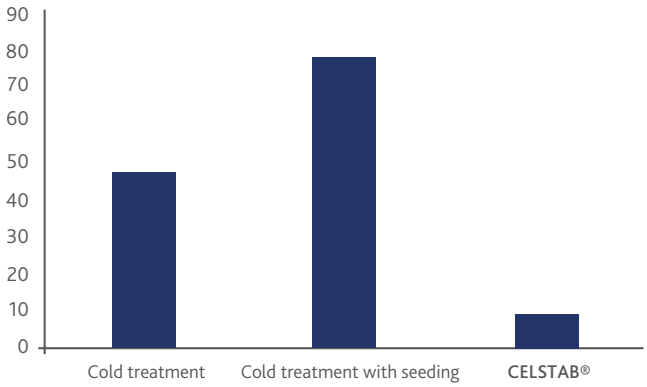

Figure 6. $AUD/1000L of wine. Values from cold treatment and cold treatment from seeding taken from Low et al (2008) in AUD based on Australian labour and electricity costs in 2008. Cost of CELSTAB® based on 2019 cost/L

CONCLUSION

CMC has been validated by the OIV since 2009 and in Australia since 2011. It is an interesting alternative to cold treatment, since requires less energy and is inexpensive and easy to implement. It is important to check the quality and suitability of any CMC product prior to its use in wine. Since it is produced from polymeric products and their physical and chemical parameters may vary considerably. **CELSTAB®** has produced and selected with optimal DP / DS for a wide range of applications.

CITATIONS

Bowyer, P.; Moine, V.; Gouty, C.; Marsh, R.; Battaglene, T. (2010) CMC: a new potassium bitartrate stabilisation tool. Aust. N.Z. Grapegrower Winemaker (558): 65–68.

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Checklist for Tartrate Stabilization with MannOstab® Liquide 200

This procedure for white and rosé wines aged more than 6 months will inhibit the precipitation of potassium bitartrate. The dose must be determined for each wine according to its unique content of protective colloids and to defined stability criteria. As neutral calcium tartrate precipitations are difficult to anticipate, treatment does not guarantee inhibition with regards to calcium salts.

IN ALL CASES, CONSULT YOUR LAFFORT TECHNICAL REPRESENTATIVE FOR ADVICE ON MANNOSTAB® TREATMENT.

DOSAGE

Doses between 50 and 150 mL/hL can be directly recommended according to wine aging conditions or calculated exactly using stability tests. Tests must be carried out after racking or pre-filtration. Any operation which modifies the wine's colloidal constitution modifies its tartaric stability and negates any prior stabilization testing.

DETERMINING DOSAGE BY THE COLD TEST

☐ Add **MANNOSTAB® LIQUIDE 200** with a micropipette to 375 mL bottles.

Treatment dose (mL/hL)	50	75	100	125	150
Volume of **MANNOSTAB® LIQUIDE 200**	187 µL	281 µL	375 µL	469 µL	562 µL

☐ Make each bottle up to 375 mL with the wine to be stabilized.

☐ Filter each sample under conditions as close as possible to those to be used for bottling (i.e., same filter porosity). For unfiltered wines, the sample must be in the same state of clarification as it will be at bottling.

☐ Store each sample at -4°C / 25°F for 6 days.

☐ After 6 days' storage at -4°C / 25°F, if crystallization has occurred in the control sample, the first sample dose in which there are no crystals is the stabilization dose. If the control does not precipitate, the wine is stable.

For red wines, it may be necessary to filter the samples after cold storage, the presence or absence of crystals on the membranes being used to determine the required stabilization dose (use prefilters with 2.5-to-3-micron nominal pore size, the objective being simply to retain any crystals that may have formed).

TREATMENT DOSAGE = STABILIZATION DOSAGE + 25 mL/hL.

IMPLEMENTATION

The addition of **MANNOSTAB® LIQUIDE 200** is carried out between preparation filtration and bottling filtration, on the day before bottling. After determination of the treatment dosage and **MANNOSTAB® LIQUIDE 200** addition, only SO_2, ascorbic acid or **STABIVIN®** (gum arabic) can be added to the wine.

IMPLEMENTATION

- Incorporate **MANNOSTAB® LIQUIDE 200** into the wine to be treated using a dosing pump or a Venturi system.
- Fully homogenize the **MANNOSTAB® LIQUIDE 200** in the tank (mix at least 1.5 volumes of the tank).

NON-FILTERED WINES

MANNOSTAB® LIQUIDE 200 must be added to the wine on the day before bottling, following the methods described above.

FILTERED WINES

☐ Good wine filterability is essential for a successful treatment procedure. Any blocking of the filtration media brings about modifications to the wine's colloidal structure and/or retention of **MANNOSTAB® LIQUIDE 200** and thus a decrease in the treatment efficiency.

☐ The turbidity (NTU) and clogging index (CI) measures are essential for defining adequate filtration conditions.

☐ Temperature of the wine during treatment and during bottling filtration must be above 15°C / 59°F. Avoid all thermal shocks (temperature variations > 5°C / 41°F) after bottling and for 72 hours post-bottling.

☐ If Clogging Index (CI) is greater than 20 after **MANNOSTAB® LIQUIDE 200** addition, pre-filtration at nominal 1.5 micron will be required before membrane filtration at bottling line. Consult your Technical Representative with additional questions.

☐ It is highly recommended to not exceed a differential pressure of 0.8 Bar (between the inlet and outlet of the filter) to avoid colloid and/or **MANNOSTAB® LIQUIDE 200** retention on the membrane.

STORAGE

Must be stored in original unopened packaging in dry, cool conditions (4°C/39.2°F - 20°C/68°F). Use within the specified use-by date. After opening, the products must be used within 24-48 hours if kept cool.

The vendor's responsibility is limited to the supply of a product which conforms to the corresponding technical data sheet. Since the usage of the product requires additional treatments that are the responsibility of the user, the vendor cannot be held responsible for results which do not conform to the preliminary trials.

Checklist for Potassium Tartrate Stabilization with CELSTAB®

STABILITY OF WINES

LAFFORT® offers a revolutionary solution for stabilizing potassium tartrate (KHT) in white and rosé wines. Please follow the checklist below to ensure that your wine is properly prepared for trial and production use of **CELSTAB®**. Please keep in mind that **CELSTAB®** should be the last treatment to your wine before final filtration and bottling, excluding **STABIVIN®**, SO_2, CO_2 and ascorbate.

Red wines > **CELSTAB®** is not recommended, consider **MANNOSTAB®** for tartrate stabilization of red wines.
White wines > **CELSTAB®** is recommended as a cost effective and efficient potassium tartrate stabilization treatment.

PRE-FERMENTATION

- ☐ Calcium concentration should be below 60 mg/L. Elevated calcium levels can cause calcium tartrate precipitation, and **CELSTAB®** is efficient in preventing only potassium tartrate precipitation.

POST-FERMENTATION

- ☐ Wine must be protein (heat) stable as measured on filtered wine held at 80°C for 30 minutes minimum with a final ΔNTU of less than 2.0.
- ☐ In the case of a late addition of finishing tannins, specifically those added after the addition of bentonite for protein stability, it is recommended to perform a protein stability test again.
- ☐ **CELSTAB®** may form a haze in wines treated with Lysozyme. If wines are treated with Lysozyme after heat stability is verified, perform an additional heat stability test.
- ☐ Prepare all sample wines for lab trials in the same manner as the bottling protocol.
- ☐ To verify the appropriate use of **CELSTAB®** treatment use the Checkstab Mini Contact Test.
- ☐ For 'Unfiltered' wines, the clarity of the wine must be < 3.0 NTU prior to **CELSTAB®** addition.
- ☐ For wines that will be passed through a membrane filter at the bottling, prior pad or crossflow filtration is highly recommended.
- ☐ Add **CELSTAB®** at 1 mL per liter of wine.
- ☐ Add **CELSTAB®** after pad or crossflow filtration and at least 24 hours before membrane filtration and bottling.
- ☐ Dilute **CELSTAB®** in 2 times the dosage volume with wine and mix into wine thoroughly.
- ☐ At membrane filtration and bottling the wine temperature should be >15°C and the membrane differential pressure should not exceed 0.8 bar.

ROSÉ WINES

CELSTAB® is recommended as a cost effective and efficient potassium tartrate stabilization treatment.

- ☐ Adhere to the checklist of all items above.
- ☐ Confirm color stability for treatment.

√ Prepare 100 mL of 0.65 µm filtered wine with 100 µL **CELSTAB®**, measure.

Record turbidity NTUi.

√ Place in refrigerator at 4°C for 48 hours, then remove and allow 30 minutes at room temperature.

Record turbidity NTUf.

- **If (NTUf – NTUi) < 5**
  the wine color is stable.
- **If (NTUf – NTUi) < 20**
  the wine color is moderately unstable.
- **If (NTUf – NTUi) > 20**
  the wine color is unstable.

In case of an unstable wine, treat with fining agent (**GECOLL® SUPRA**, **OENOCELL®**) or **STABIVIN®** until the test is positive

VALIDATION OF CELSTAB® PERFORMANCE

- ☐ To verify the efficacy of **CELSTAB®** treatment use the Checkstab Mini Contact Test .

STABILITY PRODUCT STORAGE AND PREPARATION*

PRODUCT	PREPARATION	SHELF LIFE UNOPENED AND OPENED	ADDITION TIMING	SPECIAL CONSIDERATIONS
CELSTAB®	Dilute in twice its volume in wine then homogenize in full wine volume.	2 years from production date, use immediately	24 hours before bottling	Consult Checklist for proper implementation.
LYSOZYM®	Dissolve into 10 x its weight in must or wine, then homogenize into full wine volume.	3 years from production date, use immediately	Biocontrol: Beginning of fermentation. MLF inhibition: End of alcoholic fermentation	Increases protein (heat) instability; analyze prior to bottling.
MANNOSTAB® LIQUIDE 200	Add slowly directly from bottle, then fully homogenize by mixing at 1.5 volumes of tank.	2 years from production date, use immediately	At least 48 hours prior to bottling	Consult Checklist for proper implementation.
MICROCOL® FT	Dissolve in roughly 10 times its weight in hot (50°C / 122°F) water, stirring continuously and vigorously for 2 hours. Swell for 12 - 24 hours. Mix vigorously in order to obtain a homogenous preparation before incorporation.	4 years from production date, use immediately	Juice stage for early fining.	Use inline dosing system.
MICROCOL® ALPHA			End of aging for protein stabilization	Use inline dosing system.
MICROCONTROL®	Dissolve in 10 x its weight in water or wine	3 years from production date, use immediately	After completion of MLF and several weeks before a racking. Optional: Prior to MLB addition if MLF is not desired.	Racking necessary after several weeks to contribute to reduction of microbial load through fining.
OENOBRETT® OENOBRETT® ORG	Dissolve in 10 x its weight in water or wine	3 years from production date, use immediately	After primary and ML fermentation	Do not rack for optimal performance. If racking is required, allow minimum of 8 days on the product.
SORBISOL K	Dissolve into 5 x its weight in water, use within 1 hour.	3 years from production date, use immediately	End of desired fermentation	Only use in conjunction with sulfur dioxide.
STABIMAX®	Add slowly directly from bottle, then fully homogenize by mixing at 1.5 volumes of tank.	30 months from production date, use immediately	After polishing filtration	Consult Checklist for proper implementation.
STABIVIN®	Add slowly directly from bottle, then fully homogenize by mixing at 1.5 volumes of tank.	2 years (1 L) or 30 months (5 L, 20 L, 1000 L) from production date, use immediately	After polishing filtration	Must be used on perfectly clarified wines.
STABIVIN® SP	Add slowly directly from bottle, then fully homogenize by mixing at 1.5 volumes of tank.	30 months from production date, use immediately	After polishing filtration	Must be used on perfectly clarified wines.

**All LAFFORT ® stability products can be stored at moderate temperatures in a clean, odor-free place.*

Once opened, liquid products may last up to three months if refrigerated. Note that trials must be performed with the same products that will be used in the cellar, whether fresh or previously opened. Anti-microbial products should not be stored after opening. Bentonite may be tightly sealed and kept in an aroma-free area.

LAFFORT

l'œnologie par nature

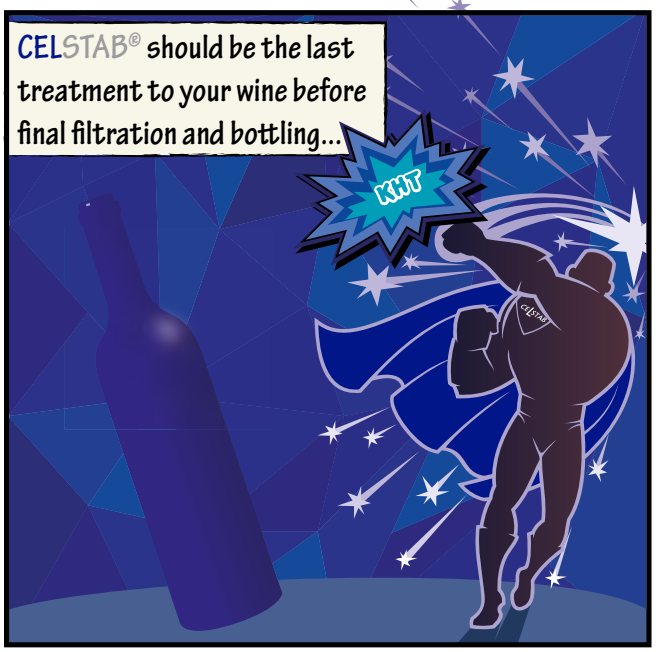
PRESENTS:

LAFFORT® HEROES

IN

CELSTAB®

THE CRYSTAL QUEST



SPARKLING WINES

SPARKLING WINES

BASE WINE PREPARATION

JUICE PREPARATION

BIOProtection and SO_2 reduction:
ZYMAFLORE® EGIDE™

Reducing SO_2 levels in sparkling base wine can give a better wine environment for secondary yeast fermentation.

Colonize the medium without fermentation activity to limit indigenous flora without any SO_2.

Add ZYMAFLORE® EGIDE™ directly to juice or grapes, no re-hydration necessary.

Dosage: 20 – 30 ppm

Juice clarification:
LAFAZYM® 600 XL ICE

Purified pectolytic enzyme for quick juice settling and complete depectinization.

LAFAZYM® 600 XL ICE is efficient at low temperatures (41°F and above) and low pH (2.9 – 4.0).

Dosage: 0.5 – 2 mL/hL

Fining and Color Adjustment of Juice:
POLYMUST® PRESS

Precise fining of each press fraction

Removes the oxidized and oxidizable phenolics, preserving color and aroma during aging.

DOSAGE: 200 – 600 ppm

CHARBON ACTIF PLUS GR

Color adjustment for hard press fractions

Activated carbon for decolorization of tinted juice.

Dosage: 200 – 600 ppm

BASE WINE FERMENTATIONS

Yeast choice for primary fermentation contributes significantly to the style and personality of the wine. Base wine has a low potential alcohol, which is an easy environment for all yeast strains in the **LAFFORT®** range. Choose a strain with an aromatic profile to match your desired wine style.

MALOLACTIC FERMENTATION

LACTOENOS® B16 STANDARD

***Oenococcus oeni* strain selected for low pH base wines.**
Very robust strain particularly adapted to low pH levels found in base wines. Pre-acclimatization is necessary. See product data sheet for a step by step protocol.

TARTARIC STABILIZATION

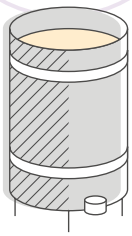
CELSTAB®

Solution of cellulose gum for inhibiting crystallization of potassium bitartrate. **CELSTAB®** is a 10% liquid solution for easy incorporation into the base wine. Addition is made entirely before tirage.
Dosage: 1 mL/L

SECONDARY FERMENTATION

Yeast Build Up Preparation

Base wine



YEAST BUILD UP PREPARATION

SUPERSTART® spark

Yeast rehydration preparation adapted to sparkling wine conditions (patent FR2736651).

- Combination of growth and survival factors to ensure a strong, clean, and complete "prise de mousse".
  Dosage: 200 ppm

TRADITIONAL METHOD (ELEGANCE, FINESSE, COMPLEXITY)

Chardonnay, Pinot Noir, Pinot Meunier

ZYMAFLORE® spark

Yeast strain recommended for elegance, finesse and full body sparkling wines.

- Develops tertiary aromas for fine, complex and elegant sparkling wines.
- Fast autolysis for optimal tirage aging.
- High resistance to alcohol and SO_2.

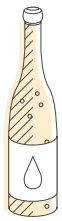
Tested and validated by the microbiological laboratory of the CIVC (Comité Interprofessionnel des Vins de Champagne).

CHARMAT METHOD (FRESHNESS, AROMATIC, SOPHISTICATION)

ZYMAFLORE® X5

Yeast strain selected for fresh and aromatic wines

- High production of varietal and secondary aromas (boxwood, grapefruit, exotic fruits).



ZYMAFLORE® X16

Yeast strain recommended for modern sparkling wines with high aromatic profile (white flowers, peach, orange blossom).

Prise de mousse

ASK ABOUT OUR YEAST STARTER CALCULATOR FOR TIRAGE

OPTIMIZED LEES AGING

For both traditional and charmat method added at tirage

FRESHAROM®

Specific preparation of inactivated yeast promoting the assimilation of glutathione precursors (5.3%) for aroma preservation. Excellent for Charmat Method:

- Delays the development of oxidized notes.
- Participates actively to the bubble finesse and foam persistence.
- **Dosage: 300 ppm**

OENOLEES®

Specific preparation of yeast cell wall extract (Patent EP1850682) with a high sapid peptide content giving a sweetness perception (Hsp12).

- Accelerates the development of "on lees" aging characters.
- Optimizes foam finesse and persistence.
- Traditional method - **Dose: 100 ppm**
- Charmat method - **Dose: 200 – 300 ppm**

TIRAGE MIXTURE

Traditional Method

TAN spark

Combination of gallic and ellagic tannin in liquid form.

- Protects base wine from oxidation during tirage process and aging on lees.
- Works with riddling agent (**CLEANSPARK®**) in the formation of compact yeast sediment for optimal riddling.
- Add to base wine before adding the riddling agent (**CLEANSPARK®**).
- **Dosage: 20 – 60 mL/hL**

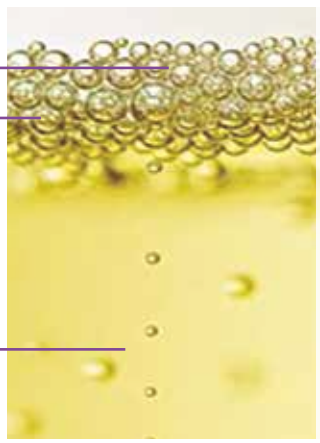
CLEAN spark

Riddling adjuvant (bentonite and alginate) for automatic and manual riddling.

- **CLEANSPARK®** envelops the yeast, preventing the yeast and solids from adherence to glass surface.
- Quick and complete removal of particles and sediments in bottles after aging on lees, forming a tight yeast plug for disgorging process.
- Add sugar, yeast, and all other components of the tirage mixture before adding **CLEANSPARK®**. **CLEANSPARK®** is the last addition to the tank.
- **Dosage: 60 – 100 mL/hL**

QUALITY OF BUBBLES

The quality of the foam is essential for customer satisfaction



INCREASES BUBBLE QUALITY

- Vegetal protein
- Macromolecule from yeast origin; mainly high molecular weight mannoprotein.
  (Ferreira et al., 2000 ; Dambrouk et al., 2004)

DECREASES BUBBLE QUALITY

- Lipids
- Fatty acids
  (Gallart et al., 2002 ; Dusseau et al., 1994)

EXPEDITION LIQUEUR (DOSAGE)

The best kept secret of every house and the final touch to give an identity to the product is the expedition (dosage) liqueur. It is the last opportunity to improve and adapt the product to the needs of each market; softness, mouthfeel, elegance and finesse, foam quality, fresh fruit aromas or aromatic complexity.

ARABIC GUM

STABIVIN®

Filtered and purified solution of 100% Verek arabic gum to stabilize the coloring matter in red and rosé sparkling wines. Add directly into expedition liquor.
Dosage: 70 – 100 mL/hL (for total wine volume)

MOUTHFEEL AND FOAM PERSISTENCE

Alternatives to sugar for giving a perception of sweetness

MANNOSPARK®

Liquid preparation of specific yeast cell wall mannoproteins (Patent 2726284):

- Reinforces the stabilization of tartrates and colloids in wine.
- Refines the size of bubbles to ensure their elegance and promotes persistence of foam at the surface of the glass.
- Adds mouthfeel and perception of sweetness.
- Traditional method: Add **MANNOSPARK®** directly to wine mixture used for expedition liquor.
- Charmat method: Add **MANNOSPARK®** to clarified base wine 48 hours before final filtration and carbonation.
- **Dosage: 100 – 200 mL/hL (for total wine volume).**

AUTOLEES®

Specific preparation of yeast cell wall extract rich in sapid peptides (Hsp12) and polysaccharides (Patent EP 1850682):

- Contributes to sweetness perception, allowing a lower quantity of sugar for dosage.
- Tool to help balance acidity and bitterness in the last stage of winemaking.
- Traditional Method: dissolve directly into wine mixture used for expedition liquor.
- Charmat Method: dissolve **AUTOLEES®** in 5 times its weight in water and mix into clarified base wine 24 hours before final filtration and carbonation.
- **Dosage: 50 – 200 ppm (for total wine volume)**

QUERTANIN® RANGE

High quality, oak tannin preparations to contribute subtle oak character for complexity and mouthfeel (similar to a brandy addition in dosage). Each tannin in the range has a unique profile, created with the different barrel toast levels as reference. Quertanins can be used individually or in combination and bench trials are required.

- Traditional method: Dissolve directly into wine mixture used for expedition liquor.
- Charmat method: Dissolve **QUERTANINS®** directly into clarified base wine 24 hours before final filtration and carbonation.
- **Dosage: 1 – 10 ppm (for total wine volume).**

ARTICLE

EFFECT OF THE ADDITION OF MANNOPROTEINS DURING THE PRISE DE MOUSSE ON THE LOSSES OF DISSOLVED CO_2 AND THE FOAM COLLAR OF ROSÉ SPARKLING WINE GLASSES.

Clara **CILINDRE**[1], Néréa **ITURMENDI**[2], Barbara **POTY**[1], Eric **RUPPÉ**[2], François **BOTTON**[2], Gérard **LIGER-BELAIR**[1], Virginie **MOINE**[2].
[1]Equipe Effervescence, Champagne et Applications, GSMA UMR CNRS 7331, Université de Reims Champagne. [2]LAFFORT.

[1] *Cilindre C, Liger-Belair G, Villaume S, Jeandet P, Marchal R,* Analytica Chimica Acta, 2010, 660, 164.
[2] *Liger-Belair G, Conreux A, Villaume S, Cilindre C, Food Research* International, 2013, 54, 516.
[3] *Liger-Belair G, Polidori G, Zéninari V, Analytica Chimica Acta,* 2012, 732, 1.

INTRODUCTION

Champagne or sparkling wines elaborated through the same traditional method, which consists in two major yeast-fermented steps, typically hold about 10 to 12 g/L of dissolved CO_2 after the second fermentation in a sealed bottle. Hundreds of molecules and macromolecules originating from grape and yeast cohabit with dissolved CO_2; they are essential compounds contributing to many organoleptic characteristics (such as effervescence, foam, aroma, taste and colour...). Indeed, the second alcoholic fermentation (called prise de mousse) and the ageing on lees (which may last from 12 months up to several years) both induce various quantitative and qualitative changes in the wine through the action of yeast [1].

In recent years, much interest has been devoted to better understand and depict each and every parameter involved in the release of gaseous CO_2 from glasses poured with champagne or sparkling wines [2,3]. Here, the impact of yeast mannoproteins on the progressive losses of dissolved CO_2 from a rosé sparkling wine was closely examined, under standard tasting conditions. The contribution of each yeast preparation, added during the 2nd alcoholic fermentation, to the collar height and to the bubble size was simultaneously evaluated.

EXPERIMENTAL PROCEDURE UNDER STANDARD TASTING CONDITIONS

A rosé base wine was elaborated according to the traditional method and divided into four different batches. Each wine was supplemented with three distinct preparations of yeast mannoproteins, namely: MP1, MP2 and MP1+MP2. The control wine was not supplemented with any preparation. The 2nd fermentation and ageing were carried out during 12 months.

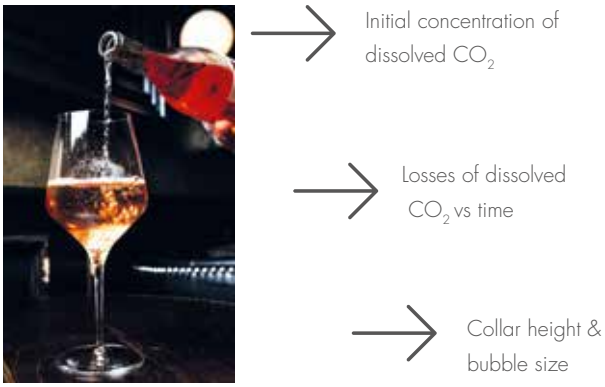


Figure 1. Flute poured with 100 mL of a rosé sparkling wine, served at 12°C/53.5°F. (photograph P. Thomas, Sipa press).

100 ± 4mL of rosé sparkling wine (12°C/53.6°F) were carefully poured into a laser-etched flute to promote bubble formation (Figure 1). All wines were examined with regard to their loss of dissolved CO_2 all along the first 10 minutes following pouring. Initial wine concentrations of dissolved CO_2, after pouring, were chemically assessed using carbonic anhydrase [1]. The total cumulative mass loss experienced by the flute poured with 100 mL of wine was recorded by a precision weighing balance (Sartorius, Secura 324 1S). A series of snapshots was taken, under the same tasting conditions, in order to follow the collar height and the bubble size.

LOSSES OF DISSOLVED CO_2 WITH TIME

The concentration of dissolved CO_2 directly impacts: the visually appealing frequency of bubble formation in the glass, the growth rate of rising bubbles, the tingling sensation in mouth and the aromatic perception of sparkling wines.

All batches of wines were found to initially hold (at t=0, after pouring) a concentration of dissolved CO_2 of about 7.51 ± 0.67 g/L (n=4).
As displayed in Figure 2, no significant difference appears between the four cumulative CO_2 mass loss-time curves.

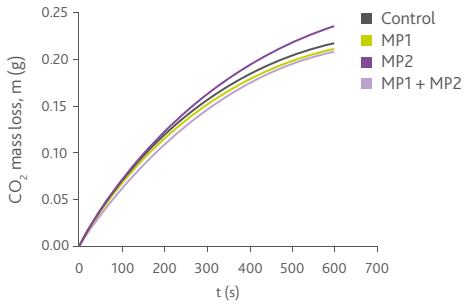


Figure 2: Cumulative CO_2 mass loss-time series corresponding to four rosé sparkling wines.

The progressive loss of dissolved CO_2 concentration with time, denoted ΔC(t), may finally easily be accessed by retrieving the following relationship:

$$\Delta C\ (t) = -\frac{M(t)}{V\ (flute)}$$

It is worth noting that, for a given rosé sparkling wine, the concentration of dissolved CO_2 found within a flute progressively decreases all along the 10 min following pouring.
The total loss of dissolved CO_2 concentration, at the end of tasting, was similar between the four rosé sparkling wines (Table 1).

Table 1: Total loss of dissolved CO_2, at the end of tasting (g/L) Means connected by same letter are not significantly different (P < 0.05).

Wine	Δ[CO_2] (t600 - t0), (g/L
Control	2.18 ± 0.38a
MP1	2.20 ± 0.21a
MP2	2.35 ± 0.23a
MP1+MP2	2.08 ± 0.29a

The addition of yeast mannoproteins during the prise de mousse, thus did not influence the loss of dissolved CO_2, under our standard tasting.

FOAM COLLAR HEIGHT DURING TASTING

The collar behaviour of the four rosé sparkling wines was followed during 10 min. The MP1, MP2 and MP1+MP2 rosé sparkling wines produced a significant thicker collar than the control wine. The collar of these three wines remained also stable until the end of tasting (as seen in Figure 3).

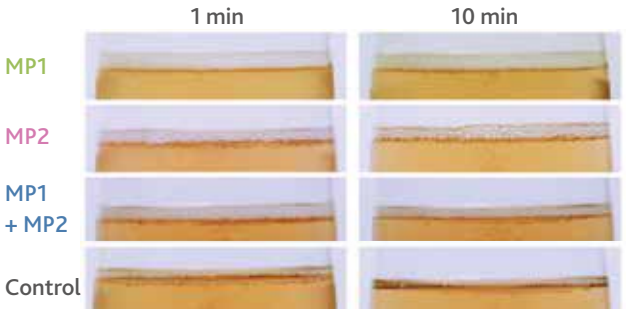


Figure 3. Closeup of the collar, at 1 min and 10 min after pouring, from the four rosé sparkling wines.

The photographs displayed in Figure 3 compare the collar height from the four rosé sparkling wines. It is clear that the bubble's size distribution is different among the four wines.
Indeed, as seen in Figure 4, MP1 showed significantly smaller bubbles, whereas larger bubbles are observed for MP2, all along the 10 min following pouring conditions.

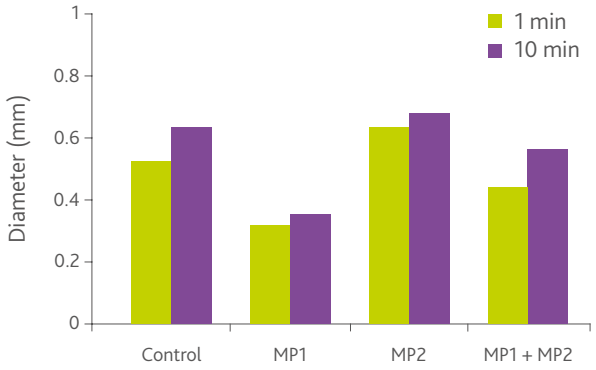
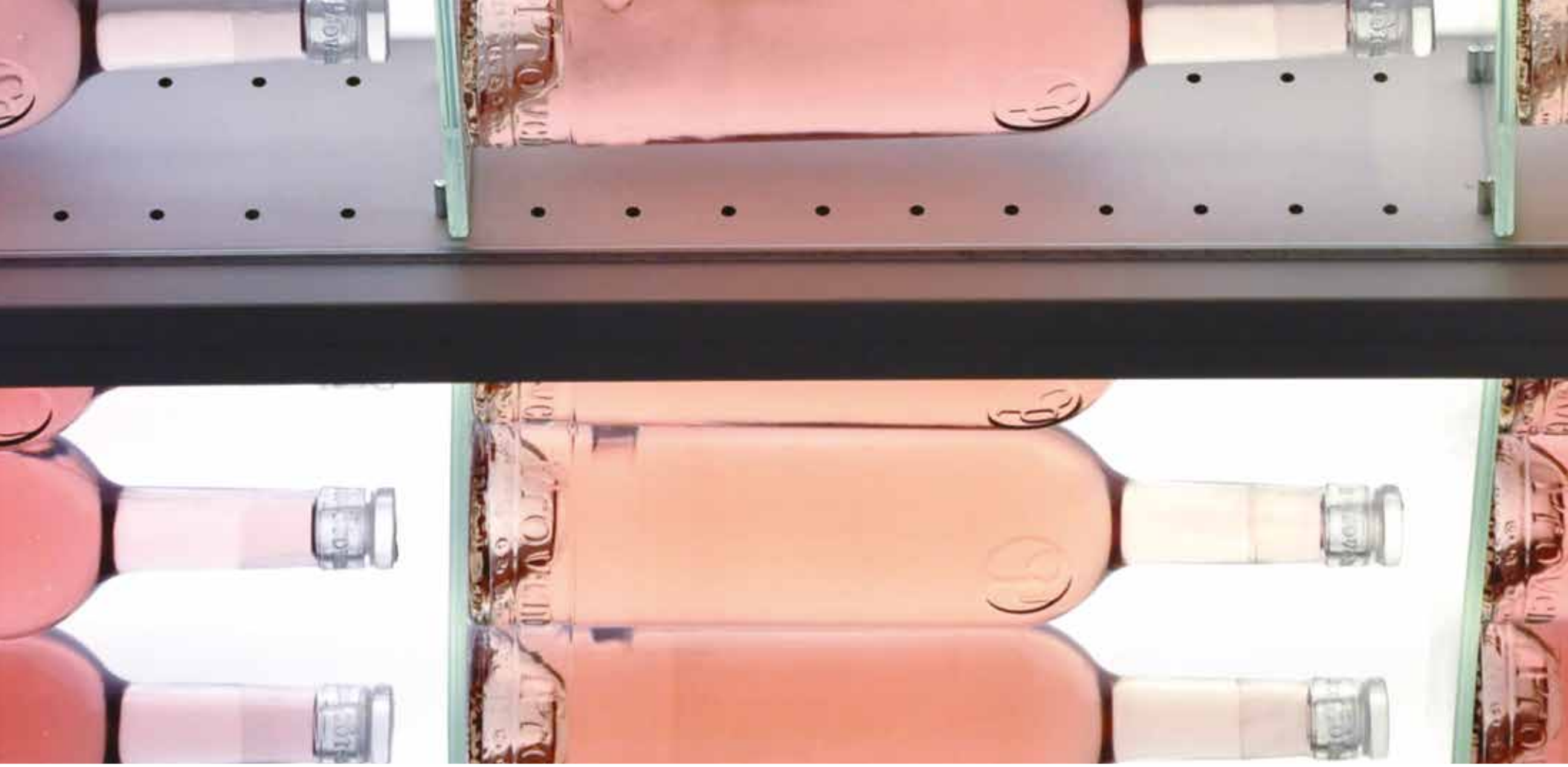


Figure 4: Diameter of bubbles in the foam collar of the four rosé sparkling wines.

It is well known that yeast mannoproteins impact organoleptic qualities of wine. Here, the contribution of yeast mannoproteins, added during the prise de mousse, to the foaming properties (collar height and bubble size) of a rosé sparkling wine has been evidenced, for the first time, in real tasting conditions.

ROSÉ



ROSÉ

ARTICLE

VEGAN ROSÉ - LESSONS FROM PROVENCE

With rising international demand, rosé remains one of the fastest growing wine categories. **Christophe ROSSI**, rosé manager and oenologist at **LAFFORT® FRANCE**, and **Alana SEABROOK** and **Andrew MARIANI**, from **LAFFORT® AUSTRALIA**, examine the latest production trends from Provence.

Grapegrower and Winemaker - February 2019, Issue 661. www.winetitles.com.au

Worldwide rosé consumption has increased by 8% over the past 10 years, and production is following suit. At present France produces almost a 3rd of the world rosé volume, 42% of which is produced within the Provence region[6]. And although this growth is unparalleled in the white wine market, producers have noted that consumers are becoming more demanding of vegan/vegetarian friendly options, prompting even the most conservative of Provence rosé producers to adapt their winemaking practices. Given current global consumption and population (and assuming an annual growth of 1%) the market will need an additional 2 to 3 millions hL (52 - 78 Mgal) of rosé wine each year (CIVP/France Agrimer). Here we aim to discuss some of the learning from the most current processing trends occurring in the global heart of rosé production.

IS IT POSSIBLE TO MAKE A PREMIUM QUALITY VEGAN - VEGETARIAN FRIENDLY ROSÉ?

Yes.With such a large amount of rosé being produced in Provence, they are pioneering the way in terms of vegan rosé production. Where once casein and PVPP were heavily used, increased understanding of phenolic pick up and improved processing have led to the availability and use of more targeted vegetable fining agents such as potato protein (patatin), pea protein and combinations with PVPP.

HARVESTING – DOES THIS INFLUENCE WHAT KIND OF FINING AGENT USED?

Every process in the harvesting and processing stage that allows phenolic compounds to oxidise, (as well as the varietal in question) will impact on the type of phenolic's in the must and the quantity required for removal. Critically, if these phenolic's aren't removed, they can oxidise compounds responsible for:

1) Turning the wine brown.
2) Oxidising key volatile aroma compounds.
3) Consuming oxygen scavengers that protect volatile aroma compounds.

Ideally, handpicking by night would minimise the amount of grape damage and subsequent enzymatic oxidation. Obviously, this is not practical or possible in the vast majority of cases, therefore managing the different must fractions is critical. This can be done in a variety of ways including;

1) Handpicking and chilling the grapes down can slow down the enzymatic oxidation process.
2) Or with machine harvested grapes, separation of the juice fraction from the whole berries in the hopper, minimising uncontrolled maceration, and the consequential extraction of phenolic compounds.

In the case of a highly oxidised fraction, a broad spectrum of fining activities in higher dosages may be required to replace animal based fining agents like casein or gelatine. However, for a free run fraction that has been protected from oxidation (and has lower levels of phenolic compounds available to oxidise) one vegetable based fining agent may be sufficient.

IN THE PRESS – HOW CAN I MAXIMISE COLOUR AND AROMA PRECURSORS WITHOUT EXTRACTING AND OXIDISING PHENOLIC COMPOUNDS?

The more mechanical disruption and pressing the grapes undergo, the more phenolic compounds will be released into

the juice. 'Saignee' technique introduces a lot of phenolic compounds via maceration, thus requiring more management of these compounds. In Provence, Premium rosé production utilises a dedicated press using gentle press enzymes that will increase the amount of free run fraction and critically allow for the extraction of aroma precursors from the skin without having to mechanically force them out. The quicker these aroma precursors (specifically thiol precursors) are extracted into the juice from the skin, the more limited the oxygen pick-up and extraction of phenolic compounds. Practically and empirically speaking from experience out of Provence, if oxidation and phenolic extraction isn't managed it is possible to achieve strawberry or raspberry fruit aromas, but impossible to get delicate aromas such as flowers, white peach, grape fruit, passion fruit or even boxwood aromas from red grapes. The press cycle itself is also critical, by the adoption of champagne press cycles which minimise rotations of the press and incrementally increase the pressure without deflating (which would promote oxidation). Common practice in Provence is to layer the fruit with compressed CO_2 pellets, enabling slow release of CO_2, and maintaining its inert environment.

CAREFULLY HARVESTED, PRESSED FREE RUN JUICE – DO I NEED TO BOTHER MANAGING PHENOLICS?

Removal of specific phenolic compounds (namely phenolic acids) will ensure that the colour does not change, but also that aroma compounds like volatile thiols are not oxidised and lost. It is worth noting the two types of oxidation, chemical and enzymatic. SO_2 and ascorbic acid have some control over enzymatic oxidation but chemical oxidation can still happen over time. This makes it critical to remove any phenolic acid that can oxidise over time.

WHEN TO ADD FINING AGENTS...JUICE OR DURING FERMENTATION?

To fine in the juice stage and be effective, the fining agent would normally need to be introduced via venturi when the juice is still cold. There is a high chance of oxygen being introduced in this process, due to the process itself and the fact that at cold temperatures the juice can absorb more oxygen. Flotation can be an effective method of introducing fining agent at this stage without the subsequent ingress of oxygen. Many Provence based winemakers add their fining agent in the first third of fermentation. This ensures optimal homogenisation at a higher temperature, utilising the dynamics of fermentation.

DOES IT MATTER WHICH FINING AGENT I USE? ARE THEY ALL THE SAME?

Each fining agent has a different specificity. Figure 1 is a gel of different fining agents and the different sizes of the fining agents. The furthest to the left is a ladder giving an indication of size. The green box depicts protein fractions that are able to interact with polyphenols in wine, whilst the red box at approximately 14 kDa indicates proteins that will interact with polymerised tannins[5]. The darker the bar, the greater the quantity. Fractions of different fining agents may be selected for particular applications. Combinations of proteinaceous fining agents may be used in conjunction with PVPP to remove the entire spectrum of phenolics that could oxidise both colour and aroma. When only using vegan friendly products, it is important to choose a broad spectrum of agents ideally including PVPP. Casein and PVPP complexes become critical when the juice has oxidised and turned colour towards the orange spectrum. This makes it critical to ensure all handling prior to is minimising oxygen pickup if the producer prefers to only use vegan vegetarian friendly products. Many Provence based producers are using PVPP and vegetable combinations (reference Figure 3) in high rates in the fermentation on free run fractions to ensure that the removal of all phenolic compounds that could cause damage. If the appropriate amount is used there is usually no requirement for further fining in the wine as this can be quite stripping on the flavour and aroma.

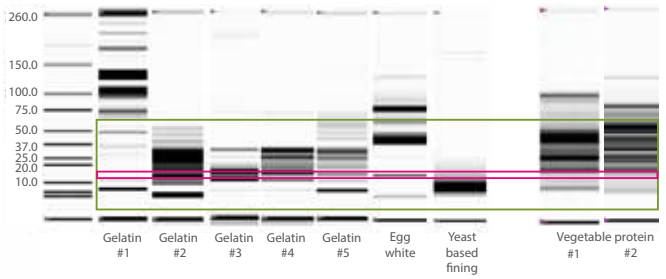


Figure 1. Electrophoresis gel with a range of animal, yeast and vegetable based fining agents which demonstrate the different sizes and quantity of the various protein fractions and importantly which ones will interact with polyphenol compounds in wine (data ex - EXCELL laboratory).

WHAT ARE THE FACTORS CRITICAL TO MAKING A PREMIUM ROSÉ FULL OF AROMATIC COMPOUNDS?

High quality fruit is important in the production of premium rosé. Interestingly, red varietals can have as many thiol precursors as premium Sauvignon Blanc grapes[3]. In red wines however, these thiols tend to be bound immediately by phenolics and rendered non-volatile. In rosé, if these phenolics are taken away and the precursors are extracted from the grape, thiols may be converted by thiol producing yeast and preserved in the wine.

Depending on the precursor in question, these precursors may be found predominantly in the skin or pulp of the grape[2]. Esters can be modulated by enzymes produced by yeast, making them a function of the yeast strains that conduct primary fermentation. In short, grape quality, absence of oxidised and oxidizable phenolics and choice of yeast strains can all affect the aromatic and flavour profile of the finished rosé.

HOW CAN I INCREASE MOUTHFEEL AND AROMA IN MY ROSÉ?

Processes like stabulation in the whole juice phase (keeping the whole juice cold at 0°C/32°F for 7 to 14 days and circulating the lees with dry ice pellets or nitrogen twice a day) can increase the amount of polysaccharides present in the wine and subsequently impacting its mouthfeel[1]. Press cycles, use of press enzymes and yeast strain selection can also all impact flavour production. The press cycle and enzyme used will mostly determine the level of aroma precursors and total phenolic compounds extracted from the skin. Different yeast strains fermented at different temperatures will produce more or less yeast modulated aroma compounds such as thiols (**ZYMFLORE®X5** and **ZYMFLORE®DELTA** are high thiol producers whilst **ZYMFLORE®X16** is more ester driven, Figure 2) and their enzymatic activities may also contribute to ester production and terpene liberation.

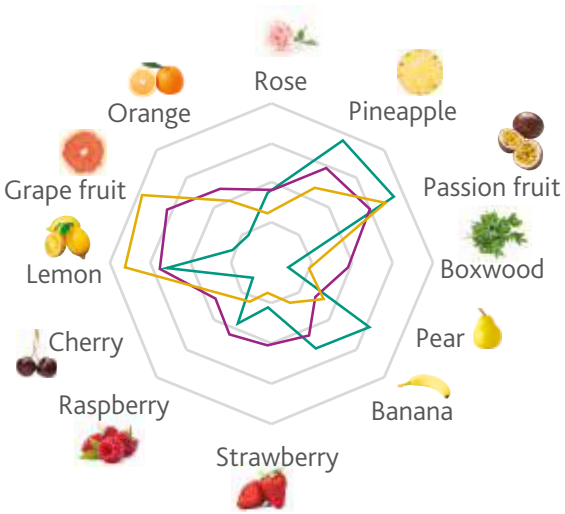


Figure 2. Variation between yeast strains in terms of aroma production.

ALL ROSÉ CHANGES COLOUR AFTER TIME DOESN'T IT?

If all phenolic compounds responsible for the oxidation of aroma and colour compounds are removed, there should be nothing left to oxidise. If these aren't removed, over time compounds responsible for colour may oxidise and become brown/orange.

HOW CAN I MANAGE COLOUR IN MY ROSÉ WINE?

It cannot be underestimated how important the management of colour is in Rosé winemaking, as it dramatically impacts the style and aesthetics of the final product. Colour will vary depending on:

- Variety,
- Fining products used,
- Starting SO_2 concentrations,
- Harvesting conditions,
- Processing methods including use of enzyme,
- Crushing/destemming and/or lack thereof.

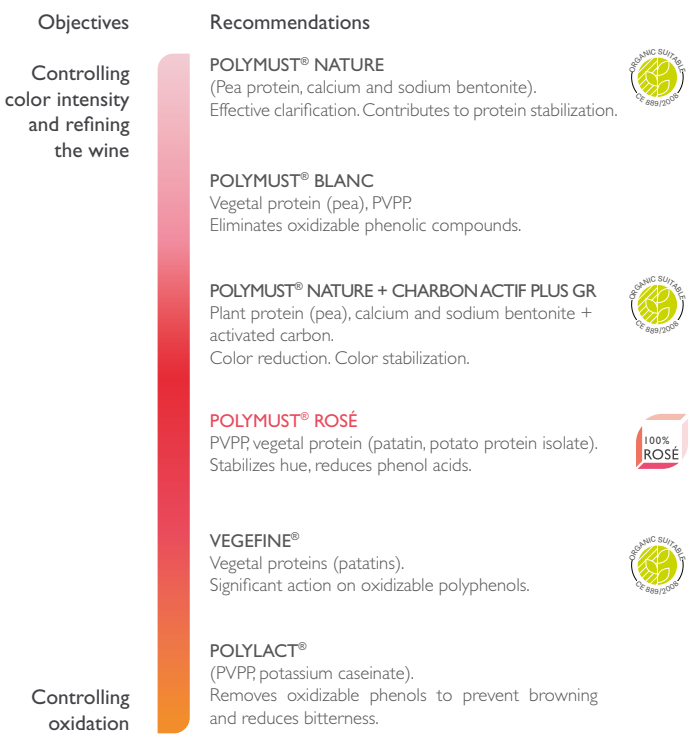


Figure 3. Rosé colour chart-fining agent recommendation based on the colour of the juice.

It is typical in Provence to have a number of different tanks with varying levels of colour in them, which will all have been treated with varied combinations of fining agents. Consequently, after fermentation, and final stabilisation processes all these tanks will have different levels of stable colour within the wine. These can then be used as blending components to achieve the winemaker's optimum spectrum without destabilising the colour.

PROTEIN AND TARTRATE STABILISATION – WHAT PRACTICES DO THEY USE IN PROVENCE AND WOULD IT WORK FOR AUSTRALIAN ROSÉ?

Sodium bentonite is primarily used (MICROCOL®ALPHA), added during fermentation at a rate of approximately 200 ppm. After filtration and blending the wines are usually checked for the presence of heat unstable proteins and have a second addition of sodium bentonite as required.

CELSTAB® (cellulose gum) is widely used in Provence to cold stabilise rosé, approved for winemaking in Australia in 2011. If the wine has been adequately fined during fermentation there should be no interaction between CMC and colouring matter.

TAKE HOME POINTS CRITICAL FOR ROSÉ PRODUCTION IN PROVENCE

1. Protection against oxidation - To avoid the oxidation of polyphenols into quinones and to protect aromas, it is essential to implement all available techniques: evaluation of good practices in wineries (avoid air outlets, check the gaskets fittings,...), rigorous process checks and use of inert gases.
2. Refrigeration and cooling capacity - Cold conditions limit enzyme activity in terms of colour extraction and oxidation by polyphenol oxidases. It is therefore essential to work on these pre-fermentation phases as quickly as possible at low temperature.
3. Pressing - The objective is to ensure that rosé wines are pressed quickly and achieve a qualitative release of juices to obtain the best aromas without extracting colour. It is strongly recommended that enzymes are used during the filling of the press.
4. Fermentation - The choice of yeast strain and nutrition both help to create and optimise the aromatic profile of a wine according to the style desired by the winemaker.
5. Fining - Early fining of rosé wines, on must or during alcoholic fermentation, helps act on the phenolic compounds that trap aromas, and allows wine colour to develop and modify wine structure. An appropriate fining will help you produce high quality rosé wines.
6. Stabilisation - At the end of process, certain choices can alter the aromatic profile or colour of wines; therefore, it is essential to understand the stabilisation options available that respect the wine

For further information please visit https://laffort.com/en/protocols-and-itineraries/

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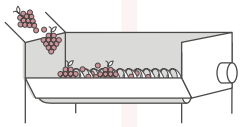
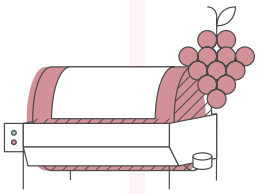
Super Premium Rosé Wine Protocol

Grape arrival



- Grapes must be healthy and clean, with potential alcohol between 12.5 and 13.5% v/v.
- Maximum grape temperature 54 - 58°F, use cooling if necessary, to slow down uncontrolled enzymatic reactions.
- Sprinkle bins with **SUPRAROM®** at 100 - 250 ppm to avoid uncontrolled skin extraction and juice oxidation; use of double bottom bins (with drain screens) recommended.

Pressing



- Separate juice from the bins; they can be blended back after separate fermentation if the quality is high enough.
- Use inert gas or dry ice during the entire grape reception process (destemmer, pump, press, and tank). Use CO_2 gas or dry ice during press filling, usually 3 to 5 lbs per ton of grapes (4 lbs of dry ice = 240 gallons of CO_2 released). Nitrogen may also be used.
- Add pressing enzyme at 30 g per ton grapes. Use **LAFAZYM® PRESS** for faster and greater free run juice yield at lower pressures with less maceration.
- Split SO_2 addition if possible between press filling and juice pumping to tank. Add total of 50 ppm.
- Increase pressure levels using the Champagne cycle, with maximum 3 rotations for the total pressing cycle.
- Separate free run and press juices: normally noted by pressure and tasting but also possible by pH.

Stabulation

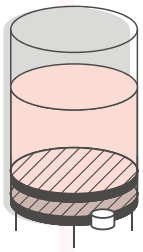
Stabulation is the process of keeping juice in contact with "juice lees", and holding the juice cold to avoid fermentation. This process can increase the aroma precursors in the juice, specifically the Thiol aromas, giving more passion fruit and grapefruit in the finished wine, and enhance mouthfeel and sucrosity. The length of stabulation varies with temperature and tank needs, lasting 4 days to 4 weeks.

- Add 25 ppm **ZYMAFLORE® ÉGIDE**^{TDMP} to the juice at tank filling.
- If less than 5 days for stabulation, then add **LAFAZYM® THIOLS**$^{[+]}$ at 50 ppm.
- Keep cooling jackets set as low as cooling capacity allows. Ideal is between 28 - 38°F.
- Stir tank twice daily with addition of dry ice pellets or inert gas sparge through bottom valve.
- Keep tanks with minimal headspace and gas headspace daily.
- Monitor tank for signs of fermentation.
- When stabulation period is over, stop stirring 36 hours before desired racking time. Juice lees will settle quickly.

TEMPERATURE	PRODUCTS	STABULATION TIME
32 - 36°F	25 ppm **ZYMAFLORE® KHIO**^{MP}	1 to 3 weeks
43 - 46°F	25 ppm **ZYMAFLORE® ÉGIDE**^{TDMP} 50 ppm **LAFAZYM® THIOLS**$^{[+]}$	48 hours to 5 days

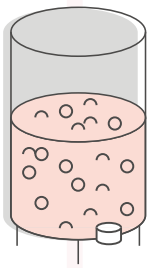
Super Premium Rosé Wine Protocol

Settling



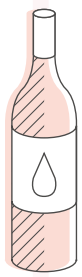
- If not using stabulation, use **LAFAZYM® 600XL ICE** at 0.5 – 2 mL/hL.
- Use inert gas in the destination tank when racking juice to this tank. Rack when juice turbidity is between 100 and 150 NTU (200 to 250 NTU if fermenting with **ZYMAFLORE® DELTA**).
- Filter lees and add filtered lees back into the juice.
- When using stabulation:
  - Turn off cooling and allow the tank temperature to rise up to 46 - 50°F before racking and monitor closely for signs of indigenous fermentation.
  - Rack as above.

Fermentation



- For yeast preparation, use 200 ppm of **SUPERSTART® BLANC & ROSÉ** along with 200 ppm of **ZYMAFLORE® X5, X16 or DELTA.** Add the yeast preparation to the tank when the temperature difference is lower than 10°C.
- Adjust fermentation temperature between 61 and 65°F.
- Adjust assimilable nitrogen if necessary, split in two additions. (see nutrition decision tool on www.laffort.com).
- Adjust juice acidity with 1/3 malic acid and 2/3 tartaric acid, depending on needs.
- Protect aromas with 300 ppm of **FRESHAROM®** after one-third of fermentation (an inactivated yeast preparation rich in glutathione and protective metabolites).
- For juice fining, add a fining agent after fermentation start (1/3 fermentation):
  - Free run juice fining: 400 to 600 ppm of **POLYMUST® ROSÉ** to prevent quinones formation, which can trap aromas.
  - Press juice fining: 500 to 800 ppm of **POLYLACT**.
- Incorporate 200 to 300 ppm of **MICROCOL® ALPHA.**

End of fermentation and aging steps



- Add SO_2 at 5g/hL (50 ppm) six days after the end of alcoholic fermentation during the first racking (to avoid residual sulfite-reductase activity).
- After blending and filtration, test protein stability. In case of tartaric stabilization with **CELSTAB®**, it is recommended to perform a cold test (6 days at -4°C (24°F)) to test tartaric stability while taking into account potential interaction between CMC and colouring matter. According to results, treat with 1 mL/L (1000 ppm) of **CELSTAB®**. Wait 48 hrs before membrane filtration and bottling.

This protocol is a standard recommendation. It is necessary to adjust this in regards to the grape varietal, cellar equipment, wine objectives, etc.
Feel free to contact your LAFFORT® representative to discuss.

ARTICLE

STABULATION - SERIOUSLY ROSÉ OR SAUVIGNON BLANC?

If your goal is to improve the aroma and complexity of a rosé or white wine, Alana **SEABROOK** and Tertius **VAN DER WESTHUIZEN** of Laffort Australia provide their insights into a tool that could be useful.

Grapegrower and Winemaker - February 2018, Issue 649. www.winetitles.com.au

If you are making 'Provence style' Rosé in 2018, or even if you just want to make an aromatic wine (for example Sauvignon Blanc) with increased levels of thiols and mouthfeel, stabulation is a tool that can be used to achieve that.

Stabulation is the process of keeping whole juice on juice bottoms/lees for a period of time, generally at cooler temperatures ideally for at least 2 weeks at -2°C/28.4°F and 0°C/32°F to avoid uncontrolled fermentation, mixed periodically in inert conditions. The principal is to maintain contact between the juice and the juice lees. The aim is to extract the good compounds (precursors of thiols and esters as well as unknown compounds which could contribute to mouthfeel) from the juice lees into the juice, so you have to stir the lees every 12 hours (with CO_2 or dry ice). This process is highly beneficial for Sauvignon Blanc as well as other aromatic whites where an increase in thiols and esters is desired. In the case of Rosé, a significant amount of trials have been carried out demonstrating not only an increase in thiols and esters due to higher levels of precursors extracted during stabulation (Figure 1), but increased colour stabilisation as well as a decrease in fatty acids.

KEY RESULTS AFTER STABULATION

- Increased aroma (Increase in thiols and esters).
- Significant increase in mouthfeel.
- Colour stabilisation for Rosé.

STABULATION PROTOCOL

The following protocol may be followed:

1. Cool down and maintain temperature of the juice without racking between -2°C/28.4°F and +3°C/37.4°F for a stabulation of 10 days or more (optimal) otherwise follow time/temperature combinations as per beside (table).
2. Mix fine juice lees by addition of dry ice each 12 hrs or recirculating the fine lees.

Temperature (°C)	Stabulation time
10 - 12°C / 50-53.6°F	24 h
8°C / 46.4°F	48 h
0 - 2°C / 32-35.6°F	4 days to 3 weeks
<7°C / <44.6°F	5 days with ZYMAFLORE® EGIDE™

3. Turn off cooling and allow the tank temperature to rise up to 8–10°C / 46.4–50°F before racking to avoid risk of oxidation.
4. Either rack off gross solids prior to fermentation or float at this point using inert gas in the destination tank. Rack when juice turbidity is between 100 and 150 NTU (200 to 250 NTU if fermentation with strains that prefer a higher turbidity like **ZYMAFLORE® DELTA**). Proceed to inoculate with desired yeast strain.

** EGIDE™ (Non-fermentative combination of Torulaspora delbruckii and Metschnikowia pulcherrima) can be held at less than 7°C/44.6°F for 5 days in addition to the above combinations (Please find more information at https://www.laffort.com/en/products/zymaflore-egide)*

Stabulation can be a tool to increase the level of aroma and complexity in a Rosé or a white wine. For more information please refer to https://www.laffort.com/en/ranges/rose-wine/ for product information.

1a.

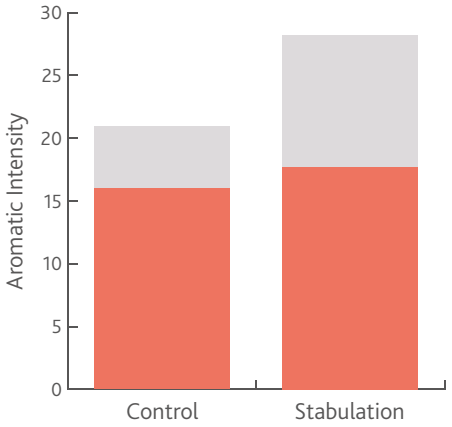


Figure 1. a. Effect of stabulation on Rosé in 2013 trials on thiol production;

1b.

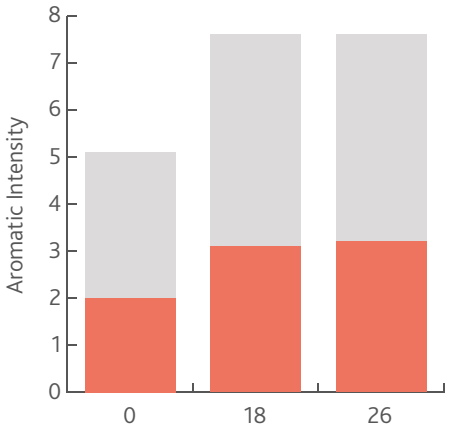


Figure 1. b. Effect of stabulation after 0, 18 and 26 days at 0°C on Rosé in 2014 trials on thiol production

1c.

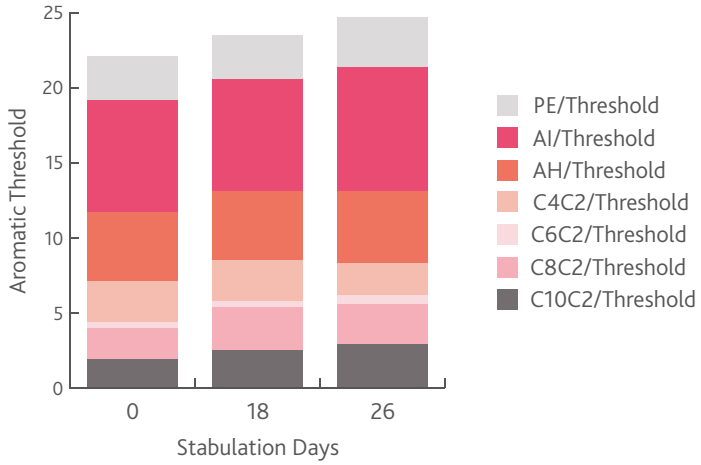


Figure 1. c. Effect of stabulation after 0, 18 and 26 days at 0°C on Rosé in 2014 trials on ester production.

1d.

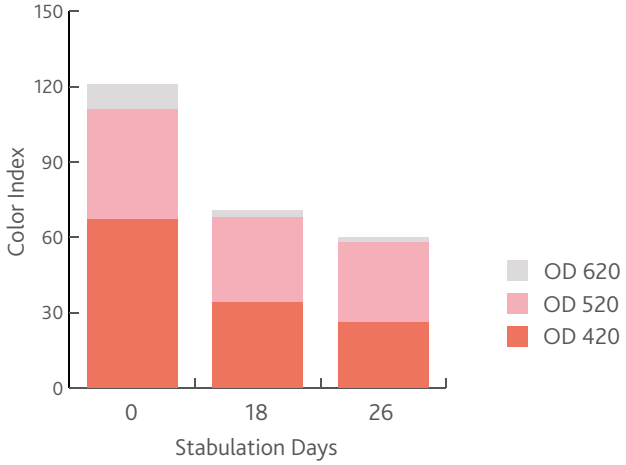


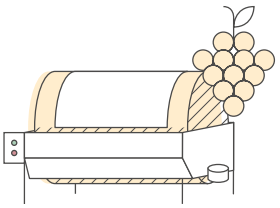
Figure 1. d. Effect of stabulation after 0, 18 and 26 days at 0°C on Rosé in 2014 trials on colour stabilisation.

WINEMAKING PROTOCOLS

WINEMAKING PROTOCOLS

Thiol Optimization Protocol

1 Pressing



To limit harvest bruising and to maximize free-run yields, extract a maximum of juice at low pressures.
LAFAZYM® PRESS (30 g/ton) **Or LAFASE® XL PRESS** (30 mL/ton).

Flotation / Static Settling

2 In special conditions such as low maturity of the grapes, hard-to clarify grapes or in order to accelerate depectinization before flotation:

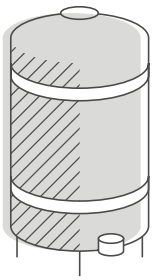
LAFAZYM® 600XL ICE (0.5 - 1 mL/hL) on must after pressing.

2.2 Stabulation

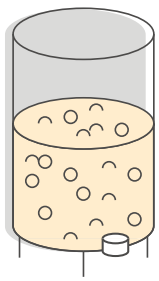
Hold juice cold on juice lees for extended time to extract more aroma precursors from the juice solids.

See rosé protocol for more information on the "stabulation" process.

Enzyme: **LAFAZYM® THIOLS**[+] (30 - 60 ppm) on must after racking and before yeasting.



3 Fermentation



Yeasting

√ **ZYMAFLORE® ALPHA:** non-*Saccharomyces* yeast to increase aromatic complexity (300 ppm).

√ **SUPERSTART® BLANC & ROSÉ**: Enhances the overall yeast potential of aroma production and revelation. To be added to the Saccharomyces yeast rehydration water (200 ppm).

√ Thiol revealing yeast*: **ZYMAFLORE® X5**, **ZYMAFLORE® DELTA**, **ZYMAFLORE® VL3** (200 ppm).

Inoculate the S.cerevisiae 24h to 72h after* **ZYMAFLORE® ALPHA.

Nutrition

NUTRISTART® AROM: complete nutrient (organic and mineral nitrogen), lifts the aromatic complexity. (200 - 600 ppm according to nitrogen needs).

LAFFORT & YOU
SHARED RESOURCES & EXPERTISE

Nutrition Decision-making tool please check our nutrition calculator online: www.laffort.com

Thiol Optimization Protocol

3 Fermentation

- **Fining during alcoholic fermentation (Add at 1/3 fermentation completion)**

VEGECOLL®:
Vegetable protein (patatin) to prevent oxidation and eliminate oxidized phenolic compounds. (30 - 200 ppm on free-run juice; 200 - 300 ppm on press juice)

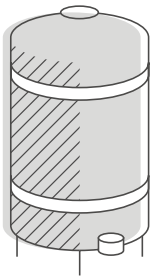
Or

POLYMUST® ROSÉ:
PVPP and vegetable protein (patatin) to preserve color and eliminate oxidized compounds (300 - 800 ppm).

Aromatic protection

FRESHAROM®: specific formulation of inactivated yeast with high protective power, rich in glutathione (200 - 300 ppm).

4 Aging



- **Enzyme**

LAFAZYM® AROM (ß-glucosidase): strengthens the aromatic complexity and the thiol perception through the revelation of terpenes (20 ppm).

Winemaking Protocol for Sauvignon Blanc, Aromatic Whites, and Rosé

Pressing Enzyme Treatment

Increase free run juice yields, shorten press cycles, decrease phenolic extraction, and extract aroma precursors from the skins.

LAFAZYM® PRESS
Granulate option.
Dosage: 30 g/ton

Applied to grapes before pressing

LAFASE® XL PRESS
Liquid option.
Dosage: 30 mL/ton

Juice Fining

Preventative treatment of oxidation, preserving aromatic profile. Eliminate oxidized phenolics, prevent browning and pinking during aging period.

POLYMUST® ROSÉ
Vegetable protein (patatin) and PVPP
Dosage: 300 – 500 ppm

OR

POLYLACT®
Casein and PVPP
Dosage: 200 – 400 ppm

Thiol Revelation

LAFAZYM® THIOLS[+]
Pectinase blend specific for releasing more aromatic potential during fermentation.
Add just after fermentation begins.
Dosage: 30 - 50 ppm

Add to juice settling tank or add during fermentation

Yeast Choice - 200 ppm

ZYMAFLORE® X5
Modern
Bright, clean fermentation profile, helps express thiols (grapefruit, boxwood).
Alc. Tol.: 16%, Temp: 55 - 68°F

ZYMAFLORE® VL3
Classic
High expression of grapefruit & passion fruit, enhances mouthfeel (Hsp12 peptide).
Alc. Tol.: 14.5%, Temp: 59 - 70°F

ZYMAFLORE® DELTA
Terroir
High expression of citrus & lychee, gives sweetness sensation and mouthfeel.
Alc. Tol.: 14.5%, Temp: 57 - 72°F

Yeast Rehydration

SUPERSTART® BLANC
Yeast rehydration product rich in sterols and minerals that help yeast develop more intense fermentation esters and thiols.
Timing: dissolve in yeast rehydration water at 104°F before adding yeast.
Dosage: 200 ppm.

Aroma Protection & Mouthfeel - Glutathione

FRESHAROM®
Glutathione based product to help protect wine aromatics from oxidation during cellar aging and bottling, giving the wine greater aging potential.
Timing: add at 1/3rd fermentation completion (~15 brix).
Dosage: 300 ppm

Fermentation Nutrition

THIAZOTE® PH
Diammonium phosphate (DAP) and thiamine.
Dosage: 100 - 500 ppm

NUTRISTART® AROM
Complex yeast nutrient, composed of organic nitrogen, DAP and thiamine to optimize wine aroma.
Dosage: 200 - 600 ppm

NUTRISTART® ORG
100% organic nitrogen from yeast origin.
Dosage: 300 - 600 ppm

Winemaking Protocol for Chardonnay and Full-Bodied Whites

Juice Clarification Enzyme Treatment

Pectolytic enzymes for faster clarification, more compact lees volume, and efficient post fermentation settling and filtration.

LAFAZYM® CL
Granulate option.
Dosage: 5 - 20 ppm

Added to juice after pressing

LAFASE® XL CLARIFICATION
Liquid option.
Dosage: 1 - 3 mL/hL

Juice Fining

Eliminates phenolics, preserves aromatics, prevents browning and removes bitterness.

POLYLACT®
Casein and PVPP
Dosage: 200 – 400 ppm

Add to juice settling tank or add during fermentation

POLYMUST® PRESS
PVPP, calcium bentonite and vegetable protein (patatin).
Dosage: 400 - 1000 ppm

Yeast Choice - 200 ppm

ZYMAFLORE® X16
Modern
High aromatic producer; pear, pineapple, & peach.
Alc. Tol.: 16.5%, Temp: 54 - 64°F

ZYMAFLORE® CX9
Classic
Meyer lemon, fresh almond & hazelnut aromas with creamy mouthfeel.
Alc. Tol.: 16%, Temp: 57 - 72°F

ZYMAFLORE® VL2
Terroir
Peach, pear, & toasted bread aromas. High polysaccharide production.
Alc. Tol.: 15.5%, Temp: 57 - 68°F

Yeast Rehydration

SUPERSTART® BLANC
Yeast rehydration product rich in sterols and minerals that help yeast develop more intense fermentation esters and thiols as well as help prevent stuck ferments.
Timing: dissolve in yeast rehydration water at 104°F before adding yeast.
Dosage: 200 ppm

Aroma Protection & Mouthfeel

OENOLEES
Eliminates bitterness, enhances mouthfeel and adds perception of sweetness. Adds rich *sur lie* aging character.
Timing: add at 1/3rd fermentation completion (~15 brix).
Dosage: 200 - 300 ppm

Fermentation Nutrition

THIAZOTE® PH
Diammonium phosphate (DAP) and thiamine.
Dosage: 100 - 500 ppm

NUTRISTART®
Complex yeast nutrient, organic nitrogen, DAP and thiamine.
Dosage: 200 - 600 ppm

NUTRISTART® ORG
100% organic nitrogen from yeast origin.
Dosage: 300 - 600 ppm

Winemaking Protocol for Pinot Noir

Fruit Forward Pinot Noir	*Full - bodied* Pinot Noir

Cold Soak

ZYMAFLORE® ALPHA^{TD}
Torulaspora delbrueckeii strain.
Add during cold soak to prevent spoilage organisms in must.
Reduce VA production during cold soak and fermentation.
Compatible with all *Saccharomyces cerevisiae*.

Pectolytic enzymes for efficient aroma, color and tannin extraction

LAFASE® FRUIT
Dosage: 30 g/ton
For enhanced fruit aromas, gentle extraction and minimization of cold soak time.

LAFASE® HE GRAND CRU
Dosage: 30 g/ton
For maximizing color, tannin and polysaccharide extraction.

Yeast Choice (200 - 300 ppm)

ZYMAFLORE® RB2
Classic
Burgundian strain giving notes of cherry, cranberry & raspberry.
Contributes to elegant mouthfeel and preserves color.
Alc. Tol.: 15%, Temp 68 - 90°F

ZYMAFLORE® XPURE
Finesse
Aromatic purity, low production of negative sulfur compounds, enhances mouthfeel with polysaccharides and notes of cherry & blackberry.
Alc. Tol.: 16%, Temp: 59 – 86°F

SUPERSTART® ROUGE
Yeast rehydration nutrient rich in sterols to help build healthy yeast membranes for greater temperature and alcohol resistance. Timing: dissolve in yeast re-hydration water at 104°F before adding yeast.
Dosage: 200 - 300 ppm

Color Stabilization

TANIN VR COLOR®
Fermentation tannin, high in catechin specific for stabilizing coloring matter. Contributes to overall structure and balance of the wine.
Dosage: 100 - 300 ppm

TANIN VR GRAPE®
100% grape catechin tannin for color stability and compensates for natural grape tannin deficiency.
Can be used during fermentation or aging.
Dosage: 100 - 300 ppm

Fermentation Nutrition

POWERLEES® ROUGE
Inactivated yeast rich in mannoprotein and Hsp12 peptides, plus ß - glucanase enzymes, to maximize mouthfeel and fruit flavors.
Will give a perception of sweetness to the finished wine.
Timing: Add any time during fermentation or aging.
Dosage: 150 - 300 ppm

THIAZOTE® PH
Diammonium phosphate (DAP) and thiamine.
Dosage: 100 - 500 ppm

NUTRISTART®
Complex yeast nutrient, organic nitrogen, DAP and thiamine.
Dosage: 200 - 600 ppm

NUTRISTART® ORG
100% organic nitrogen from yeast origin.
Dosage: 300 - 600 ppm

Winemaking Protocol for Fruit Forward Reds

Maceration Enzyme Treatment

Pectolytic enzymes for efficient aroma, color and tannin extraction from grape skins.
Increases pressing yields and aids in more efficient post fermentation settling and filtration.

LAFASE® FRUIT
Granulate option
Dosage: 30 g/ton


Add to must at destemmer.

LAFASE® XL EXTRACTION
Liquid option
Dosage: 20 mL/ton

Fermentation Tannin - Protection & Structure

Helps prevent oxidation, preserving aromatic profile.
Eliminate oxidized phenolics, prevent browning during barrel aging and remove bitterness.

TANIN VR SUPRA® ELEGANCE
Fermentation tannin, blend of skin, seed, and wood tannins. For enhancing structure, stabilizing color, and inhibiting oxidative enzymes (laccase) from mold infection.
Dosage: 200 - 400 ppm


Add to must at first tank mixing.

TANIN VR COLOR®
Fermentation tannin, high in catechin tannin specific for stabilizing coloring matter. Contributes to overall structure and balance of the wine.
Dosage: 200 - 400 ppm


Add during first 1/3rd of fermentation.

Yeast Choice (200 - 300 ppm)

ZYMAFLORE® RX60
Modern
Very high aroma production. Raspberry, blueberry & blackberry. Excellent fermentation kinetics.
Alc. Tol.: 16.5%, Temp: 68 – 86°F

ZYMAFLORE® XPURE
Finesse
Aromatic purity, low production of negative sulfur compounds, good mouthfeel with notes of cherry & blackberry.
Alc. Tol.: 16%, Temp: 59 - 86°F

ZYMAFLORE® F83
Terroir
Isolated in Tuscany, high production of red fruits, and high glycerol production. Excellent choice for Mediterranean varieties.
Alc. Tol.: 16.5%, Temp.: 68 - 86°F

Yeast Rehydration

SUPERSTART® ROUGE
Yeast rehydration nutrient rich in sterols to help build healthy yeast membranes for greater temperature and alcohol resistance.
Timing: dissolve in yeast rehydration water at 104°F before adding the yeast..
Dosage: 200 - 300 ppm

Fermentation Nutrition

POWERLEES® ROUGE
Inactivated yeast rich in mannoprotein and Hsp12 peptides, plus ß - glucanase enzymes, to maximize mouthfeel and fruit flavors.
Will give a perception of sweetness to the finished wine.
Timing: Add any time during fermentation or aging.
Dosage: 150 - 300 ppm

THIAZOTE® PH
Diammonium phosphate (DAP) and thiamine.
Dosage: 100 - 500 ppm

NUTRISTART®
Complex yeast nutrient, organic nitrogen, DAP and thiamine.
Dosage: 200 - 600 ppm

NUTRISTART® ORG
100% organic nitrogen from yeast origin.
Dosage: 300 - 600 ppm

Winemaking Protocol for Big Structured Reds

Maceration Enzyme Treatment

Pectolytic enzymes for efficient color and tannin extraction from grape skins.
Allows for earlier pressing, higher pressing yields, and more efficient post fermentation settling and filtration period.

LAFASE® HE GRAND CRU

Granulate option
Dosage: 30 g/ton

Add to must at destemming.

LAFASE® XL EXTRACTION

Liquid option
Dosage: 30 mL/ton

Fermentation Tannin - Protection & Structure

Helps prevent oxidation, preserving aromatic profile.
Eliminate oxidized phenolics, prevent browning during barrel aging and remove bitterness.

TANIN VR SUPRA®

Fermentation tannin, blend of skin, seed, and wood tannins. For enhancing structure, stabilizing color, and inhibiting oxidative enzymes (laccase) from mold infection.
Dosage: 200 - 400 ppm

Add to must at first tank mixing.

TANIN VR COLOR®

Fermentation tannin, high in catechin tannin specific for stabilizing coloring matter. Contributes to overall structure and balance of the wine.
Dosage: 200 - 400 ppm

Add during first 1/3rd of fermentation.

Yeast Choice (200 - 300 ppm)

ZYMAFLORE® FX10

Modern
High polysaccharide production contributes to midpalate length, masks green character, and preserves fruit.
Alc. Tol.: 16%, Temp: 68 - 95°F

ZYMAFLORE® XPURE

Finesse
Aromatic purity, low production of negative sulfur compounds, good mouthfeel with notes of cherry & blackberry.
Alc. Tol.: 16%, Temp: 59 - 86°F

ZYMAFLORE® F15

Terroir
Produces fruity, well balanced wines. High glycerol production for increased mid palate weight.
Enhances darker fruit characters.
Alc. Tol .: 16%, Temp: 68 - 90°F

Yeast Rehydration

SUPERSTART® ROUGE

Yeast rehydration nutrient rich in sterols to help build healthy yeast membranes for greater temperature and alcohol resistance.
Timing: dissolve in yeast rehydration water at 104°F before adding yeast.
Dosage: 200 - 300 ppm

Fermentation Nutrition

THIAZOTE® PH

Diammonium phosphate (DAP) and thiamine.
Dosage: 100 - 500 ppm

NUTRISTART®

Complex yeast nutrient, organic nitrogen, DAP and thiamine.
Dosage: 200 - 600 ppm

NUTRISTART® ORG

100% organic nitrogen from yeast origin.
Dosage: 300 - 600 ppm

Building Mouthfeel - Hsp 12 Peptide

OENOLEES®

Specific preparation of yeast cell walls for eliminating bitterness and adding perception of sweetness (Hsp12 peptide).
Will enhance mouthfeel, giving rich *sur lie* aging character. Timing: add at any time during or post fermentation.
Dosage: 200 - 300 ppm

Lowering SO_2 Additions during Winemaking

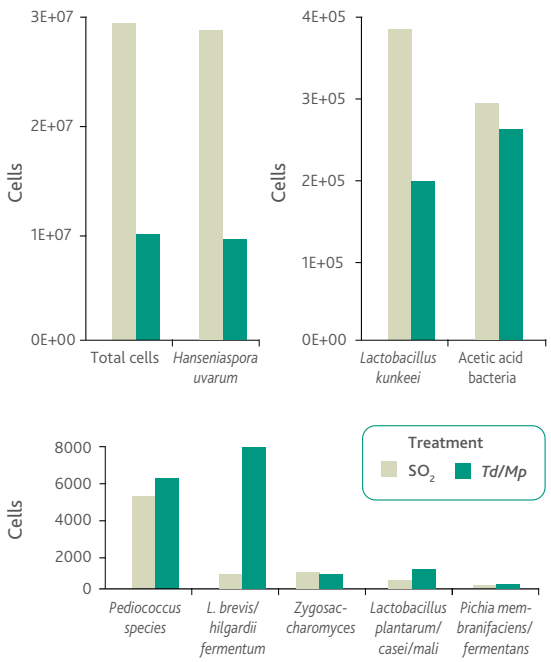
1 Mechanical equipment & grape harvest

Grape Bio Protection:

√ ZYMAFLORE® ÉGIDE™: *Torulaspora delbrueckii and Metschnikowia pulcherrima* - to be used with or without rehydration:

- In the bins of harvesting machines.
- In the transportation bins (add it on the bottom of the bin or in layers, during the addition of grapes to the bin).
- During grape crushing.
- During cold soak: perform a thorough homogenization of the tank when the tank has been filled up.

MICROORGANISM RESPONSE TO TREATMENT



Overall, the addition of Td/Mp (a blend of Torulaspora delbrueckii and Metschnikowia pulcherrima) performed better than a standard addition of SO_2 at controlling wine spoilage organisms in the research winery. Note that the cell counts are the sum of all the measurements taken.

WARNING

Precautions to take

- Harvest fruit in optimal sanitary state.
- Control the temperature (the lowest possible).
- Avoid berry crushing/bruising.
- Ensure maximum hygiene in the cellar.
- Protect all tanks with inert gas.

Enological goals

√ Control of the indigenous microbial flora:

BIO PROTECTION

- Colonization of the must with microorganisms naturally present in grapes.
- Inhibiting the development of spoilage microorganisms.
- Create a favorable environment for the implantation of *Saccharomyces* yeast.

During pre-fermentation phase, the dose of ZYMAFLORE® ÉGIDE™ should be adjusted regarding the time of contact with the must, the temperature and the microbial pressure:

~ The maximum dose is recommended in case of strong microbial pressure and in low temperatures.
~ Lighter doses for long pre-fermentation phase and or mild temperatures.

Lowering SO_2 Additions during Winemaking

2 Grape Processing & Fermentation

Enzyme addition:

√ Choose an enzyme according to the desired wine style.

- For fast juice clarification with white & rosé must, use **LAFAZYM ®CL** or **LAFASE® XL EXTRACTION**.
- For fast color and tannin extraction in red must and better settling post fermentation, use **LAFASE® FRUIT**, **LAFASE® HE GRAND CRU**, or **LAFASE® XL EXTRACTION.**

Tannin addition to grapes:

√ Using **TANIN GALALCOOL®** for whites, and **TANIN VR SUPRA®** or **VR SUPRA® ELEGANCE** for reds can replace traditionally used SO_2 for anti-oxidation activity. Tannins are especially important in the case of rot and subsequent laccase activity.

Adjust acidity:

√ Acidulate must or juice to lower pH and limit growth of spoilage microorganisms.

Active Dry Yeast:

√ Select strains producing low SO_2.

√ **ZYMAFLORE® XPURE**.

- For highly aromatic red wines, with black fruit aromas, release of Hsp12 and very low SO_2 production.

WARNING

Precautions to take

- Manage temperature carefully.
- Conduct strict cellar hygiene.
- Protect tanks with inert gas before AF.
- Minimize wine movement.

Enological goals

- Protection against oxidation. Use inert gas cover for all juice and wine movements.
- Color extraction and protection with reds.
- White/Rosé juice clarification and fining.
- Excellent oxygen/aeration management.
- Minimize the time gap between AF - MLF to avoid undesirable microbial proliferation.

Add NOBILE® FRESH GRANULAR 24M (2 g/L)

- Enhances complexity and roundness, preserving the fruit – can be used during fermentation phases.

Fining of white and rosé must during fermentation:

√ Remove oxidizable phenolics to prevent browning or pinking of wine during aging and preserve aromatic potential.

√ **POLYMUST® PRESS** (300 – 500 ppm)

PVPP, Vegetal Protein (patatin), & bentonite, non-allergenic, GMO-free.

√ **VEGECOLL®** (20 – 100 ppm)

Vegetal Protein (patatin), non - allergenic, GMO-free.

Addition of glutathione:

√ **FRESHAROM®** provides glutathione, a powerful anti-oxidant for whites and rosé wines (200-300 ppm).

Lowering SO_2 Additions during Winemaking

Co - inoculation or sequential inoculation with *Œnococcus œni*:

√ LACTOENOS® 450 PREAC or LACTOENOS® B7 DIRECT

- Bacteria highly effective for direct inoculation, active over a wide pH, alcohol, and temperature range.

3 Aging in Cellar

Tannin additions – protect wines from oxygen:

√ QUERTANIN® Range (additions of 10 to 20 ppm every month, during the entire aging period).

- TANFRESH® specifically formulated for white and rosé wines. Dosage: 10 - 30 ppm.

Microbial Control – protect wines from microbial spoilage:

Preventive treatments

√ MICROCONTROL® (100 ppm)

- Chitosan and inactivated yeasts.
- Reduces the overall pressure of spoilage microorganisms (yeasts and bacteria).

Curative or Preventive treatments

√ OENOBRETT® (100 ppm) or OENOBRETT ORG (100ppm)

- OENOBRETT® is Chitosan and *ß-glucosidase* enzyme.
- OENOBRETT ORG® is 100% chitosan.
- Both products can decrease spoilage organisms such as *Brettanomyces*.

WARNING

Precautions to take

- Implement thorough wine chemistry analysis on regular basis with a close watch on VA numbers.
- Taste wines often watching for signs of oxidation.
- Limit wine transfers to the minimum possible.
- Constant wine protection with inert gas.
- Regular topping program for cooperage and tanks.

Enological goals

- Excellent oxygen management.
- Microbiological control and management.
- Shape wine to be ready for bottling early.
- Fining treatments, clean racking, mannoprotein additions.
- Consider early bottling and commercial release of the wine.

Prepare wine for early bottling - Build mouthfeel and finesse:

√ POWERLEES® ROUGE (200 ppm)

- Specific formulation of inactive yeast and ß-glucanase used for wine fining and building mid-palate weight and sweetness perception in the wine. Use during fermentation or aging on all wine types.

√ MANNOFEEL® (30 - 150 mL/hL)

- Mannoprotein in liquid form for smoothing tannins or astringency and building mid-palate weight.
- Can be used during aging or just before bottling on all wine types.

Fermentation Management of Rot Infected Grapes

STEP 1 // Estimate level of rot in U/mL

Add 80 – 100 ppm of SO_2 depending on the laccase activity as determined either visually or with the **BOTRYTEST®**.

Level of Rot (%)	<1	1 to 5	6 to 10	11 to 25	26 to 50	51 to 100
Laccase activity (U/mL)	0.39	0.78	2.25	6.56	8.12	15.86

STEP 2 // Must preparation

Reductive cover (CO_2) asap, then add:

U/mL	2 - 5	5 - 10	>10
TANIN VR SUPRA® (ppm)	150	300 - 400	500 - 800

On *Botrytis*-affected harvest, add the total dosage as early as possible, before breaking skins.

STEP 3 // Yeast

Rehydrate the wine yeast (250 ppm) with **SUPERSTART® ROUGE** at 300 ppm to ensure a strong fermentation finish. Compensate for nitrogen deficiency in the must, if necessary, by adding **THIAZOTE® PH**, **NUTRISTART®** or/and **NUTRISTART® ORG** (use nutrient online tool, **LAFFORT®** Website).

Recommended yeast: **ZYMAFLORE® XPURE**, **ZYMAFLORE® RX60**.

STEP 4 // Fermentation

- Accelerate extraction by using an extraction enzyme, as soon as fermentation starts: **LAFASE® HE GRAND CRU** or **LAFASE® FRUIT** (according to the style objective) at 30 g per ton of grapes.
- Use **TANIN VR COLOR®** at 1/3rd through ferment at 250 ppm to stabilize the color.
- Limit pumping-over and cap punch down (mechanical activity). Tank transfers are not recommended at this stage.
- At mid-fermentation, add under the cap **EXTRALYSE®** preparation containing the ß-glucanase required for degrading the *Botrytis* glucans. A dosage of 60 - 100 ppm is recommended according to the tannin content and infection level.
- Limit time on skins to the minimum.
- Transfer free-run anaerobically into a tank with inert gas cover. Maintain anaerobic conditions until all laccase activity has disappeared.
- Press wines have a higher laccase activity and will have a low filterability index due to a high colloidal content. Treatment with a mixed enzyme preparation of pectinase/ß-glucanase such as **EXTRALYSE®** at 100 ppm will be very important to improve the filterability of the wine.

Fermentation Management of Rot Infected Grapes

STEP 1 // BIOProtection on grapes & materials

In order to control the indigenous microflora on botrytized grapes, the use of a yeast preparation for BIOprotection should be considered.

Apply in dry form or by spraying, **ZYMAFLORE® EGIDE**^{TDMP} on the harvesting machine, the grape transport bucket, and the cellar equipment at the reception.

Dosage: 20 - 30 g per ton as dry weight of **ZYMAFLORE® EGIDE**^{TDMP}.

Ask the **LAFFORT®** team about the technical information for the sprayer use for BIOprotection.

STEP 2 // Estimate level of rot in U/mL

Level of Rot (%)	<1	1 to 5	6 to 10	11 to 25	26 to 50	51 to 100
Laccase activity (U/mL)	0.39	0.78	2.25	6.56	8.12	15.86

STEP 3 // Pressing

Reductive cover (CO_2) as soon as possible, then add:

Level of Rot (%)	Low rot contamination	Medium rot contamination	High rot contamination
U/mL	2 - 5	5 - 10	> 10
SULFITES (ppm)	80 - 100		
TANIN GALALCOOL® (ppm)	50 - 70	80 - 150	100 - 200

TANIN GALALCOOL® will reduce the natural enzymatic oxidation activity due to its high affinity towards the laccase protein, complementing the activity of SO_2. Use as soon as possible after crush & SO_2 addition.

Fermentation Management of Rot Infected Grapes

STEP 4 // Pressing

Addition of enzymes on must in tank after pressing:

U/mL	2 - 5	5 - 10	> 10
LAFAZYM® CL* (ppm)	10 - 20	20	20 - 30
or			
LAFAZYM® 600 XL^{ICE}* (mL/hL)	1 - 2	2	2 - 3

* Purified enzymes selected for their ability to not produce vinyl phenols, important as these can mask fruit.

Cool juice to 10°C, then add:

U/mL	2 - 5	5 - 10	>10
POLYLACT ® (ppm)	50 - 70	100 - 200	300 - 500
or			
POLYMUST® PRESS (ppm)	100 - 200	300	400 - 500
Supplementary addition in case of excess oxidation: CASEI PLUS			250 - 300

Recommended racking after minimum of 6 hours.

STEP 5 // Fermentation

Rehydrate the wine yeast (250 ppm) with **SUPERSTART® BLANC** at 300 ppm to ensure a strong fermentation finish.

Compensate for nitrogen deficiency in the juice, if necessary, by adding **THIAZOTE® PH**, or **NUTRISTART®** range. Use nutrient online tool (**LAFFORT®** Website).

Recommended yeast: **ZYMAFLORE® CX9, ZYMAFLORE® X5**, **ZYMAFLORE® X16** or **ACTIFLORE® BO213**.

It is recommended to carry out a secondary fining during fermentation to remove the residual oxidized and / or oxidizable phenolic compounds.

U/mL	2 - 5	5 - 10	> 10
POLYLACT® (ppm)	50 - 70	100 - 200	300 - 500
or			
POLYMUST® PRESS (ppm)	100 - 200	200	200 - 300

To improve the spectrum of elimination of oxidized and / or oxidizable phenolic compounds, it is advised to alternate the fining products according to what was carried out on the must.

The recommended doses are determined for the application of a double fining on the must and during fermentation. If only one fining will be performed, the doses can be increased.

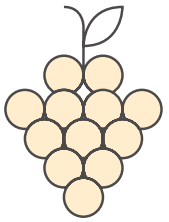
Maintain anaerobic conditions until all laccase activity has disappeared. Press wines will have a higher laccase activity resulting in a low filterability index due to a high colloidal content. Treatment with an enzyme preparation of pectinase / ß-glucanase such as **EXTRALYSE®** at 100 ppm in the last 1/3 of alcoholic fermentation will help to improve the filterability of the wine.

Protocol for Fruit Harvested in a Heat Wave

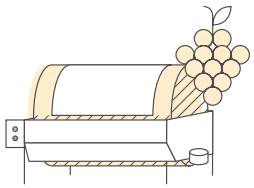
Extreme heat spells during the late stages of grape ripening can lead to many difficulties in wine cellars. There is often a compression of harvest dates among different varieties, leading to a congestion in many cellars and a need to turn tanks over quickly. Some key negative side effects of a heat wave that impact wine quality are:

1. High brix without grape maturity (green tannins).
2. Sunburn on fruit.
3. Low malic acid levels, resulting in difficult MLF conditions.
4. Acid balance TA/pH out of normal range.
5. Low juice yields from dehydrated fruit.
6. High phenolic extraction during white grape pressing.
7. Musts/juices that are low in YAN, but high in protein content.

White Grapes



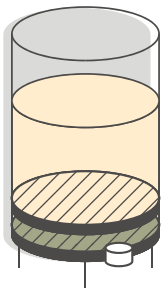
With sunburn or dehydrated white fruit, the biggest threat to the finished wine is high phenolic content which gives bitterness and early oxidation (browning/off flavor) potential. There is also a low juice yield. Juice from heat shock growing conditions have high protein levels and low malic acid. Here are suggestions for modifying your standard protocol for working with white fruit with sunburn/dehydration:



1 Before Pressing

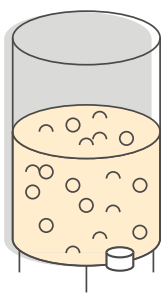
- We recommend using a pressing enzyme, **LAFAZYM® PRESS**, to try to improve your juice yields. Add **LAFAZYM® PRESS** at 45 g/ton to fruit at least one hour before starting press cycle.

2 Juice Settling



- Add 50 ppm SO_2 to the juice.
- Juice from sunburn fruit can be difficult to settle, so we also recommend using a strong pectinase enzyme, **LAFAZYM® CL**, on the juice for settling. Add **LAFAZYM® CL** at 15 ppm to the juice.
- To reduce the phenolic compounds in the juice, a fining treatment at juice settling with **POLYLACT®**, **CASEI PLUS** or **POLYMUST® PRESS** at 200 - 600 ppm.
- The protein content of the juice will be higher than normal. A bentonite addition can help reduce the protein levels and lower the amount of bentonite needed to stabilize the wine after fermentation. It is important to allow at least 6 hours between the **LAFAZYM® CL** (enzyme) addition and the bentonite addition, as the bentonite will inactivate the enzyme activity. Recommended bentonite addition is 350 ppm of **MICROCOL® FT**.

3 Fermentation



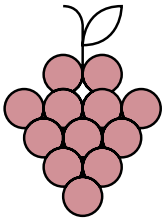
- Low YAN levels should be corrected with **NUTRISTART®**, a combination of organic nitrogen, DAP, and Thiamine. It is important to use a complex nutrient, not only DAP. You want at least 150 ppm YAN in the juice before adding the yeast.
- It is important to use **SUPERSTART® BLANC**, yeast re-hydration nutrient, to boost the yeast and maximize fermentation aromatics. Add to re-hydration water (104°F) at 250 ppm.
- Use a yeast strain with strong fermentation capacity, such as **ZYMAFLORE® X5**, **ZYMAFLORE® X16** or **ACTIFLORE® BO213**.
- Consider doing co-fermentation for any wines that you want to complete ML fermentation. Use a strong strain like **LACTOENOS® B7 DIRECT.**

4 Post fermentation

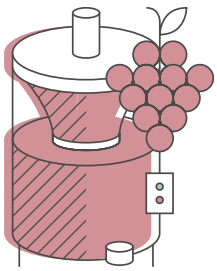
- To help build midpalate weight and remove astringent characters from the wine, add **OENOLEES®** at 200 - 400 ppm.

Protocol for Fruit Harvested in a Heat Wave

Red Grapes

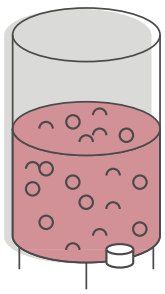


With sunburn or dehydrated red fruit, the sugar levels are high but the tannin and phenolic maturity are not optimal. There is the risk of extracting herbaceous character from the skins and seeds. The under ripe fruit can have lower anthocyanins, resulting in wines with lighter color. Juice conditions are generally lower in YAN and malic acid, but higher in protein. The higher protein content will bind up more natural tannin from the skins. Here are suggestions for modifying your standard protocol for working with red fruit with sunburn/dehydration:



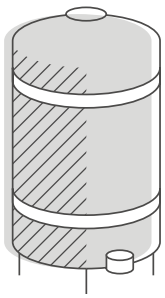
1 Processing

- Add 50 ppm SO_2 to the juice.
- Use a fermentation tannin such as **TANIN VR SUPRA® ÉLÉGANCE** to bind with the increased protein in the must. This will preserve your skin tannin and help build up the backbone structure of the wine. **TANIN VR SUPRA® ÉLÉGANCE** added at the crusher at 200 - 300 ppm.
- Enzymes are key for improving juice yield and for getting extraction of anthocyanins quickly from the skins. In the case of green character, it is recommended to extract early and press off early. **LAFASE® FRUIT** is a pectinase that will increase juice yield, extract anthocyanin, and improve settling post fermentation. Add **LAFASE® FRUIT** at 40 g/ton at first tank mixing.
- Addition of oak chips/granular during fermentation will help to mask herbaceous character in the wine. **NOBILE® FRESH** or **NOBILE® SPICE** are good choices for fermentation oak, use at 3 g/L.



2 Fermentation

- Measure acid levels after a couple days and adjust twice if needed. The buffering capacity of the juice will be out of balance, and acid in juice can drop significantly after time in contact with skins.
- Low YAN levels should be corrected with **NUTRISTART®**, a combination of organic nitrogen, DAP, and Thiamine. It is important to use a complex nutrient, not only DAP. You want at least 150 ppm YAN in the juice before adding the yeast.
- It is important to use **SUPERSTART® ROUGE**, yeast re-hydration nutrient, to boost the yeast and maximize fermentation aromatics. Add to re-hydration water (104°F) at 250 ppm.
- Use a yeast strain with strong fermentation capacity, such as **ZYMAFLORE® FX10**, **ZYMAFLORE® RX60** or **ACTIFLORE® BO213**.
- Consider doing co-fermentation for any wines that you want to complete ML fermentation. Use a strong strain like **LACTOENOS® B7 DIRECT.**



3 Post Pressing

- Early fining of press fractions with **POLYMUST® PRESS** will help remove astringent and/or bitter phenolics. **POLYMUST® PRESS** addition range 200 - 400 ppm.
- Help build midpalate weight and remove astringent characters from the wine, add **OENOLEES®** at 200 - 400 ppm.

During aging, for both red and whites, evaluate wines early and do fining trials to remove bitterness and astringency. Also adjust any acid imbalance with tartaric acid. Oak aging can add length and mask herbaceous characters from the sub-optimal maturity of the fruit.

Alcoholic Fermentation Restart Protocol

For 100 hL of wine in stuck AF

1 PRELIMINARY OPERATION ON STUCK WINE

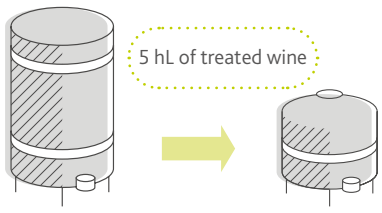
- •Rack/centrifuge avoiding air.
- Adjust wine temperature to 20°C (68°F).
- Adjust Free SO_2 to 10-20 ppm.
- Add: → For white wines: **BI-ACTIV®:** 40 g/hL (400 ppm).
  → For red wines: **OENOCELL®:** 40 g/hL (400 ppm).
- Mix wine anaerobically every 12 hours for 24 hours.
- Move on to step 2.

Stuck fermented wine
100 hL

2 PREPARATION OF THE YEAST INOCULUM

2.1. Preparation of the wine for the yeast inoculum

- Take 5 hL of the volume of the treated stuck wine from step 1.
- Adjust the alcohol to 8%, the sugar content to 20 g/L and the temperature to 20°C (68°F).
- Add **THIAZOTE® PH:** 20 g/hL (200 ppm) to this volume of wine and mix thoroughly.



Wine in stuck AF prepared in step 1
95 hL

Wine for 5 hL starter
5 hL

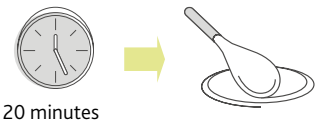
2.2. Yeast preaparation

- Prepare 60 L of water at 40°C (104°F).
- Add the yeast rehydration nutrient **SUPERSTART® SPARK** or **SUPERSTART® ROUGE**: 30 g/hL (300 ppm) of the volume of wine to be treated, and homogenize.
- Add **ACTIFLORE® B0213**: 30 g/hL (300 ppm) of the volume of wine to be treated, and homogenize.

60 L Water

Water + SUPERSTART® SPARK/ROUGE + ACTIFLORE® BO213

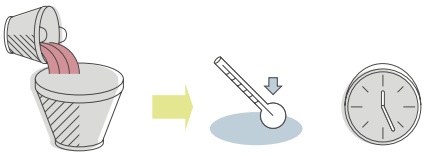
- Wait 20 minutes, and homogenize.



20 minutes

- Add immediately 20 L of treated wine from step 2-1.
- Wait 10 minutes, let cool to 20°C (68°F) and **maintain the temperature between 20-25°C (68°F-77°F).**
- The total time of the yeast rehydration must not exceed 45 minutes.

**Check with a thermometer.*



Add 20 L of treated wine prepared in step 2.1

20 - 25°C
68 - 77°F*

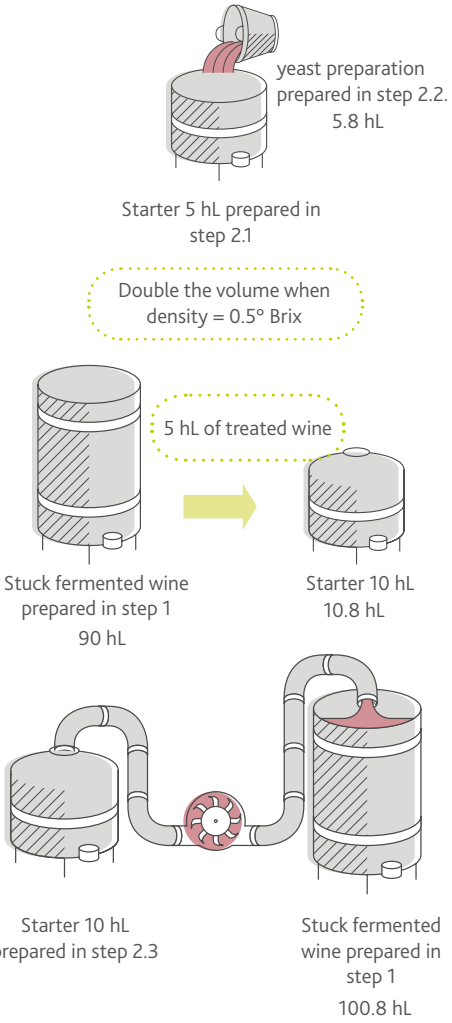
10 minutes

LAFFORT & YOU
SHARED RESOURCES & EXPERTISE

Alcoholic Fermentation Restart Protocol

2.3. Acclimation of the yeast preparation

- Add the yeast preparation (Step 2.2) to the prepared wine for the yeast inoculum (step 2.1).
- Measure the Brix and maintain the inoculum at 20°C (68°F) with aeration until 0.5°Brix (avoid the total exhaustion of sugars in the inoculum and a fall in the yeast activity). Aerate as soon as AF starts.
- Double the volume with treated wine (step 1) at 20°C (68°F).
- Measure the Brix and maintain again the inoculum at 20°C (68°F) until 0.5°Brix. Aerate again when fermentation becomes active.



3 INCORPORATION OF YEAST INOCULUM IN THE TANK

- Add the yeast innoculum to the treated wine (step 1), maintain at 20°C (68°F).
- Add 30 g/hL (300 ppm) of **NUTRISTART® ORG** to the total volume of the tank to the treated wine (Step 1).

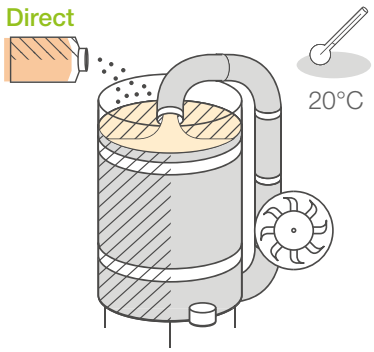
To learn more: discover our **RESTARTING FERMENTATION (AF) Decision Making Tool** on our website, in the **LAFFORT & YOU** section.

LAFFORT & YOU
SHARED RESOURCES & EXPERTISE

Malolactic Bacteria Protocol

LACTOENOS® DIRECT

DIRECT INOCULATION



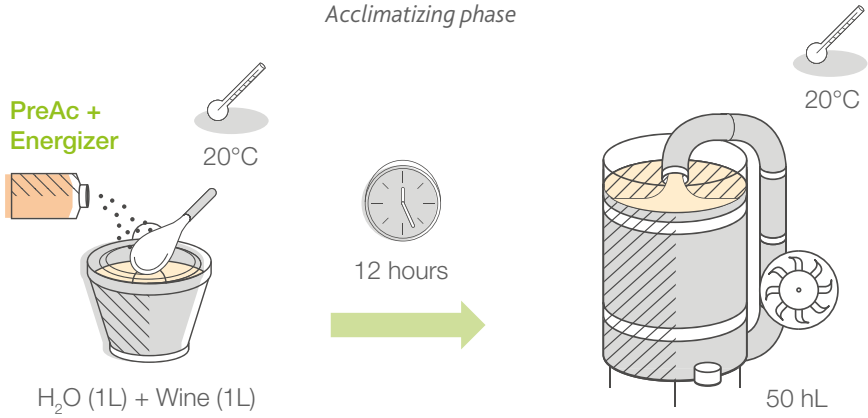
- Early co-inoculation
  24 - 48 h after start of AF.
- Late co-inoculation
  0 - 4 Brix.
- Sequential inoculation
  After AF, on running off.
- Curative inoculation

LACTOENOS® PREAC

INOCULATION WITH ACCLIMATIZATION

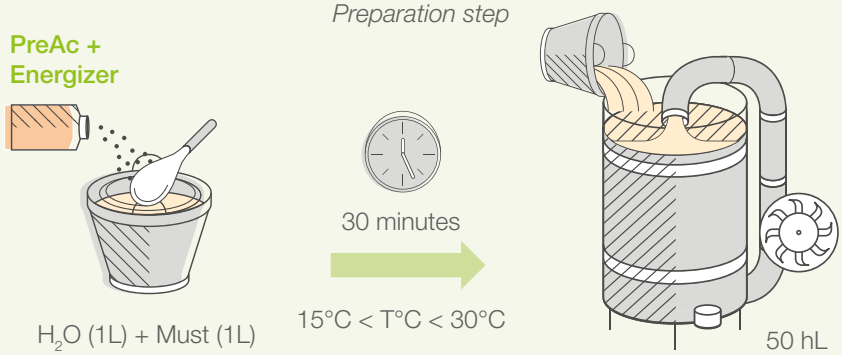
LATE CO-INOCULATION or SEQUENTIAL INOCULATION

Acclimatizing phase



Specific case : EARLY CO-INOCULATION

Preparation step



FIND OUT MORE: Discover our **MALOLACTIC FERMENTATION RESTART PROTOCOL** on our website, at **LAFFORT & YOU** section.

Managing Diacetyl in Alcoholic Fermentation

	BUILDING MORE DIACETYL	PREVENTING DIACETYL
Bacterial strain	**LACTOENOS® SB3 DIRECT**	**LACTOENOS® 450 PREAC** or **LACTOENOS® B7 DIRECT**
Dosage rate	Lower	Higher
Timing	Sequential inoculation	Co-inoculation
Duration of MLF	Long MLF	Fast MLF
Wine chemistry	Higher pH & warmer wine temperature	Lower pH & cooler temperature
Lees management	Rack off lees before MLF	MLF on lees
Stirring	Yes	No

1 BACTERIAL STRAIN

- **LACTOENOS® SB3 DIRECT** is a fast diacetyl producer and diacetyl is at its maximum level just at the end of MLF.
- Bacterial Strain: **LACTOENOS® 450 PREAC** and **LACTOENOS® B7 DIRECT** are slow diacetyl producers, and usually have not reached the production peak at the end of MLF.

2 DOSAGE RATE

- For higher diacetyl production, use a lower dosage rate.
- For lower diacetyl production, use a higher dosage rate.

3 WHEN TO ADD BACTERIA

- For maximum diacetyl impact, it is best to add bacteria sequentially after primary fermentation.
- For reducing diacetyl, co-inoculation of yeast and bacteria is recommended. The diacetyl that is produced at the beginning of MLF fermentation can be reduced to acetoin by the active yeast completing primary fermentation.

4 DURATION

- More diacetyl is produced during slower malolactic fermentation.
- Less diacetyl is produced during faster malolactic fermentations.

5 WINE CHEMISTRY

- Slower malolactic fermentations favor higher diacetyl production, lower pH and cooler temperature conditions create a more difficult environment for the bacteria so the fermentation rate will be slow.
- Faster malolactic fermentations favor lower diacetyl production, higher pH and warmer temperature conditions create a more favorable environment.

For more information on diacetyl production, check out the technical paper on citric acid metabolism in lactic bacteria and controlling the diacetyl content in wine on page 43 - 47.

Malolactic Fermentation Restart Protocol

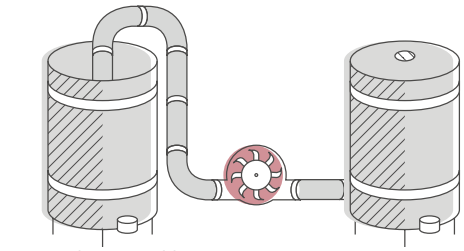
If *Brettanomyces bruxellensis* is present and depending on the level of contamination, consider a racking, or even filtration (1 µm) to eliminate this undesirable population. The medium will then be depleted in the lees necessary for lactic acid bacteria nutrition and the addition of a nutritional supplement will be essential.

MALOLACTIC FERMENTATION RESTART PROTOCOL

A

If contaminated with *Brettanomyces*:
- Rack/centrifuge anaerobically.

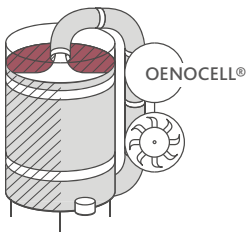
Note: if Brettanomyces population is higher than 10^3 cell/mL, filter the wine (1 µm).



Wine with MLF problems

B

- Incorporate **OENOCELL®** (20 g/hL - 200 ppm).
- Mix wine anaerobically every 12 hours for 48 hours, or continuously if possible.



C

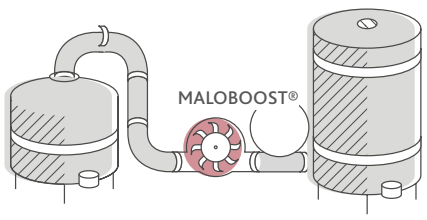
- Prepare the **LACTOENOS® B16 STANDARD** reactivation medium by following steps 1 and 2 in the "Protocol for reactivation of **LACTOENOS® B16 STANDARD** in wine" available in the product data sheet.
- Inoculate the starter with this reactivation medium by following step 3 of the same protocol.

Reactivation medium:
LACTOENOS® B16 + reactivator

Starter with
MALOBOOST®

D

Inoculation and nutrition:
- When the starter is ready (see protocol), add to the wine prepared in step B.
- Add **MALOBOOST®** (20 to 40 g/hL / 200 to 400 ppm).
- Mix thoroughly in a closed circuit.



Starter prepared in step C

Wine prepared in step B

Important: maintain a stable temperature, between 18°C - 25°C (64 - 77 °F), during all stages and until the end of MLF.

Flotation Protocol with **VEGECOLL®**

Many factors influence the flotation process and hence its success. The parameters of the protocol have been specially adapted for an easy flotation. Do not hesitate to contact **LAFFORT®**'s team before your flotation trials in order to explore the potentially inhibiting parameters and find the appropriate solutions.

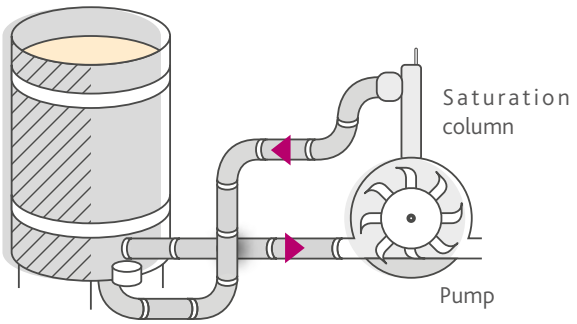
1 Preparing of the juice

- Clarification by flotation involves migration of the particles of the must to the surface of the tank. This migration is prevented in the presence of pectins. The addition of pectolytic enzyme directly after grape pressing is necessary to accelerate the process.
  - → **LAFASE® XL FLOT** - 1 - 4 mL/hL.
  - → **LAFAZYM® 600XL ICE (allows complete depectinization at low temperatures)** 1 - 2 mL/hL.
- In the case of must particularly difficult to clarify (variety, maturity, ...) or to accelerate the depectinization the use of **LAFASE® BOOST** at 1 mL / hL is recommended.
- Check the completion of the depectinization before starting the flotation. Use our **PECTIN TEST**, easy and fast.

2 Connecting the flotation pump

- For an easy flotation, the filling of the tank should not exceed 85 to 90% of the total volume.
- The temperature of the must should be between 15 and 18°C (59-65°F). The colder the must, the higher the viscosity, the more difficult the flotation process.
- Connect the pump inflow to racking valve, and the pump outflow to lees valve.
- For the best results, total hose length should not exceed 3 m (inflow and outflow).
- Make sure all the air is out of the saturation column before closing the tap.

MUST FOR CLARIFICATION

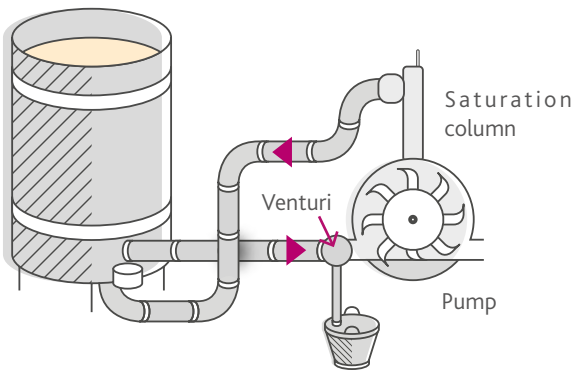


3 Adding the VEGECOLL®

- Start the pump without gas injection.
- Check that the saturation pressure is between 2 and 3 bar (the size of the tank does not matter).
  Prepare **VEGECOLL®** in a clean, inert container **following LAFFORT® recommendations**.
- The recommended dose of **VEGECOLL®** is 20 - 100 ppm. (the dose can be adjusted according to the characteristics of the must).
- Place into the **VEGECOLL®** preparation the pipe dedicated to the venturi section provided on the flotation system.
- **Inject VEGECOLL® as slowly as possible.**
- Mix the tank for 20 to 25 minutes at a saturation pressure of 2 to 3 bar, without addition of gas.

WARNING

MUST FOR CLARIFICATION

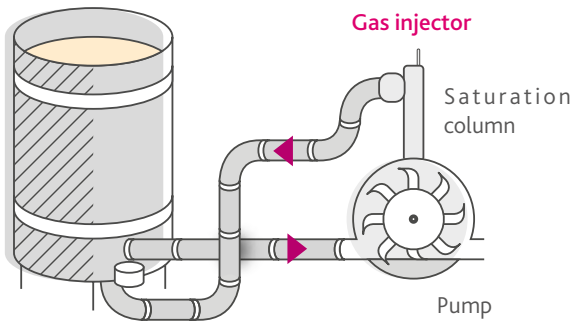


Flotation Protocol with **VEGECOLL®**

4 Starting the flotation process

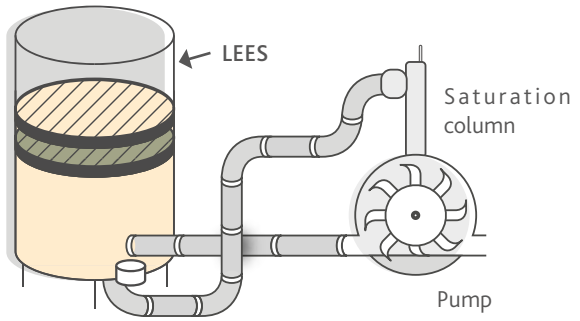
- Once the tank is homogenised, open the gas injection valve.
- The nitrogen inlet pressure should be between 5 and 7 bar.
- The gas flow rate must be between 25-60 L/min (depending on flotation setup). The saturation pressure must be adjusted to 5 bar.
- Check the quality of the flotation. To do this, take a sample at the tap of the saturation column.
- Remember to readjust the saturation pressure between 5 and 7 bar after sampling.
- The circulation time for flotation is between 60-150 min. Depending on the volume of the tank.
- Pump the equivalent of 1 to 2 volumes of the tank - 1.5 times is usually enough.

MUST FOR CLARIFICATION



5 Completion of flotation process and waiting time

- Once the flotation process is complete, stop the pump.
- Close the gas.
- Close all the valves in the tank.
- Leave the tank for 60 to 120 minutes so that lees can rise to the surface.
- **Do not leave the tank longer than 240 minutes. Gravitational force can cause lees separation and resuspension of the lees if the waiting time is too long.**
- Check the turbidity of the clarified batch.



Find out more: Discover our *FLOTATION* video on our website, at **LAFFORT & YOU**.

LAFFORT & YOU
SHARED RESOURCES & EXPERTISE

Dealing with Under-Ripe Grapes & Green Character

Concerns about making red wine from under-ripe fruit

- Limited extractability from the skins (reduced color and mouthfeel).
- Unripe and green seed tannins (astringency).
- Green character in the wine ("green pepper").

1 Issue: Limited extractability from skins

Under-ripe grapes often have thicker skins, which have a limited extractability. Anthocyanins, tannins and aroma precursors will be harder to release from the inside of the grape cell.

→ Solution: The use of an enzyme will help break open the cell wall structure and release the anthocyanins, aroma precursors, and soft tannins from the grape cell. Getting color and soft tannin extraction before the alcoholic phase of maceration will help reduce the level of astringent character in the finished wine.

- Add **LAFASE® FRUIT** enzyme during first pump over at 40 g/ton.

2 Issue: Under-ripe tannin and green seed tannin

When phenolic maturity does not happen at the same rate as sugar accumulation the fruit can have harsh tannin. When this happens in conjunction with seed tannin extraction, the result can be an increase in final wine astringency.

→ Solution: Limit the maceration time and the extraction regime during the alcoholic phase of fermentation. This shorter maceration time can result in lower overall tannin content and lower color intensity and stability. Use fermentation tannins to build structure, stabilize color, and help reduce green character perception in the wine.

- Add at grape processing: **TANIN VR GRAPE®** (100% grape skin and seed tannin): 200 – 400 ppm OR **TANIN VR SUPRA® ÉLÉGANCE** (blend of ellagic and proanthocyanidic tannin) 200 – 400ppm.
- Add at 1/3rd alcoholic fermentation: **TANIN VR COLOR®** (blend of tannin sources, rich in catechin for color stabilization): 200 – 400 ppm.

3 Issue: Green Character in the wine

Fruit with under ripe phenolic maturity can impart a green or veggie character into the finished wine.

→ Solution: It is possible to reduce or mask the green character during fermentation and after fermentation with different tools:

- Use toasted oak granular during fermentation: NOBILE® **SWEET VANILLA** (3 - 4 g/L).
- Use a yeast strain that produces lots of fermentation esters: **ZYMAFLORE® FX10** or **ZYMAFLORE® RX60** (200 ppm).
- Use a yeast derived product rich in mannoproteins and polysaccharides: **POWERLEES® ROUGE** (200 – 400 ppm).
- Do an early fining treatment on all press wine: **POLYMUST® PRESS** (200 – 400 ppm).
- Use toasted oak chips during aging (2 - 3 g/L):
  - NOBILE® **SWEET VANILLA**: Red fruits, vanilla, toasted marshmallow.
  - NOBILE® **INTENSE**: Dark fruit, mocha, toasted almond.

LAFFORT® LINKS

Use your phone's camera to access these videos in English, Spanish or French through the QR codes below.

1 Yeast rehydration.

This video includes specific time, temperatures, techniques, and equipment needed for carrying out a yeast rehydration for healthy and clean fermentations. A great tool for training your cellar crew before harvest.

2 Flotation video

Flotation is a dynamic clarification technique that saves time and energy in the cellar and optimizes juice quality. **LAFFORT®** offers two products especially adapted to this technique: a high-performance enzyme preparation for depectinization and a fining agent for effective flocculation. This video illustrates the steps needed to clarify the must within a few hours and which can quickly be inoculated with yeast.

3 Barrel Sulfur Additions with SO_2 tablets video

A quick and efficient way to add SO_2 to your wine. The **OENOSTERYL®** tablets are self - dissolving tablets of potassium metabisulfite and potassium bicarbonate. Each tablet is sealed individually to keep them fresh until use. Watch a video illustrating how easy and efficient they are to use.

4 Smoke Taint

Forest fires and exposure of grapes to smoke has become a major winemaking issue. Wines that are 'smoke tainted' receive negative comments from both winemakers and consumers, such as smoky, burnt, campfire, and ashtray. There are several winemaking techniques that can be used to mitigate the effects of smoke character in wines, and are addressed by the following protocols.

5 Frost Taint

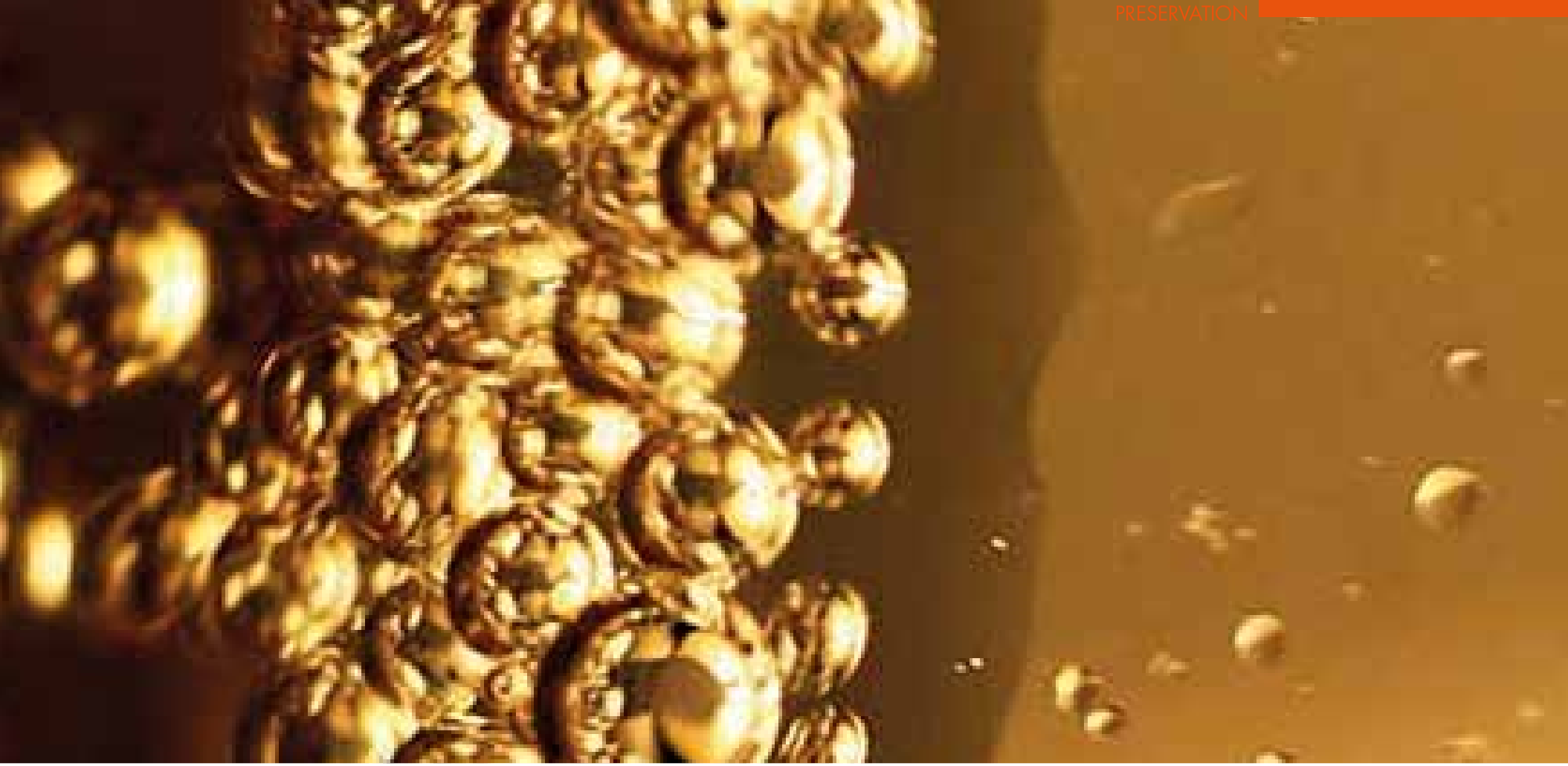
When wine grapes are exposed to frost and extreme cold temperatures, an unusual and unpleasant floral note is found in red wines made from grapes picked after the frost. This is a protocol on how to mitigate this character in wines made from frost exposed fruit.

6 Nutrient Calculator

Laffort has an easy-to-use calculator for fermentation nutrition.

7 Tools for acidification in must and wines

Tartaric, malic and lactic acid are all authorized for use in wine. They differ in their structure, their acidification capacity and their sensory impact. This protocol lets you make the right choices based on your wine's parameters and the desired objective.



PRESERVATION

Q&A

PRESERVATION WITH SULFUR DIOXIDE

Preservation with Sulfur Dioxide is one of the oldest processes in technological winemaking. **LAFFORT®** offer liquid SO_2 solutions, effervescent SO_2 tablets, and burnable sulfur discs and wicks.

OENOSTERYL®

*1. Why should I use **OENOSTERYL®** tablets?*

OENOSTERYL® is a premeasured SO_2 tablet composed of Potassium Metabisulfite and Potassium Bicarbonate to accurately dose your barrels with SO_2 with a released effervescence to homogenize into the wine.
For a 60 - gallon barrel, **OENOSTERYL®** will dose at:

- 2 g = 9 ppm
- 3 g = 15 ppm
- 5 g = 22 ppm

*2. Can I break the **OENOSTERYL®** tablets in half to deliver a smaller dose?*

OENOSTERYL® effervescent tablets are easy to break up into smaller pieces, which is useful to subdivide into smaller vessels. Do take care to use proportional measurements when dividing tablets as there is a high risk of over sulfuring to small vessels such as carboys.

3. Can the tablets get stuck in heavy lees?

There is a chance, especially with the heavier 5 g tablets, that the tablet will sink directly to the bottom of the barrel and get stuck in the heavy lees. You can certainly break up the tablet to allow the smaller pieces more surface area so that they effervesce faster.

4. Should I add the tablet after I top the barrel or before I top the barrel?

For best results, add tablet 1-2 hours prior to topping wines, or ensure there is enough headspace, as the effervescence briefly expands the wine as it dissolves into solution.

SULFUR DISC

1. How much sulfur do I need to burn to preserve barrels.

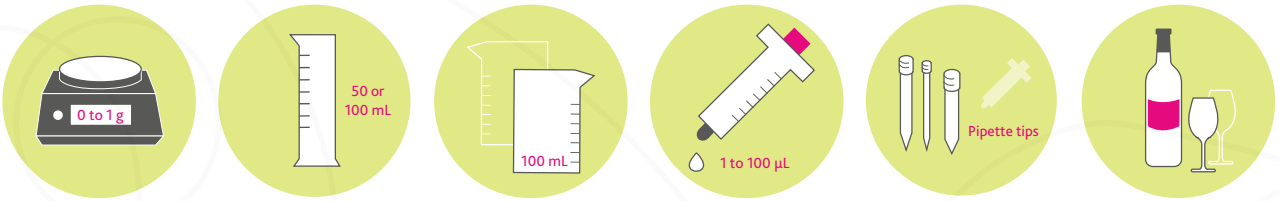
Most barrels can be adequately preserved by burning 1 g of Sulfur per 100 L of capacity, that is, a 2.5 g sulfur disc will be sufficient for up to a 250 L barrel. Repeat the treatment each four weeks. In practice, we find that almost all barrel preservation is done with 5 g sulfur discs over the same period.

BENCH TRIALS

Bench trials are an essential step in determining dosage rates for treatments during wine aging. With fining treatments, you want to add just enough product to remove the unwanted character, but not over-fine the wine and remove aromatics or desired texture. With tannin and mannoprotein treatments, each wine has a "sweet spot" where the mouthfeel comes into balance and this can only be found through bench trialing different dose rates.

The team at LAFFORT® wants to make the bench trial process as painless as possible.
With the correct tools, bench trials can be quick and easy.

TOOLS



TIPS

1 - For powder or granulate products:
Prepare a 5% solution 2 hours ahead (exception with **ICHTYOCOLLE®** – prepare a 1% solution).

2 - For liquid products:
Use product directly (no dilution necessary).

3 - Make a plan:
Write down your dosage rates and calculate the volume of bench trial solution needed for each sample.

4 - Organize the workspace:
Label all wine sample bottles/glasses before adding the bench trial solution.

5 - Homogenize bench trial solutions
right before pipetting into wine sample.

6 - Mix wine samples thoroughly after addition.

7 - Fining agents require 2 – 4 days of settling. Look for compact lees layer at bottom of sample bottle, then decant clean wine for sensory analysis.

8 - Structure building tannins:
TANIN VR GRAPE®, **TAN'COR GRAND CRU®**, **TAN'FRESH®**, **TANIN GALALCOOL SP®** are best evaluated after 48 hours of contact time with the wine.

9 - Finishing products:
QUERTANIN® range, **AUTOLEES®**, **MANNOFEEL®**, and **STABIVIN SP®** can be added and tasted immediately after mixing into wine sample.

LAFFORT® CONVERSION CHART

ppm or mg/L	100	200	300	400	500	600	700	800	900	1000
g/hL	10	20	30	40	50	60	70	80	90	100
lbs/1,000 gal	0.8	1.7	2.5	3.3	4.2	5.0	5.8	6.7	7.5	8.3

mL/hL	1	5	10	20	30	40	50	100	200	300
mL/1000 gal	38	189	379	757	1,136	1,514	1,893	3,785	7,570	11,355
mL/gal	0.04	0.19	0.38	0.76	1.14	1.51	1.89	3.79	7.57	11.36

1 gal = 3.785 L	12 x 750 mL case = 2.37753 gal
1L = 1000 mL	1 barrel = 225 L = 59 gal
1hL = 100L = 26.40 gal	1 ton = 165 gal approx
1 lbs = 454 grams	1g/L = 0.1%
1 US ton = 2000lbs = 907 kg	1 metric ton = 1000 kg = 2205 lbs

LAFFORT® BENCH TRIAL DOSAGE - GRANULATES

Soluble products, such as the **QUERTANIN®** range and **AUTOLEES®**, can use this table for direct addition and tasting immediately afterwards.

Fining treatments need time to settle before evaluating. In general, 2 - 4 days is the recommended settling time. Look for a compact lees layer at the bottom of the sample bottle, then decant clean for sensory evaluation.

Dosage	Volume of Wine Sample			
	50 mL	100 mL	250 mL	375 mL
10 ppm	10 µL	20 µL	50 µL	75 µL
20 ppm	20 µL	40 µL	100 µL	150 µL
30 ppm	30 µL	60 µL	150 µL	225 µL
40 ppm	40 µL	80 µL	200 µL	300 µL
50 ppm	50 µL	100 µL	250 µL	375 µL
60 ppm	60 µL	120 µL	300 µL	450 µL
70 ppm	70 µL	140 µL	350 µL	525 µL
80 ppm	80 µL	160 µL	400 µL	600 µL
90 ppm	90 µL	180 µL	450 µL	675 µL
100 ppm	100 µL	200 µL	500 µL	750 µL
125 ppm	125 µL	250 µL	625 µL	938 µL
150 ppm	150 µL	300 µL	750 µL	1125 µL
200 ppm	200 µL	400 µL	1000 µL	1500 µL

* µL = microliters.

FOR POWDER OR GRANULATE PRODUCTS.

Prepare a 5% solution, e.g. 2.50 grams dissolved in 50 mL water. Mix thoroughly and allow solution to swell for two hours before use.

Using the table at left, add the indicated number of microliters of the solution to the trial sample to achieve the specified ppm.

Exception – for **ICHTYOCOLLE®**, prepare a 1% solution and multiply the volume indicated by 5.

Tannin and Autolees samples can be dissolved in a 12% alcohol solution instead of water when making the 5% bench trial solution.

LAFFORT® BENCH TRIAL DOSAGE - LIQUIDS

Liquid products, such as **STABIVIN® SP**, can use this table for direct addition and tasting immediately afterwards.

Fining treatments, such as gelatins, need time to settle before evaluating. In general, 2 - 4 days is the recommended settling time. Look for a compact lees layer at the bottom of the sample bottle, then decant clean for sensory evaluation.

Dosage	Volume of Wine Sample			
	50 mL	100 mL	250 mL	375 mL
10 mL/hL	5 µL	10 µL	25 µL	38 µL
20 mL/hL	10 µL	20 µL	50 µL	75 µL
30 mL/hL	15 µL	30 µL	75 µL	113 µL
40 mL/hL	20 µL	40 µL	100 µL	150 µL
50 mL/hL	25 µL	50 µL	125 µL	188 µL
60 mL/hL	30 µL	60 µL	150 µL	225 µL
70 mL/hL	35 µL	70 µL	175 µL	263 µL
80 mL/hL	40 µL	80 µL	200 µL	300 µL
90 mL/hL	45 µL	90 µL	225 µL	338 µL
100 mL/hL	50 µL	100 µL	250 µL	375 µL
125 mL/hL	63 µL	125 µL	313 µL	469 µL
150 mL/hL	75 µL	150 µL	375 µL	563 µL
200 mL/hL	100 µL	200 µL	500 µL	750 µL

* µL = microliters.

FOR LIQUID PRODUCTS.

Use directly.

Using the table at left, add the indicated number of microliters to the trial sample to achieve the specified dose rate in mL/hL.

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