oenom@g
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Editorial

"Natural" Wines?

"Natural Wines." Just a simple sign on a restaurant wall. But it triggered a totally unexpected reaction: a government agent ordered the restaurant owner to remove the offending sign. According to the notice received, "The term 'natural' can be applied only to a product which is found in nature or as close as possible to its natural state, untreated, and which has only normal components with no additives." While we could ponder for a moment the meaning of "normal components," an important issue has been raised nonetheless.

Although the terms "natural" and "organic" wine are part of everyday language, they may be controversial and, depending on the country, complicated to define. For some wine drinkers, a natural wine would be one made entirely without added sulphites, while other natural wine aficionados would accept a light sulphiting, no matter what the law says. Most laws have lots to say about how organic wines should be made. For this reason, autolysates and inactivated yeasts could not be used in the 2014 vintage of certified "organic" wines. So what is a winemaker to do when a must has a nutrient deficiency? Opt for diammonium phosphate (DAP), a synthetically produced chemical, instead of organic nitrogen nutrition based on inactivated yeast?

"Natural" is also the term we use in our logo "Natural solutions that add value to the world of winemaking." And that is the Lallemand promise: To select the best Nature can offer to provide tools for the quality of your wines. Technical solutions, through the natural potential of micro-organisms – alive or not – to accompany the winemaking process.

In this issue of Oenomag, you will discover a new and specific bacteria nutrient for red wines with a high concentration of tannins – ML Red Boost™. Plus, we will show how recent research could lead to a new way to limit the risk of volatile phenols.

Cheers!

1 High Phenol Wines: When Bacteria Get Involved...

Among the main concerns of winemakers and oenologists, the faults linked to volatile phenols in wine were always significant. Through advances in research and the improvement of winery practices, much progress has been made. Effective and natural new solutions have been developed in the battle against *Brettanomyces bruxellensis*. Recent studies have revealed a way that will further reduce the risk of phenolic faults.

What are phenolic faults?

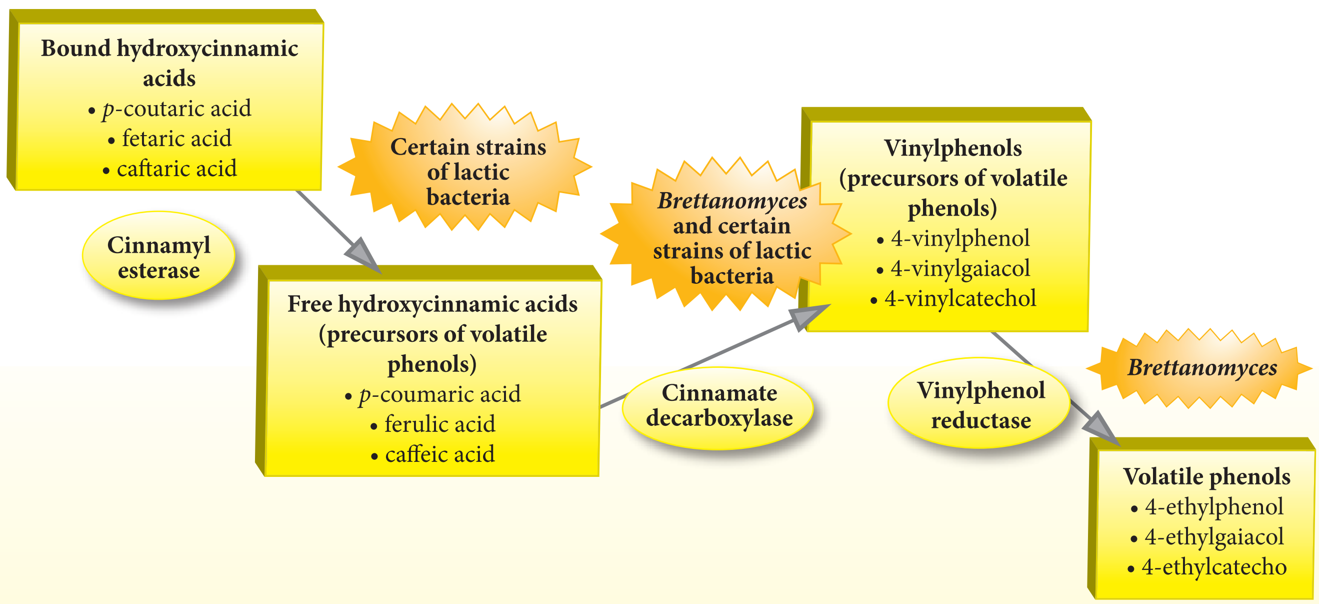
What we generally call phenolic faults corresponds to three molecules, clearly identified as responsible for this type of odours, whose perception threshold can vary according to the type of wine:

- 4-ethylphenol, whose perception threshold is about 500 µg/L in wine; characterized by animal, stable, horse sweat, band-aid or ink odours, and stable over time, is the molecule found mainly in contaminated wines
- 4-ethylgaiacol, whose perception threshold is about 100 µg/L in wine; a little less frequent, this molecule is characterized by the odour of cloves
- 4-ethylcatechol, with a smoky or camphor odour.

Even under these thresholds, the positive aromas of wine can be masked by the presence of small quantities of one or more of these molecules, without going as far perceiving the phenolic fault.

The biosynthesis pathways for volatile phenols

The main pathway (Figure 1) for producing these compounds comes from the metabolization of certain precursors present in the grape, called hydroxycinnamic acids, by the *Brettanomyces bruxellensis* spoilage yeast. These acids are naturally present in the bound form (*p*-coutaric, fetaric and caftaric acids, for example) and the free form (*p*-coumaric, ferulic and caffeic acids), but only the free forms of these precursors can be transformed by *B. bruxellensis* into malodourous volatile phenols (Schopp *et al.* 2013). In fact, any conversion from the bound forms to the free forms of these precursors by the enzymatic activity of cinnamyl esterase present in certain micro-organisms (figure 1) will increase the production of volatile phenols when contaminated by *B. bruxellensis* (Osborne *et al.* 2013).



2 High Phenol Wines: When Bacteria Get Involved...

***Brettanomyces* is not the only culprit!**

Researchers at the Oregon State University (United States) recently showed that certain lactic bacteria, including *Oenococcus oeni*, have this cinnamyl esterase enzymatic activity and could therefore increase the quantity of free precursors, made usable by *Brettanomyces* to produce volatile phenols (Burns and Osborne 2013). During their experiments, they compared three *O. oeni* bacteria used to carry out malolactic fermentation (MLF) on a Pinot Noir wine, and measured various parameters: degradation of *p*-coutaric acid, production of *p*-coumarc acid, and the production volatile phenols after contamination by *B. bruxellensis*. The results clearly show that the choice of wine bacteria for the realization of MLF is not inconsequential. Indeed, one of the *O. oeni* strains (#1) transformed 6.8 mg/L of *p*-coutaric acid into 4.9 mg/L of *p*-coumaric acid, while these concentrations remained unchanged with the other two bacteria (#2 and #3). What's more, after contamination with *B. bruxellensis*, the concentration of 4-ethylphenol in the wine obtained with bacteria #1 (which has this cinnamyl esterase enzymatic activity and had therefore freed hydroxycinnamic acids) is clearly higher than in the two other wines, respectively 1579.5 mg/L compared to 263.3 mg/L, nearly six times more! At the same time, the concentration of 4-ethylgaiacol was increased by a factor of three!

In fact, certain lactic bacteria in the *Lactobacillus* and *Pediococcus* genera (Fras *et al.* 2014, and Couto *et al.* 2006) are prone to produce volatile phenols directly from free hydroxycinnamic acids, like *B. bruxellensis*.

"Phenol-negative": a new prime criteria for wine bacteria

In light of recent findings, we must consider MLF even more carefully, especially when there is a risk of contamination by *B. bruxellensis*. It appears particularly risky to carry out MLF with indigenous bacteria, as the probability of finding at least one "phenol-positive" wine bacteria liable to increase the quantity of volatile phenol precursors is very high.

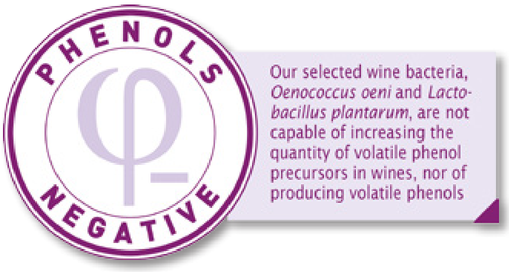
Given these results, Lallemand launched a screening program for all our selected wine bacteria to assess their cinnamyl esterase activity, i.e., their capacity to increase or not increase the level of free hydroxycinnamic acids (phenol precursors) from bound hydroxycinnamic acids naturally present in the grape.

The current list of our selected bacteria tested and characterized as "phenol-negative," i.e., incapable, in metabolic terms, of increasing the quantity of volatile phenol precursors, or of volatile phenols directly, is as follows: Lalvin VP41® (MBR® and 1-Step®), Lalvin PN4 1-Step®, Lalvin 31®.

The other wine bacteria selected by Lallemand are under study and the results will be communicated to you as soon as possible.

This new criteria, which we believe is very important for the final quality of wines and the security of winemaking, is now an integral part of the Lallemand selection process.

3 High Phenol Wines: When Bacteria Get Involved...



Act at the source by selectively eliminating *Brettanomyces* populations: The force of chitosan

The most radical way to eliminate any risk of producing volatile phenols with *Brettanomyces* is to eliminate this spoilage yeast. For that, there is a new natural oenological tool that is biodegradable, easy to use, non allergenic and non toxic: fungal source chitosan. Admitted in July 2009 by the Organisation Internationale de la Vigne et du Vin (OIV) as a new practice in Oenological Codex, and by the European Union in December 2010, this natural polymer derived from chitin is obtained by an original process patented by the Kitozyme company. First, double-acting chitosan specifically attracts *Brettanomyces* cells and binds to them (a form of fining), then chitosan disrupts the energy metabolism of the cells, triggering cell death, with formidable effectiveness (figure 2).

Incorporated into the wine after MLF at a rate of 4 g/hL, then eliminated after 10 days of action, this product is a veritable revolution in the mastery of *Brettanomyces* contamination.

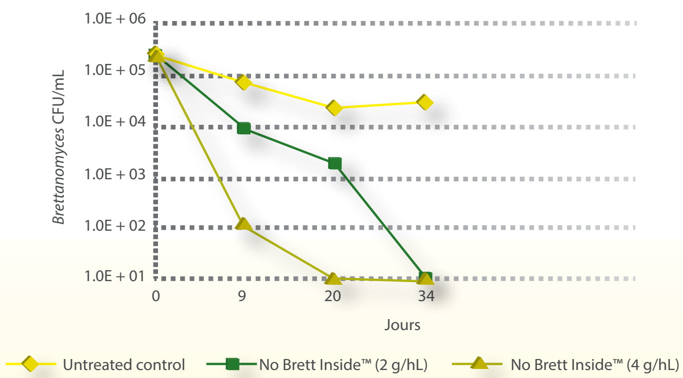


Figure 2. Trial on a Merlot mine contaminated with Brettanomyces yeast (2.25 x 106 CFU/mL): comparison of two different dosages of **No Brett Inside**™ (2 and 4 g/hL).

Prevention and good practices in the winery: Don't forget the basics!

The existence of such tools is no reason to skip the fundamentals in the fight against *Brettanomyces*: impeccable hygiene and perfect microbiological control, through preventive actions and certain winemaking rules, particularly regarding the phases of fermentation.

Brettanomyces loves slow and stuck fermentations! Many cases of phenolic deviation could be avoided by correctly managing the alcoholic fermentation (AF), if only through the correct handling of the selected yeasts. This includes good rehydration practices and adding the yeast as early as possible! Systematically inoculate with the yeast as soon as the must is in the vats, because *Brettanomyces* can establish its presence in only a few hours.

With especially ripe grapes, don't hesitate to use yeast protectors high in sterols (the NATSTEP® process such as Go-Ferm Protect™) to not only provide the selected yeast with a good chance of survival in the high alcohol wine at the end of AF, but to give it an advantage from the start of AF, through the action of minerals and vitamins, which activate enzymatic reactions.

In cases where these precautions are not enough to limit *Brettanomyces* at the end of AF, the practice of co-inoculation (inoculating the must with wine bacteria at the beginning of AF) has been proven effective on controlling populations of contaminating bacteria such as *Brettanomyces*.

Generally, early inoculation with lactic bacteria is the main way to control these risks, because otherwise there is a window of opportunity for these spoilage yeasts between the AF and the MLF.

Fungal source chitosan is commercialized under the name **No Brett Inside**™.

1 ML RED BOOST

For concentrated red wines: ML RED BOOST™ opens the way to malolactic fermentation

Certain red wines, notably those made from varietals like Merlot or Tannat, are regularly resistant to malolactic fermentation, but we can't blame the usual suspects like ethanol, pH, SO_2, or even the temperature. Although it often appears that poor nutrition is the problem, new research highlights the inhibiting action of certain polyphenols vis-à-vis lactic bacteria. Are these obstacles insurmountable? Probably not, thanks to the development of a specific nutrient for red wines with concentrated tannins.

Polyphenols in wine: inhibiters or stimulators?

In practice, successful MLF is hard to achieve in red wines high in polyphenols, as is generally the case with wines made with Merlot grapes, Tannat grapes in the South-West of France, and Graciano grapes in Spain, and, more broadly, wines made through thermo-vinification.

Much research has explored the impact of polyphenols on the growth and viability of lactic bacteria, and on the metabolism of malic acid degradation, with sometimes contradictory results. These effects vary from stimulating growth to inhibiting it, with bacterial activity according to the bacteria strain and according to the nature and concentration of the polyphenols.

Moreover, numerous tannins are polymerized with other molecules, reducing their toxic effects. MLF is even harder to trigger in a wine containing mainly weakly polymerized tannins. When exogenous oenological tannins are added to classic doses, they do not appear to present this type of inhibition.

For the moment, very little data on the molecular mechanisms in play is available, but research continues.

Understanding the inhibitions by reconstructing red wines from a white wine

Recent experiments have focused on the impact of polyphenolic extracts on the operation of MLF (Lonvaud *et al.* 2013).

The extracts were obtained from three grape varietals, Merlot, Cabernet Sauvignon and Tannat, then were added to a Chardonnay wine (characteristics: ethanol 12.0%/vol., pH 3.5 and total SO2 <20 mg/L) at a concentration similar to the polyphenol concentration in the initial wine.

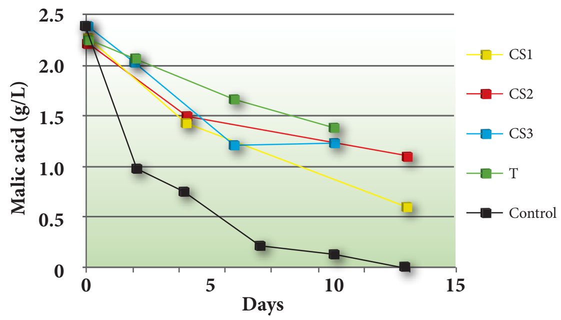


Figure 3. Malolactic fermentation kinetics (malic acid concentration) in a Chardonnay wine (control) and in the same wine after adding extracts of Cabernet Sauvignon (CS1, CS2 and CS3) and Tannat (T).
Source: Lonvaud 2013

The results show that for the two *Oenococcus oeni*, the bacterial growth is inhibited by the addition of extracts from the Cabernet Sauvignon or the Tannat grapes. The result is a significant slowing of the malic acid degradation (figure 3). Although MLF completed in 10 days in the Chardonnay control wine, from 1 to 1.5 g/L of malic acid remained in the wines to which extracts were added.

Removing the inhibition: The role of inactivated yeasts

With the aim of inhibiting the effects on MLF, the addition of two preparations of inactivated yeasts – Y1 and Y2 – were studied.

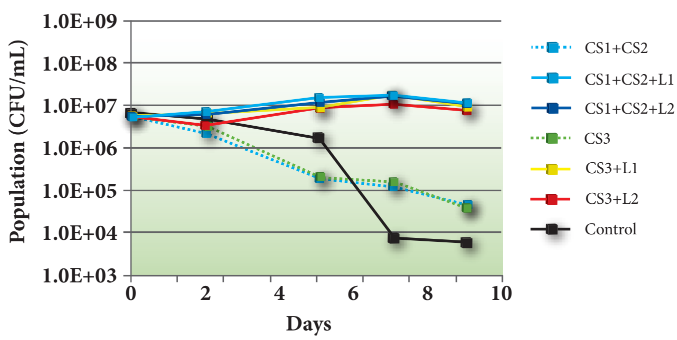


Figure 4. Bacterial growth in a Chardonnay wine (control) to which extracts of Cabernet Sauvignon (CS1, CS2 and CS3) were added, and the same wines after adding the inactivated yeasts Y1 and Y2.
Source: Lonvaud 201

2 ML RED BOOST

Compared to the Chardonnay control wine, the inactivated yeast preparations contribute to stimulating MLF and removing the inhibition linked to the addition of the polyphenolic extracts (figure 4). These inactivated yeast preparations could act on several levels. The polysaccharides from the yeast derivatives could bind with the tannins and make them less toxic for the bacteria. The redox potential measured would also indicate that the redox potential would decrease with the addition of two nutrients, also contributing to the stimulation of bacterial growth. Indeed, these activators contribute to enriching the nutritional profile of the wine and, through this, make it more likely to support bacterial growth.

And now, an MLF activator specific to red wines: ML RED BOOST™

Different nutrients from specific inactivated yeast were tested to verify their impact on the growth of the wine bacteria *O. oeni* and *L. plantarum* (figure 5). One of these nutrients clearly boosted the multiplication of bacteria whatever the strain, demonstrating the specific action of certain yeast fractions on bacterial development.

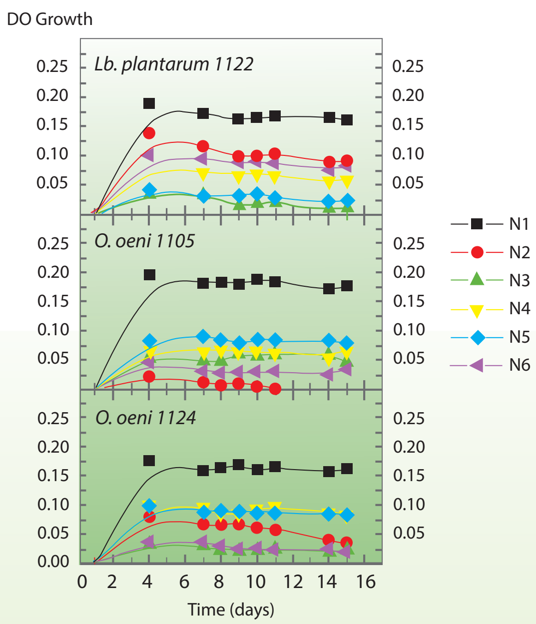


Figure 5. Growth of three oenological bacteria in the presence of various nutrients in a model wine Source: Mira de Orduña 2011-2012 (a Lallemand-Cornell University collaboration).

Concentrated red wines from the 2012 and 2013 harvests underwent trials with the addition of the specific activator ML Red Boost™. For three wine bacteria under consideration, the ML Red Boost™ addition was made 24 hours before the model wine was inoculated with the bacteria in order to make the medium more conducive to the growth of *O. oeni* (thereby decreasing the inhibiting action of tannins).

Eight days after inoculation, the bacteria population was 60% to 700% higher in wines treated with ML Red Boost™, compared to the trials with no activator. These population differences were accentuated two weeks after inoculation and led to very short durations for MLF, in all the test wines with nutrients (figure 6).

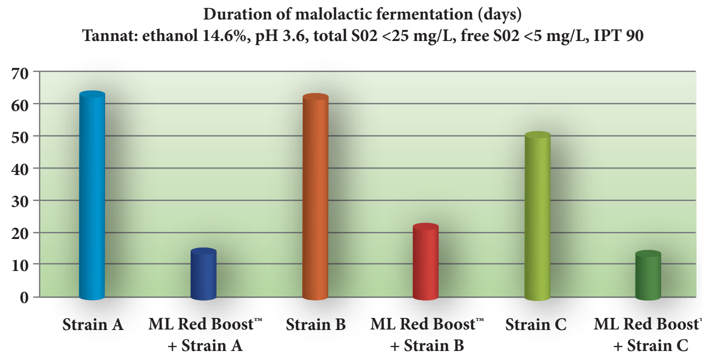


Figure 6. Duration of malolactic fermentation with three different bacteria strains, with and without the ML Red Boost™ activator.

The idea that L-malic acid is sufficient to meet the energy needs associated with the development of O. oeni is widespread. However, the reality is quite different. The absence of certain nutrients essential to the implantation, growth and metabolism of *O. oeni* may be the reason behind slow or even stuck MLF. Nutritional deficiencies and bacteria inhibiters vary from one medium to another. Their negative impact can be limited by adding fermentation activators, with the choice differing according to the type of wine. Designed for high polyphenol red wines, the ML Red Boost™ activator contributes to encouraging their implantation in the medium.

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Coming up in the next Lallemand oenomag

Every year a new wine vintage, and they're never the same. The same goes for Oenomag, , so don't miss the next issue! The innovation and research at the heart of our craft, to meet and anticipate your needs. The next Oenomag will be the occasion to reveal the unpublished results of our experiments during the 2014 harvest! Targeted selections, innovative processes and optimizations are sure to interest you.

New discoveries coming soon! Don't miss it!

Summary

Some recent studies show us how to improve the fight against phenolic faults in wines. Researchers have shown that certain lactic bacteria were prone to increase the quantity of precursors for these phenols, and therefore the phenols themselves, in the case of contamination by *Brettanomyces bruxellensis*. The choice of a "phenol-negative" bacteria to conduct MLF becomes a major risk-management tool in the winemaker's toolbox.

The winemaker now has powerful means to prevent these faults, by utilizing these new data and respecting the basic principles of hygiene and microbiological control, to which can be added fungal source chitosan, formidably effective for the specific elimination of *Brettanomyces* populations!

"For wine is the mirror of man"

Alcaeus, Greek lyric poet

A new yeast that decreases alcohol level

The result of a collaboration between Lallemand and the INRA in Montpellier, a new non-GMO *Saccharomyces cerevisiae* wine yeast has been selected, which lets us make wines with lower alcohol levels and no production of undesirable compounds. The young researcher Valentin Tilloy, under the direction of Sylvie Dequin (INRA Sup Agro) received the *2014 Entretiens Scientifiques Lallemand-Michel Feuillat prize*, which recognizes the greatest scientific advances in microbiology applied to oenology.

This new yeast presents a very unusual metabolism, with a reduction in the final alcohol level of up to 1.3%/vol., and the difference is compensated by an increased production of glycerol, a natural metabolite known for enhancing the wine's volume in the mouth. No undesirable compounds are produced, and the level of volatile acidity is particularly low. The next challenge for Lallemand Oenology is to produce this yeast while ensuring it maintains its remarkable winemaking properties.

Caption:
Valentin Tilloy
with Anne Ortiz-Julien
and Sandra Escot
(Lallemand Oenology)