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LALLEMAND

WINEMAKING UPDATE

Number 2 – 2014

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NEWS FLASH

❖ The **Institute of Masters of Wine** and **Lallemand** are delighted to announce the winner of the **2014 Lallemand Award**: Cath Oates of Mount Barker, Australia. Established in 2010, each year the award bestows a bursary on a student in the Institute's study program for the quality of their work. Exceptionally, this year the award includes an invitation to attend the Institute's 8th International Symposium, in Italy. "I am proud to win the Lallemand Award," said Ms. Oates. "And I'm absolutely over the moon about attending the MW Symposium in Florence. For me, and for our relatively remote viticultural region of Australia, it couldn't be a better end to what has been an already fabulous 2014 vintage." Ms. Oates won the award for the quality of her 1,000-word essay on the following subject: *In the context of and according to current market trends, discuss the winegrowing practices that winemakers can use to modulate the aroma profile of wines and the objectives that can achieved through these practices.*

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WINEMAKING UPDATE

WINEMAKING UPDATE is published by Lallemand to inform oenologists and winemaking staff about the latest news and applications arising from research. To request previous issues, or to send your questions or comments, contact us at:

Lallemand S.A.S.
Piet Loubser
P.O. Box 3542
7602 Matieland, South Africa
Tel.: +27 21 913 7555
ploubser@lallemand.com

www.lallemandwine.com

Wine Bacteria to Control Volatile Phenols and *Brettanomyces*

From the vineyard to the bottle, every step of winemaking impacts the quality in the wine. Climate change also affects the quality of grapes, especially the sugar levels and pH, and, consequently, winemaking conditions must adapt. The increase in sugar levels and pH also influence the micro-organisms present on the grapes and, of course, the yeast and bacteria populations. The interactions between these micro-organisms are very complex and winemakers must manage alcoholic and malolactic fermentation taking into account the evolution of grape ecology under these new conditions. The growth of one of the yeasts, *Brettanomyces*, is considered a contaminant and must be controlled. This issue of *Winemaking Update* explores a natural way to control *Brettanomyces* yeast and the volatile phenols they produce, with selected wine bacteria used to conduct malolactic fermentation.

1. *Brettanomyces* – A Recurrent Culprit

Brettanomyces/Dekkera yeasts are well known wine spoilage micro-organisms that can damage wine quality, from increasing haziness to producing volatile phenols – aromatic compounds associated with medicinal, band-aid, barnyard, horsey and mousy off-odours (Fugelsang et al. 1993, and Heresztyn 1986). Controlling the precursors of volatile phenols and the growth of this spoilage yeast in the winery is a major challenge, as it can develop even in difficult conditions, such as high alcohol, high pH, nutritional depletion, high sulphur dioxide (SO_2), etc. Although *Brettanomyces* can be detected at any stage of the winemaking process, it is typically detected after alcoholic fermentation (AF) and before spontaneous malolactic fermentation (MLF) or during barrel aging (figure 1).

2. Ethylphenol Metabolism

The off-odours are caused principally by 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP). With *Brettanomyces*, these compounds are produced during the biotransformation of the hydroxycinnamic acids, *p*-coumaric acid and ferulic acid, which are precursors naturally present in grapes in the bound or free form. Only the free form is used by *Brettanomyces*. The transformation of these free precursors into 4-EG and 4-EP (figure 2) occurs in two steps: first with the cinnamate decarboxylase enzyme, followed by the vinylphenol reductase enzyme. Several factors influence the concentration of these precursors, ranging from the varietal, to viticultural conditions (hot climate, cold climate) and winemaking practices. According to a recent study by Schopp et al. (2013), *Brettanomyces bruxellensis* can metabolize only the free form of *p*-coumaric

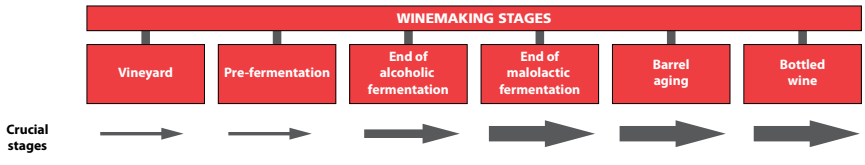

Figure 1. Crucial stages of winemaking when *Brettanomyces* is most often detected

Continued

and ferulic acids. In fact, any conversion of coutaric acid (in the bound form) by the cinnamyl esterase enzyme, to *p*-coumaric acid (in the free form) by other wine micro-organisms (figure 2) can contribute to the increased production of ethyl phenols by *B. bruxellensis* (Osborne et al. 2013).

It is interesting to note that *Brettanomyces* is not the only micro-organism that can produce volatile phenols. Some lactic acid bacteria, such as *Pediococcus* and *Lactobacillus* (Couto et al. 2006) are also naturally able to produce volatile phenols from free hydroxycinnamic acid (*p*-coumaric and ferulic acids). Similar results were observed with some strains of *Lactobacillus plantarum* during research by Fras et al. (2014).

A recent study by Burn and Osborne (2013) showed that certain wine bacteria of the *Oenococcus oeni* species can metabolize coutaric acid into *p*-coumaric acid, through the action of one of their enzymes, cinnamyl esterase (figure 2), thereby increasing the levels of the volatile phenol precursors available for *Brettanomyces*.

3. Selected Wine Bacteria against *Brettanomyces*

The first step to controlling *Brettanomyces* is respecting winemaking best practices. It is important to have an integrated strategy that takes into account the interdependence of diverse wine parameters, such as grape quality, SO_2, pH, wine temperature, nutrients, oxygen, barrel condition and oenological practices. Good cellar hygiene, reducing the lag phase between the end of AF and the beginning of MLF, and early stabilization, along with proper SO_2 dosage, greatly minimize the risk of microbial spoilage. The winemaker's strategy to limit the risk of developing *Brettanomyces* has three key factors: the presence of precursors for volatile phenols, the growth phases of *Brettanomyces* and the wine conditions. Selected wine bacteria can prevent *Brettanomyces* development by taking into consideration these three aspects.

3.1 Wine condition

Secure, fast and complete alcoholic and malolactic fermentations, combined with early stabilization, help preserve the quality of the wine and limit the residual nutrients that *Brettanomyces* utilizes to survive and develop.

3.2 Preventing the presence of precursors for volatile phenol

Osborne et al. (2012) investigated the capacity of wine bacteria (*O. oeni* and *L. plantarum*, including selected bacteria) to degrade hydroxycinnamic acids bound to tartaric ester present in the wine into the free form, the precursors for volatile phenol production by *Brettanomyces*. The trials were done in Pinot Noir wine inoculated with selected wine bacteria, and the results compared to a control sample in which MLF was blocked. The researchers assessed the concentrations of hydroxycinnamic acids (esterified and free) after MLF. The variation in the concentrations of hydroxycinnamic acids indicates whether the wine bacteria can degrade certain acids, and make them available to the *Brettanomyces* for the production of volatile phenols.

They found that some strains of *O. oeni* wine bacteria clearly have the capacity to increase the level of coumaric acid (free form) in the wine and thus generate an increase in the level of ethylphenols in the presence of *Brettanomyces*.

This study sheds new light on the metabolic pathway of certain *O. oeni* strains which possess the cinnamyl esterase enzyme and can degrade coutaric acid into coumaric acid, for example.

Following these observations, we sought to characterize all our selected wine bacteria. The results presented in table 1 show there is no change in the concentration of the hydroxycinnamic acids (both bound and free) in the wines inoculated with our bacteria compared to the control wine, where MLF was blocked.

Thus, our selected wine bacteria cannot degrade coutaric acid into coumaric acid, or any other bound hydroxycinnamic acid, which is the origin of the volatile phenol precursors responsible for the development of the off-odours associated with *Brettanomyces*. This led to the conclusion that the cinnamyl esterase enzyme, which is responsible for for-

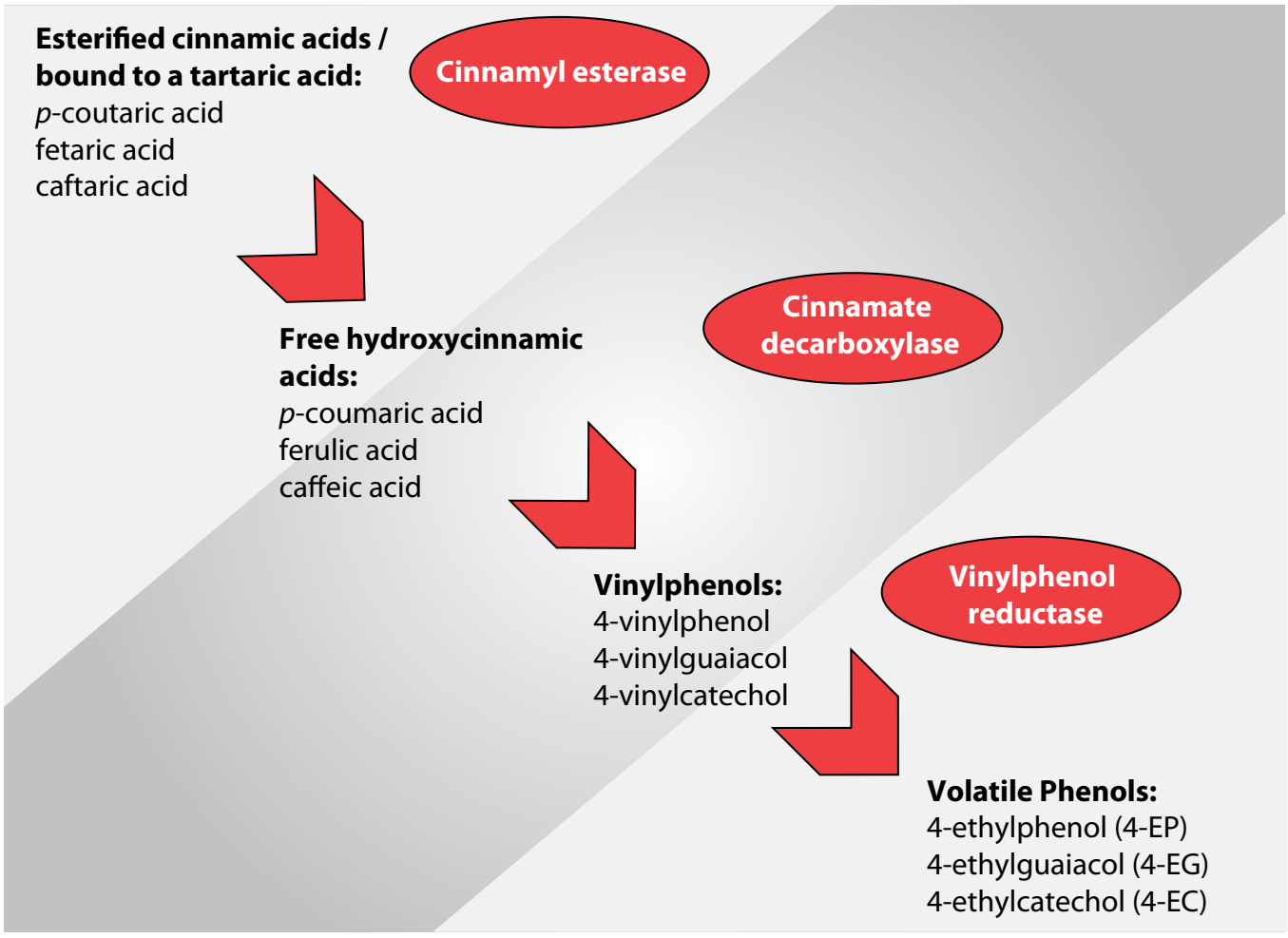

Figure 2. Ethylphenol production

	Caftaric acid	Coutaric acid	Caffeic acid	*p*-coumaric acid	Ferulic acid
PN4	23.2 ± 0.4	6.6 ± 0.1	2.4 ± 0.2	0.9 ± 0.1	3.5 ± 0.2
***O. oeni* 1**	24.1 ± 1.3	6.9 ± 0.4	3.0 ± 0.1	1.0 ± 0.1	3.3 ± .03
Beta	25.0 ± 2.2	7.0 ± 0.6	2.6 ± 0.5	0.8 ± 0.3	4.2 ± .05
V22	25.8 ± 1.3	7.1 ± 0.3	2.4 ± 0.1	0.6 ± 0.1	3.8 ± .01
Control without MLF	25.1 ± 1.1	6.8 ± 0.5	2.2 ± 0.2	0.9 ± 0.2	4.1 ± .03

Table 1. Hydroxycinnamic acid concentration in samples of Pinot Noir wine four weeks after inoculation with different selected wine bacteria

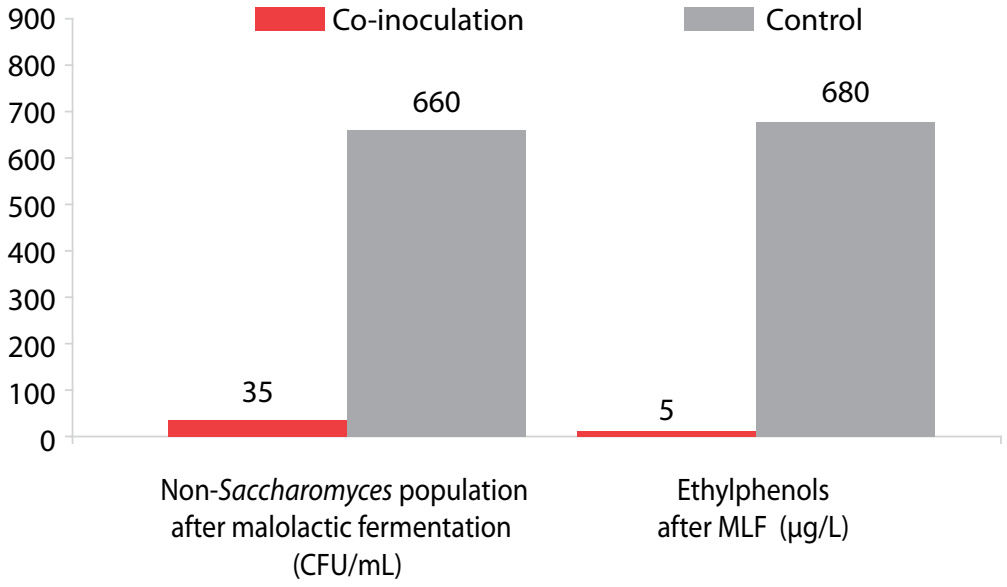

Figure 3. Microbial population and ethylphenols in Cabernet Franc wines (France) at the time of co-inoculation

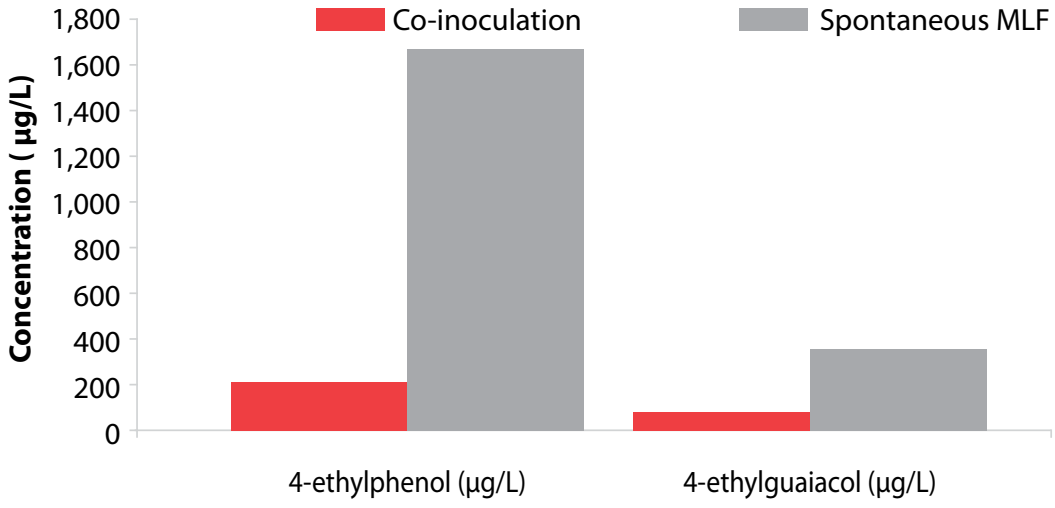

Figure 4. Ethylphenol levels after malolactic fermentation in a Cabernet Franc (France)

ming *p*-coumaric acid from coutaric acid, or any other free hydroxycinnamic acid is absent from our selected wine bacteria, which means they can be considered "**phenol negative**."

The complete list of our selected wine bacteria characterized as phenol negative includes Lalvin VP41, PN4, Beta, Alpha, Lalvin 31, which are all in the species *O. oeni*, as well as our *Lactobacilus plantarum* V22. Therefore, the winemaker can choose one of these bacteria for MLF at no risk of producing precursors to volatile phenols.

3.3 Preventing *Brettanomyces* growth with secure and fast malolactic fermentation and no lag phase

The utilization of a selected yeast and proper yeast nutrition ensures the rapid onset, effective and complete AF, which, as we know, is part of an integrated strategy to prevent the development of *Brettanomyces*. Yet that does not guarantee results. The period from the end of AF to the start of MLF is particularly conducive to the development of *Brettanomyces*: the wine is not protected by SO_2, there are still some nutrients available to the spoilage yeast, and competition from other wine micro-organisms is hardly a threat, as the yeast has finished and is dying off and the indigenous lactic bacteria are not yet established. The use of selected wine bacteria is a solution to shorten the time lapse between AF and MLF and thereby prevent the development of *Brettanomyces*. Early inoculation with the wine bacteria, either right after AF or in co-inoculation (24 hours after inoculation with yeast), has proven to be a simple and effective method for preventing the development of *Brettanomyces*. In a study by Pillet et al. (2011), a Cabernet Franc from the Gironde region of France underwent co-inoculation trials with Inoflore that ensured rapid MLF. The analysis of the trial results led to an interesting discovery: the population of the non-*Saccharomyces* yeast (later revealed as *Brettanomyces*) was significantly lower in the samples of co-inoculated wine, as shown in figure 3. During this trial, it was observed that the co-inoculation prevented *Brettanomyces* development and, consequently, volatile phenol production.

During a co-inoculation trial on a Cabernet Franc must (in the Languedoc-Roussillon region of France), compared to a spontaneous MLF wine, co-inoculation once again resulted in lower ethylphenol levels. The level of 4-ethylphenol is eight times higher in the control wine compared to the co-inoculated wine, and 4-ethylguaiacol is four times greater (figure 4).

To prevent the development of *Brettanomyces* and the problems associated with this micro-organism, the winemaker can use not only co-inoculation, but early or sequential inoculation with wine bacteria right after AF. In a study by Gerbaux et al. (2009) in a Burgundy Pinot Noir, it was shown in laboratory and cellar trials that early inoculation with wine bacteria, right after AF, was useful in controlling the proliferation of *Brettanomyces*. The pH and temperature can negatively impact the onset and progress of MLF, and increase the risk of producing volatile phenols. Launching MLF by inoculating with selected wine bacteria, instead of relying on the spontaneous onset of MLF, avoids exposing the wine unnecessarily to the risks of developing *Brettanomyces*, which are particularly high during the period preceding MLF. The results presented in table 2 show that MLF began much sooner in the wines inoculated with two different wine bacteria, which contributed to a shorter duration for the process and significantly reduced the concentrations of volatile phenols. The data from inoculation trials, done at two different cellar temperatures, were compared to the data from the control wine, which underwent spontaneous MLF. In all probability, the greater the risk of *Brettanomyces* growth, the earlier the wine should be inoculated with malolactic bacteria.

	CELLAR REGULATED AT 18 -19 °C			CELLAR REGULATED AT 14 -15 °C		
	Control*	Bacteria 1	Bacteria 2	Control*	Bacteria 1	Bacteria 2
Time required for MLF (days)	58	16	13	124	31	27
Volatile phenol level (µg/L)						
4-ethylguaiacol	404	8	7	551	20	15
4-ethylphenol	870	17	9	1119	46	32
Average sensory score (on a scale of 1 to 10)						
Visual quality	5.6	6.0	6.0	6.0	5.1	5.1
Aroma quality	3.8	5.1	4.7	3.4	4.8	5.0
Taste quality	3.8	4.9	4.3	3.5	4.9	4.5
Overall quality	3.4	4.7	4.3	3.5	4.9	4.5
Intensity of animal defect	3.8	0.7	0.9	4.4	0.4	1.0

* Not inoculated with lactic acid bacteria

Table 2. Volatile phenol production in Pinot Noir wines (Burgundy, France) after malolactic fermentation induced by inoculation with wine bacteria versus a control wine which underwent spontaneous fermentation

TO SUMMARIZE...

Winemakers now have more information on the best way to prevent – and even treat – *Brettanomyces* contamination in wines. Inoculation with selected wine bacteria to induce and accelerate malolactic fermentation has been shown to be an effective means to prevent contamination. We know that inoculating the wine with a dose of $>10^6$ cells/mL of selected wine bacteria will stop the growth of this spoilage yeast. Managing the winemaking process through secure alcoholic fermentation and malolactic fermentation is a good starting point to prevent the development of undesirable indigenous flora. It is very important to carefully choose the selected bacteria based on its capacity to inhibit the production of free hydroxycinnamic acids, such as *p*-coumaric acid, precursor to volatile ethylphenols by *Brettanomyces*. Lallemand wine bacteria, including **Lalvin VP41, PN4, Beta, Alpha, Lalvin 31, which are all in the species *O. oeni*, as well as our *Lactobacilus plantarum* V22** do not have the cinnamyl esterase enzyme that leads to the transformation of this precursor in the free form, making it available to *Brettanomyces*. We call these Lallemand wine bacteria **phenol negative**. Moreover, appropriate inoculation strategies (co-inoculation, and early or sequential inoculation right after AF) have been shown to be effective tools to prevent the development of *Brettanomyces*. By choosing a phenol-negative wine bacteria and by carefully selecting the timing of inoculation with this bacteria, the winemaker can adopt an even more effective strategy to protect against the production of volatile phenols by *Brettanomyces*.

For more information on this topic, contact your Lallemand representative.

References available upon request.