

NEWS FLASH

❖ The XXII^{es} *Entretiens Scientifiques Lallemand* technical meetings were a two-part event focused first on hot-climate red varieties and understanding their sensory development, and, second, on rosé winemaking and the impact of different techniques on the wine style, including a presentation on the rosé wine market. This year, the top scientists in the field presented on these topics to an international crowd, including winemakers from Eastern Europe. The event was also an opportunity to hand out the Lallemand awards. The student award, the *Prix Michel-Feuillat - Entretiens Scientifiques Lallemand*, was presented to Dr. Guillaume Antalick, Université de Bordeaux II, for his work on "Biochemical and sensory changes associated with fruity notes in red wines during malolactic fermentation. The importance of esters." The Lallemand - Institute of Masters of Wine bursary was awarded to Sharon Wild, a second-year Master of Wine student from Australia for her essay responding to "Discuss the evolution of rosé wine styles and consumer preferences globally over the past five years." Also on hand were the winners of the ML Wines competition (Madrid 2011), who received their prizes from the president of Lallemand, Mr. Jean Chagnon. The proceedings of the 2011 *Entretiens Scientifiques* are now available.

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Sculpting the Aromatic Profile of Wines through Diacetyl Management

In addition to carrying out the bio-deacidification of wine, malolactic (ML) bacteria influence aroma and flavour through various mechanisms, including the production of volatile grape- and yeast-derived metabolites. In wine, one of those volatile compounds – diacetyl – has important stylistic implications. This diketone, also known as 2,3-butanedione, is associated with the "buttery" character of wine and is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid to 2,3-butanediol (figure 1). The formation and degradation of diacetyl is closely linked to the growth of such ML bacteria as *Oenococcus oeni* and the metabolism of sugar, malic acid and citric acid. Yeasts are also able to synthesize diacetyl during alcoholic fermentation (AF). However, most of this diacetyl is further metabolized to acetoin and 2,3-butanediol. This issue of *Winemaking Update* will review winemaking practices and the latest findings to help modulate diacetyl content in wine through malolactic fermentation (MLF).

gly depend on the style and type of wine, and varies from 0.2 mg/L for Chardonnay wine, 0.9 mg/L for Pinot Noir, to 2.8 mg/L for Cabernet Sauvignon wine (Rankine et al. 1969 and Martineau et al. 1995). When present at a high concentration (exceeding 5-7 mg/L), diacetyl is regarded by consumers to be undesirable in the wine, whereas at around 1-4 mg/L, and depending on the style and type of wine, it is considered to contribute a desirable "buttery" or "butterscotch" flavour character (Rankine et al. 1969 and Davis et al. 1985).

1. Do I smell butter?

Diacetyl at low concentrations – and in combinations with other wine aroma compounds – will impart yeasty, nutty and toasty aromas (Peynaud 1947 and Etievant 1991). At high concentrations, diacetyl has a characteristic buttery aroma associated with a lactic character. The sensory threshold of diacetyl in wine has been shown to stron-

2. Shaping the diacetyl content in wine

Several winemaking procedures can influence the diacetyl content in wine and, therefore, influence the wine style desired. Bartowsky and Henschke (2004) have presented the winemaking factors that affect diacetyl content.

2.1 The inoculation ratio of bacteria affects the timing of induction and completion of MLF. It has been observed that a lower inoculation ratio, such as 10⁴-10⁵ cfu/mL, can result in higher diacetyl accumulation in wine, up to an eight-fold increase.

2.2 The conversion of α-acetolactate to diacetyl is a non-enzymatic decarboxylation that is enhanced by the presence of oxygen. A trial done by Nielson and Richelieu (1999) showed that the amount of diacetyl that accumulated in the wine varied greatly,

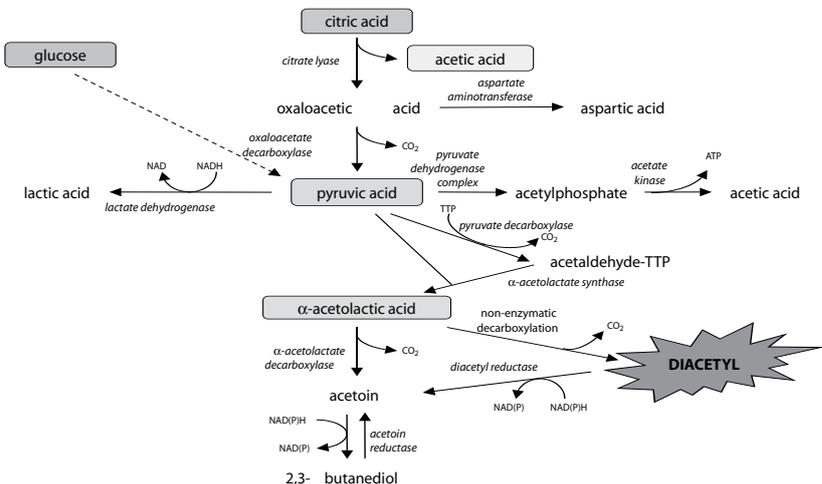


Figure 1. Pathway for citric acid metabolism by *Oenococcus oeni*. Eveline Bartowsky, adapted from Ramos et al. 1995.

with the formation of 2 mg/L under anaerobic conditions and 12 mg/L under semi-aerobic conditions.

2.3 Most strains of *O. oeni* are able to metabolize citric acid during MLF. The metabolism of citric acid is highly strain-dependant relative to that of malic acid, and consequently the depletion of citric acid in the wine may not occur until after malic acid depletion. Higher peak concentrations of diacetyl generally correlate with an elevated concentration of citric acid.

2.4 When MLF is conducted at lower temperatures, such as 18°C rather than 25°C, it tends to be slower, but the wines accumulate a higher concentration of diacetyl.

2.5 SO₂ is able to interact with diacetyl in a reversible manner. In the presence of SO₂, diacetyl is reduced and the concentration of free diacetyl in the wine lowered. However, as the SO₂ content decreases, as for example during aging, the ratio of free diacetyl will increase again, thus increasing its sensory impact.

3. Choosing the right malolactic bacteria for sequential inoculation

When MLF is desired, the ML bacteria appear to be an important control point to influence the final diacetyl concentration. The formation and degradation of diacetyl are closely linked to the growth of ML bacteria and the metabolism of sugar, malic acid and citric acid. Certain ML bacterial strains (table 1) have been observed to produce a higher residual concentration of diacetyl in wines than other strains, principally when sequential inoculation is used for MLF. In a study done at the AWRI by Bartowsky (2010) on Cabernet Sauvignon wines in Southern Australia, the results show that some ML bacteria will produce significantly different concentrations of diacetyl during sequential inoculation (figure 2). It was also found that the later *O. oeni* use citric acid during MLF, the less diacetyl they produce (table 1).

4. How important is the ability to use citric acid?

Some ML bacteria strains are called “citrate negative” and were thought to remove any possibility of diacetyl production, as diacetyl is a by-product of citric acid degradation via pyruvic acid and α-acetolactic acid (figure 1). However, this is not the only mechanism involved in the production of diacetyl, as pyruvic acid is an intermediate metabolite, which can also derive from the glucose

metabolism in the must. In a study done on Chardonnay from Baden in Germany, we compared diacetyl production from different selected ML bacteria. We know that the concentration is strain dependant in sequential inoculation (see section 3), but it does not appear that the citrate-negative characteristic significantly lowered the level of diacetyl. Both citrate-negative strains behaved similarly to strains VP41 and PN4, which are citrate positive and considered low- and medium-producers of diacetyl (figures available upon request).

5. Co-inoculation to reduce diacetyl content

Co-inoculation of the wine with selected yeast and ML bacteria also has important stylistic implications in terms of diacetyl production. Whether or not to use co-inoculation for MLF is probably the most important decision during this step of winemaking.

Co-inoculation with yeast and ML bacteria (inoculation with the ML bacteria within 24 hours after yeast inoculation), allows an acclimatization of the ML bacteria during AF and an early start of malic acid degradation towards the end of or immediately after AF, when yeast cells are still alive. Under these reductive conditions generated by active yeast cells, which consume part of the oxygen available, diacetyl is immediately reduced to acetoin then to 2,3-butandiol, which has little sensory impact.

In co-inoculation situations, we also have an earlier stabilization of the wines. Less citric acid is then consumed and, consequently, diacetyl is also lower. Our studies show that co-inoculation often results in more fruit-driven wine styles as opposed to the lactic,

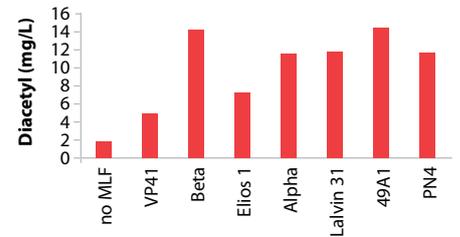


Figure 2. Diacetyl concentration of Cabernet Sauvignon wines from Adelaide Hills, Australia, that have undergone sequential malolactic fermentation with different selected malolactic bacteria.

buttery or nutty styles that result when MLF begins upon completion of AF. For example, in figure 3, the Beta starter produces less diacetyl in co-inoculation (48 hours) than in delayed inoculation (2/3 of AF) or sequential inoculation (post-AF). The impact of the ML bacteria strain is not as strong in co-inoculation, as the wines will show repeatedly low levels of diacetyl with this technique with different ML bacteria.

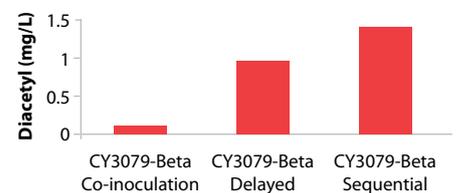


Figure 3. Diacetyl production in Chardonnay 2010 (Val de Loire) with different timing of inoculation for malolactic fermentation

6. Some guidelines

The recommendations in table 2 summarize the actions that can be undertaken to shape the diacetyl content of wines.

MT01	VP41	Elios 1	Alpha	Lalvin31	PN4	Beta
Standard build-up culture	Very late attack of citric acid - Only attacks citric acid after completion of malic acid	Late attack of citric acid, at the end of MLF	Medium attack of citric acid during MLF	Medium attack of citric acid during MLF	Early attack of citric acid (mid MLF)	Very early attack of citric acid (beginning/mid-MLF)
No production of diacetyl	Very low production of diacetyl	Low production of diacetyl	Medium production of diacetyl	Medium production of diacetyl	Medium production of diacetyl	High production of diacetyl in sequential inoculation

Table 1. Impact of different malolactic bacteria on the production of diacetyl

<p>Buttery aroma</p> <p>Sequential inoculation with Beta, PN4</p> <p>Eliminate as much as possible yeast lees</p> <p>Lower temperature during MLF</p> <p>Quick stabilization with SO₂ at end of MLF</p>	<p>Fruit driven-style</p> <p>Co-inoculation with Beta, Alpha, VP41, new selection (upcoming)</p> <p>Sequential with Lalvin 31, VP41, new selection (upcoming)</p> <p>Temperature during AF/MLF 18-20°C</p> <p>Yeast lees contact</p> <p>Delayed SO₂ addition (minimum 1 week)</p>
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Table 2. Shaping the diacetyl content of wine

TO SUMMARIZE...

Many factors influence the diacetyl content of wine and, therefore, the buttery character in wine. To properly modulate the contribution of this character, several winemaking procedures can be worked on to achieve the desired effect, from a major impact to a slight one. Two of the most important factors are the choice of ML bacteria used to achieve MLF, and the timing of inoculation with those bacteria. Our program of ML bacteria characterization for diacetyl allows us to better understand ML bacteria and recommend those required to influence the production of the desired compounds. In sequential inoculation, some strains of ML bacteria, such as the VP41 and Lalvin 31, are recognized as low producers, whereas PN4 and Beta are higher producers. The timing of inoculation with bacteria starters is very important, as co-inoculation will always yield lower diacetyl levels in wines, so low in fact that we can propose very low diacetyl solutions through co-inoculation. To choose the right ML bacteria for your wine, as well as the right procedure, please consult your Lallemand representative.

References available upon request.