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LALLEMAND

WINEMAKING UPDATE

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FLASH NEWS

- The "YSEO™ yeast" has arrived! Resulting from years of research, naturally strengthened selected yeast strains are now available. A new generation of *Saccharomyces cerevisiae*, they are the same yeast strains, but a unique growth process increases their capacity to adapt to such difficult fermentation conditions as high alcohol, low temperatures and nutrient deficiencies. Called YSEO™ yeast, they ensure more stable and reliable alcoholic fermentation. YSEO™ yeast reduces the risk of sensory deviations while allowing the full expression of varietal aromas.
- In other news, the *XVIIes Entretiens Scientifiques Lallemand* technical meeting in 2005 was held at La Guardia (La Rioja), Spain. Over 180 participants came to listen to the scientists presenting their latest research results regarding the sensory contributions of yeast and the impact of selected yeast on biodiversity. A round table discussion followed, with winemakers from 10 countries expressing their views on yeast inoculation. The proceedings of this scientific gathering will soon be available from your Lallemand representative.

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WINEMAKING UPDATE

WINEMAKING UPDATE is a Lallemand Inc. publication. Its goal is to inform oenologists and winemaking staff about news and indications arising from research. To request previous issues, send your questions or comments contact us at:

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MALOLACTIC FERMENTATION: TIMING OF INOCULATION – NEW FINDINGS

Malolactic fermentation (MLF) occurs in wine as a result of the metabolic activity of certain strains of lactic acid bacteria. Wine acidity is reduced and flavour is modified due to this secondary bacterial fermentation, which is generally considered to benefit wine quality. The advantages of inducing MLF by inoculation with selected strains of lactic acid (malolactic) bacteria are twofold. First, the winemaker has greater control over the timing and duration of malic acid conversion and, secondly, MLF has a positive influence on wine flavour and quality. Sensory studies show that the flavour compounds produced by lactic acid bacteria impart recognizable changes to the flavour characteristics of the wine. The influence of the timing of inoculation can be an important factor in determining the sensory profile of the wine. This issue of *Winemaking Update* will present the latest findings on the timing of the inoculation of wine with malolactic bacteria.

1. Simultaneous inoculation with yeast and bacteria

Inoculating the must with malolactic bacteria (MLB) is usually recommended after completing alcoholic fermentation, to avoid the possible production of acetic acid and D-lactic acid, a situation referred to as "piqure lactique." If MLF occurs during alcoholic fermentation, it can occasionally cause a stuck alcoholic fermentation. In other cases, inoculation with MLB at the same time as the yeast has been advocated, because it was felt the bacteria would have a better chance of growing and acclimatizing in a low-ethanol environment. The MLB do not suffer from a shortage of nutrients nor will they be exposed to the toxic effects of alcohol.

1.1 What is the risk of producing volatile acidity?

The risk of producing acetic acid can be associated with a strong growth of bacteria that inhibits yeast growth. But, as MLB consume organic acids first (in order: malic acid, citric acid, fumaric acid, plus other acids) before using the sugars (in small quantities only), the risk of producing excessive acetic acid (volatile acidity) is greatly reduced. A direct relation has also been observed between the degradation of citric acid and a slight increase in acetic acid, as all MLB degrade citric acid into acetic acid and diacetyl. However, it must be noted that citric acid is present only in very small quantities.

Experiments conducted by the Lallemand research group have confirmed that low acetic acid is produced during growth of MLB and active MLF in certain situations. Trials conducted using simultaneous bacterial and yeast inoculation versus bacterial inoculation upon completion of alcohol fermentation have shown no significant difference in the final acetic acid concentration. In addition, the co-inoculation of wine with yeast and bacteria will result in fewer lactic and buttery flavours, resulting in a fruit-driven wine style.

1.2 Examples of simultaneous alcoholic–malolactic fermentation

Lallemand, in collaboration with Massey University in New Zealand, experimented with making wine using one yeast strain and two malolactic bacteria strains. For each yeast/bacterium combination, MLB were inoculated, either together with the yeast (simultaneous AF/MLF) or upon completion of the alcoholic fermentation (sequential AF/MLF). The wine was made from Chardonnay grapes from a commercial vineyard in the Hawke's Bay region. The results presented in Table 1 show that sequential AF/MLF always resulted in prolonged malolactic fermentation when compared to simultaneous AF/MLF. There was no significant difference in the production of acetic acid.

Sensory evaluation of the wines produced in this experiment showed more fruity wines with co-inoculation than with simultaneous inoculation.

Continued

Table 1. Time to complete malolactic fermentation and production of volatile acidity vs. inoculation sequence

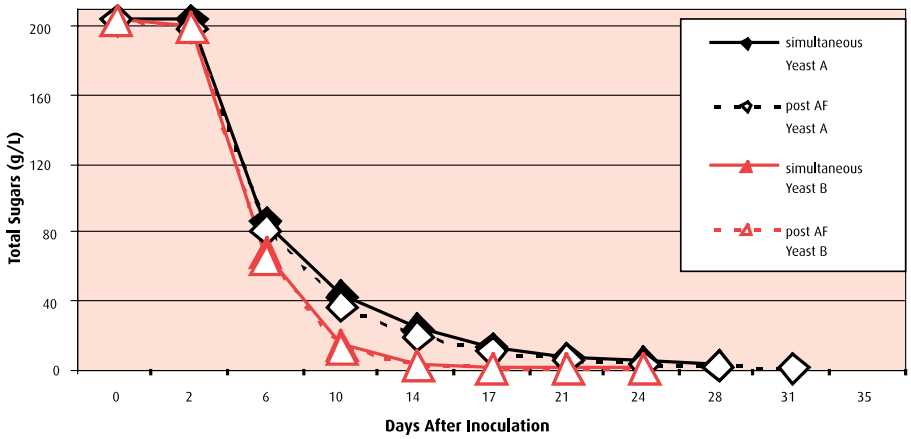
	Time to complete malolactic fermentation		Acetic acid production (g/L)	
	MLB strain A	MLB strain B	MLB strain A	MLB strain B
Simultaneous AF/MLF	26 days	19.5 days	0.195	0.187
Sequential AF/MLF	74 days (some malic acid remained)	68 days	0.147	0.168

In collaboration with the German research stations at Neustadt and Trier, Lallemand investigated the simultaneous inoculation of yeast and bacteria into Riesling wine. The results presented in Figure 1 show that simultaneous inoculation with yeast and bacteria had no influence on yeast fermentation, and no acetic acid was formed in excess (result not shown).

1.3 Inoculation with malolactic bacteria starter cultures during alcoholic fermentation

It is possible to inoculate with malolactic bacteria during the course of alcoholic fermentation, especially in situations of high pH and high alcohol potential, because at this stage most free SO_2 is bound by carbonyl compounds produced during yeast growth, and the alcohol concentration has not yet reached toxic levels. However, the most intense levels of yeast-induced antagonism by such metabolites as decanoic acid may be encountered at this stage. Rosi et al. reported a reduction of bacterial viability when inoculation was performed at the midpoint of alcoholic fermentation. They attributed this decrease to such factors as nutrient depletion, production of ethanol and a drop in pH caused by acid production. Their results further indicate that inoculation at this point during fermentation will subject the MLB to strong yeast antagonism that may be insurmountable. Research at Lallemand confirmed these findings and reinforced the fact that inoculation at the midpoint of alcoholic fermentation will always result in a marked reduction of bacterial viability and activity. Therefore careful management of the MLF is essential with this method.

Figure 1. Simultaneous addition of yeast and bacteria vs. alcoholic fermentation



Early inoculation with the malolactic starter culture resulted in a much faster MLF, and malic acid was degraded within 23 days as compared to more than 50 days (data not shown) with inoculation after AF. The timing of inoculation had an important impact on the sensory profile of the wine. In the case of simultaneous inoculation, the rapid onset of MLF allowed for malic acid degradation under the reductive conditions generated by the still active yeast cells. This reductive environment prevented the formation of buttery or lactic aromas, and the wine retained the flavours and aromas typical of Riesling fruit. Wines inoculated after alcoholic fermentation showed the buttery and nutty flavours typical of malolactic fermentation, and the flavours and aromas of the Riesling fruit were absent. The technique of inducing AF and MLF simultaneously **in low pH white wine musts** has proven very satisfactory.

2. Inoculation with malolactic bacteria starter cultures after alcoholic fermentation

Inoculation at the end of alcoholic fermentation does not pose the risk of the heterofermentative bacterial decomposition of sugars and the resultant increase in volatile acidity. The merit of inoculation at the end of alcoholic fermentation can also be related to the availability of bacterial nutrients that have arisen from the death of yeast and subsequent autolysis. However, exposure to the high levels of ethanol present at the time of late inoculation may result in delayed MLF, especially in wines produced in hot climates. For wines with high alcohol potential, it is important to use a starter culture with higher resistance to this condition.

2.1 Delayed induction of MLF

In the Burgundy region of France or in other wine regions that produce mainly Pinot Noir wines, the rapid development of MLF is contrary to their time-honoured winemaking techniques, which have traditionally relied upon spontaneous MLF in the spring. The increasing use of active, direct inoculation MLF starter cultures has led to earlier MLF, but has also resulted in a significant reduction in pigmentation. To avoid this problem, the work of Gerbaux et al. has shown the following conditions must be met:

1. Increase the time between "décuvage" (the elimination of the gross fermentation lees) and the start of MLF.
2. Reduce the speed of MLF by using lysozyme at beginning of AF and/or right after AF, cooling the wine below 12°C, and/or with a moderate addition of SO_2.
3. Delay the addition of SO_2 until after completion of MLF.

Generally speaking, all winemaking techniques that inhibit or delay MLF – higher SO_2 levels, temperatures below 12°C or the addition of lysozyme at any stage of wine production – will help stabilize colour loss in lightly pigmented wines, such as Pinot Noir or Sangiovese.

LITERATURE

Rosi, I., G. Fia, and V. Canuti. 2003. Influence of different pH values and inoculation times on the growth and malolactic activity of a strain of *Oenococcus oeni*. *Austr. J. of Grape and Wine Research*. 9:194-199.

Gerbaux, V., and C. Briffox. 2003. L'Influence de l'ensemencement en bactéries lactiques sur l'évolution de la couleur des vins de Pinot noir pendant l'élevage. *Revue des œnologues*. 103:19-23.

TO SUMMARIZE ...

There is no universal approach for inducing MLF by inoculation. The most desirable time for inoculation depends upon a myriad of vinification factors, the most important of which are juice composition, the yeast strain used to produce the wine and the winemaking techniques, as well as the style of wine desired. Some key parameters have to be studied, including juice composition (sugar level and pH), fermentation conditions (yeast strains, temperatures and SO_2 levels) and wine style (fruity, vin de garde or maintenance of colour). Please consult your Lallemand representative for assistance in making the best decision.