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WINEMAKING UPDATE

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FLASH NEWS

❖ ***Ochratoxin Removal by Yeast Strains*** Ochratoxin A (OTA), a toxin produced by some fungi, is now being carefully regulated in the European Union and in other countries to limit its concentration in such food products as cereal and coffee. OTA has also been found in wine and grape juice, and appears to be more predominant in Mediterranean-type wines. It was found that different oenological yeasts can reduce OTA at different levels via an adsorption mechanism (Bejaoui *et al.*, 2004). Higher yeast biomass concentration was also found to reduce more OTA. Another study carried out by the ICV in collaboration with Lallemand has shown that OTA reduction was also related to the yeast strains. For example, ICV D80 *Saccharomyces cerevisiae* had the highest OTA reduction of all six yeast strains tested. More studies are underway to verify if OTA can be potentially released back into the wine during aging. The malolactic bacteria *Oenococcus oeni* can also play a role in degrading OTA. The Lalvin 31 strain was shown to do this very successfully.

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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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SPECIAL EDITION OF WINEMAKING UPDATE

Yeast Inoculation Strategies – Getting The Best Fermentation Possible!

Good alcoholic fermentation management is the key to avoiding stuck or sluggish fermentations while making quality wine. Selecting the most appropriate yeast strains based on the must conditions and the type of wine style desired is an important winemaking decision. The proper strain, carefully rehydrated and at the correct inoculation dose for fermenting the must, should be foremost in the winemaker's decision to help avoid problems with stuck or sluggish fermentation and aromatic deviations

This special edition of Winemaking Update will describe the basics behind determining the inoculation rate to minimize such problems, will define the conditions that will get you the most out of fermentation under high potential alcohol conditions, will explain the use of sequential inoculation and dynamic synergy, and will provide a brief description of nutrient strategies to obtain a smooth and complete fermentation.

1. Why use a minimum of 25 g/hL?

During wine fermentation, yeast cells will usually undergo at least five cell divisions (doubling the yeast population with every cell division). One of the most important factors in obtaining a steady and complete fermentation is the presence of an adequate cell population when the yeast has finished growing, which is normally when 30% to 50% of the sugars remain to be fermented. To complete fermentation and minimize the risk of fermentation problems, at this stage you need a minimum yeast cell population of approximately 120-150 x 10^6 cells/mL. At inoculation densities below 5 x10^6 cell/mL, the likelihood of achieving a successful fermentation decreases significantly. In part, this is due to the demand placed on the initial yeast cell population to divide for more than five or six generations.

The suggested 25 g/hL rate of yeast inoculation is based on the fact that a proper initial cell density is required for the onset and the completion of fermentation. Before the onset of fermentation, the winemaker should keep the lag phase as short as possible to avoid microbial spoilage and the production of undesirable compounds. The length of the lag phase is significantly influenced by the initial yeast cell density or inoculation rate, as well as factors like juice clarity, sugar and SO_2 concentrations, which will also impact on the relation between lag phase duration and inoculation rate. At 25 g/hL (or 2 pounds per

Continued

FLASH NEWS

❖ ***H_2S Production by Wine Yeasts*** In a study done in collaboration with Dr. Seung Park at the University of California – Davis, the relative hydrogen sulphide (H_2S) production of different yeast strains was studied under different levels of nitrogen in the must. The study found that under low nitrogen conditions (60 ppm), yeast strains had a tendency to produce more H_2S compared to nitrogen levels of 170 ppm. The yeast strains could be classified as low, medium or high producers. This information will help determine the proper yeast and yeast nutrition under specific must conditions.

1,000 gallons), the inoculation will provide approximately 5×10^6 cells/mL with the majority of selected and dried natural yeast preparations. Increasing the inoculum density will result in decreased lag time, but is ineffective beyond a certain point due to the crowding effect. With that amount of cells at the beginning, you significantly increase your chance to obtain the required $120\text{-}150 \times 10^6$ cells/mL to successfully complete fermentation. Note that some yeast strains will require higher inoculation rates (30-50 g/hL) to complete fermentation because their metabolism is very different.

The following results (Figure 1) illustrate the importance of an inoculum of 25 g/hL versus 10 g/hL. A study done in

FIGURE 1. **Chardonnay (215 g/L sugars, pH 3.17, T.A. 6.5 g/L) fermentation at 16°C comparing inoculation rates of 10 g/hL and 25 g/hL of EC-1118 yeast strain with Fermaid added at mid-fermentation (30 g/hL).**

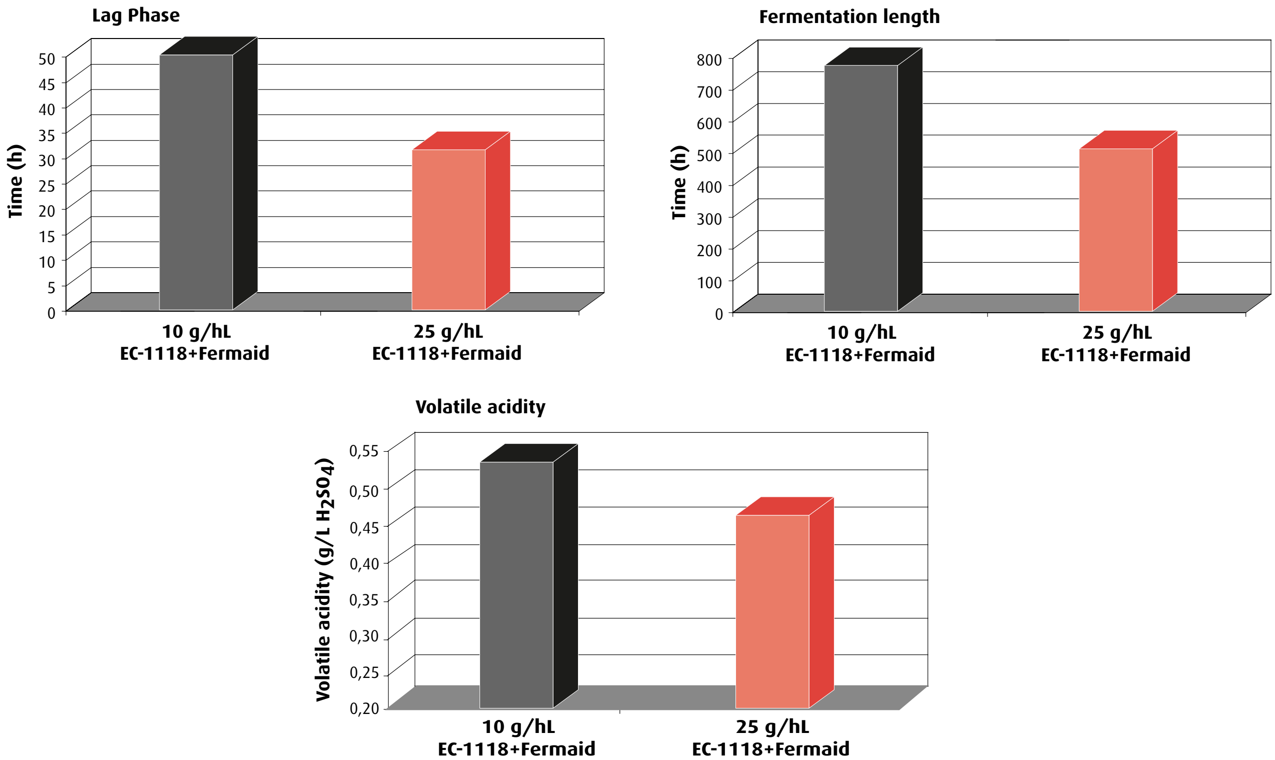
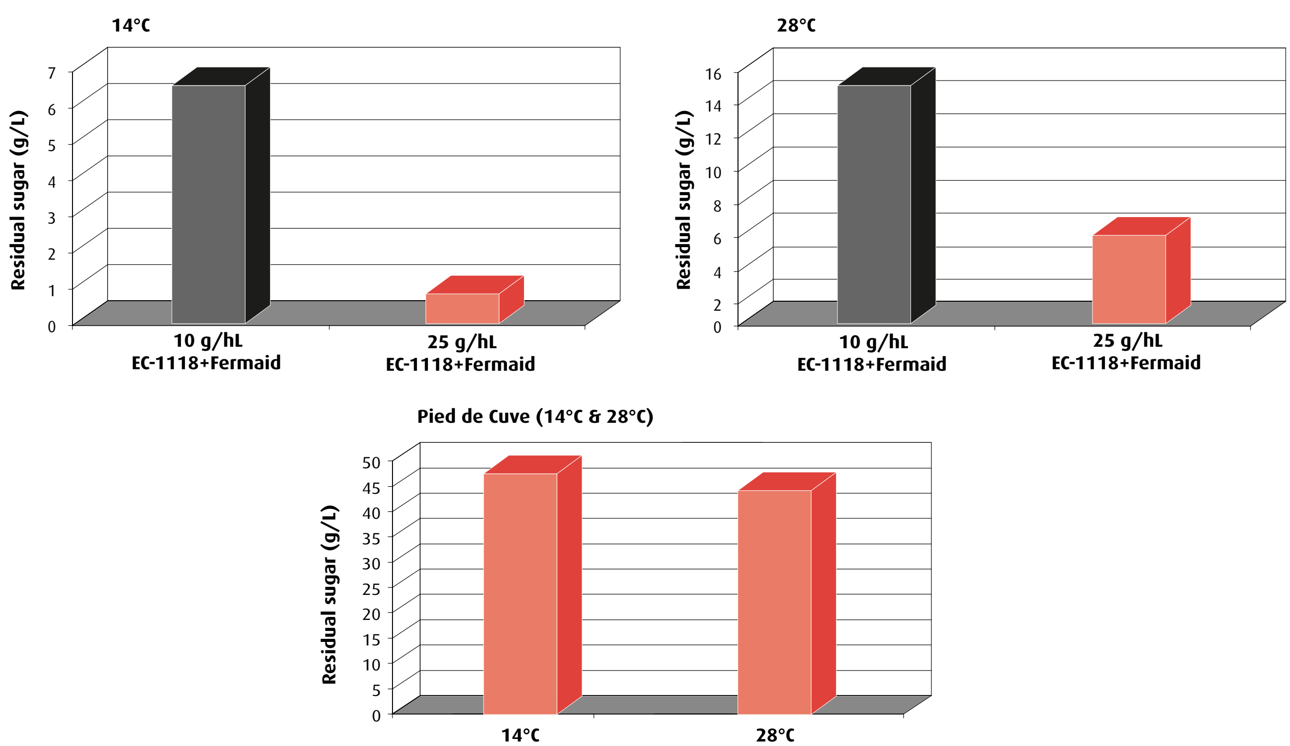


FIGURE 2. **Residual sugar of a Bourboulenc-Grenache (14°C and 28°C, 15.4% vol., <u>258 g/L sugar</u>, 220 NTU, 70 mg/L assimilable N) fermentation with 10 and 25 g/hL of EC-1118 yeast and with the *pied de cuve* technique.**



France by INRA Pech Rouge in collaboration with Lallemand (Ortiz-Julien, 2003) clearly showed the benefits of inoculating a "normal" Chardonnay must with an alcohol potential of 12.7% with 25 g/hL of the EC-1118 yeast strain. This resulted in a significant reduction of lag phase time, fermentation length and volatile acidity.

In another study, under more difficult conditions, the must of Bourboulenc-Grenache (15.4% vol., **258 g/L sugar**, 220 NTU, 70 mg/L assimilable N), was fermented with 10 g and 25 g/hL of the EC-1118 yeast strain and compared to the use of the *pied de cuve* technique at both 14°C and 28°C. The levels of residual sugars were shown to be significantly higher with the *pied de cuve* inoculation (up to 48 g/L) compared to the inoculation rate of 25 g/hL as shown in Figures 2a and 2b. Therefore, working with 25 g/hL of inoculum is a good strategy to avoid sluggish or stuck fermentation, even in difficult situations (i.e., low nitrogen, high sugar).

Although recent results also suggest that a high inoculation rate may allow a reduction in the amount of SO_2 used in fermentation, it is too early to give an accurate estimation of the SO_2 dosage as a function of inoculation rate.

2. What to do in high potential alcohol (high sugar) situations?

2.1 High sugar musts require more yeast inoculum

High sugar musts are increasingly common, especially for New World winemakers and in hot climates, and are often coupled with nitrogen deficiencies. In such situations, fermentation management requires particular attention. Not only is proper nutrition during fermentation and rehydration essential for a steady and complete event, the appropriate inoculation rate should receive consideration. As we saw in Figure 2 A, the levels of residual sugars are lower when 25 g/hL were used in a must containing 258 g/L of sugar (approximately 25° Brix). The volatile acidity was also significantly lower, the lag phase duration was reduced and the fermentation rate was faster (results not shown) in the Bourboulenc-Grenache wine fermented with 25 g/hL of EC-1118 yeast.

In an even higher sugar must for icewine or sweet wines (*vins liquoreux*), higher inoculation rates are required. In the case of very high sugar content juices, the hyperosmotic stress placed on the yeast can reduce yeast biomass and ultimately generate a stuck or sluggish fermentation, as well as increase the production of volatile acidity. Therefore, when the Brix level is above 30° Brix, an inoculation rate of 50 g/hL is required. For example, in a study carried out on icewine (35 to 42° Brix) at the Cool Climate Oenology and Viticulture Institute at Brock University in Canada, it was shown that a higher inoculation rate (50 g/hL vs. 20 g/hL) along with the utilisation of Go-Ferm in the rehydration water significantly reduced the fermentation time as well as the rate of volatile acid produced as a function of sugar consumed (Kontkanen et al, 2004).

2.2 Other strategies: sequential inoculation and dynamic synergy

A novel approach to ensure a steady and complete fermentation in over-mature grapes (28° Brix) is based on a study of red wines conducted by Professor Edmundo Bordeu at the Universidad Católica de Chile in 2004 on the use of sequential inoculation. The principle is the following: the selected yeast strain is inoculated in the must at 30 g/hL in two stages, first at the beginning of fermentation and then when the brix has reached a certain point, but before symptoms of stuck or sluggish fermentation are apparent. At first, 15 g/hL of the selected yeast is rehydrated in water supplemented with Go-Ferm, then used for inoculation. When the alcohol level reaches about 4%, the other 15 g/hL of yeast (also rehydrated in water and supplemented with Go-Ferm) is used for inoculation. This method works well as it is always very difficult to restart a stuck fermentation. When reinoculated early, there was a clear advantage in terms of lower residual sugars and lower volatile acidity.

A dynamic synergy is also a good alternative. This new concept is based on the use of two or more selected yeast strains particularly well adapted to each other in that they complement sensory properties and fermentation kinetics in perfect synergy. After many months of research at Lallemand, our studies have shown that the proper combination of certain strains in the proper ratio can help carry out fermentation in difficult conditions, such as a high sugar situation. For example, a yeast strain that is particularly interesting in terms of sensory contribution is often desired by winemakers. However, this yeast strain is very sensitive to difficult winemaking conditions and may end up having difficulty finishing the fermentation. This yeast is thus associated with another yeast strain that is quite resistant to difficult winemaking conditions and reliable for completing the fermentation. The result is a wine that possesses the sensory characteristics that were wished for by using the first yeast strain, but without any worries regarding fermentation kinetics or completion, thanks to the second yeast strain. Such mixtures for use in a single inoculum are in development and will soon be available to winemakers.

3. Good fermentation practices – a quick review

The followings are other good fermentation practice considerations when dealing with high-Brix musts, and Table 1, on page 4, summarizes the different inoculation strategies.

In reds:

- Aeration or oxygen additions when the cap forms (usually when 15 g/L sugar is fermented,) as well as nutrient addition at one third sugar depletion.
- Temperature management during yeast rehydration, the initial phase of fermentation and at the peak of fermentation.
- Regular movement of the yeast near the end of fermentation and into the yeast cell decline phase.

In whites:

- Initial juice turbidity level optimum between 80 and 100 NTU.
- Aeration or oxygen addition as soon as the fermentation is active (usually when 15 g/L sugar is fermented), as well as nutrient addition at one third sugar depletion.
- Temperature management during yeast rehydration and yeast inoculation
- Regular movement of the yeast near the end of fermentation and into the yeast cell decline phase.

REFERENCES

Bejaoui, H., P. Mathieu, P. Taillandier and A. Lebrihi, 2004. OTA removal in

TABLE 1. Different inoculation strategies in different must conditions

Must conditions	Inoculation rate	Go-Ferm	Fermaid
"Normal" conditions (red, white, rosé) below 25°Brix	25 g/hL	30 g/hL	High N > 250 mg/L: No addition
		30 g/hL	N 150 < N < 250 mg/L: Add 30 g/hL at 1/3 through fermentation
		30 g/hL	Low N < 150 mg/L: Add 15 g/hL at inoculum and 15 g/hL at 1/3 through fermentation
Sweet wines/ Icewine 30-42° Brix	50 g/hL	60 g/hL	Regardless of nitrogen content, add 30 g/hL at inoculum and 20 g/hL at 1/3 through fermentation
High sugar must/ over mature grapes over 25° Brix	25-50 g/hL. (The higher the sugar content, the higher the inoculation rate)	30-60 g/hL	High N > 250 mg/L : 30 g/hL at 1/3 through fermentation
			N 150 < N < 250 mg/L: Add 50 g/hL at 1/3 through fermentation
			Low N < 150 mg/L: Add 30 g/hL at inoculum and 20 g/hL at 1/3 through fermention
	30 g/hL in sequential inoculation : 15g/hL at the beginning and 15 g/hL at approximately 4% alcohol	20 g/hL for each inoculation	Regardless of nitrogen content, add 20 g/hL at each inoculation

synthetic and natural grape juice by selected oenological *Saccharomyces* strains. *J App Microbiol.* 97:1038-1044.

Bordeu, E., 2005. Inoculation of yeast during alcoholic fermentation to face problems of stuck fermentations. Internal report.

Kontkanen, D., D.L. Inglis, G.J. Pickering and A. Reynolds, 2004. Effect of yeast inoculation rate, acclimatization and nutrient addition on icewine fermentation. *Am J Enol Vitic*. 55(4):363-370.

Monk, P., 1997. Optimum usage of active dry wine yeast. ASVO Seminar. *Advances in Juice Clarification and Yeast Inoculation*. 22-23

Ortiz-Julien, A., 2003. Inoculation rate study. INRA Pech-Rouge. Internal report.

Ortiz-Julien, A., 2002. Inoculation rate study. INRA Montpellier. Internal report.

Park, S., 2005. UC Davis. Internal report.

Querol, A., E. Barrio, T. Huerta and D. Ramón, 1992. Molecular monitoring of wine fermentations conducted by active dry yeast. *App Environ Microbiol.* 58 (9):2948-2953.

TO SUMMARIZE …

Every must requires particular attention, and as every vintage brings its own variability, reducing the risks of fermentation deviation is important. Keep in mind that in a very short time a naturally selected yeast in a dry form has to become an actively functioning unit in the wine environment, which can be hostile (high sugar, low pH, high SO_2), and one of the keys to good fermentation management is the proper inoculation rate.

The points to remember are the following:

1. A minimum of 25 g/hL is necessary to obtain the proper cell density for a smooth and complete fermentation.
2. The inoculation dosage has to be adapted to the must conditions and the wine type: higher sugar musts requires higher inoculation, and sweet-wine types require a much higher inoculation rate.
3. The nutritional status of the must, as well as the nitrogen/oxygen requirements of the yeast strains have to be considered. Rehydrating the dried yeast in warm water and Go-Ferm, as well as supplying the yeast with Fermaid during fermentation, are part of good fermentation management.
4. Other strategies can be investigated with over-mature grapes, such as sequential inoculation or the use of dynamic synergy products. Consult your Lallemand representative for the best conditions adapted to your particular situation.