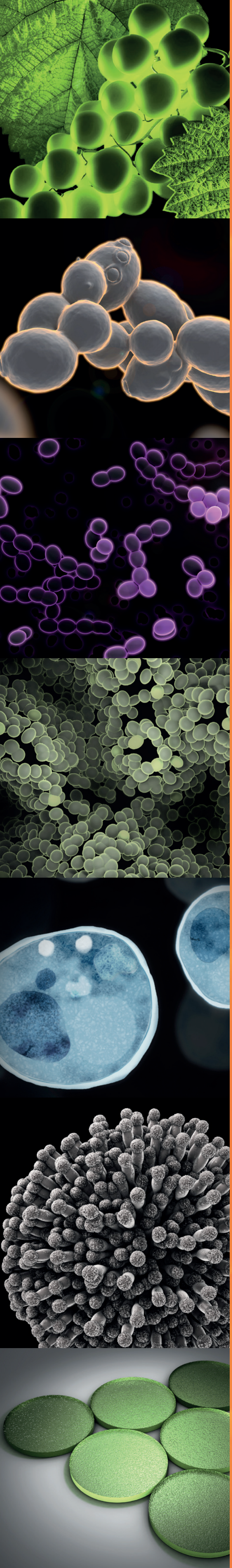

Alcoholic and malolactic fermentations: what impact on freshness?

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In the context of climate change, increased pH and alcohol content can result in heavier wines, while some consumers are moving towards a lighter, fresher style of wine. Beyond the notion of acidity, the sensory aspect must also be taken into account (fresh fruit aromas, vegetal notes, etc.). From veraison to bottling, each step can have an impact on the different layers of a wine's freshness. This article aims to present recent results and tools related to fermentation management and the search for freshness in winemaking.

Impact of selected natural *Saccharomyces cerevisiae* yeasts on acidity in wine

The acidity of a wine is one of its basic characteristics, both from an analytical and sensory point of view. In particular, this parameter affects the progress of the malolactic fermentation (MLF), the effectiveness of the sulfur dioxide added (more active at low pH) and the preservation of the wine. On tasting, there is no direct correlation between the pH and the perceived acidity, but a knowledge of the pH provides information about the sensory properties of the wine.

The change in pH during the alcoholic fermentation (AF) is not linear. After a slight fall at the start of the AF linked to assimilation of nitrogen by the yeast, the pH then rises due to a physicochemical phenomenon linked to the presence of ethanol (Akin, 2008).
The correlation between pH, acid concentration and freshness is complex. However, malic acid plays a central role in the acidity and its perception.

Malic acid is the second acid present in musts after tartaric acid. For some yeasts, such as *Schizosaccharomyces pombe* or *Hanseniaspora occidentalis*, malic acid transport is performed by specific membrane transporters. In contrast, with *Saccharomyces cerevisiae*, it is transported by simple diffusion. The more malic acid there is in the medium, the more this phenomenon will be important and the more of it the yeast will consume. Temperature, pH and the initial nitrogen content seem to play a minor role in the degradation of malic acid. There is, however, a large intrinsic variability amongst different *Saccharomyces cerevisiae* (figure 1). Indeed, even if transport is by simple diffusion, malic acid can take different metabolic pathways to lead to succinic acid, ketoglutaric acid, acetic acid or ethanol. The variability between yeasts rests on these different metabolic pathways.

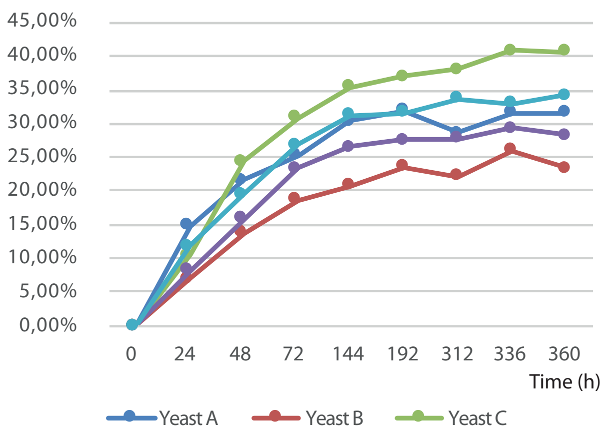

Fig. 1: Consumption of malic acid by different yeasts (synthetic must)**.** ***Source: R&D Lallemand***

The yeast Ionys$_{WF}$™, the result of a selection by progressive adaptation undertaken in a collaboration between Lallemand and INRA Montpellier, constitutes a special case for *Saccharomyces cerevisiae*. The specific nature of its tricarboxylic acid cycle leads to significant production of several organic acids, including succinic, α-ketoglutaric and malic acids (patent pending W02015/11411).

Control of acetaldehyde during fermentations and aromatic freshness

In addition to the acidity, aromas present in wines also have an impact on the perception of freshness in a wine. Controlling of the indigenous flora present during alcoholic and malolactic fermentations prevents the development of faults that would eventually mask the freshness sensation. In addition, the choice of yeast and selected wine bacteria can have an impact on the sensory profile of a wine and its freshness. Acetaldehydel, in particular, is a key compound in the perception of aromatic freshness. Produced by the yeast during the AF, there is a production/consumption equilibrium that varies for each *Saccharomyces cerevisiae*. The final acetaldehyde content at the end of the AF can therefore be ranked according to the yeast selected (figure 2). Its production increases with additions of sulfites but can also be influenced by low pH, nutritional deficiency or low temperature during the AF.

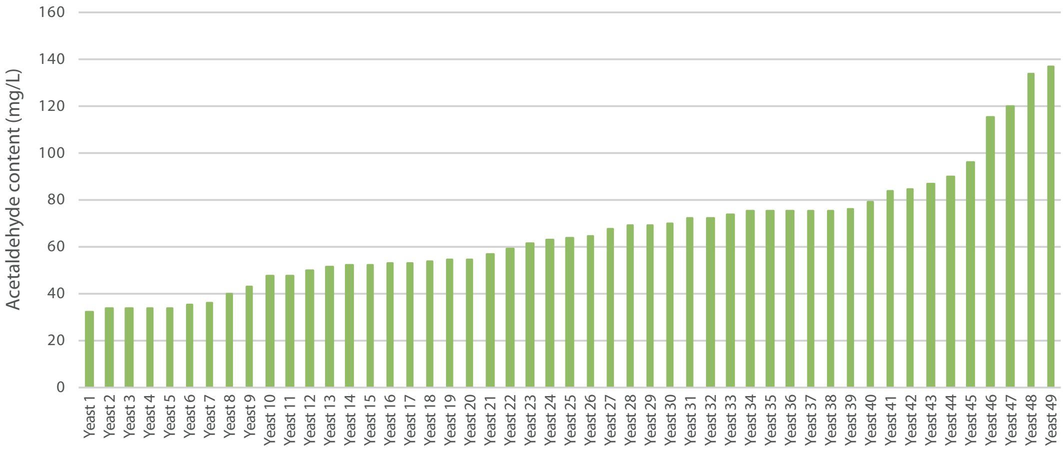

Fig. 2: Ranking of dierent yeasts as a function of the ethanal present at the end of AF (synthetic must)**. *Source: R&D Lallemand***

The ICV group, Lallemand, SupAgro and INRA Montpellier undertook a study to develop an innovative technique to select yeasts producing very low levels of SO_2, H_2S and acetaldehyde. The first part of this work consisted in identifying the metabolic pathways and, above all, the genetic basis for the production of SO_2, H_2S and ethanal by the yeast. It was thus possible to identify two regions of the genome (Quantitative Trait Loci) and transfer them by backcrossing to a target strain, chosen for its fermentation capacity and its oenological interest. This approach has allowed development of an innovative technique for selecting yeasts that produce almost no SO_2, H_22S or acetaldehyde (patent pending PTC/IB220131050623). By having a wine yeast producing lower to no acetaldehyde can therefore help with the perception of freshness in wine.

Wine bacteria also influence the acetaldehyde content, as they reduce this compound during the malolactic fermentation (MLF). Since a large part of the added SO_2 combines with the acetaldehyde present in the medium, it is possible to reduce the quantity of bound SO_2 by co-inoculating the wines or by waiting about one week after the end of the MLF before stabilizing the wines (Table 1).

Chardonnay		pH 3.2	pH 3.35	pH 3.5	pH 3.65
Final acetaldehyde content (mg/L)	**After AF**	**29.6 ± 0**	**30.4 ± 0.5***	**16.0 ± 4**	**12.6 ± 0**
	Co-inoculation	**19.0 ± 1**	**12.5 ± 0.1**	**15.4 ± 0.1**	**7.3 ± 0.4**
Final bound SO_2 content (mg/L)	**After AF**	**71.5 ± 15**	**84.5 ± 11**	**64.5 ± 4**	**64 ± 2**
	Co-inoculation	**59.5 ± 7**	**57 ± 7**	**59 ± 4**	**45 ± 6**

Source: Cornell University

Malolactic fermentation and its impact on freshness

In contrast to *Saccharomyces cerevisiae* yeasts, *Oenococcus oeni* wine bacteria have a gene encoding a malate permease (Tourdot-Maréchal *et al.*, 1993). Malic acid thus enters the cell through a specific membrane permease, but also by passive diffusion that can correspond to 50% of the malic acid at a pH of 3.2. The malic acid is then converted to lactic acid and excreted into the medium, resulting in an increase in pH (of between 0.1 and 0.3) and a decrease in the total acidity.

Lactobacillus plantarum ML Prime® is a special case. Used in co-inoculation, this microorganism very quickly consumes the malic acid (in 3 to 7 days), without production of acetic acid due to its homo-fermentative metabolism of hexoses. Because of the speed of the MLF, all the malic acid present in the must is converted to lactic acid, before the yeasts performing the alcoholic fermentation have a chance to consume it. The final lactic acid content is therefore greater in wines co-inoculated with *Lactobacillus plantarum* ML Prime® than in a wine co-inoculated with a strain of *Oenococcus oeni*. This corresponds to a positive impact on freshness (figure 3).

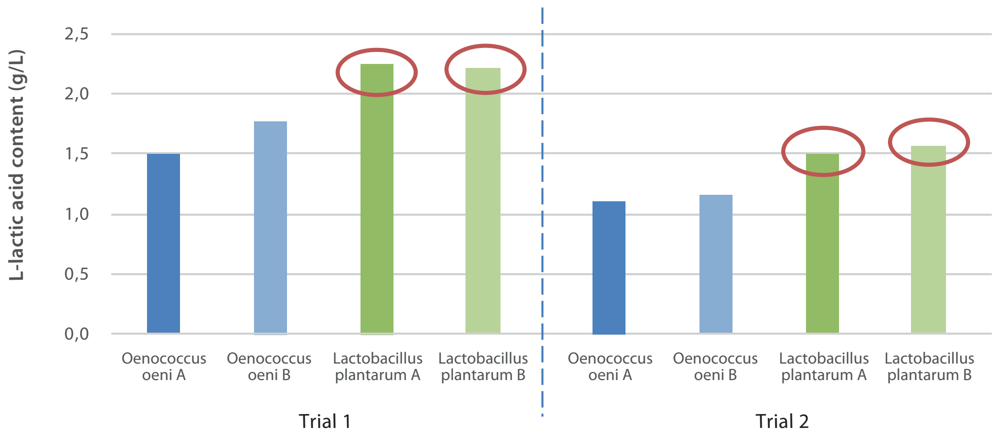

Fig. 3: Final L-lactic acid content at the end of MLF
(wine made from Barbera, Italy, 2017)

Selected wine bacteria and diacetyl

As stated previously, the choice of selected wine bacteria can have an impact on the sensory profile of a wine. Diacetyl in particular is a key compound which, when present in excess, can make wines heavier. The final diacetyl content depends on several parameters: temperature, speed of the malolactic fermentation, lees contact, etc. The choice of the wine bacteria and the time of inoculation are also key factors. For *Oenococcus oeni*, production of diacetyl is mostly due to degradation of citric acid (figure 4), for which there is strong variability between strains. Similarly, the timing of inoculation is key, as in co-inoculation the diacetyl is immediately converted by the yeast into 2,3-butanediol, an odorless compound. In white and rosé wines, if malolactic fermentation is wanted, the "diacetyl" parameter should be taken into account if there is a wish to preserve aromatic freshness.

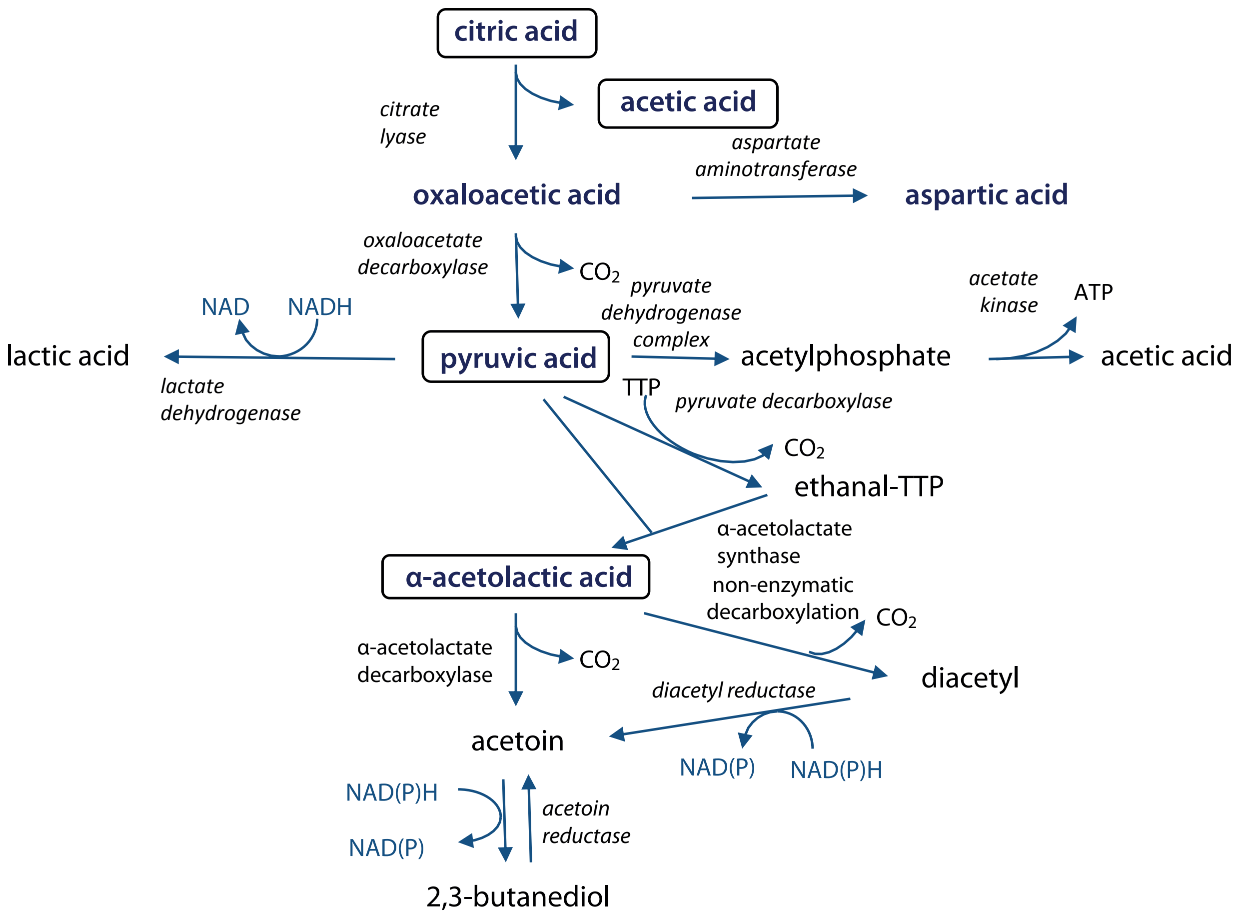

Fig. 4: Citric acid metabolism for *Oenococcus oeni*
Source: Eveline Bartowski, AWRI 2004

Conclusion

The freshness of a wine can be influenced by its acidity, but also by various sensory compounds and their interactions. Detailed characterization of the intrinsic properties of yeasts and wine bacteria is therefore indispensable. Similarly, an understanding of the parameters that can influence these microorganisms is essential. An overall knowledge of these different factors provides winemakers with various biotechnological tools to achieve a chosen sensory profile.