Malolactic fermentation: chemical and biological aspects

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Wine is a biologically complex ecosystem, in which a wealth of interlocking chemical and biochemical reactions take place. Among the fundamental biological processes that take place inside wine there are alcoholic fermentation and malic acid degradation. Yeasts, through alcoholic fermentation, yield ethanol and carbon dioxide from glucose and fructose; lactic bacteria are instead responsible for the so-called “malolactic fermentation”, in which L-malic acid is consumed to form L-lactic acid and carbon dioxide. The 'so-called' qualifier is due to the improper use of the term “fermentation”, since the malolactic process is merely the degradation of L-malic acid (Figure 1).

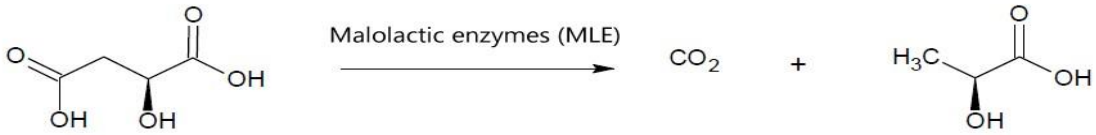

Figure 1

The L-malic acid degradation process performed by the lactic bacteria is anyway accepted by oenology under the label “fermentation”.

What are then the effects of the malolactic fermentation on wine? What are its controlling parameters? How can it be controlled? This topic opens on a vast, and somewhere fuzzy, panorama. The L-malic acid degradation is a key process for red wines, which are stabilized under both the biological and organoleptic aspects. Red wines lose acidity, gaining in suppleness and body. On the other hand, this same process is usually avoided in white wines, since they are appreciated for their fruitiness , acidity and fresh notes.

Evolution of lactic bacteria in wine

From the grapes to the wine, bacteria populations can vary wildly both in numbers and variety of bacteria strains. The bacteria that can be found in the must/wine system belong mostly to the *Pediococcus*, *Lactobacillus* and *Oenococcus* strains. The *Oenococcus Oeni* is the one that is most capable of adapting to the hostile chemical-physical environment of wine.

During the first phases of vinification, the bacterial biomass may be present in variable measure depending on environmental conditions and sanitation state of the grapes. After the crushingdestemming step, the bacterial biomass grows, to decrease again when the alcoholic fermentation begins and the yeast biomass, more suited to the “must” environment, increases. During the whole alcoholic fermentation phase, bacteria are kept in a latent state by the preponderance of yeasts and the unsuitability of the “must” environment (acidity, pH and presence of sulphur dioxide). All the above is true for a normal vinification process; sometimes issues hindering the start of the alcoholic fermentation, together with chemical-physical conditions favourable to bacterial development, allow the simultaneous presence of both yeasts and bacteria. This condition is

extremely dangerous for the organoleptic qualities of the wine-to-be, since lactic bacteria are capable of degrading sugars causing a significant increase in concentration of acetic acid and D-lactic acids, a phenomenon that is known with the name of "lactic disease" (*piqûre lactique*). The final phase of alcoholic fermentation involves a decrease in the yeast populations, with a marked increase in cellular lysis leading to the release in the vinification medium of vitamins, peptides and amino-acids that contribute to the start of the malolactic fermentation. A key factor is the production by yeasts of short-chain fatty acids, mainly octanoic and decanoic acids. These yeast metabolites, in addition to hindering the alcoholic fermentation process, may also compromise the subsequent malolactic phase. Their action on the bacteria takes place at the cell membrane level, where these compounds infiltrate and destabilize the phospholipidic bilayer, finally causing cell death.

A normal red vinification process involves therefore as its next step after alcoholic fermentation, the starting of the malolactic fermentation process, which involves removal of L-malic acid and formation of L-lactic acid. This reaction causes an increase in the pH of the medium and a decrease in overall acidity, since L-malic acid (a dicarboxylic acid) is replaced by L-lactic acid, which is a monocarboxylic acid. This increase in the pH value is marked by a colour change towards mauve, caused by the acid-base reaction of anthocyanins. A decrease in L-malic acid concentration by anywhere from 10% to 30% may also take place during alcoholic fermentation through the maloalcoholic fermentation mechanism (Figure 2):

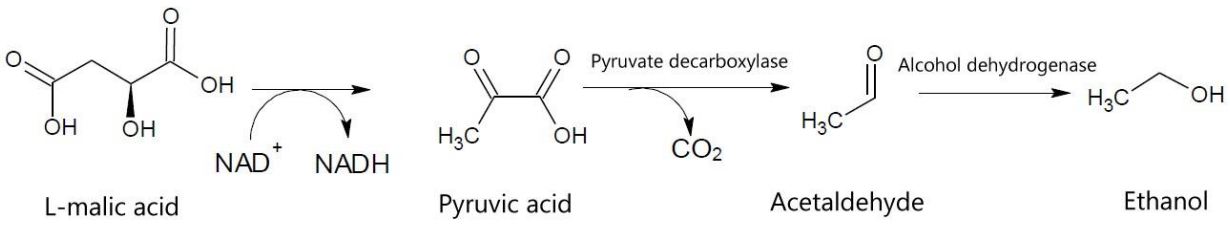

Figura 2

The lactic bacteria, in addition to degrading L-malic acid, also metabolize citric acid, albeit at a markedly lesser speed. The metabolic pathways by which citric acid gets used are mainly two: one oriented towards production of membrane bilayer phospholipids, while the other is used by bacteria as a detoxifying mechanism, leading to the synthesis of compounds such as acetoine, 2,3-butandiole and diacetyl (Figure 3). Among these, diacetyl is surely the most important, since it affects the organoleptic qualities of the wine.
Indeed, when this compound is present at a concentration of about 2-4 ppm, it brings out a buttery flavour that can considerably improve the *bouquet* and aroma of the wine. If its concentration increases above around 6 ppm, its influence may become negative. Diacetyl synthesis rate is strongly connected with bacterial growth speed; this is because in conditions of low growth speed, bacterial metabolism will orient towards the production of detoxifying compounds (Figure 3), while conversely in conditions of high growth speed, the anabolic needs will steer citric acid consumption towards lipid synthesis.

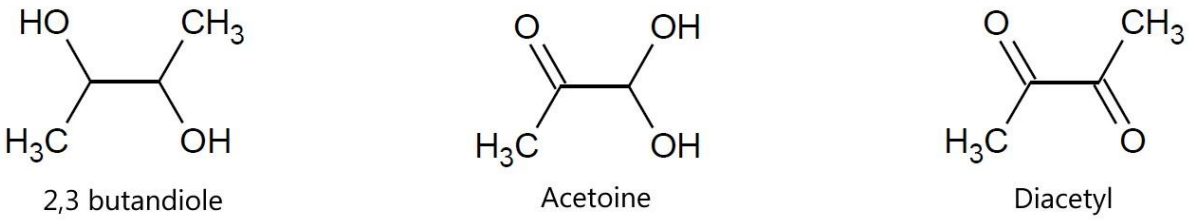

Figure 3

It is also during malolactic fermentation that biogenic amines are formed. Among these, the most important is histamine, but lower concentrations of putrescine, cadaverine, thyramine and phenylethylamine may also be found. These biogenic amines are responsible for adverse physiological reactions such as headache and various allergic reactions. These molecules have a fermentative origin, being formed by decarboxylation of their precursors. Histamine, for instance, is formed by histidine decarboxylation starting with alcoholic fermentation, but its concentration rises more markedly during the subsequent malolactic fermentation. While we do not yet fully know the causes and the chemical and biological factors that come into play during the biogenic amine synthesis by bacteria, this process is strongly connected with the specific bacterial strain that drives malolactic fermentation.

Chemical and physical factors of the bacterial growth

The chemical and physical parameters that drive lactic bacteria growth in wine are pH, temperature, sulphur dioxide concentration and alcoholic strength. These parameters are strongly interconnected. The growth of a lactic bacteria population is favoured by the pH being above 3.5, while a lower pH impairs bacterial development by making it more difficult for these organisms to maintain an intracellular pH at the optimal level for malolactic enzyme activity. Higher pHs, therefore, are favourable to malolactic fermentation, but on the other hand they make the process more fragile from a microbiological viewpoint, since the variety of the microbial strains might be wider.
Sulphur dioxide concentration is also a very important parameter for the starting of the malolactic phase. It is present in the wine in both free and bound forms; the first is represented by the bisulphite ion (HSO_3^-), as well as by sulphur dioxide proper (SO_2), commonly called “molecular sulphur”. The sulphite ion (SO_3^{2-}) is practically absent at the wine pH. The *bound* form instead is made up of that percentage of sulphur dioxide which is combined with several organic compounds with a carbonyl functional group; among these, the most important sulphur dioxide binding compound is represented by acetaldehyde. The total sulphur dioxide is then given by the sum of free and bound form. Total sulphur dioxide being the same, malolactic bacteria inhibition depends on the binding degree in wine; indeed, the greater the binding, the lower the concentration of the free form in the medium. And it is molecular sulphur dioxide only that has a strong inhibitory effect on malolactic bacteria. Molecular sulphur dioxide, in turn, increases when the pH decreases, since the acid-base equilibrium of the bisulphite ion HSO_3^- is shifted leftward, further inhibiting bacterial growth:

$$SO_2 + H_2O \rightleftharpoons H^+ + HSO_3^-$$

Temperature also influences the growth of all kinds of micro-organisms, since chemical reaction speed, and in particular biochemical reaction speed, is strongly correlated with temperature. The optimum temperature interval for malolactic fermentation is 20 – 22 °C. Lower temperatures are liable to slow it down and, if low enough, stop it altogether. On the other hand, at temperatures above 25 °C there is a risk of a dangerous increase in volatile acidity.
As it occurs with yeasts, high ethanol concentrations also have a toxic effect on bacteria, and therefore hinder malolactic fermentation. Ethanol slips inside the cellular membranes and disrupts their lattice, thereby reducing assimilation of L-malic acid.
The concentration of L-malic acid is in turn very important for the starting of the malolactic process; concentrations below 1g/L can make it difficult, and it is for this very reason that an accurate analytical assay of this acid in the pre-fermenting phase is very important in order to handle the wine-making process that will follow.

Analytical control of the malolactic fermentation

Modern malolactic fermentation control methods are based on assaying the two organic acids involved in the process, that is L-malic and L-lactic acids. The main chemical methods are the enzymatic spectrophotometric analysis and the HPLC (High Performance Liquid Chromatography) analysis. These two methods allow a precise monitoring of the ongoing malolactic fermentation (Figure 1). Of these, the L-lactic acid enzymatic spectrophotometry is surely the best method to detect the starting steps of MLF; this is because the standard HPLC analysis is influenced not only by the L-lactic acid but by its D isomer as well, which was formed by the yeasts during the previous alcoholic fermentation step. For this reason, HPLC analysis does not allow a precise quantification of the L-isomer concentration, which is the sole tell-tale parameter of the starting of the malolactic fermentation.
L-lactic acid concentrations above the threshold of 0.1 g/L reveal that the malolactic fermentation has begun. After verifying this, the decrease in L-malic acid concentration can then be monitored until its virtual disappearance below a threshold of about 0.2 g/L.
An L-malic acid assay therefore helps the oenologist not only in monitoring the optimal evolution of the malolactic step, but in forecasting its optimal starting. It is for this reason that an L-malic acid assay on the grape juice is of capital importance. Indeed this information allows the oenologist to evaluate with significant advance what the L-malic acid concentration at the beginning of the malolactic stage is likely to be. Thereby making it possible to plan how to best ensure the optimality of the fermentation process.