

Mini Review

Malolactic Fermentation-a Secondary Fermentation to Enhance Quality of Grape Wines

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Abstract

A variety of elements (vineyard management practices, grape composition at maturity, fermentation, and culture selection) have a part in the conversion of grapes to wine in winemaking. Wine fermentation is a complex heterogeneous microbiological process that involves the successive development of numerous yeasts and other microorganisms found in musts, such as lactic acid and acetic acid bacteria.

Key words: MaloLactic Fermentation, Lalvin, Bacteria, Dicarboxylic acid.

Introduction

Wine making has been a region-specific fermentation process in which either natural (skin) microflora has been used in European countries or tailor-made *Saccharomyces* species together with specialist yeasts for creating fragrance compounds have been used in American and Australian continents. MaloLactic Fermentation (MLF) is caused by lactic acid bacteria found on the surface of grape berries, in addition to yeasts. *Lactobacillus sp.*, *Pediococcus sp.*, and *Oenococcus oeni* are the most common bacteria that catalyse secondary fermentation. *O.oeni* is the only one of these strains that can handle strong alcohol (>13.0% v/v) and a low pH (3.20). Because of these two factors (high alcohol and low pH), as well as the presence of SO₂, low temperature, and limited nutrients, biological deacidification is slow [1]. MLF is currently considered a requirement in almost all commercial red and white winemaking. Private businesses such as Lallemand Co., Canada, have developed malolactic cultures sachets containing MLF cultures under the names "VP" and "Lalvin." As a result, the selection of lactic acid bacteria from grape berries, particularly *Lactobacillus sp.* (with malolactic encoding enzymes) and their implications in quality winemaking have become a subject for new research investigations focused on the various elements impacting MLF development. Furthermore, India's wine industry is still in its infancy, with production limited to Maharashtra, Karnataka, and Goa, with practically all wineries relying on foreign yeasts and MLF bacteria. Furthermore, there are almost no wineries in North India. To create wineries in North India and develop local wine cultures, innovative and indigenous cultures (yeasts and LAB) must be isolated and examined for their respective characteristics in order to develop inoculum for the manufacture of high-quality wines [2].

Literature Review

Malolactic Fermentation (MLF) is a secondary fermentation process that results in the biological conversion of malic acid into lactic acid and carbon dioxide, as well as a decrease in acidity and an increase in pH, as well as a variety of aromatic and sensory alterations in the wine [3]. The synthesis of secondary compounds during MLF results in significant changes in the quality and composition of the wine, as well as providing microbiological stability to the final product.

MLF is now widely regarded as an important component of fermentation, particularly in the production of red and white wines [4]. The increased oenological interest in the MLF process and its implications for improving wine quality has led to new research investigations focusing on the various elements impacting MLF development, such as inoculation stage, bacterial culture selection, nutrient supplementation, and so on.

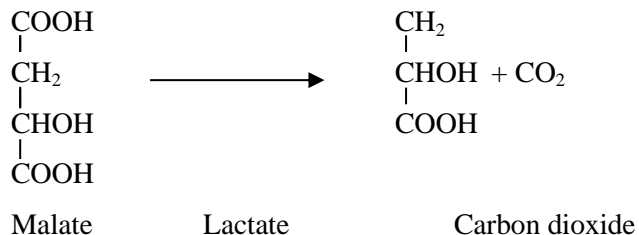
Lactic Acid Bacteria (LAB) perform MLF in a variety of conditions; nevertheless, several components of the wine, such as ethanol, acidic pH, phenolic compounds, and sulphur dioxide, might stress them. MLF is a naturally occurring biological response in wine that is initiated by inoculation with specific bacterial starters or triggered by indigenous LAB [5]. The conversion of L-malic acid to L-lactic acid and carbon dioxide, as well as subsequent alterations in fragrance, taste, and organoleptic characteristics, describe MLF in wine.

Malic acid deacidification

Malate is a dicarboxylic acid while Lactate is monocarboxylic, with one carboxyl group only.

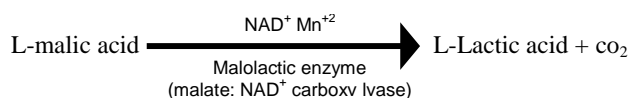
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As a result, the decarboxylation of malate to lactate produces one molecule of CO₂ for every molecule of lactate. Lactate has only one proton that can be released, whereas dicarboxylic acids have two acidic groups that can release protons [6]. When malate is converted to lactate, one of the free protons in the system is fixed. The titratable acidity can be reduced by 0.01 to 0.03 g tartaric acid equivalents/L by fixing hydrogen ions on lactate. The pH level is also raised by up to 0.3 units. This is critical because if a wine's pH is low (below 3.5) the lactic acid bacteria's metabolic activity can elevate the pH to a level that supports the growth of many additional species.



During MLF, Lactic acid bacteria have been found to degrade L-malic acid to L-lactic acid via three different pathways:

- The first involves three enzymes: malate dehydrogenase, oxaloacetate decarboxylase, and L-lactate dehydrogenase, and proceeds through the intermediates oxaloacetic acid and pyruvic acid.
- A second process, which uses pyruvic acid and a mixture of malic enzyme and lactate dehydrogenase, works *via* pyruvic acid.
- A single malolactic enzyme is used in the third method. This enzyme (malate: NAD⁺carboxylase) catalyses the direct conversion (decarboxylation) of the dicarboxylic acid L-malic acid to the monocarboxylic acid L-lactic acid, and requires cofactors NAD⁺ and Mn⁺². The enzymatic basis for this process in wine malolactic bacteria, notably *Leuconostoc oenos* (*Oenococcus oeni*) ML34, was not fully understood until the work of Kunkee and Morenzoni in the 1970s.



Inoculation time

The timing of the ML fermentation depends upon the conditions of the juice and whether or not the temperature, pH and nutrients are permissive for all organisms [7].

The consequences of different timings for MLF inoculation are explained below:

Pre-fermentation Inoculation

Pre-fermentative inoculation of ML bacteria can reduce the amount of nutrients necessary for yeast development and ethanolic fermentation, resulting in stalled or sluggish fermentation. During the pre-fermentation inoculation for MLF, off-characters are frequently produced [8]. Lactic acid bacteria create chemicals that hinder yeast growth and fermentation, which has long been known (Bisson 2001b).

Simultaneous with yeast inoculation

When ML bacteria and yeast were inoculated together, winemakers typically saw an increase in acetic acid production. There is also a decline in the viable populations of both organisms. Under these conditions, yeast can recoup more quickly than bacteria, but the culture may still be susceptible to arrest. This inoculation is greatly influenced by strain characteristics [9-11]. Some commercial yeast strains appear to be unaffected by the ML bacteria's action, while many strains of the bacteria may be extremely suppressive. Other commercial strains are substantially more susceptible to fermentation arrest upon bacterium injection.

Mid-Fermentation

Inoculating bacteria in the middle or late stages of ethanolic fermentation, but before the end, can be dangerous because there are little nutrients remaining for the bacteria at this point. At this time in the inoculation process, the ethanol content may be low and not inhibiting to ML [12]. So, inoculating ML may seem like a good idea, especially in high Brix musts and juices, but the yeast will continue to metabolise and raise the alcohol concentration. Because the rate of carbon dioxide synthesis is low at this moment, sulphur dioxide produced by yeast fermentation may be at its peak. As a result, SO₂ loss owing to CO₂ evolution has slowed. Alternatively, because the yeast is most reliant on available oxygen in the dust and fatty acids required for ethanol tolerance at this time, the addition of biomass may quickly deplete the fermentation of essential survival components [13].

Post Fermentation

Another successful technique is post-fermentation inoculation, which prevents the malolactic bacteria from inhibiting the yeast, but it can be problematic if the ethanol content is too high. Nutrients have also been depleted at this point. Other inhibitory substances produced by the yeast could have an effect on the ML bacteria. Post-fermentation inoculation improves temperature control by allowing the initial ethanolic fermentation to take place at a temperature that preserves grape volatile characteristics but is too low for the ML bacteria to develop. Another critical decision for the winemaker is the ML strain to be utilised during the MLF.

Most common decision is to inoculate selected ML bacteria at the end of ethanolic fermentation, to avoid an excess development of LAB that can give high quantities of acetic acid. In literature, a co-inoculum of selected yeasts and bacteria has been proposed to induce simultaneous ethanolic fermentation and MLF to increase the adaptation of LAB to wine, particularly in concern of adaptation to high ethanol levels. Co-inoculation at different times has been studied by some authors [14]. In the case of co-inoculum and when the selected ML bacteria are inoculated at the end of ethanolic fermentation, the yeast-bacteria interaction also to be considered. Studied the interactions between *Saccharomyces cerevisiae* and *Oenococcus oeni* in wine and showed that yeasts can oppose or stimulate MLF. Lead to a finding a strain of *Saccharomyces cerevisiae* that produce a peptide responsible for inhibiting MLF. A successful co-inoculum of yeast and ML bacteria strongly depends on the selection of suitable yeast-bacterium combinations.

To avoid an excessive development of LAB, which can produce large amounts of acetic acid; it is usual practise to inoculate selected ML bacteria near the end of ethanolic fermentation [15]. A co-inoculum of selected yeasts and bacteria has been proposed in the literature to produce simultaneous ethanolic fermentation and MLF to improve LAB adaptation to wine, especially in the case of high ethanol levels. Some authors have looked into co-inoculation at

different times. When using co-inoculum and inoculating selected ML bacteria near the end of ethanolic fermentation, the yeast-bacteria interaction must also be taken into account. The interactions of *Saccharomyces cerevisiae* and *Oenococcus oeni* in wine and discovered that yeasts can either inhibit or enhance MLF.

Changes in quality of wines by MLF

MLF improves the aroma and flavour of the wine while also providing it a smooth texture. Through the generation of volatile secondary metabolites and alterations to grape and yeast generated metabolites, LAB affect the aroma and flavour of the wine found a difference between MLF and non-MLF wines, reporting decreased acidity levels in MLF wines despite no significant changes in flavor [16]. Certain LAB breakdown citric acid into a variety of metabolic products, the most notable of which being acetoin compounds,

CONCLUSION

Winemaking is a microbiological process that involves a complicated system. The microorganisms that live in grape must, as well as the chemical composition and temperature of the must, have an impact on the criteria that determine wine quality. Wine has a wide range of yeast and bacterium communities. During Alcoholic Fermentation (AF), different yeast species take over, while others fade into obscurity or vanish entirely. The evolution of indigenous microbial populations is determined by the four key factors of pH, alcohol, temperature, and CO₂, which can be tracked by winemakers from the beginning to the end of AF. Interactions between microorganisms are recognized to be important in fermentation, although assessing these interactions is difficult. Using yeast starter cultures to massively grow the population is a simple and reliable technique to master the system's microbiological composition. The

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