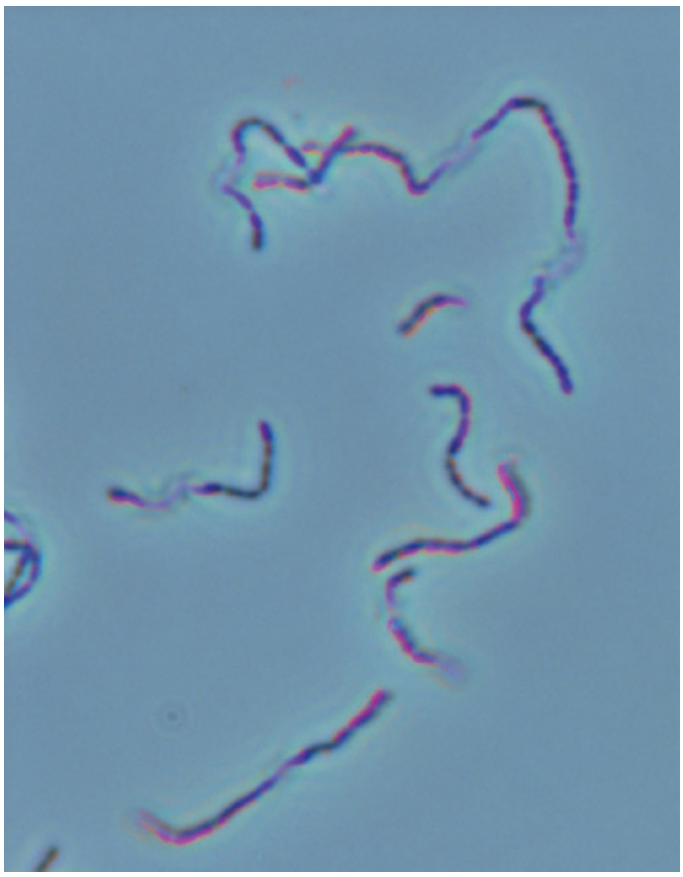
Malolactic Fermentation

Eveline J. Bartowsky and Graham H. Fleet



Oenococcus oeni at 1000x magnification

Authors

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MLF

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1 Introduction

It has been known since the early 1900s that, after alcoholic fermentation by yeasts, many wines undergo another fermentation which is widely known as the malolactic fermentation (MLF). This fermentation is conducted by lactic acid bacteria, and a key outcome is the deacidification of wine by the conversion of the di-carboxylic acid, L-malic acid, to L-lactic acid (a mono-carboxylic acid) through a decarboxylation reaction (Figure 1). For some winemakers, particularly those producing high acid wines, the MLF is a positive occurrence because this decrease in acidity softens the sensory character of an otherwise harsh, acid product. For winemaking in general, however, the MLF is a reaction that needs to be properly managed and controlled because it has the potential to occur in wines after bottling, causing gassiness and turbidity and, effectively, giving a spoiled product.

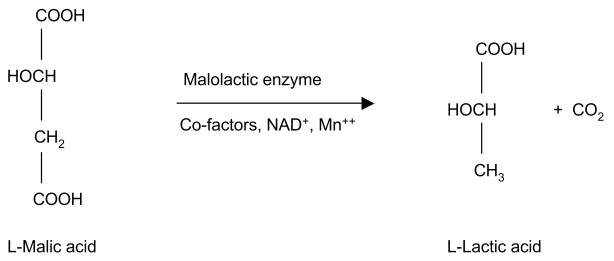

Figure 1. The malolactic reaction in wine.

During the past 50 years, there have been substantial efforts by winemakers and researchers to understand the science, technology and significance of MLF in wine production. As a consequence of much research, it is now evident that the MLF has impacts that extend beyond the basic concepts of wine deacidification and wine spoilage. Rather, it is a very significant microbiological and biochemical process that has a diversity of positive and negative influences on wine quality and on the efficiency of wine production. Whereas MLF was often a chance, spontaneous reaction in the total production chain, we now have a reasonably good understanding of the many factors that affect its occurrence and impact. With this knowledge, the modern winemaker can more effectively control and manage MLF to achieve a desired outcome.

This chapter gives an overview of the importance of MLF in wine production, the scientific basis of the process and practical guidelines for its control and management in the winery. As mentioned already, a substantial amount of fundamental and applied research has been conducted on the MLF. Early studies focused on the microbiology of MLF and factors that affected the occurrence and growth of malolactic bacteria in wines. These studies provided the foundations for subsequent biochemical, physiological and molecular research, designed to understand how malolactic bacteria grow in wine, how they change the chemical composition of wine and how these changes impact on wine sensory quality and acceptability. This information has been extensively reviewed over the years and the reader is referred to articles by (Bartowsky 2005, Beelman and Gallander 1979, Davis et al. 1985, Henick-Kling 1995, Henick-Kling 1993, Henschke 1993, Kunkee 1967, Kunkee 1974, Kunkee 1991, Liu 2002, Lonvaud-Funel 1999, Versari et al. 1999, Wibowo et al. 1985).

2 Occurrence and significance of MLF

2.1 Occurrence and microbiology

Generally, MLF commences as a natural or spontaneous reaction about 1–3 weeks after completion of the alcoholic fermentation, and lasts about 2–6 weeks. Delays in wine production and decreased process efficiency have significant negative impacts if the MLF fails to occur within a reasonable time-frame. Lactic acid bacteria resident in the wine are responsible for the MLF but, today, winemakers can choose to encourage this reaction by inoculation of commercial cultures of *Oenococcus oeni*, formerly known as *Leuconostoc oenos*. Strategies to specifically induce and control the MLF are discussed in section 4. Lactic acid bacteria indigenous to wines originate from the grapes and winery equipment, although there is very little definitive research on this topic (Amerine and Kunkee 1968, Fleet 1993, Bae et al. 2006). Fresh grape juice, crushed and extracted under commercial conditions, gives lactic acid bacteria at populations of 10^2–10^4 cfu/mL. Species of *Leuconostoc*, *Lactobacillus* and *Pediococcus* are the main types found at this stage. Acetic acid bacteria, *Gluconobacter*

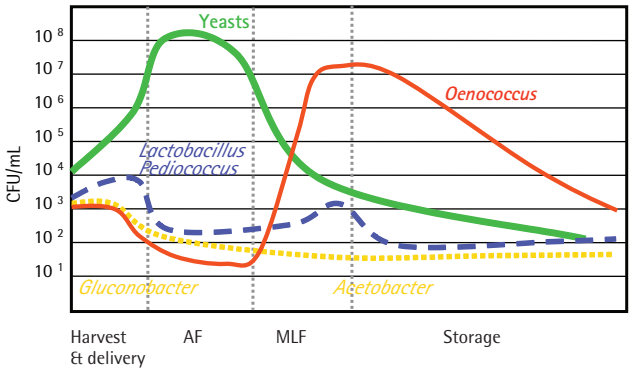

Figure 2. Dynamics of the growth of different microorganisms throughout grape vinification (adapted from Wibowo et al. 1985). AF, alcoholic fermentation; MLF, malolactic fermentation.

and *Acetobacter* species are also indigenous bacteria in grape must and wine, however they play no role in malolactic fermentation and thus will not be discussed further. Lactic acid bacteria undergo little or no growth during subsequent alcoholic fermentation and tend to die off because of their inability to compete with the growth of yeasts (Fleet et al. 1984, Wibowo et al. 1985; Figure 2). However, they are capable of abundant growth in the juice and, if yeast growth is delayed, they could grow to spoil the juice and contribute to stuck alcoholic fermentation (Bisson 1999, Lonvaud-Funel 1999). Soon after the alcoholic fermentation is completed, the surviving lactic acid bacteria commence vigorous growth to conduct the MLF. Final populations of between 10^6–10^8 cfu/mL can eventually be produced (Figure 2). The onset, duration and ecology of this growth are determined by many factors, which include viticultural (and hence juice compositional factors) and vinification conditions, the properties of the wine, and influences of other microorganisms (Table 1). Consequently, the natural occurrence of MLF and its completion by the preferred species, *O. oeni*, can be unpredictable. Although various species of *Leuconostoc*, *Lactobacillus* and *Pediococcus* can conduct the malolactic reaction, many also give undesirable, off-flavours and spoil the wine (Sponholz 1993). Generally, this is not the case with *O. oeni* and, consequently, it has emerged as the species of choice for conducting the MLF (van Vuuren and Dicks 1993). As will be discussed later, there is great diversity in the phenotypic and genotypic properties of isolates (strains) of *O. oeni* and, consequently, their growth responses in wines can be quite variable. It is not surprising, therefore, that natural MLF may not be the result of a single strain, and that several different strains of *O. oeni* may grow throughout this process (Reguant et al. 2005a,b, Bridier et al. 2010).

Wine pH, concentration of ethanol, and concentration of sulfur dioxide have strong influences on the growth of lactic acid bacteria. Different species, and even strains within species, show different responses to these properties

Table 1. Factors affecting the growth of malolactic bacteria in wine

Grape berry damage	Sulfur dioxide concentration
Pesticide residues	Ethanol concentration
Yeast influences	pH
Temperature	Bacteriophages

(Davis et al. 1988). Moreover, one property may moderate the impact of another property. For example, malolactic bacteria may be less tolerant of low pH and high sulfur dioxide when the concentration of ethanol is higher (Britz and Tracey 1990). Wines of low pH (e.g. pH 3.0), high ethanol content (> 12% v/v), and high total sulfur dioxide (> 50 mg/L) are less likely to support the growth of lactic acid bacteria and may not undergo successful MLF. Strains of *O. oeni* are more tolerant of low pH than those of *Leuconostoc*, *Pediococcus* and *Lactobacillus* species, and, generally, predominate in wines of pH 3.0–3.5. Wines with pH values exceeding 3.5 tend to have a mixed microflora, consisting of *O. oeni* and various species of *Pediococcus* and *Lactobacillus*. The latter are more tolerant of higher concentrations of sulfur dioxide than *O. oeni*, and more likely to occur in wines with higher amounts of this substance (Davis et al. 1988; Davis et al. 1986b). Thus, winemaker management of pH and sulfur dioxide content is important if it is desired to have MLF conducted solely by *O. oeni*.

Temperature is one of the most significant external factors that affect the natural or spontaneous occurrence of MLF. The optimum temperature for growth of *O. oeni* and other malolactic bacteria is near 25° C (Henick-Kling 1993). As the temperature of the wine decreases below 20° C, the possibility of a healthy, vigorous MLF decreases. For this reason, many winemakers in European countries aim to have this reaction completed before the onset of autumn-winter, and might even heat their cellars in order to achieve this goal.

Microbiological factors that affect the growth of *O. oeni* in wine and successful completion of MLF include excessive growth of moulds and acetic acid bacteria on grapes, yeasts responsible for the alcoholic fermentation, and bacteriophages. Substances produced by the growth of fungi or acetic acid bacteria on damaged grapes could either stimulate or inhibit malolactic fermentation (Joyeux et al. 1984, Wibowo et al. 1985, Lonvaud-Funel 1999). Pesticides and fungicides applied to grapes in the vineyard have two consequences for malolactic bacteria. First, they could affect the survival and growth of these bacteria on the grape surface and their subsequent carryover into the juice as indigenous flora. Second, pesticide or fungicide residues in the juice could affect the growth of these bacteria during MLF (Bae et al. 2005). During alcoholic fermentation and subsequent autolysis, yeasts release nutrients that are believed to encourage the growth of lactic acid bacteria. However, the

growth of some strains of *Saccharomyces cerevisiae* during alcoholic fermentation can be inhibitory to the subsequent growth of *O. oeni* (Wibowo et al. 1988, Patynowski et al. 2002, Alexandre et al. 2004, Fleet 2003, Nehme et al. 2008). Production of high concentrations of sulfur dioxide, inhibitory proteins, or toxic fatty acids (hexanoic, octanoic, decanoic, and dodecanoic) by these yeast strains may inhibit malolactic bacteria (Alexandre et al. 2004, Fleet 2003). The toxicity of fatty acids towards malolactic bacteria is increased in the presence of higher concentrations of ethanol (Carrete et al. 2002). It is not known how the growth of non-*Saccharomyces* species might impact on development of the malolactic fermentation. Bacteriophages active against *O. oeni* have been isolated from wines and can interrupt and delay the MLF (Davis et al. 1985; Lonvaud-Funel 1999). Lysogeny (i.e. carriage of bacteriophage) of *O. oeni* is common, and bacteriophage-resistant strains have been described (Davis et al. 1985, Poblet-Icart et al. 1998).

2.2 Significance

As mentioned already, the function and significance of MLF in wine production extends beyond the original concepts of wine deacidification and wine microbial stability. This section outlines the broader implications of MLF in winemaking (Table 2). Subsequent sections provide biochemical and molecular explanations of these influences and practical strategies for achieving desired MLF outcomes.

2.2.1 Deacidification

Wine deacidification is one of the original and main reasons for conducting the MLF. As explained before, malolactic bacteria decarboxylate L-malic acid to L-lactic acid, giving a decrease in acidity and an increase in wine pH by about 0.2–0.5 units, depending on how much L-malic acid has been transformed. It is well known that wines produced from grapes cultivated in cool climate regions have higher concentrations of L-malic acid (2–8 g/L; pH 3.0–3.5) that gives them a harsh taste and can mask their grape varietal character (Iland and Gago 2002). A decrease in this acidity by MLF significantly improves the sensory appeal and quality of these wines (Beelman and Gallander 1979, Davis et al. 1985, Kunkee 1974). However, MLF does not necessarily benefit wines produced from grapes grown in warmer climate regions. Such grapes have less L-malic acid (< 2 g/L, pH > 3.5), and further reduction in acidity by MLF may give an insipid wine with weak sensory balance and appeal. Moreover, it may increase the pH of these wines to values exceeding 3.75, thereby increasing their ability to support the growth of spoilage bacteria.

2.2.2 Microbiological stability

Wines that have not undergone MLF before bottling/packaging, risk the possibility that this reaction will spontaneously occur at some later stage in the bottle/package. If this happens, the wine becomes gassy and turbid, and is considered spoiled. For this reason, many winemakers prefer to have the MLF completed before bottling or packaging. This view even prevails among producers of warmer climate, low acid wines, where the acidity of the wine may need to be adjusted by addition of tartaric acid after the MLF. Two mechanisms have been proposed to explain the increased microbial stability of wines after MLF. First, growth of bacteria during MLF uses up residual nutrients in the wine, leaving little, if any, substrates for further microbial growth and potential spoilage. Second, there is evidence that some strains of *O. oeni*, as well as other malolactic bacteria, produce bacteriocins which may contribute to increased microbiological stability. Bacteriocins are small-sized proteins with antibiotic-like properties that are released into the medium by some bacteria (Edwards et al. 1994, Navarro et al. 2000). However, stability after MLF varies with the wine and its composition. There are reports that *O. oeni* and various species of *Lactobacillus* and *Pediococcus* can re-establish growth in wines that have already completed the MLF (Edwards et al. 1994, Wibowo et al. 1988).

Table 2. Consequences of malolactic fermentation in wines

- Deacidification, increase in wine pH
- Increased microbiological stability
- Spoilage if it occurs after wine packaging
- Flavour enhancement
- Flavour taints
- Colour changes
- Biogenic amine production
- Possible contribution to ethyl carbamate formation.

2.2.3 Flavour and colour modification

MLF not only affects the taste of wine through deacidification, but it also contributes other flavour characteristics that may either enhance or detract from overall acceptability. Wine flavour is usually associated with the presence of volatile compounds, but non-volatile components also influence the palate or mouthfeel of the wine (Iland and Gago 2002). Sensory impressions such as buttery, vanilla-like, nutty, spicy, fruity, vegetative, toasty, sweaty and ropy have been used on different occasions to describe MLF influences (Bartowsky et al. 2002a, Bartowsky and Henschke 1995, Davis et al. 1985, de Revel et al. 1999, Henick-Kling 1995, Lonvaud-Funel 1999, Sauvageot and Vivier 1997). These flavour changes will be determined by the wine constituents metabolised by the malolactic bacteria, and the nature, concentration and sensory threshold of the metabolic products they generate. Some biochemical reactions underlying these changes are listed in Table 3.

There are several mechanisms by which the MLF may affect wine flavour. First, there is the deacidification effect

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