INTRODUCTION

Acidity in wine originates mainly from two sources. The first is the organic acids extracted from grapes into the must during harvesting and crushing; L-tartaric, L-malic and citric acids are the predominant acids (Boulton *et al*., 1996). The chemical composition of harvested grapes therefore strongly influences the composition of must at the onset of vinification and ultimately the final quality of the bottled wine. Secondly, the combined metabolism of yeasts and bacteria during subsequent fermentation steps contributes to the pool of wine acids. The net contribution of these microorganisms to wine acidity is the sum of both the degradation of some grape acids and the biosynthesis of some unique organic acids by yeasts and bacteria during and after alcoholic fermentation. Succinic acid is the major acid produced by yeast during fermentation. Lower levels of other tricarboxylic acid (TCA) cycle intermediates are also present (Ribéreau-Gayon *et al*., 2000). Lactic acid is mainly produced by lactic acid bacteria (LAB) during malolactic fermentation, but small amounts can also be synthesised by yeast. Several cellar procedures such as maceration and cold stabilisation also influence the final acid composition of wine due to precipitation phenomena (Jackson and Schuster, 1997).

The conversion of grape sugars to ethanol and carbon dioxide is often described as the fundamental biochemical reaction involved in winemaking, but an intricate ensemble of biological and spontaneous chemical reactions all contribute to the final product. Besides the importance of flavour compounds, the presence or absence of organic acids in wine plays a pivotal role in the production of quality wines. Acidity in wine directly or indirectly affects several different levels of the winemaking process and ultimately determines wine quality in terms of the perceived organoleptic and aesthetic character. Wine acidity also influences the ageing potential or the shelf-life of wine, as it determines the physical, biochemical and microbial stability of wine. In addition, wine acidity and pH affect the timely succession of cellar events and the effectiveness of several techniques applied by winemakers (Margalit, 1997).

Winemakers often experience problems when some wine acids exceed acceptable concentration levels. Since the artificial manipulation of sugars and flavourants in wine is detrimental to wine quality and prohibited in most wine producing countries, winemakers can only modify the acidity component of wine by adding or removing certain acids. The adjustment of acidity in must or wine is complex and a number of factors must be taken into account to ensure the correct method and timing for rectifying wine acidity. Winemakers routinely employ bacterial malolactic fermentation to deacidify wine (Henick-Kling, 1993). Although this step is considered the most natural method for wine acidity adjustment, which also contributes to microbial stability and organoleptic complexity, there are a number of pitfalls associated with this biological process.

This review discusses, with special reference to L-malic acid, the origin and evolution of organic acids in grapes and wine, thew5.lic

ORGANIC ACIDS IN GRAPES

The principal organic acids in grapes are L-tartaric and L-malic acid (see Table 1), accounting for more than 90% of the grape berry's acid content (Boulton *et al*., 1996). Although L-malic and L-tartaric acids have similar chemical structures, they are synthesised from glucose via different metabolic pathways in grape berries. L-Malic acid is formed via glycolysis and the TCA cycle, while ascorbic acid is the principle intermediary product of L-tartaric acid biosynthesis. Slight differences in grape acidity among different grape varieties are usually found, affecting especially the ratio between L-tartaric acid and L-malic acid in different grape cultivars (Kliewer *et al*., 1967). L-Tartaric acid is usually present in grapes at average concentrations of 5 to 10 g/L (Ruffner, 1982), while mature grapes contain between 2 and 6.5 g/L L-malic acid (Boulton *et al*., 1996; Ribéreau-Gayon *et al*., 2000). Excessive amounts of malic acid (15 to 16 g/L) may be present in grapes harvested during exceptionally cold summers in the cool-climate viticultural regions of the world (Gallander, 1977). Although tartaric acid is often found at higher concentrations than L-malic acid and is the stronger acid of the two, its concentration is relatively constant. It is the fluctuating concentration of L-malic acid that usually poses problems to winemakers (Margalit, 1997).

Evolution of L-malic acid during grape berry development

The development of the grape berry displays a double-sigmoidal growth pattern (Kanellis and Roubelakis-Angelakis, 1996), characterised by three successive phases: Phase I is the green or herbaceous stage immediately after flowering (see Fig. 1). The berries are hard and green, and undergo a short period of cell division and cell enlargement resulting in rapid expansion of the berry (Kanellis and Roubelakis-Angelakis, 1996; Terrier *et al*., 2001). Characteristic of Stage I is the increase in vacuolar size of the grape berry cells due to the rapid storage of L-malic and L-tartaric acid (Fillion *et al*., 1999; Pratelli *et al*., 2002). Stage II comprises a short lag phase during which berry growth ceases and berry acidity reaches a maximum due to the continued accumulation of L-malic and L-tartaric acid. Following the lag phase, there is a second period of 'berry growth' (Stage III). The entry into Stage III begins with the sudden onset of ripening or "*véraison*", which generally starts between 6 to 8 weeks after flowering and lasts for 35 to 55 days depending on the grape cultivar (Coombe, 1992; Pratelli *et al*., 2002; Ribéreau-Gayon *et al*., 2000).

Véraison, which seems to be a stress-associated process, is characterised by several drastic physical and biochemical changes in the grape berry (Coombe, 1992; Davies and Robinson, 2000). The second most significant biochemical change during *véraison* is the rapid reduction of grape berry acidity, which coincides with the change in sugar composition of the grape berry. Grape berries respire actively during the early stages of growth, but the intensity of respiration slows down as they advance in age. During *véraison*, the availability of the respiratory substrate, sucrose (via photosynthesis), becomes limited due to the degradation of chlorophyll. The berry is therefore forced to shift its metabolism from sugar to L-malic acid respiration. Prior to the onset of *véraison*, L-malic acid is the most abundant organic acid (up to 25 g/L) in the grape berry vacuole, resulting in the low internal pH of 2.5 of grapes (Ruffner, 1982; Ribéreau-Gayon *et al*., 2000). With the onset of *véraison*, the L-malic acid concentration rapidly decreases to between 4 and 6.5 g/L, or even as low as 1 to 2 g/L, with a concomitant increase in internal berry pH (pH of ca. 3.5).

The biochemistry related to the accumulation and rapid respiration of L-malic acid in grapes has been studied in detail (see Fig. 2). L-Malic acid accumulates in the berry vacuole before *véraison* (Stages I and II, see Fig. 2) via the collective activities of the phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) enzymes (Blanke and Lenz, 1989; Diakou *et al*., 2000; Or *et al*., 2000). The cytosolic PEPC enzyme, well known for its photosynthetic role in C_4- and CAM-plants, catalyses the β-carboxylation of phosphoenolpyruvic acid to yield oxaloacetic acid and inorganic phosphate. The resulting oxaloacetic acid is further reduced by the NAD-dependent malate

TABLE 1

Organic acids present in grapes and wine (Boulton *et al*., 1996).

Fixed acids		Volatile acids	
Major acids	**Minor acids**	**Major acids**	**Minor acids**
L-tartaric acid (5-10 g/L)	pyruvic acid	acetic acid	formic acid
L-malic acid* (2–6.5 g/L)	α-ketoglutaric acid		propionic acid
L-lactic acid (1-3 g/L)	isocitric acid		2-methylpropionic acid
citric acid** (0.5-1 g/L)	2-oxoglutaric acid		butyric acid
succinic acid (0.5–1.5 g/L)	dimethyl glyceric acid		2-methylbutyric acid
amino acids	citramalic acid		3-methylbutyric acid
	gluconic acid***		hexanoic acid
	galacturonic acid		octanoic acid
	glucuronic acid		decanoic acid
	mucic acid		
	coumaric acid		
	ascorbic acid		

*15 –16 g/L L-malic acid has been reported in cool climate regions

**> 0.3 g/L when wines are stabilised for metal precipitation

***present in wine with *Botrytis cinerae* infection

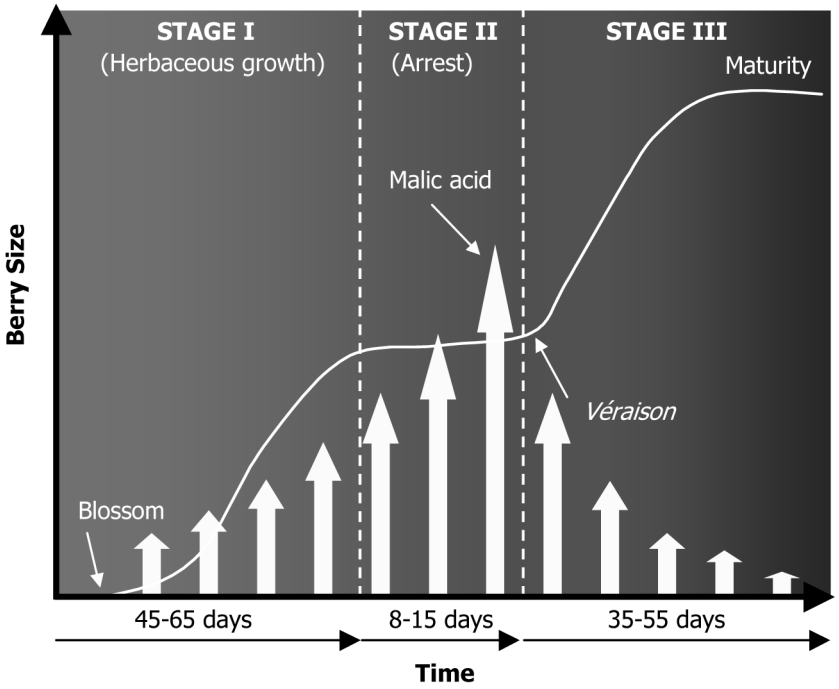

FIGURE 1

The double-sigmoid model of grape berry development indicates the three stages of herbaceous growth, temporary growth arrest and *véraison* (adapted from Jackson and Schuster, 1997). The thick arrows denote the simultaneous pattern of L-malic acid synthesis and accumulation as well as rapid decrease via respiration during grape berry development.

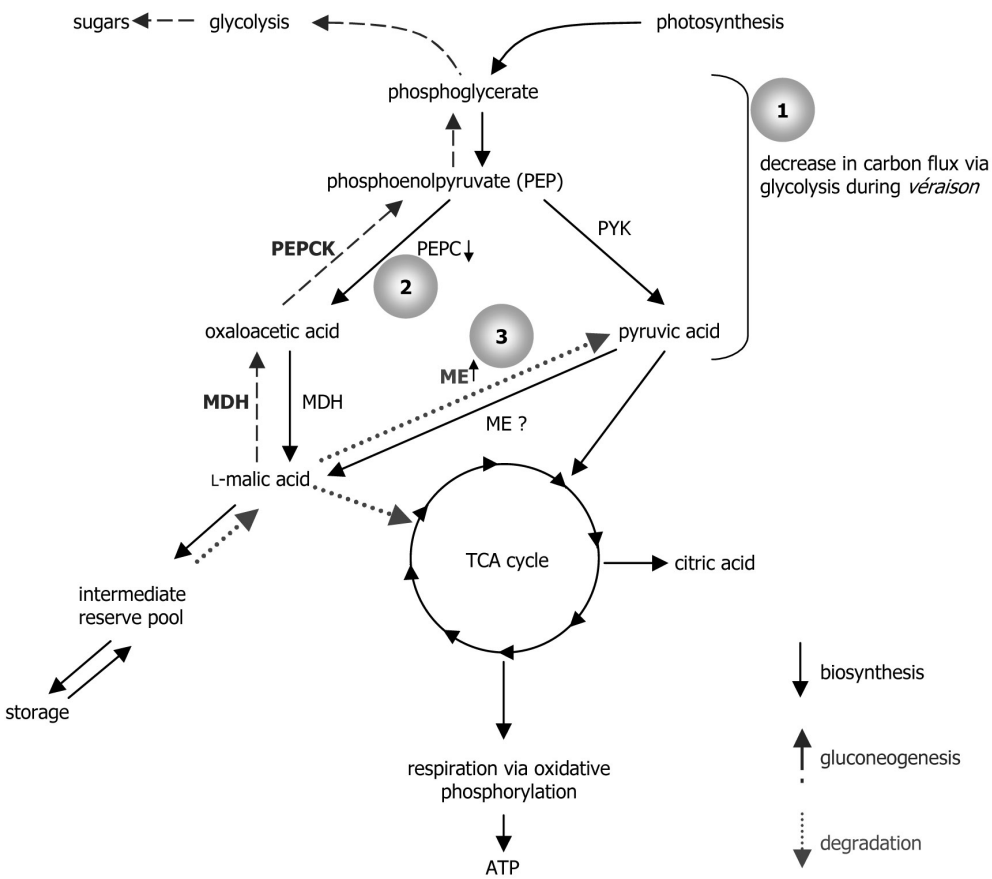

FIGURE 2

The biochemical pathways involved in the biosynthesis, dissipation and regulation of L-malic acid in grape berries (adapted from Kanellis and Roubelakis-Angelakis, 1996).). ME = malic enzyme; PEPC = phosphoenolpyruvate carboxylase; PEPCK = phosphoenolpyruvate carboxykinase; MDH = malate dehydrogenase. The decrease in L-malic acid is due to (1) a decrease in carbon flux via glycolysis, (2) a decrease in L-malic acid biosynthesis via PEPC, and (3) an increase in L-malic acid respiration via the malic enzyme.

dehydrogenase to produce L-malic acid. Oxaloacetic acid and L-malic acid can enter the TCA cycle to produce citrate as well as other metabolites (Diakou *et al*., 2000). The β-carboxylation of phosphoenolpyruvic acid plays an important role as an anapleurotic CO_2 fixation step that supplies carbon skeletons for other cellular processes such as osmolarity regulation, pH regulation and nitrogen assimilation (Diakou *et al*., 2000). Although a high malic enzyme (ME) activity during the accumulation phase of malic acid has been noted, the actual contribution to L-malic acid concentration via the reverse malic enzyme reaction, i.e. pyruvic acid carboxylation, was found to be insignificant (Ruffner *et al*., 1984; Kanellis and Roubelakis-Angelakis, 1996).

The rapid decrease in L-malic acid concentration inside the grape berry during *véraison* is the result of a significant decrease in L-malic acid biosynthesis synchronised with a sharp increase in L-malic acid degradation via respiration. Initially the concentration of L-malic acid in the berry vacuole is diluted due to the influx of water during berry expansion in the second growth phase (Stage III, see Fig. 1). Secondly, the slowing down of glycolytic carbon flow during *véraison* results in the increase of glucose and fructose in the berry vacuole, and also a decrease in L-malic acid synthesis via pyruvic acid in the tricarboxylic acid (TCA) cycle. The biosynthesis of L-malic acid via the PEPC enzyme is also reduced due to a lack of PEPC gene transcription, which correlates with the start of *véraison* (Or *et al*., 2000).

The rate of respiration of stored L-malic acid significantly increases during *véraison* due to a higher demand for respiratory substrates in the grape berry. L-Malic acid is degraded in grape berries via two pathways, mainly by the cytosolic NADP-malic enzyme (ME) (Ruffner *et al*., 1984) and, to a lesser extent, by the PEP carboxykinase (PEPCK) (see Fig. 2) (Ruffner and Kliewer, 1975). There is also evidence that the malate dehydrogenase (MDH), especially the mitochondrial iso-enzyme, plays a putative role in the degradation of L-malic acid in the grape berry. Gene expression profiles and enzyme activities of the ME and MDH enzymes increase at the onset of *véraison*, descriptive of the rapid depletion scenario of L-malic acid (Or *et al*., 2000). L-Malic acid degraded via the NADP-malic enzyme fuels the required biosynthetic (in particular the provision of NADPH) and respiratory pathways (Ruffner *et al*., 1984), whereas a small percentage of L-malic acid (<5%) is converted back to phosphoenolpyruvate via MDH and PEPCK for glucose synthesis via gluconeogenesis (see Fig. 2) (Ruffner and Kliewer, 1975; Ruffner, 1982; Kanellis and Roubelakis-Angelakis, 1996).

Environmental factors: Warm climate *vs*. cool climate

Acidity in wine is a function of various exogenous factors, such as the climate or average temperature, the grape cultivar and vineyard practices (Beelman and Gallander, 1979; Ribéreau-Gayon *et al*., 2000; Zoecklein *et al*., 1995). The most important factors that influences the final sugar:organic acid and the malic:tartaric acid ratios in grapes is the prevailing climatic conditions and ambient temperature during Stage III of berry ripening (Crippen and Morrison, 1986; Kanellis and Roubelakis-Angelakis, 1996; Ruffner, 1982; Zoecklein *et al*., 1995;).

Cool-climate regions, which include parts of northern Europe, Canada and northeast USA, are characterised by shorter ripening periods and/or sub-optimal mean temperatures (9 °C to15° C) during the late ripening season (Jackson, 2001). The rate of respiration of L-malic acid is significantly slower in cold climates, resulting in "immature grapes" at harvesting, containing a high titratable acidity (TA) content and low pH. High acidity in cool-climate grapes can be exasperated by unusually cold seasons, poor vineyard locations, sub-optimal cultivar selections or poor viticultural practices such as overcropping. In these countries, L-malic acid can comprise up to 50% of the total acidity in grapes.

In contrast, the warm climates, including parts of southern Europe, California, South Africa and Australia, have longer ripening seasons and/or higher mean temperatures (16°C and above) (Pretorius, 2000). Since the prevailing temperature of the region directly influences the rate of L-malic acid respiration in grapes, grapes in the warmer climates tend to have a faster rate of L-malic acid respiration compared to those of the cooler climates. Thus, grapes from the warmer climates often contain insufficient final TA values and do not meet desirable pH values at harvest time.

THE ROLE OF MALIC ACID IN WINE

Organic acids can contribute positively to the organoleptic character of wine when in balance with the other wine components. The sour-sweet balance is well known as a required sensory quality in wine, especially in white wine (Burns and Noble, 1985; Fischer and Noble, 1994; Martin and Revel, 1999; Vannier *et al*., 1999). Acid-balanced wines are usually perceived as having refreshing or crisp sensory undertones, while descriptions such as "sharp", "green", "acidulous" or "unripe" often refer to wine with too much acidity. When present in excessive concentrations, organic acids leave a uniquely tart or sour taste, indicative of the specific acid in wine. For example, excess quantities of L-malic acid are perceived as a sour taste, resembling that of unripe apples. Furthermore, wine acidity often disguises or accentuates the perception of other wine tastes; it usually masks excess sweetness, while the perception of astringency is emphasised when coinciding with low pH values (Noble, 1998).

The specific organic acid composition of wine determines the specific pH of the wine, which in turn indirectly influences the perception of taste in wine. Since the pH of any given solution is based on the balance between the protonated and deprotonated isoforms of organic molecules, the pH of wine determines the degree of organic acid and amino acid ionisation in a wine solution. The level of ionisation of these organic building blocks influences the ionic state, solubility and biological activity of many complex molecules such as proteins, fatty acids, phenolic compounds, etc. It is thus conceivable that a minor change in wine pH (as small as 0.05 units), when coinciding with changes in total acidity (TA) of 0.2 to 0.5 g/L, significantly influences the organoleptic perception of wine (Margalit, 1997).

Organic acids and pH also play important roles in the development of specific flavour compounds during vinification. A high TA and low pH in grape must has been linked to the release of floral aroma and other flavour precursors from grape skins during the crushing stages of vinification. The release of stored organic acids, specifically L-malic acid and tartaric acid, from the grape berry during crushing is responsible for acid hydrolysis of non-volatile flavour compounds like monoterpene glycosides, some phenolic compounds, C_{13}-norisoprenoids, benzyl alcohol and 2-phenylethanol from the berry. These flavour compounds are

essential for the development of a healthy, complex flavour profile during vinification and subsequent ageing of wine (Strauss *et al*., 1987; Winterhalter *et al*., 1990). Acidity in wine, and more specifically wine pH, plays an important role in the preservation of wine aroma and flavour, as the pH of wine influences the rate of oxidation in wine. A low pH in must or wine safeguards wine and prevents or delays phenolic oxidation by maintaining the phenolic compounds in their non-ionised state, rendering them less susceptible to oxidation.

The acidity and pH of wine also influences the visible attributes of wine, such as wine colour and clarity. The colour of white wine is usually affected by pH-dependent phenolic oxidation reactions that lead to browning of the wine colour. In red wines, low pH and free sulphur dioxide are essential for the development and stability of the red colour, which can be ascribed to anthocyanins (Margalit, 1997). Furthermore, the pH of red wine also influences the degree of co-pigmentation of anthocyanins, which in turn determines the red colour density. Furthermore, wine clarity can be affected by the undesirable precipitation of acids, particularly tartaric acid, which can be considered an aesthetic imperfection, especially in young bottled wines. Excess tartaric acid is routinely removed before bottling by cold stabilisation and racking or filtering of potassium bitartrate crystals.

Wine acidity, but more importantly wine pH, has a profound effect on the microbial stability of wine as it determines the survival and proliferation of bacteria and yeast species during and after vinification. Grape must or juice with a low pH (<3.5) is usually more protected against microbial spoilage at the onset of alcoholic fermentation, as the low pH is not conducive to the growth of most spoilage bacteria and yeast species, but still permits the proliferation of the wine yeast *S. cerevisiae* (Capucho and San Romao, 1994).

Extreme pH values in wine usually have an adverse effect on the growth of yeast and bacteria during vinification, *i.e.* at pH values below 2.9 the growth of spoilage bacteria and yeasts, as well as most strains of *S. cerevisiae* will be inhibited. This may delay the onset of alcoholic fermentation and affect the success of inoculating with LAB starter cultures after alcoholic fermentation (Charoenchai *et al*., 1998). When the pH of must or juice exceeds pH 3.5, the risk of overgrowth of spoilage lactobacilli, pediococci and strains of *Oenococcus oeni* during alcoholic fermentation is increased. At elevated pH ranges, strains of these LAB can rapidly proliferate to substantial populations before the actual onset of alcoholic fermentation. Premature growth of LAB poses a serious risk to wine quality, since glucose is fermented to lactic acid and acetic acid, resulting in elevated volatile acidity levels in wine, reduced ethanol yields as well as stuck or sluggish alcoholic fermentation (Fugelsang, 1997; Narendranath *et al*., 1997).

Lastly, TA and pH also influence several winemaking operations. For example, the requirement for chemical additives is reduced in low pH wines. Wines with low pH values require lower concentrations of SO_2 due to the added protection against oxidation and microbial spoilage supplied by the low pH. The effective precipitation of pectins and heat-sensitive proteins during bentonite treatment is also enhanced by a lower pH. Since less bentonite is required at lower pH values for the effective removal of solids and proteins during racking, the loss of flavour compounds is minimised (Boulton *et al*., 1996; Margalit, 1997; Ribéreau-Gayon *et al*., 2000).

PHYSICOCHEMICAL METHODS FOR ACIDITY ADJUSTMENT IN WINE

Acidity adjustment in grape must is an essential step during vinification when the TA and/or pH of the must or wine exceed acceptable ranges. As a rule, the TA of wine increases by 1 to 2 g/L during alcoholic fermentation via the production of L-malic, succinic, acetic and lactic acids by strains of yeast and bacteria. However, significant variations might occur due to the contribution of specific yeast strains used for alcoholic fermentation and the success of the malolactic fermentation.

Several factors need to be considered to determine the timing (before or after alcoholic fermentation) and method of rectifying wine acidity, which are generally based on the initial pH of the must or juice. Traditionally, acidification of low-acid (high pH) grape must in warm viticultural regions is preferably done before the start of alcoholic fermentation. Reducing the pH is conducive to the development of optimal wine flavour during fermentation and prevents the proliferation of spoilage lactobacilli and pediococci during alcoholic fermentation. Similarly, a reduction of TA prior to fermentation is a prerequisite in grape must with a pH below 2.9, since the onset of alcoholic fermentation by strains of *Saccharomyces* will be negatively affected at such low pH extremes.

Viticulturists have several vineyard practices available to preserve or decrease the acidity of grapes. Winemakers in the warm-climate countries, for example, can alleviate the problem of low acidity wines by selecting specific cultivars with a natural higher tartaric:malic acid ratio, such as Semillon and Riesling (Lavee and Nir, 1986). In contrast, Cabernet Sauvignon is classified as an intermediate-acidity variety (Kliewer *et al*., 1967) that will relieve high acidity problems to a limited degree in the cool-climate regions. Canopy management, irrigation control and soil fertilisation have a significant influence on the acid composition of grapes. For example, nitrogen and potassium fertilisation stimulates the accumulation of L-malic acid due to increased foliage and shading in bunches. Berries maturing in densely shaded canopy interiors are generally associated with higher TA levels due to an increase in L-malic acid (Archer and Strauss, 1989; Morrison and Noble, 1990; Smart, 1985; Zoecklein *et al*., 1995). Similarly, viticulturists in the cool-climate regions can use trellising and leaf-pruning techniques, which allows grape bunches to be more exposed to sunlight and increase the microclimate temperature to ensure increased respiration of L-malic acid during *véraison.*

Several cellar operations influence the TA and pH of wine. Skin contact or maceration generally leads to a slight decrease in TA and an increase in pH in the must before the start of fermentation (Darias-Martin *et al*., 2000; Ferreira *et al*., 1995). Skin contact is often applied in red wines (and some white wines) to extract anthocyanins and other phenolic flavour compounds from the pomace to ensure the optimal colour and flavour development in red wines. However, increased extraction of potassium from the skins coincides with phenolic extraction during skin contact, which normally causes an increase in potassium bitartrate precipitation. Grape acidity can also be reduced by carbonic maceration, a maceration process in which the grapes are kept under anaerobic conditions by exposing them to CO_2 gas before crushing or pressing. In this anaerobic environment, certain intracellular fermentation reactions are stimulated in the intact berries, one of which is the oxidative decarboxylation of L-malic acid to pyru-

vic acid, catalysed by a berry NADP-dependent malic enzyme (EC 1.1.1.40) (Beelman and Gallander, 1979).

Acidification of low-acid wines

Several artificial methods for acidification of grape must are available to winemakers, the most traditional method of which is the use of raw gypsum or plaster (hydrous calcium sulphate), which reacts with potassium bitartrate and releases free tartaric acid. This method is seldom applied in modern winemaking due to the risk of increased hydrogen sulphide production by yeast during fermentation (Zoecklein *et al*., 1995). Acidification of wine can also be achieved by adding naturally occurring grape acids to wine, such as tartaric, D/L-malic, citric or fumaric acid. Although succinic acid is relatively resistant to microbial attack under fermentative conditions, it cannot be utilised as an acidulating agent due to its bitter-salty taste (Ribéreau-Gayon *et al*., 2000).

Tartaric acid is the preferred acidulating agent in low-acid wines; it is relatively more resistant to microbial breakdown and can thus be added before the onset of alcoholic fermentation without the risk of off-flavours. Acidification of grape must with L-malic acid and citric acid can be applied with some degree of success, but degradation of these acids by spoilage LAB poses a risk under winemaking conditions. L-Malic acid does not precipitate like tartaric acid, but can initiate a second round of malolactic fermentation if still present in the wine just before bottling. As a precaution, malic acid is added as a racemic mixture of D/L-malic acid to the must at the beginning of fermentation, which leads to increased TA and lower pH of the wine. The risk of bottled malolactic fermentation is significantly reduced, since most or all of the L-malic acid is removed by the first round of malolactic fermentation, leaving only the D-malic acid isoform that is resistant to microbial attack, and maintains a low pH.

Deacidification of high-acid wines

In cool viticultural regions, the removal of tartaric acid or excess L-malic acid from the wine before or after alcoholic fermentation is usually required to ensure wines with a balanced acid content and sufficient stability. Deacidification of high-acid wines can be achieved through physicochemical methods such as blending, chemical neutralisation and precipitation, or by biological means through the microbial degradation of L-malic acid, as will be discussed in a following section.

Blending of grape musts with different TA and pH indexes is one of the most elementary and effective solutions available to winemakers. However, blending of a low-pH must with a high-pH must to neutralise the pH before fermentation is not always practically achievable due to the lack of available musts with significantly opposite characteristics in the same wine-producing region. Another physical method employed to decrease the TA of high-acid musts is the process of amelioration (Kluba and Beelman, 1975), which involves the dilution of grape must with water to reduce the must TA before fermentation. Amelioration, however, has become an unacceptable winemaking practice due to its detrimental effect on wine flavour, aroma, body and colour (especially in red wines) (Margalit, 1997).

Acids become less soluble and their salts precipitate in wine under chilled conditions and increased ethanol concentrations. The TA of wine or grape must can therefore be reduced by precipitating tartaric acid and, to a lesser extent, L-malic acid salts. Tartaric acid usually precipitates in wine at the end of fermentation as potassium bitartrate crystals. To prevent the formation of tartaric acid crystals during wine ageing, cold stabilisation is applied to promote tartaric acid crystallisation and precipitation. Removal of the tartaric acid crystals during subsequent racking, filtration or centrifugation leads to a lower tartaric acid concentration, and thus a decrease in TA and an increase in pH. Potassium bicarbonate ($KHCO_3$) and calcium carbonate ($CaCO_3$) can be added to wine to assist in tartaric acid precipitation, but L-malic acid is unaffected, often leading to undesirable tartaric:malic acid ratios in wine (Ribéreau-Gayon *et al*., 2000).

An effective method to reduce the TA of high-acid grape musts before fermentation is double salt-precipitation treatment (also known as Acidex or DICALCIC treatment) (Ribéreau-Gayon *et al*., 2000). This method is used in cool climate regions and is based on the addition of a fine powder of calcium carbonate that contains a 1% calcium tartaric and malic acid salt mixture as a seeding agent. At pH values above 4.5 this additive causes the precipitation of supposedly equimolar quantities of both tartaric and L-malic acid. Usually, a pre-determined fraction of the wine is treated with Acidex and then blended back with the untreated wine to yield a wine with a lower TA and higher pH.

BIOLOGICAL DEACIDIFICATION VIA MALOLACTIC FERMENTATION

Since the physicochemical deacidification of wine is often time-consuming, requires increased labour and capital input, and is regularly associated with reduced wine quality (Pretorius, 2000), biological deacidification of wine with LAB is the traditional method of choice for the removal of excess wine acidity. During alcoholic fermentation, wine yeast strains convert the grape sugars, glucose and fructose into ethanol and various flavour compounds. Once all of the sugars are depleted, the yeast population rapidly declines, followed by the proliferation of LAB that utilise the remaining hexose and pentose sugars and performs malolactic fermentation (MLF). Modern winemaking has harnessed the benefits of the naturally occurring LAB in wine by developing pure starter cultures of selected LAB for improved efficiency and reliability of malolactic fermentation.

Various strains of LAB are regularly associated with different food and beverage-related biotopes such as wine, beer, ciders, vegetables, silage, bread (sourdough), cocoa and coffee fermentations (Hashizume and Mori, 1990; Henick-Kling, 1993) (Table 2). As the name suggests, strains of LAB have the ability to produce significant quantities of lactic acid from sugars. This is achieved either via a homofermentative metabolic pathway when only lactic acid is produced from glucose, or via a heterofermentative pathway when glucose is fermented to lactic acid, ethanol and acetic acid. Several strains of LAB, classified in *Oenococcus*, *Leuconostoc*, *Weissella*, *Pediococcus* and *Lactobacillus* genera, have the additional ability to convert L-malic acid into L-lactic acid and CO_2 by means of the malolactic enzyme (see Fig. 3). The malolactic enzyme catalyses the conversion of L-malic acid to L-lactic acid in the presence of the cofactors NAD^+ and Mg^{2+} (Bony *et al*., 1997).

Wine LAB have a complex ecology, but are usually present in low numbers (10^2–10^4 colony-forming units (CFU)/g) on grapes

TABLE 2

Lactic acid bacteria isolated from wine (Carr *et al*., 2002; Du Plessis *et al*., 2004).

Genus	Wine-related species
Oenococcus	*O. oeni* (formerly *Leuconostoc oenos*)
Leuconostoc	*L. mesenteroides*
Lactobacillus	*Lb. plantarum, Lb. sakei, Lb. fructivorans* (formerly *trichodes*), *Lb. buchneri, Lb. fermentum, Lb. casei , Lb. homohiochii, Lb. fructosus, Lb. desidiousis (reclassified as Lb. kefir), Lb. brevis, Lb. hilgardii, Lb. curvatus*, *Lb. delbrueckii*, *Lb. jensenii, Lb. kunkeei, Lb. nagelii, Lb. paracasei, Lb. vermiforme, Lb. vini, Lb. yamanasheinsis, Lb. homohiochii,*
Pediococcus	*P. damnosus* (formerly *P. cerevisiae*), *P. parvulus, P. pentosaceus*

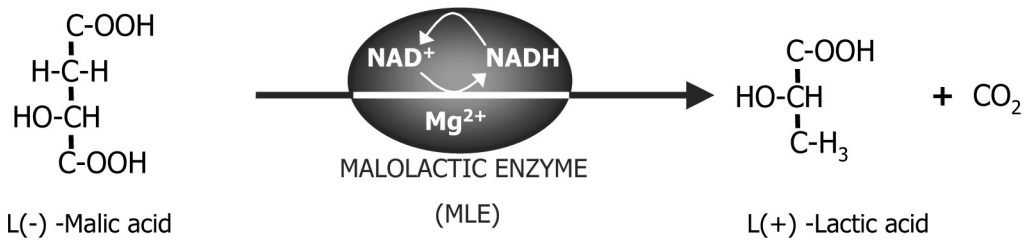

FIGURE 3

The NAD-dependent malolactic enzyme (MLE) transforms the C_4 dicarboxylic acid L-malic acid to the C_3 monocarboxylic acid L-lactic acid without any free intermediates. The conversion is a direct decarboxylation of L(-)-malic acid to L(+)-lactic acid and carbon dioxide (Pilone and Kunkee, 1970).

and in grape must at the early stages of vinification (Fugelsang, 1997; Lafon-Lafourcade *et al*., 1983; Lonvaud-Funel, 1999; Wibowo *et al*., 1985). The prevalence of LAB in grape musts is mainly correlated to the must pH; an increase in pH (pH > 3.5) leads to a higher total number of LAB. However, elevated populations of LAB can also be found in musts from damaged grapes (Lonvaud-Funel, 1999). There are mainly four different genera of LAB present in the must at the beginning of vinification (see Table 2), i.e. *Oenococcus*, *Lactobacilllus, Pediococcus* and *Leuconostoc* (Dicks *et al*., 1990; Dicks and Van Vuuren, 1988; Fugelsang, 1997; Lonvaud-Funel, 1999; Wibowo *et al*., 1985).

During the early days of yeast alcoholic fermentation, the LAB population increases to ca. 10^4 CFU/mL, but quickly declines to only a few cells/mL with the onset of ethanol production (Van Vuuren and Dicks, 1993; Fugelsang, 1997). The decline in LAB population is ascribed to a combination of low initial pH values, low temperatures, increased ethanol concentration, competitive interactions with yeasts, possible bacteriophage infections, as well as high concentrations of SO_2. At the end of alcoholic fermentation, mainly strains of *O. oeni* proliferate to a population of 10^7 CFU/mL, which coincides with malolactic fermentation (Costello *et al*., 1983; Fleet *et al*., 1984; Lonvaud-Funel, 1999; Wibowo *et al*., 1985). Strains of *O. oeni* have the unique ability to survive in a wine milieu at pH values lower than 4.2 and ethanol levels as high as 10% (v/v) (Garvie and Farrow, 1980). Strains of *Pediococcus* and *Lactobacillus* only proliferate in wines with initial high pH levels prior to or during alcoholic fermentation, and usually cause spoilage or sluggish alcoholic fermentations in wines (Costello *et al*., 1983; Fugelsang, 1997).

Advantages and disadvantages of bacterial malolactic fermentation

Malolactic fermentation is the preferred deacidification method used in most of the wine regions of the world. Red wine production in both cold- and warm-climate regions usually involves bacterial malolactic fermentation, naturally or induced, after yeast alcoholic fermentation. Natural malolactic fermentation occurs less frequently in white wines due to an average lower pH of most white cultivars and higher concentrations of SO_2 employed (Rodriquez *et al*., 1990), but it can be induced with LAB starter cultures in some styles of wine, *e.g.* Chardonnay. The malolactic fermentation is crucial in the Champagne wine region of France, where the traditional *méthode champenoise* process is used to produce sparkling wine. The grapes used during the production of base wines are usually high in acid content and require the malolactic fermentation as a primary fermentation to deacidify and mature the base wine prior to the yeast fermentation in the bottle (Pool and Henick-Kling, 1991).

The malolactic fermentation affects four different, but interrelated, aspects of wine quality: wine acidity, microbial stability, sensory complexity and the hygienic quality of wine. Under certain conditions, the contributions made by malolactic fermentation improve wine quality, but the same contributions may be considered highly undesirable under a different set of circumstances (see Table 3) as found in the cool- versus warm-climate wine regions.

Loss of acidity and increase in pH

Depending on the initial pH of the must, the removal of L-malic acid via MLF can be either advantageous or detrimental to wine quality. In high-acid/low-pH wines typically found in the cool-climate regions, a decrease in excess L-malic acid is highly favourable for the production of acid-balanced wines (Boulton *et al*., 1996; Henick-Kling, 1995; Lonvaud-Funel, 1999). MLF usually leads to a reduction in final TA of 1 to 3 g/L and an average increase in pH of 0.1 to 0.3 units in wine (Margalit, 1997). Subsequently, the rise in pH after MLF often promotes the precipitation of potassium bitartrate, leading to an additional reduction in TA (Beelman and Gallander, 1979; Ribéreau-Gayon *et al*., 2000).

TABLE 3

Influence of LAB's metabolism on wine sensory profile.

Bacterial strain	Advantage	Risk
Selected *O. œni*	• Reduction of total acidity • Reduction of ketone and aldehyde compounds (reducing SO_2 requirement) • Partial microbial stability • Reduction of grassy and vegetative notes • Increase in front-pallet volume • More diacetyl level control • Dominance over wild bacteria	• Production of volatile acidity (especially under high pH conditions, in presence of residual sugars and after L-malic acid degradation) • Loss of color • Production of ethyl carbamate
Spontaneous *O. œni*	• Reduction of total acidity • Reduction of ketone and aldehyde compounds (reducing SO_2 requirement) • Partial microbial stability • Reduction of grassy and vegetative notes • Increase in front-pallet volume	• Long lag phase involving an increase in the volatile acidity depending on the pH • Significant bacterial growth involving a high production of diacetyl • Production of spoilage aromas and flavours (mousy off-flavour, sweat, sauerkraut) • Reduction of esters (fruity characters) • Loss of varietal aromas • Color loss by direct action on polyphenols • Production of biogenic amines • Production of ethyl carbamate
L. mesenteroides	• Reduction of total acidity	• Production of viscous compounds (ropy wines) • Production of spoilage aromas and flavours
Lb. plantarum, Lb. casei	• Reduction of total acidity in must or wine • No production of acetic acid from sugar (hexose)	• Sensitive to alcohol over 5% vol. • Sluggish or stuck fermentation in high pH wine at high contamination levels • Production of spoilage aromas and flavours *(Lb.* casei*)*
P. pentosacceus ***P. damnosus***	• Reduction of total acidity in must or wine • No production of acetic acid from sugar (hexose)	• Production of viscous compounds (ropy wines) • Production of biogenic amines • Risk of sluggish or stuck fermentation at pH >3.5, with high contamination levels • Risks increase with the pH value
Lb. brevis ***Lb. hilgardii***	• Reduction of total acidity in must or wine	• Production of viscous compounds (ropy wines) • Production of biogenic amines • High production of ethyl carbamate • Production of spoilage aromas and flavours • Production of acetic acid
Lb. kunkeei		• Strong competition with yeasts during the alcoholic fermentation for nutrients • Overproduction of acetic acid

The incidence of MLF in wine with an initial high pH (or low acidity), usually found in the warmer climatic regions, has the opposite impact on wine quality. MLF in these wines leads to an additional reduction in wine acidity and a subsequent increase in pH due to the degradation of L-malic acid, and results in undesirable "bland" wines that lack adequate acidity. Under these circumstances, the aesthetics of red wine is often negatively affected in terms of red colour intensity, with a potential loss of ca. 30% in red colour due to the shift in pH (Kunkee, 1967; Vetsch and Lüthi, 1964). Furthermore, the risk of spoilage by strains of lactobacilli and pediococci is enhanced in these elevated-pH wines.

Microbial stability

Winemakers have long believed that MLF leads to an increase in microbial stability due to the depletion of essential nutrients in wine and the fastidious growth requirements of LAB, especially *O. oeni*. This might be partly true for high-acid/low-pH wines, where the antimicrobial effect of low pH dominates in inhibiting the growth of spoilage bacteria after the first round of MLF. The apparent depletion of residual nutrients, which includes L-malic acid, citric acid, amino acids, nitrogen bases, vitamins and fermentable sugars left after alcoholic fermentation, prevents the growth of other spoilage bacteria. Furthermore, LAB produce antimicrobial compounds such as lactic acid and bacteriocins (Rammelsberg and Radler, 1990) that inhibit the growth of other related bacterial species (Henick-Kling, 1993). However, spoilage by strains of LAB is often encountered, especially if low concentrations of L-malic acid remain in the wine or the wines have high initial pH levels (Costello *et al*., 1983; Davis *et al*., 1986).

The benefit of increased microbial stability due to MLF is therefore also more applicable to the cool-climate viticultural

regions where the low pH of wine remains an inhibitory factor after completion of MLF. Under these circumstances, complete removal of L-malic acid and citric acid during MLF, which serves as a relatively good nutritional resource for *O. oeni*, does minimise the risk of bacterial growth after bottling. In the warmer viticultural regions, the use of MLF is tolerated in most red and some white cultivars for the sole purpose of completely removing L-malic acid from the wine and contributing to the organoleptic profile of the wine. However, winemakers have to adjust the pH of the wine prior to MLF, usually with tartaric acid, to prevent spoilage by LAB and other bacteria.

Wine sensory modifications.

The role of MLF in improving the sensory complexity of wine is one of the more dubious benefits of MLF when compared to its role in deacidification and microbial stability of wine. Results from various chemical and sensorial studies have shown that numerous flavour-active substances are produced by LAB that are involved in the aroma changes of wine during malolactic fermentation. However, to date, only a few of these compounds and their biosyntheses have been identified (Moreno-Arrabis and Polo, 2005). The most convincing change in wine taste after MLF is the replacement of the strong "green" taste of L-malic acid with the less aggressive taste of lactic acid (Beelman and Gallander, 1979; Lonvaud-Funel, 1999). Removal of the sharp taste of excess L-malic acid is usually described in terms of the mouth feel and extended aftertaste of wine compared to non-MLF control wines (Davis *et al*., 1985; Henick-Kling, 1993; Henick-Kling *et al*., 1994; Malík, 1998). In general, wines that underwent MLF, particularly red wines, are often characterised by lower vegetative/herbaceous aromas, while the fruity and floral characters are also reduced due to the degradation of several esters and other flavour compounds (McDaniel *et al*., 1987; Laurent *et al*., 1994). Many other flavours such as 'buttery', 'lactic', 'nutty', 'oaky', 'yeasty' and 'sweaty' have been described in wines after MLF (Laurent *et al*., 1994).

The improvement of the front-palate volume and roundness in the mouth is not only due to the reduction in acidity of the wine. Certain LAB can produce metabolites that improve mouth feel either directly or by binding with bitter and astringent wine compounds. Specific metabolites synthesised during the metabolism of LAB, especially strains of *O. oeni*, have been identified as flavour compounds in wine and it is argued that these compounds play a role in improving the sensory complexity of wine (McDaniel *et al*., 1987; Rodriquez *et al*., 1990). These metabolites are synthesised in varying concentrations during MLF and include compounds such as acetaldehyde, 2,3-butanediol, acetic acid, acetoin, 2-butanol, and various other volatile esters (such as ethyl lactate, isoamyl acetate, ethyl caproate, diethyl succinate and ethyl acetate) (Meunier and Bott, 1979; Zeeman *et al*., 1982). Diacetyl, a volatile diketone and end product of citric acid metabolism by LAB, is also produced during MLF, and is often perceived as a desirable buttery or nutty flavour when present in low concentrations (Bartowsky & Henschke, 2004; Davis *et al*., 1985).

The exact sensory contribution of MLF in wine is extremely difficult to evaluate due to the intricate nature of the factors that play a role. The number of flavour compounds synthesised during MLF is greatly influenced by the initial wine pH and the fermentation temperature, which determines the rate of malolactic fermentation. When the rate of malolactic fermentation is high (high pH and temperature), the production of acetic acid is enhanced, while the production of diacetyl is favoured under low pH and temperature conditions. The flavour contribution by MLF in wine is further complicated by the large diversity of strains and species of LAB that are usually involved in MLF (see Table 2). For example, individual strains of *O. oeni* contribute different flavour changes to the wine during MLF (Henick-Kling *et al*., 1994; Zeeman *et al*., 1982).

The sensory changes incurred during malolactic fermentation are not always desirable in all wine styles and cultivars. Some delicate European white wines, such as Muscat, Riesling, Sauvignon Blanc and Gewürztraminer, are protected against natural MLF, since the LAB degrade many terpenes and other flavour molecules that diminish the varietal fruity-floral aromas revealed during alcoholic fermentation (Lonvaud-Funel, 1999 Radler, 1972). Furthermore, many of the metabolic products of LAB are usually perceived as off-flavours when produced at elevated levels. For example, the synthesis of diacetyl often masks the characteristics of white wines with heavy notes of butter or cheese that can be considered as off-flavours (Martineau *et al*., 1995). Some strains of LAB, especially the lactobacilli, have the ability to degrade L-tartaric acid, which could lead to severe deficiencies in wine TA, a spoilage defined as *tourne* (Ribéreau-Gayon *et al*., 2000). Another type of off-flavour associated with lactobacilli, *amertume*, is caused by dehydration of glycerol to 3-hydroxypropionaldehyde, which is further reduced to 1,3-propanediol (Schutz and Radler, 1984). Bitterness is caused by a combination of acrolein derived from 3-hydroxypropionaldehyde to phenolic compounds (Rentscher and Tanner, 1951). Some *Lactobacillus* spp. sometimes contributes to a mousy taint due to nitrogen heterocycle compounds produced by the acylation of lysine and ornithine, as shown in *Lactobacillus hilgardii* by Costello *et al*. (2001).

Health factors.

The wholesomeness of wine is becoming an ever-increasingly important marketing tool in the wine trade. Winemakers are therefore steering clear of procedures that could tarnish the hygienic or health image of their wines, *i.e.* the excessive use of detrimental chemicals such as SO_2. The synthesis of two undesirable compounds in wine during conventional malolactic fermentation, namely biogenic amines and ethyl carbamate, have led to a renewed interest in finding alternatives for bacterial wine deacidification (see Table 3).

Biogenic amine production during MLF

LAB are well known to produce biogenic amines during the process of fermentation of foods and beverages (Guerrini *et al*., 2002). Biogenic amines (*e.g.* histamine) are generated via the decarboxylation of naturally occurring amino acids (*e.g.* histidine) through substrate-specific enzymes (Ten Brink *et al*., 1990). The physiological role of this reaction in LAB is thought to ensure growth and survival in acidic conditions, since it increases the pH. Although the biogenic amine content of fermented foods is usually higher than that found in wine, the presence of alcohol, SO_2 and other biogenic amines has been shown to amplify the toxic effect of certain biogenic amines (Fernandes and Ferreira, 2000;Ibe *et al*., 1991; Silla Santos, 1996). For example, the presence of putrescine, the most prevalent amine in wine

(Soufleros *et al*., 1998), is known to act as a potentiator of histamine toxicity in humans (Taylor, 1986).

The concentration of biogenic amines, especially histamine, tyramine and putrescine, in wine is higher after the completion of malolactic fermentation (Lonvaud-Funel and Joyeux, 1994; Radler and Fäth, 1991; Soufleros *et al*., 1998). The biogenic amine content of wine is dependent on the amino acid composition of the wine after alcoholic fermentation and the specific microflora present in wine, but more importantly on the ability of to decarboxylate amino acids. At elevated pH levels, biogenic amines are usually found at higher concentrations in wine due to easier growth and greater bacterial diversity (Lonvaud-Funel and Joyeux, 1994). Commercial cultures of malolactic bacteria are therefore selected not to produce high levels of biogenic amines.

Ethyl carbamate (EC) production during MLF

Arginine, usually one of the most abundant amino acids in grape juice, is metabolised by yeasts to urea, which is released into wine when it accumulates in the yeast cell during or at the end of fermentation. Urea spontaneously reacts with the ethanol in wine to form EC, a naturally occurring compound present in all fermented foods and beverages. There is a general agreement that the presence of EC in wine is not desirable and must be maintained at as low a level as possible since it has been proven to be a carcinogen when administered at high dosages in animal tests (Azevedo *et al*., 2002; Liu and Pilone, 1998). Although, the main source of EC in wine is from the ethanolysis of urea synthesised during yeast metabolism, some strains of LAB can also produce precursors of EC. Most of the commercial *O. oeni* strains are able to break down arginine through the arginine deiminase pathway (ADI pathway), during which citrulline and carbamyl phosphate are excreted, and react with ethanol to produce ethyl carbamate (Liu *et al*., 1994).

Control of malolactic fermentation

Although malolactic fermentation is the most widely accepted deacidification method of wine, the bioconversion of L-malic acid to lactic acid is a difficult and time-consuming process that does not always proceed favourably under the typical conditions of wine. It can lead to spoilage of wine when it occurs after bottling and during storage of wine, especially in wines with pH values higher than 3.5. Malolactic fermentation tends to occur spontaneously in low-acid wines, where it is least wanted, and the outcome of the modified flavours is unpredictable due the proliferation of some *Lactobacillus* and *Pediococcus* spp. (Henick-Kling and Edinger, 1994; Zeeman *et al*., 1982). However, the occurrence of spontaneous malolactic fermentation in wineries is unpredictable and irregular (Boulton *et al*., 1996; Davis *et al*., 1986; Wibowo *et al*., 1985) and may take place immediately after alcoholic fermentation or only weeks or months later, or only after bottling if the wine has not been correctly stabilised (Henick-Kling, 1995). The control of LAB is crucial during winemaking, since their presence at the end of alcoholic fermentation is beneficial, but detrimental to wine quality during maturation, which is solely based on spontaneous chemical reactions that result in the modification of wine aroma and colour (Lonvaud-Funel, 1999).

Numerous physicochemical, chemical and biological conditions in wine influence the development of MLF (Britz and Tracey, 1990; Vaillant *et al*., 1995). The most important factors that influence the onset and completion of MLF are the initial wine composition, fermentation and storage temperature, as well as interactions between LAB and other wine-related microorganisms. Even with a complete understanding of MLF and the factors that play a role in the successful development of MLF, wine remains a hostile environment to LAB. Numerous advances have therefore been made in the field of MLF starter culture production as well as some other alternative technologies for the successful development of MLF in wine. Winemakers can intentionally induce MLF in "resistant" wines by blending them with wine that is undergoing, or which has already completed, MLF (Castino *et al*., 1975). Based on their survival abilities, specific strains of *O. oeni* have been selected for the commercial production of liquid, lyophilised or freeze-dried starter cultures for the inoculation of wine to ensure successful MLF (Henick-Kling, 1995). Although no official data are available on the prevalence of induced MLF in commercial wine production, roughly 75% of all red wines and 40% of white wines usually undergo induced MLF (Maicas, 2001; Ough, 1992).

Starter cultures of LAB are selected according to strict criteria (Davis *et al*., 1985; Vaillant *et al*., 1995). The strains must have a fast growth rate under winemaking conditions and should not produce any off-flavours or off-odours. Malolactic strains are further selected for their tolerance to low pH (pH < 3.0), high ethanol levels of up to 14% (v/v) and non-lysogenic characteristics after phage infection (Drici-Cachon *et al*., 1996). Lastly, the strains should have good growth characteristics and be suitable for drying to make their commercial production economically viable in terms of type and cost of culturing media (Kunkee, 1991). Strains of LAB can also be selected based on their inability to produce biogenic amines such as histamine. With the help of PCR, DNA probes and activity assays, strains of LAB can now be screened for the presence of the amino acid decarboxylase genes (Costantini *et al*., 2006; Le Jeune *et al*., 1995; Lonvaud-Funel, 2001; Marcobal *et al*., 2004;).

The availability of active bacterial starter cultures to induce MLF has certainly contributed to many successful applications of this secondary 'fermentation'. However, the completion of malolactic fermentation cannot be guaranteed and starter culture delay or failure is not unusual under certain conditions (Beelman and Gallander, 1979; Guerzoni *et al*., 1995). Reactivation conditions of freeze-dried starter cultures before inoculation into wine also play a role in the successful completion of malolactic fermentation (Nault *et al*., 1995), since direct inoculation of starter cultures in wine leads to loss of viability (Davis *et al*., 1985; Nielsen *et al*., 1996).

Alternative technologies to develop MLF in wine

Persistent problems with bacterial starter cultures have driven the search for other alternatives to ensure rapid and reliable procession of MLF in wine. The use of bioreactors with free or immobilised strains of LAB, as well as cell-free bioreactors with immobilised malolactic enzyme and cofactors, have been evaluated for application in wine deacidification (Gao and Fleet, 1994; Maicas, 2001; Spettoli *et al*., 1982, 1984, 1987). The major problems associated with these technologies involve the degree of difficulty to prepare matrixes, the use of unacceptable chemicals in wine and the unsuccessful scaling-up of free or immobilised

high-density cell bioreactors for industrial application. Furthermore, cell-free bioreactors using only immobilised enzymes have thus far not been completely effective in converting L-malic acid to lactic acid.

Genetic engineering of strains of *S. cerevisiae* to degrade L-malic acid is an important alternative that has a significant potential for the wine industry. Molecular biologists have attempted to transfer the malolactic activity of LAB into *S. cerevisiae* to enable the yeast to execute the alcoholic and malolactic fermentation simultaneously. Williams *et al*. (1984) expressed the gene for malolactic activity of *L. delbrueckii* in *S. cerevisiae*, but the recombinant strain of *S. cerevisiae* only managed to metabolise 1% and 1.5% (w/v) L-malic acid in synthetic media and grape must, respectively. The L-malic acid assimilating activity from *O. oeni* was also cloned and expressed in *E. coli* and yeast, but due to DNA stability problems the research was not further pursued (Lautensach and Subden, 1984). Nearly a decade later, the *mle*S genes of *L. lactis* and *O. oeni* were subcloned under control of the strong, constitutive 3-phosphoglycerate kinase (*PGK1*) promoter and terminator sequence of *S. cerevisiae* and successfully expressed in a laboratory strain of *S. cerevisiae* (Ansanay *et al*., 1993; Denayrolles *et al*., 1994; 1995; Labarre *et al*., 1996). Both the *E. coli* and *S. cerevisiae* strains containing the *mle*S gene produced malolactic activity, but they were still unable to efficiently degrade L-malic acid under winemaking conditions.

The main limitation of the above attempts to genetically engineer *S. cerevisiae* with a malolactic pathway is ascribed to the slow, inefficient uptake of L-malic acid via simple diffusion by the yeast (Grobler *et al*., 1995). Expression of the malolactic gene (*mle*S) in yeast was therefore not adequate to improve *S. cerevisiae*'s ability to degrade L-malic acid (Ansanay *et al*., 1996). Pre-empting the requirement of a *S. cerevisiae* with a functional malate transport mechanism, cloning and characterisation of the malate permease gene (*mae1*) and the malic enzyme gene (*mae2*) of *S. pombe* were undertaken (Grobler *et al*., 1995; Subden *et al*., 1998; Viljoen *et al*., 1994). Co-expression of the malate transporter gene (*mae1*) of *S. pombe* and the malolactic enzyme gene of *L. lactis* and *O. oeni* in a laboratory strain of *S. cerevisiae* resulted in a recombinant strain of *S. cerevisiae* that actively transported L-malic acid and efficiently converted it to lactic acid (Volschenk *et al*., 1997a, 1997b; 2004). The recombinant *S. cerevisiae* strain was able to perform alcoholic and malolactic fermentation simultaneously, rendering malolactic fermentation with LAB redundant.

The malate transporter gene (*mae1*) of *S. pombe* and the malolactic enzyme gene of *O. oeni* were subsequently stably integrated in the genomes of an industrial Prise de Mousse wine yeast strain in a pioneering endeavour to develop a commercially available wine yeast strain with the ability to degrade L-malic acid during alcoholic fermentation (Husnik *et al*., 2006). The ML01 yeast has received Generally Regarded As Safe (GRAS) status from the US FDA and has been commercialised in the USA and Moldavia. The application of the malolactic wine yeast ML01 has resulted in efficient malolactic fermentation, lower volatile acidity and improved colour properties of wine (Husnik, unpublished). Ethyl carbamate was lower in wines produced with ML01 compared to wines produced with the parental strain S92 and LAB.

CONCLUDING REMARKS

Wine acidity and pH play a crucial role in the winemaking process and the final organoleptic quality and shelf-life of wine. L-Tartaric acid and L-malic are the major grape acids, contributing to more than 90% of the TA in wine (Beelman and Gallander, 1979; Gao and Fleet, 1994; Henick-Kling, 1993; Radler, 1993). The production of premium wines depends on the optimal adjustment of wine acidity in relation to the other wine components to obtain a balanced wine with an optimum flavour and colour profile. In low acid wines, acidulating agents such as L-tartaric acid or D/L-malic acid are routinely added prior to fermentation to increase the TA of must to ensure an optimal acid:sugar ratio in grape must (Beelman and Gallander, 1979; Boulton *et al*., 1996). The traditional method to deacidify wine involves the conversion of L-malic acid to L-lactic acid and CO_2 during malolactic fermentation by strains of *O. oeni*. However, due to inherent problems associated with malolactic fermentation and its unsuitability in some fruity-floral cultivars, alternative biological methods for the deacidification of wine will be of great benefit to the wine industry. Deacidification of wine with a malo-degrading yeast strain may eliminate the use of bacterial starter cultures for malolactic fermentation, which in turn will reduce the risk of the production of hazardous compounds such as biogenic amines and ethyl carbamate. Furthermore, it should decrease delays in cellar operations due to the simultaneous completion of alcoholic fermentation and deacidification, which in turn will minimise the risk of spoilage by oxidation and the proliferation of spoilage microorganisms.

LITERATURE CITED

Ansanay, V., Dequin, S., Blondin, B. & Barre, P., 1993. Cloning, sequence and expression of the gene encoding the malolactic enzyme from *Lactococcus lactis*. FEBS Lett. 332, 74-80.

Ansanay, V., Dequin, S., Camarasa, C., Schaeffer, V., Grivet, J., Blondin, B., Salmon, J. & Barre, P., 1996. Malolactic fermentation by engineered *Saccharomyces cerevisiae* as compared with engineered *Schizosaccharomyces pombe*. Yeast 12, 215-225.

Archer, E. & Strauss, H.C., 1989. Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. S. Afr. J. Enol. Vitic. 10, 74-77.

Azevedo, Z., Couto, J.A. & Hogg, T., 2002. Citrulline as the main precursor of ethyl carbamate in model fortified wines inoculated with *Lactobacillus hilgardii*: a marker of the levels in a spoiled fortified wine. Lett. Appl. Microbiol. 34, 32-36.

Bartowsky, E.J. & Henschke, P.A., 2004. The 'buttery' attribute of wine-diacetyl-desirability, spoilage and beyond. Int. J. Food Microbiol. 96, 235-252.

Beelman, R.B. & Gallander, J.F., 1979. Wine deacidification. Adv. Food Res. 25, 1-53.

Blanke, M.M. & Lenz, F., 1989. Fruit photosynthesis. Plant Cell Environ. 12, 31-46.

Bony, M., Bidard, F., Camarasa, C., Ansanay, V., Dulau, L., Barre, P. & Dequin, S., 1997. Metabolic analysis of *S. cerevisiae* engineered for malolactic fermentation. FEBS Lett. 410, 452-456.

Boulton, R.B., Singleton, V.L., Bisson, L.F. & Kunkee, R.E., 1996. Principles and Practices of Winemaking. Chapman and Hall, New York. pp. 102-181; 244-273; 352-378.

Britz, T.J. & Tracey, R.P., 1990. The combination effect of pH, SO_2, ethanol and temperature on the growth of *Leuconostoc oenos*. J. Appl. Bacteriol. 68, 23-31.

Burns, D.J.W. & Noble, A.C., 1985. Evaluation of the separate contributions of viscosity and sweetness of sucrose to perceived viscosity, sweetness and bitterness of Vermouth. J. Texture Stud. 16, 365-389.

Capucho, I. & San Romao, M.V., 1994. Effect of ethanol and fatty acids on malolactic activity of *Leuconostoc oenos*. Appl. Microbiol. Biotechnol. 42, 391-395.

Carr, F.J., Chill, D. & Maida, N., 2002. The lactic acid bacteria: A literature survey. Crit. Rev. Microbiol. 28, 281-370.

Castino, M., Usseglio-Tomasset, L. & Gandini, A., 1975. Factors which affect the spontaneous initiation of malolactic fermentation in wines. The possibility of transmission by inoculation and its effect on organoleptic properties. In: J.G. Carr, C.V. Cutting, and G.C. Whiting (eds.). Lactic acid bacteria in beverages and food. Academic Press, New York. pp. 139-148.

Charoenchai, C., Fleet, G. & Henscke, P.A., 1998. Effects of temperature, pH and sugar concentration on the growth rates and cell biomass of wine yeasts. Am. J. Enol. Vitic., 49, 283-288.

Costantini, A., Cersosimo, M., Del Prete, V. & Garcia-Moruno, E., 2006. Production of biogenic amines by lactic acid bacteria: screening by PCR, thin-layer chromatography, and high-performance liquid chromatography of strains isolated from wine and must. J. Food Protec. 69, 391-396.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43, 101-110.

Costello, P., Lee, T.H. & Henschke, P.A. 2001. Ability of lactic acid bacteria to produce N-heterocycles causing mousy off-flavour in wine. Aust. J. Grape and Wine Research, 7, 160-167.

Costello, P.J., Morrison, G.J., Lee, T.H. & Fleet, G.H., 1983. Numbers and species of lactic acid bacteria in wines during vinification. Food Technol. Austr. 35, 14-18.

Crippen, D. & Morrison, J.C., 1986. The effect of sun exposure on the compositional development of Cabernet Sauvignon berries. Am. J. Enol. Vitic. 37, 235-242.

Darias-Martin, J.J., Oscar, R., Diaz, E. & Lamuela-Raventós, R.M., 2000. Effect of skin contact on the antioxidant phenolics in white wine. Food Chem. 71, 483-487.

Davies, C. & Robinson, S.P., 2000. Differential screening indicates a dramatic change in mRNA profiles during grape berry ripening: cloning and characterization of cDNAs encoding putative cell wall and stress response proteins. Plant. Physiol. 122, 803-812.

Davis, C.R., Wibowo, D.J., Eschenbruch, R., Lee, T.H. & Fleet, G.H., 1985. Practical implications of malolactic fermentation: A review. Am. J. Enol. Vitic. 36, 290-301.

Davis, C.R., Wibowo, D.J., Lee, T.H. & Fleet, G.H., 1986. Growth and metabolism of lactic acid bacteria during and after malolactic fermentation of wines at different pH. Appl. Environ. Microbiol. 51, 539-545.

Denayrolles, M., Aigle, M. & Lonvaud-Funel, A., 1994. Cloning and sequence analysis of the gene encoding the *Lactococcus lactis* malolactic enzyme: relationships with malic enzymes. FEMS Micriobiol. Lett. 116, 79-86.

Denayrolles, M., Aigle, M. & Lonvaud-Funel, A., 1995. Functional expression in *Saccharomyces cerevisiae* of the *Lactococcus lactis mle*S gene encoding the malolactic enzyme. FEMS Microbiol. Lett. 125, 3744.

Diakou, P., Svanella, L., Raymond, P., Gaudillère, J. & Moing, A., 2000. Phosphoenolpyruvate carboxylase during grape berry development: protein level, enzyme activity and regulation. Aust. J. Plant Physiol. 27, 221-229.

Dicks, L.M.T. & van Vuuren, H.J.J., 1988. Identification and physiological characteristics of hetero-fermentative strains of *Lactobacillus* from South African red wines. J. Appl. Bacteriol. 64, 505-513.

Dicks, L.M.T., van Vuuren, H.J.J. & Dellaglio, F., 1990. Taxonomy of *Leuconostoc* species, particularly *Leuconostoc oenos*, as revealed by numerical analysis of total soluble cell protein patterns, DNA-base composition, and DNA-DNA hybridizations. Int. J. Syst. Bacteriol. 40, 83-91.

Drici-Cachon, Z., Guzzo, J., Cavin, J.-F. & Diviès, C., 1996. Acid tolerance in *Leuconostoc oenos*. Isolation and characterization of an acid-resistant mutant. Appl. Microbiol. Biotechnol. 44, 785-789.

Du Plessis, H.W., Dicks, L.M.T., Pretorius, I.S., Lambrechts, M.G. & Du Toit, M. 2004. Identification of lactic acid bacteria isolated from South African brandy base wines. Int. J. Food Microbiol. 91, 19- 29.

Fernandes, J.O. & Ferreira, M.A., 2000. Combined ion-pair extraction and gas chromatography-mass spectrometry for the simultaneous determination of diamines, polyamines and aromatic amines in port wine and grape juice. J. Chromatogr. A. 886, 183-195.

Ferreira, B., Hory, C., Bard, M.H., Taisant, C., Olsson, A. & le Fur, Y., 1995. Effects of skin contact and settling on the level of the C18:2, C18:3 fatty acids and C_6 compounds in Burgundy Chardonnay musts and wines. Food Qual. Pref. 6, 35-41.

Fillion, L., Ageorges, A., Picaud, S., Coutos-Thévenot, P., Lemoine, R., Romieu, C. & Delrot, S., 1999. Cloning and expression of a hexose transporter gene expressed during the ripening of grape berry. Plant. Physiol. 120, 1083-1093.

Fischer, U. & Noble, A.C., 1994. The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. Am. J. Enol. Vitic. 45, 6-10.

Fleet, G.H., Lafon-Lafoucade, S. & Ribéreau-Gayon, P., 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl. Environ. Microbiol. 48, 1034-1038.

Fugelsang, K.C., 1997. In: Wine Microbiology. Chapman and Hall, New York.

Gallander, J.F., 1977. Deacidification of Eastern table wines with *Schizosaccharomyces pombe*. Am. J. Enol. Vitic. 28, 65-68.

Gao, C. & Fleet, G.H., 1994. The degradation of malic acid by high-density cell suspensions of *Leuconostoc oenos*. J. Appl. Bacteriol. 76, 632-637.

Garvie, E.I. & Farrow, J.A., 1980. The differentiation of *Leoconostoc oenos* from non-acidophilic species of *Leuconostoc*, and the identification of five strains from the American Type Culture Collection. Am. J. Enol. Vitic. 31, 154-157.

Grobler, J., Bauer, F., Subden, R.E. & van Vuuren, H.J.J., 1995. The *mae1* gene of *Schizosaccharomyces pombe* encodes a permease for malate and other C_4 dicarboxylic acids. Yeast 11, 1485-1491.

Guerrini, S., Mangani, S., Granchi, L. & Vincenzini, M., 2002. Biogenic amine production by *Oenococcus oeni*. Curr. Microbiol. 44, 374-378.

Guerzoni, M.E., Sinigaglia, M., Gardini, F., Ferruzzi, M. & Torriani, S., 1995. Effects of pH, temperature, ethanol, and malate concentration on *Lactobacillus plantarum* and *Leuconostoc oenos*: Modeling of the malolactic activity. Am. J. Enol. Vitic. 46, 368-374.

Hashizume, T. & Mori, E.E.M., 1990. Evaluation of apple cultivars for cider making. Colet. Inst. Technol. Aliment. 20, 73-82.

Henick-Kling, T., 1993. Malolactic fermentation. In: G.H. Fleet (ed.). Wine Microbiology and Biotechnology. Harwood Academic Publishers, Switzerland. pp. 289-326.

Henick-Kling, T., 1995. Control of malolactic fermentation in wine: energetics, flavour modification and methods of starter culture preparation. J. Appl. Bacteriol. S79, 29S-37S.

Henick-Kling, T., Acree, T.E., Krieger, S.A., Laurent, M.H. & Edinger, W.D., 1994. Modification of wine flavour by malolactic fermentation. Wine East. March/April, 8-15; 29-30.

Henick-Kling, T. & Edinger, W.D., 1994. Malolactic starter cultures for wine. In: T. Henick-Kling (ed.). Proc. 23rd Ann. New York Wine Ind. Works, New York State Agricultural Experiment Station, Cornell University, Geneva. pp. 107-115.

Husnik, J.I., Volschenk, H., Bauer, J., Colavizza, D., Luo, Z. & van Vuuren, H.J.J., 2006. Metabolic engineering of malolactic wine yeast. 2006. Metabol. Eng. 8, 315-323.

Ibe, A., Saito, K., Nakazato, M., Kikuchi, Y., Fujinuma, K. & Nishima, T., 1991. Quantitative determination of amines in wine by liquid chromatography. J. Assoc. Off. Anal. Chem. 74, 695-698.

Jackson, D.I., 2001. Climate-monographs in cool climate viticulture 2. Daphne Brasell Associates Ltd., Wellington, New Zealand

Jackson, D. & Schuster, D., 1997. The grape and its growth. In: The Production of Grapes & Wine in Cool Climates. Lincoln University Press, Canterbury, New Zealand. pp. 50-54.

Kanellis A.K. & Roubelakis-Angelakis, K.A., 1996. Grape. In: G.B. Seymour, J.E. Taylor, G.A. Tucker (eds.). Biochemistry of Fruit Ripening. Chapman and Hall, London. pp. 189-234.

Kliewer, W. M., Howarth, L. & Omori, M., 1967. Concentration of tartaric acid and malic acid and their salts in *Vitis vinifera* grapes. Am. J. Enol.Vitic. 18, 42-54.

Kluba, R.M. & Beelman, R.B., 1975. Influence of amelioration on the major acid components of musts and wine from four French-hybrid grape cultivars. Am. J. Enol. Vitic. 26, 18-24.

Kunkee, R.E., 1967. Control of malolactic fermentation induced by *Leoconostoc citrovorum*. Am. J. Enol. Vitic. 18, 71-77.

Kunkee, R.E., 1991. Some roles of malic acid in the malolactic fermentation in wine making. FEMS Microbiol. Rev. 88, 55-72.

Labarre, C., Guzzo, J., Cavin, J.-F. & Diviès, C., 1996. Cloning and characterization of the genes encoding the malolactic enzyme and the malate permease of *Leuconostoc oenos*. Appl. Environ. Microbiol. 62, 1274-1282.

Lafon-Lafourcade, S., Lonvaul-Funel, A. & Carré, E., 1983. Lactic acid bacteria of wines: stimulation of growth and malolactic fermentation. Antonie Van Leeuwenhoek. 49, 349-352.

Laurent, M.H., Henick-Kling, T. & Acree, T.E., 1994. Changes in the aroma and odor of Chardonnay due to malolactic fermentation. Wein Wissensch. 49, 3-10.

Lautensach, A. & Subden, R.E., 1984. Cloning of malic acid assimilating activity from *Leuconostoc oenos* in *E. coli*. Microbios. 39, 29-39.

Lavee, S. & Nir, G., 1986. Grape. In: S.P. Monselise (ed.). CRC handbook of fruit set and development. CRC Press, Boca Raton, Florida, USA. pp. 167-191.

Le Jeune, C., Lonvaul-Funel, A., Ten Brink, B., Hofstra, H. & van der Vossen, J.M., 1995. Development of a detection system for histidine decarboxylation lactic acid bacteria based on DNA probes, PCR and activity test. J. Appl. Bacteriol. 78, 316-326.

Liu S.Q. & Pilone, G.J., 1998. A review: arginine metabolism in wine lactic acid bacteria and its practical significance. J. Appl. Microbiol. 84, 315-327.

Liu, S.Q., Prichard, G.C., Hardman, M.J. & Pilone, G.C., 1994. Citrulline production and ethyl carbamate (urethane) precursor formation from arginine degradation by wine lactic acid bacteria *Leuconostoc oenos* and *Lactobacillus buchneri*. Am. J. Enol. Vitic. 45, 235-242.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek. 76, 317-331.

Lonvaud-Funel, A., 2001. Biogenic amines in wines: role of lactic acid bacteria. FEMS Microbiol. Lett. 199, 9-13.

Lonvaud-Funel, A. & Joyeux, A., 1994. Histamine production by wine lactic acid bacteria: isolation of a histamine-producing strain of *Leuconostoc oenos*. J. Appl. Bacteriol. 77, 401-407.

Maicas, S., 2001. The use of alternative technologies to develop malolactic fermentation in wine. Appl. Microbiol. Biotechnol. 56, 35-39.

Malík, F., 1998. Bases of enology. In: M. Berovic (Ed.). Bioprocess engineering course. National Institute of Chemistry, Ljubljana. pp. 182-206.

Marcobal, A., de las Rivas, B., Moreno-Arribas, M.V. & Muñoz, R., 2004. Identification of the ornithine decarboxylase gene in the putrescine-producer *Oenococcus oeni* BIFI-83. FEMS Microbiol. Lett. 239, 213-220.

Margalit, Y., 1997. In: J. Crum (ed.). Concepts in Wine Chemistry. Wine Appreciation Guild Ltd., San Fransisco, USA. pp. 16-18; 76-82.

Martin, N. & Revel, G., 1999. Sensory evaluation: scientific bases and enological application. J. Int. des Sciences de la Vigne et du Vin, Special Issue: Wine tasting, June, 81-93.

Martineau, B., Henick-Kling, T. & Acree, T., 1995. Reassessment of the influence of malolactic fermentation on the concentration of diacetyl in wines. Am. J. Enol. Vitic. 46, 385-388.

McDaniel, M., Henderson, L.A., Watson, B.T. & Heatherbell, D., 1987. Sensory panel training and screening for descriptive analysis of the aroma of Pinot Noir wine fermented by several strains of malolactic bacteria. J. Sens. Stud. 2, 149-167.

Meunier, J.M. & Bott, E.W., 1979. Das berhalten vershiender Aromastoffe in Bergunder-weinen im verlauf des biologischen Säureabbaus. Chem. Mikrobiol. Technol. Lebensm. 6, 92-95.

Moreno-Arrabis, M.V. & Polo, M.C., 2005. Winemaking biochemistry and microbiology: Current knowledge and future trends. Crit. Rev. Food Sci. Nutr. 45, 265-286.

Morrison, J.C. & Noble, A.C., 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. Am. J. Enol. Vitic. 41, 193-200.

Narendranath, N.V., Hynes, H.S., Thomas, K.C. & Ingledew, W.M., 1997. Effects of lactobacilli on yeast-catalyzed ethanol fermentations. Appl. Environ. Microbiol. 63, 4158-4163.

Nault, I., Gerbaux, V., Larpent, J.P. & Vayssier, Y., 1995. Influence of pre-culture conditions on the ability of *Leuconostoc oenos* to conduct malolactic fermentation in wine. Am. J. Enol. Vitic. 46, 357-362.

Nielsen, J.C., Prahl, C. & Lonvaul-Funel, A., 1996. Malolactic fermentation in wine by direct inoculation with freeze-dried *Leuconostoc oenos* cultures. Am. J. Enol. Vitic. 47, 42-48.

Noble, A.C., 1998. Why do wines taste bitter and feel astringent? In: A.L. Waterhouse and S.E. Ebeler (eds.). Chemistry of wine flavour. Oxford University Press, Washington, DC, USA. pp. 156-165.

Or, E., Baybik, J., Sadka, A & Ogrodovitch, A., 2000. Fermentative metabolism in grape berries: isolation and characterization of pyruvate decarboxylase cDNA and analysis of its expression throughout berry development. Plant Sci. 156, 151-158.

Ough, C.S., 1992. Winemaking basics. Haworth Press, Binghampton, New York.

Pilone, G.J. & Kunkee, R.E., 1970. Carbonic acid from decarboxylation by "malic" enzyme in lactic acid bacteria. J. Bacteriol. 103, 404-409.

Pool, R. & Henick-Kling, T., 1991. Production methods in Champagne. New York State Agricultural Experiment Station, Cornell University, New York.

Pratelli, R., Lacombe, B., Torregrosa, L., Gaymard, F., Romieu, C., Thibaud, J. & Sentenac, H., 2002. A grapevine gene encoding a guard cell K^+ channel displays developmental regulation in the grapevine berry. Plant Physiol. 128, 564-577.

Pretorius, I.S., 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16, 675-729.

Radler, F., 1972. Problems in malolactic fermentation. Weinberg. Keller 19, 357-370.

Radler, F., 1993. Yeasts-metabolism of organic acids. In: G.H. Fleet (ed.). Wine Microbiology and Biotechnology. Harwood Academic Publishers, Switzerland. pp. 165-182.

Radler, F. & Fäth, K.P., 1991. Histamine and other biogenic amines in wine. In: J.M Rantz (ed.). Proc. Int. Symp. Nitrogen Grape Wine. , Am. Soc. Enol. Vitic., Davis, California. pp. 185-195.

Rammelsberg, M. & Radler, F., 1990. Antibacterial polypeptides of *Lactobacillus* species. J. Appl. Bacteriol. 69, 177-184.

Rentscher, H. & Tanner, H. 1951. Das bitterwerden der rotweine. Mitt.Gebeite Lebensmittelunster 42,463-475.

Ribéreau-Gayon, P., Dubourdieu, D., Donéche B. & Lonvaud, A., 2000. Handbook of Enology. Wiley & Sons, Ltd., Chichester, England.

Rodriquez, S.B., Amberg, E. & Thornton, R.J., 1990. Malolactic fermentation in Chardonnay: Growth and sensory effects of commercial cultures of *Leoconostoc oenos*. J. Appl. Bacteriol. 68, 139-144.

Ruffner, H.P., 1982. Metabolism of tartaric and malic acids in *Vitis*: a review – part B. Vitis. 21, 346-358.

Ruffner, H.P. & Kliewer, W.M., 1975. Phosphoenolpyruvate carboxykinase activity in grape berries. Plant Physiol. 56, 67-71.

Ruffner, H.P., Possner, D., Brem, S. & Rast, D.M., 1984. The physiological role of malic enzyme in grape ripening. Planta 160, 444-448.

Schutz, H. & Radler. F. 1984. Anaerobic reduction of glycerol to propoanediol 1,3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. Syst. Appl. Microbiol. 5, 169-178.

Silla Santos, M.H., 1996. Biogenic amines: their importance in foods. Int. J. Food Microbiol. 29, 213-231.

Smart, R.E., 1985. Principles of grapevine canopy microclimate manipulation with implications of yield and quality – a review. Am. J. Enol. Vitic. 36, 230-239.

Soufleros, E., Barrios, M.L. & Bertrand, A., 1998. Correlation between the content of biogenic amines and other wine compounds. Am. J. Enol. Vitic. 49, 266-278.

Spettoli, P., Bottacin, A., Nuti, M.P. & Zamorani, A., 1982. Immobilization of *Leuconostoc oenos* ML34 in calcium alginate gels and its application to wine technology. Am. J. Enol. Vitic. 33, 1-5.

Spettoli, P., Nuti, M.P., Crapici, A. & Zamorani, A., 1987. Technological improvement of malolactic fermentation in wine by immobilized microbial cells in a continuous flow reactor. Ann. N.Y. Aca. Sci. 501, 385-389.

Spettoli, P., Nuti, M.P. & Zamorani, A., 1984. Properties of malolactic activity purified from *Leuconostoc oenos* ML34 by affinity chromatography. Appl. Environ. Microbiol. 48, 900-901.

Strauss, C.R., Gooley, P.R., Wilson, B. & Williams, P.J., 1987. Development of precursors of C_{13}-norisoprenoid flavorants in Riesling grapes. Am. J. Enol. Vitic. 38, 23.

Subden, R.E., Krizus, A., Osothsilp, C., Viljoen, M. & van Vuuren, H.J.J., 1998. Mutational analysis of malate pathways in *Schizosaccharomyces pombe*. Food Res. Int. 31, 37-42.

Taylor, S.L., 1986. Histamine food poisoning: toxicology and clinical aspects. Crit. Rev. Toxicol. 17, 91-128.

Ten Brink, B., Damink, C., Joosten H.M.L.J. & Huis in't Veld, J.H.J., 1990. Occurrence and formation of biologically active amines in foods. Int. J. Food Microbiol. 11, 73-84.

Terrier, N., Sauvage, F.X., Ageorges, A. & Romieu, C., 2001. Changes in acidity and in proton transport at the tonoplast of grape berries during development. Planta 213, 20-28.

Vaillant, H., Formisyn, P. & Gerbaux, V., 1995. Malolactic fermentation of wine: study of the influence of some physio-chemical factors by experimental design assays. J. Appl. Bacteriol. 79, 640-650.

Vannier, A., Brun, O.X. & Feinberg, M.H., 1999. Application of sensory analysis to champagne wine characterisation and discrimination. Food Qual. Pref. 10, 101-107.

Van Vuuren, H.J.J. & Dicks, L.M.T., 1993. *Leuconostoc oenos*: a review. Am. J. Enol. Vitic. 44, 99-112.

Vetsch, U. & Lüthi, H., 1964. Decolorisation of red wines during biological decomposition of acids. Mitt. Geb. Lebensm. Hyg. 55, 93-98.

Viljoen, M., Subden, R.E., Krizus, A. & van Vuuren, H.J.J., 1994. Molecular analysis of the malic enzyme gene (*mae2*) of the yeast *Schizosaccharomyces pombe*. Yeast 10, 613-624.

Volschenk, H., Viljoen, M., Grobler, J., Bauer, F., Lonvaud-Funel, A., Denayrolles, M., Subden, R.E. & van Vuuren, H.J.J., 1997a**.** Malolactic fermentation in grape musts by a genetically engineered strain of *Saccharomyces cerevisiae*. Am. J. Enol. Vitic. 48, 193-196.

Volschenk, H., Viljoen, M., Grobler, J., Petzold, B., Bauer, F., Subden, R.E., Young, R.A., Lonvaud, A., Denayrolles, M. & van Vuuren, H.J.J., **1997b.** Engineering pathways for malate degradation in *Saccharomyces cerevisiae*. Nature Biotech. 15, 253-257.

Volschenk, H., Viljoen–Bloom, M., van Staden, J., Husnik, J. & van Vuuren, H.J.J., 2004. Genetic engineering of an industrial strain of *Saccharomyces cerevisiae* for malic acid degradation via an efficient malo-ethanolic pathway. S. Afr. J. Enol. Vitic. 25, 63-73.

Wibowo, D., Eschenbruch, R., Davis, C.R., Fleet, G.H. & Lee, T.H., 1985. Occurrence and growth of lactic acid bacteria in wine: A review. Am. J. Enol. Vitic. 36, 302-313.

Williams, S.A., Hodges, R.A., Strike, T.L., Snow, R. & Kunkee, R.E., 1984. Cloning the gene for the malolactic fermentation of wine from *Lactobacillus delbrueckii* in *Escherichia coli* and yeasts. Appl. Environ. Microbiol. 47, 288-293.

Winterhalter, P., Sefton, M.A. & Williams, P.J., 1990. Volatile C_{13} norisoprenoid compounds in Riesling wine are generated from multiple precursors. J. Agric. Food Chem. 41, 277-283.

Zeeman, W., Snyman, J.P. & van Wyk, C.J., 1982. The influence of yeast strain and malolactic fermentation on some volatile bouquet substances and on the quality of table wines. In: A.D. Webb (ed.). Proc. Univ. of Calif., Grape Wine Cent. Symp. University of California, Davis. pp. 79-90.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. & Nury, F.S., 1995. Wine Analysis and Production. Chapmann & Hall, New York.