Growth and Metabolism of Lactic Acid Bacteria during and after Malolactic Fermentation of Wines at Different pH

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Commercially produced red wines were adjusted to pH 3.0, 3.2, 3.5, 3.7, or 4.0 and examined during and after malolactic fermentation for growth of lactic acid bacteria and changes in the concentrations of carbohydrates, organic acids, amino acids, and acetaldehyde. With one exception, Leuconostoc oenos conducted the malolactic fermentation in all wines and was the only species to occur in wines at pH below 3.5. Malolactic fermentation by L. oenos was accompanied by degradation of malic, citric, and fumaric acids and production of lactic and acetic acids. The concentrations of arginine, histidine, and acetaldehyde also decreased at this stage, but the behavior of hexose and pentose sugars was complicated by other factors. Pediococcus parvulus conducted the malolactic fermentation in one wine containing 72 mg of total sulfur dioxide per liter. Fumaric and citric acids were not degraded during this malolactic fermentation, but hexose sugars were metabolized. P. parvulus and species of Lactobacillus grew after malolactic fermentation in wines with pH adjusted above 3.5. This growth was accompanied by the utilization of wine sugars and production of lactic and acetic acids.

Lactic acid bacteria make a significant contribution to the quality of wines by conducting malolactic fermentation and by causing spoilage at later stages of vinification (3, 17, 18, 20). Wine composition and, consequently, wine quality are altered by these bacteria, because some wine components are utilized as substrates for growth and metabolic end products are excreted into the wine.

Many qualitative studies (for a review, see references 3, 17, and 18) and more recent quantitative data (9, 11, 19) have shown that Leuconostoc oenos is the species most frequently occurring in wine and is mainly responsible for the malolactic fermentation. Species of Pediococcus and Lactobacillus have been isolated to a lesser extent and are more likely to occur at stages after malolactic fermentation in wines of higher pH (3.5 to 4.0). A major weakness of the ecological studies so far reported has been the infrequency and irregularity of analysis of wines during the critical periods when lactic acid bacteria grow. Such a problem has arisen because wineries from which wine samples are obtained are generally located at considerable distances from research laboratories (9). Consequently, a complete ecological description of the growth of lactic acid bacteria in a particular wine at close time intervals during vinification is still lacking; this ecology would be particularly complex in the higher-pH wines, in which the successive growth and death of several species may occur (9, 11).

The chemical changes in the composition of wine that are caused by lactic acid bacteria are not well defined, except for the conversion of malic acid to lactic acid during malolactic fermentation by L. oenos and to a lesser degree by species of Pediococcus and Lactobacillus (3, 17, 18, 20, 27). Isolated studies suggest that wine carbohydrates (10, 24, 28) and amino acids (3, 23, 34) may be utilized by these bacteria during the malolactic fermentation and that this metabolism as well as that of organic acids (3, 17, 20, 27, 28) can lead to changes in the concentration of constituents which affect the sensory quality of wines. The metabolism of wine components by lactic acid bacteria after malolactic fermentation is largely unstudied. Quantitative studies that correlate the kinetics of bacterial growth in wines with changes in the concentration of specific wine constituents have not been conducted. Consequently, the biochemical mechanisms by which lactic acid bacteria grow in wines are still poorly understood.

In this paper we report the enumeration, isolation, and identification of lactic acid bacteria from several wines at close time intervals during and after malolactic fermentation. The growth of isolated species is correlated with their utilization of wine sugars, organic acids, and amino acids. The effect of wine pH on both the species isolated and their utilization of wine components is also reported.

MATERIALS AND METHODS

Wine samples. Shiraz wine was taken from wineries A and B (50 liters from each) immediately after completion of alcoholic fermentation (about 7 days after the crushing of the grapes) and was transported to the University of New South Wales laboratory for storage and analysis. The two wineries are located in the Hunter Valley district of New South Wales, Australia. At the laboratory, the wines were racked off the lees and dispensed as 4-liter volumes in sterile glass flasks that were fitted with air locks. Duplicate volumes of each wine were adjusted to the desired pH by the addition of either sterile 50% tartaric acid or 5 M potassium hydroxide. The pH of one set of duplicates remained unadjusted. Wines were stored at 20°C and were sampled regularly for analysis of lactic acid bacteria, organic acids, monosaccharides, pH, and (at some sampling times) acetaldehyde and amino acids. Samples (30 ml) for analysis were taken from the center of the flask after thorough mixing of the contents. The flasks were flushed with CO₂ at each sampling. Microbiological and pH analyses were conducted immediately after sampling. The

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sample was then filtered through a membrane (pore size, 0.45 μm) and frozen until required for chemical analysis.

Wines from several other wineries were obtained and stored as described above, but without pH adjustment.

**Enumeration and identification of lactic acid bacteria.** Lactic acid bacteria were enumerated by spread-inoculating 0.1 ml of the wine sample (diluted if necessary) over the surface of plates of MRS agar (Oxoid Ltd., London, England) and tomato juice agar (9, 25). Plates were incubated in an atmosphere of 5% CO₂ for 7 days at 30°C. Plating media contained 100 μg of cycloheximide per ml to suppress the growth of yeasts. Colonies that grew on the surface of the plates and which were gram positive and catalase negative (9, 25) were counted and identified as lactic acid bacteria. Several representatives of each colony type were subcultured and identified to genus and species level by the tests described previously for *L. oenos* (13), for *Pediococcus parvulus* (2, 14), and for isolates of *Lactobacillus* (29, 30).

Carbohydrate fermentation tests were conducted in API 50 CH galleries (La Balmes les Gottes-38390, Montalieu Vercin, France).

**Chemical analyses.** Hexose and pentose sugars were separated and determined by gas-liquid chromatography (GLC) of their per-o-acetyladonitrile derivatives. Glucitol and myo-inositol were also determined by GLC but as acetate derivatives. Procedures for the derivatization of the sugars have been previously described (35). The derivatized sugars were separated by injection into a 2100 Aerograph chromatograph (Varian Associates, Palo Alto, Calif.) equipped with a glass column (1 m by 3 mm inner diameter) packed with 10% DEGS on Chromosorb W/AW 80/100; nitrogen was the carrier gas at a flow rate of 60 ml/min. Injector and detector temperatures were 190 and 250°C, respectively, and the oven temperature was programmed from 140 to 190°C at 1°C/min. Before derivatization, wine samples were treated to remove substances that interfered with the resolution of the sugars by GLC. This involved passage of 1.0 ml of the wine sample first through a small column (1.0 ml) containing mixed-anion (Bio-Rad AG-1 × 8, acetate form, 100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) and cation (Dowex-1 × 8, H⁺ form, 100/200 mesh) resins and then through a C18 Sep-Pak (Waters Associates, Inc., Milford, Mass.). The columns were eluted with distilled water, and the eluate containing the sugars was concentrated by freeze-drying. The dried residue was used for derivatization. Studies with spiked wine samples indicated that the pretreatment and derivatization procedures gave greater than 85% recovery of each sugar. The identification of wine carbohydrates was confirmed by combined GLC-mass spectrometry on a Finnigan 4021 instrument at the Australian Wine Research Institute.

GLC performed by the method described above did not satisfactorily separate either glucose from mannitol or mannose from 2-deoxyglucose. Consequently, the concentrations of glucose and mannose, as well as those of fructose and glycerol, were determined enzymatically by using kits from Boehringer GmbH, Mannheim.

Organic acids were measured by high-pressure liquid chromatography (HPLC) with a chromatograph (Waters Associates) equipped with an ion exclusion, cation exchange column (Bio-Rad HPX-87H) and a detector operating at 210 nm. The column was eluted with 0.1% phosphoric acid at 55°C at a flow rate of 0.5 ml/min. Before HPLC, wine samples were filtered through a membrane (pore size, 0.45 μm) and passed through a C18 Sep-Pak. This procedure yielded >95% recovery of the organic acids found in wine.

The concentration of malic acid was also determined by an enzymatic method with a kit from Boehringer.

The free amino acids were separated by HPLC and estimated by post-column derivatization with orthophthalaldehyde (16). The technology and equipment used for these analyses were from Waters Associates. The chromatograph was equipped with an amino acid analysis column (Waters Associates) that was gradient eluted with sodium eluent A and sodium eluent B buffers (Waters Associates). Acetaldehyde was measured enzymatically by using a kit from Boehringer.

**RESULTS**

**Shiraz wine from winery A.** The alcohol and total sulfur dioxide (SO₂) concentrations of the Shiraz wine from winery A were 14.2% and 11.2 mg/liter, respectively. Samples were adjusted to pH 3.0, 3.2, 3.7, or 4.0, and one sample remained unadjusted at pH 3.5. The growth of bacteria in the wines at pH 3.2 and 3.7 is shown in Fig. 1 and 2 along with some changes in wine composition.

*L. oenos* was present at initial levels of 10 to 100 cells per ml, and after a lag period of 1 to 2 days, grew to a maximum population of 10⁷ to 10⁸ cells per ml in wines at pH 3.2, 3.5,
concentration of malolactic fermentation of Shiraz wine (pH 3.7) from winery A. (a) L. oenos (○), P. parvulus (△), and Lactobacillus cellobiosus (□). Symbols for panels b, c, and d are the same as for Fig. 1.

3.7, and 4.0. The growth rate increased as the wine pH was increased. The maximum population was achieved at 44 days after crushing in wine at pH 3.2 and at 17 days after crushing in wine at pH 4.0. No growth occurred in wine at pH 3.0. The malolactic fermentation occurred in conjunction with the growth of L. oenos and was indicated both by the complete degradation of malic acid and by a parallel increase in concentration of lactic acid to about 1.8 g/liter. The pH of the wines increased by approximately 0.3 U during this period. No species other than L. oenos was detected in the wines during the malolactic fermentation.

Other chemical changes that occurred during malolactic fermentation in wines at pH 3.2 and 3.7 are shown in Fig. 1 and 2, respectively. The trends were the same for the wines at pH 3.5 and 4.0, although the time scales were different. Citric and fumaric acids were completely degraded, and the concentration of acetic acid increased by about 0.1 g/liter. Glucose concentration doubled from 90 to approximately 160 mg/liter by the end of the fermentation, and fructose concentration also increased, although to a lesser extent. Both arabinose and mannose concentrations decreased during malolactic fermentation. However, the concentration of galactose, ribose, xylose, rhamnose, glycerol, myo-inositol, glucitol (data not shown), and an unidentified monosaccharide (U1) remained unchanged. Mass spectral data indicated that U1 is probably a hexose glycoside, but further identification studies are required. Increases in the concentrations of glucose and fructose and decreases in the concentrations of mannose and arabinose were also observed in the wine at pH 3.0, in which there was no bacterial growth and no malolactic fermentation.

The microbiological and chemical changes that occurred in the wines after malolactic fermentation depended upon pH. Data for the wines at pH 3.2 and 3.7 are presented in Fig. 1 and 2, respectively. L. oenos survived at a level of approximately 10^6 cells per ml in the wine at pH 3.2 and was the only species isolated from this wine. The concentrations of glucose and fructose continued to increase, and those of mannose and arabinose continued to decrease. The concentrations of ribose, xylose, and, to a lesser extent, rhamnose increased slightly.

L. oenos remained at a level of 10^6 cells per ml in the wine at pH 3.5 during the postmalolactic period. However, an unidentified species of Lactobacillus was also isolated from this wine at 60 days after crushing, and it subsequently grew to a population of 10^7 cells per ml to coexist with L. oenos. The growth of this species coincided with small but significant decreases in the concentrations of glucose, fructose, myo-inositol, and ribose, indicating that they were being utilized as growth substrates. The concentrations of arabinose and mannose continued to decrease in the same fashion as did the wine at pH 3.2 and did not specifically correlate with the growth of the Lactobacillus sp.

Both P. parvulus and Lactobacillus cellobiosus were isolated from the wine at pH 3.7 during the postmalolactic fermentation period. These species grew to populations of 10^6 to 10^7 cells per ml, and when this occurred, L. oenos was no longer isolated from the wine (Fig. 2). The growth of P. parvulus and Lactobacillus cellobiosus was accompanied by substantial changes in the chemical composition of the wine. Lactic and acetic acid concentrations increased from 1.8 and 0.4 g/liter, respectively, at the end of malolactic fermentation to 3 and 1.65 g/liter, respectively, and wine pH decreased from 3.95 to 3.72. The concentrations of glucose, fructose, myo-inositol, glycerol, ribose, xylose, and U1 decreased markedly (Fig. 2). The concentration of arabinose also decreased, although not in coincidence with bacterial growth. Mannose, after virtually complete utilization, later increased to a level between 25 and 30 mg/liter.

P. parvulus and an unidentified species of Lactobacillus grew in the wine at pH 4.0 during the period of postmalolactic fermentation and achieved populations of 10^7 cells per ml by 30 and 45 days, respectively, after grape crushing. However, both L. oenos and the Lactobacillus sp. disappeared by 71 days, leaving P. parvulus as the surviving organism. The chemical changes that occurred in this wine were similar to those of the wine at pH 3.7 except that galactose was also utilized.

Acetaldehyde, present at 15 mg/liter at the end of alcoholic fermentation, was partially degraded in all wines during malolactic fermentation. The proportion degraded increased from 13.6 to 29% as the wine pH increased from 3.2 to 4.0.

Table 1 shows the concentration of some amino acids in the wine at pH 3.7 just before malolactic fermentation, at the end of this reaction (62 days) and at a later stage (100 days) of storage. With the exception of histidine and arginine, which were completely utilized during malolactic fermentation, the concentrations of all individual amino acids increased slightly during this fermentation and storage. Similar data were found for the wine at pH 3.2, except that the increases in concentrations were slightly smaller.

Shiraz wine from winery B. Shiraz wine from winery B
TABLE 1. Effect of malolactic fermentation and subsequent conservation on the concentration of some amino acids of a Shiraz wine at pH 3.7

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Conc (mmol/liter)</th>
<th>Before MLF&lt;sup&gt;a&lt;/sup&gt; (10 days)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>After completion of MLF (62 days)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>After storage (100 days)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>0.015</td>
<td>0.055</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Threonine + asparagine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.085</td>
<td>0.125</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Serine + glutamine&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.085</td>
<td>0.175</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.08</td>
<td>0.16</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Glycerine</td>
<td>0.085</td>
<td>0.16</td>
<td>0.205</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.15</td>
<td>0.235</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.005</td>
<td>0.06</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.005</td>
<td>0.035</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.015</td>
<td>0.115</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.03</td>
<td>0.056</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>ND</td>
<td>0.045</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>0.085</td>
<td>0.145</td>
<td>0.195</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>0.01</td>
<td>0.095</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.035</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>0.02</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> MLF, Malolactic fermentation.
<sup>b</sup> Days after crushing (Fig. 2).
<sup>c</sup> Acids not resolved on chromatogram; concentration expressed as millimoles of threonine per liter.
<sup>d</sup> Acids not resolved on chromatogram; concentration expressed as millimoles of serine per liter.
<sup>e</sup> ND, Not detected.

measured for a Shiraz wine from the McLaren Vale district, South Australia. The concentration of ribose, arabinose, myo-inositol, and particularly of U1 decreased during malolactic fermentation; the concentration of the other sugars, including glucose and fructose, remained constant. *L. oenos* (10<sup>6</sup> cells per ml), *P. parvulus* (10<sup>5</sup> cells per ml), and *Lactobacillus brevis* (10<sup>5</sup> cells per ml) were isolated from one Shiraz wine at pH 3.85 on completion of the malolactic fermentation.

**DISCUSSION**

The growth cycle of lactic acid bacteria in wines is complex and consists of distinct phases that occur during alcoholic fermentation, malolactic fermentation, and conservation after malolactic fermentation (11, 19, 28). In this study we give a detailed, quantitative description of the ecological and chemical behavior of lactic acid bacteria during and after malolactic fermentation. Overall, our data strengthen and extend the conclusions of previous studies (3, 17, 18, 20).

The effect of pH on the growth rate of lactic acid bacteria in wines is well demonstrated in the literature (4, 5, 22) and by the data in Fig. 1 and 2. Generally, the rate of bacterial growth and malolactic fermentation increased as wine pH was increased from 3.0 to 4.0. In addition, pH had a
parvulus was the only species able to grow, and it conducted wines. Usually, L. oenos was the only species isolated from under practical winery conditions, however, is determined (28), Pediococcus and Lactobacillus spp. rarely grow in bacteria at this stage seems antagonistic to the survival of L. been completed by L. oenos (Fig. 2). The growth of these enological significance warrant further study.

The concentration of SO2 may also selectively influence the species of lactic acid bacteria that grow in wines. This is well illustrated by the Shiraz wine from winery B, which had a total SO2 concentration of 72 mg/liter and did not support the growth of any lactic acid bacteria until the pH was adjusted to 4.0. Despite the initial presence of L. oenos, Lactobacillus buchneri, and P. parvulus in this wine, P. parvulus was the only species able to grow, and it conducted the malolactic fermentation (Fig. 3). According to other studies (18, 33), a total SO2 concentration of >50 mg/liter generally restricts the growth of lactic acid bacteria in wines, especially at the lower pH values, when a greater proportion of the SO2 is in the undissociated, antimicrobial form. The frequent occurrence of Pediococcus spp. in Australian wines (9, 25) may be indicative of wines with a high concentration of SO2 and a high pH (e.g., 3.5 to 4.0).

The populations of 10^6 to 10^7 cells per ml formed by lactic acid bacteria either during or after malolactic fermentation were quantitatively significant and produced measurable changes in the concentrations of some wine components. Although published evidence is not strong, it is generally considered that wine sugars are utilized as carbon substrates for the growth of lactic acid bacteria (18, 24, 28). Our data provide experimental evidence to support this conclusion, but also show that the behavior of sugars in some wines is quite complex.

The concentrations of glucose, fructose, and the unidentified monosaccharide U1 decreased in direct proportion to the growth of P. parvulus during the malolactic fermentation of the Shiraz wine at pH 4 from winery B (Fig. 3), and it may be concluded that these carbohydrates were metabolized by this species. Similar conclusions can be made for the utilization of glucose, fructose, U1, myo-inositol, glycerol, ribose, and xylose during the growth of pediococci and lactobacilli after malolactic fermentation of the Shiraz wine at pH 3.7 from winery A (Fig. 2). Metabolism of the hexose and pentose sugars (18, 28) probably accounted for the large increases in the concentration of acetic and lactic acids also observed at this stage. The metabolism of glycerol by lactic acid bacteria was related to production of the bitter substance acrolein (10, 18). The utilization of myo-inositol by wine lactic acid bacteria is not well documented in the literature, but was reported earlier by Peynaud and Demerçq (26). Further studies are required to understand the metabolisms of this substance as well as that of U1, myo-inositol, U1, ribose, and arabinose, but not glucose or fructose, were also metabolized by L. oenos during malolactic fermentation of the Shiraz wine from the McLaren Vale district.

Data for the Shiraz wines (winery A, pH 3.2 or 3.7) (Fig. 1 and 2) suggest that the growth of L. oenos during malolactic fermentation was not accompanied by the specific utilization of any hexose or pentose sugars. Strains of L. oenos isolated from these wines fermented glucose and fructose as determined by the API tests, but, contrary to expectation, the concentrations of these two sugars increased significantly during malolactic fermentation. These increases were not directly related to growth, as might appear from Fig. 1 and 2, because they occurred in other wines (e.g., the same Shiraz wine at pH 3.0 and the Shiraz wine described in Fig. 3), in which there was no growth of L. oenos. Other researchers have also noted increases in the concentrations of some wine sugars during malolactic fermentation (8, 10), but the mechanism of this behavior has not yet been satisfactorily explained. Residual enzymatic activities from grapes and yeasts could be involved. The hydrolysis of sucrose would produce both glucose and fructose, but sucrose was not found in our wine samples even before malolactic fermentation (data not shown). Trehalose is another important wine disaccharide (32) whose hydrolysis would yield glucose. We did not specifically analyze this sugar, but some preliminary measurements indicated that its concentration decreased during malolactic fermentation.

The hydrolysis of phenolic glycosides of wine (18) could also lead to an increase in the concentration of monosaccharide sugars. However, the utilization of glucose and fructose by L. oenos still remains a possibility, since they may be generated at a rate faster than they are utilized.

Decreases in the concentration of mannose and arabinose during malolactic fermentation (Fig. 1 and 2) were not specifically related to the growth of L. oenos, as they also occurred under conditions at which no bacterial growth was evident (e.g., in the same wine at pH 3.0 and in the Shiraz wine described in Fig. 3). Moreover, the strains of L. oenos isolated from the wines at this stage were not able to ferment arabinose, as measured by the API tests. We are not able to provide a satisfactory explanation for these decreases; further studies are needed. Increases in the concentration of mannose at later stages of vinification (Fig. 2 and 3) are also difficult to interpret, but could possibly arise from the hydrolysis of mannans polysaccharide of the yeast cell wall during autolysis (1).

As expected from what is now a well-established fact (3, 17), malic acid was degraded by L. oenos and P. parvulus during malolactic fermentation, with the concomitant production of lactic acid. The degradation of citric and fumaric acids and production of acetic acid were important secondary reactions during the malolactic fermentation by L. oenos but not during the malolactic fermentation by P. parvulus. Other researchers (6, 7, 10, 28, 31, 36) have also observed these secondary reactions; there is probably direct metabolism of citric acid to acetic acid. The metabolic pathway of this reaction has been studied for dairy lactic acid bacteria (15). The metabolism of citric acid in wine is also related to an increase in the concentration of diacetyl and acetoin (12, 28, 37).

Significant changes in the concentration of wine amino acids have been reported to occur during malolactic fermentation. Some amino acids decrease in concentration, whereas others increase, but no consistent trends have emerged for any individual amino acid, except for a decrease in arginine, which is probably converted to ornithine (3, 23, 28, 34). We were able to confirm the utilization of arginine by L. oenos during malolactic fermentation, and in addition, we noted the utilization of histidine. It is possible that histidine was decarboxylated to histamine, which is also known to occur in wines (18). An increase in the concentration of all
other amino acids could be explained by the degradation of wine proteins by proteases produced by lactic acid bacteria, but the production of such enzymes by these bacteria has not yet been studied. Dairy lactic acid bacteria are well known for their protease production (21). Increases in the concentration of amino acids might also occur through yeast autolysis (1). Amino acid metabolism by wine lactic acid bacteria has not been studied. By analogy to dairy fermentation (21), such metabolism is likely to affect wine quality. The HPLC technology that now permits rapid measurement of free amino acids will facilitate further study of the behavior of amino acids during vinification and should enable a better understanding of their contribution to wine quality.

The degradation of acetaldehyde by L. oenos is significant, since SO₂, which is strongly bound to this component, is freed to become active against further bacterial growth (33).

The data described in this paper, together with those reported by others (18, 20, 33), have provided further evidence of the important influence of pH and SO₂ concentration upon the conduct of the malolactic fermentation and, consequently, upon the quality of red wines. They also indicate the potential of bacteria to grow in wines after conclusion of the malolactic fermentation and the need for sound winemaking practices to prevent spoilage at this stage. Furthermore, our findings suggest that the metabolism of wine components, especially the carbohydrates, during and after malolactic fermentation is more complex than previously thought and requires more detailed investigation.

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LITERATURE CITED


