

Article

Indigenous Malolactic Starter Cultures as Innovative Tools to Modify the Sensory Profile of a Wine: An Oenological Challenge

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Abstract: Malolactic fermentation conducted by lactic acid bacteria is essential for enhancing wine's sensory qualities. Although this process can occur spontaneously through the action of native lactic acid bacteria (LAB) from the grapes and cellar, it carries risks such as increased volatile acidity, consumption of residual sugars, and the formation of undesirable metabolites like biogenic amines. This study evaluated the oenological potential of three innovative native malolactic fermentation starters (MLFS) on a pilot scale, aiming to offer local wineries cost-effective MLFS with native LAB strains. Alcoholic fermentation of Malbec grapes was carried out using a commercial yeast starter, followed by a sequential inoculation of native malolactic starters formulated with (i) mesophilic *Lactiplantibacillus plantarum* UNQLp11 and *Oenococcus oeni* UNQOe73.2, both from the Province of Río Negro; (ii) psychrotrophic *Lentilactibacillus hilgardii* UNQLh1.1 and *Oenococcus oeni* UNQOe19, both from the Province of Río Negro; (iii) mesophilic *Lactiplantibacillus plantarum* UNQLp1001, from the Province of Buenos Aires; and (iv) a commercial malolactic started, which served as a control. Malic acid was consumed by all starters. RAPD-PCR using M13 primer showed the native LAB's capability for implantation in wine. A sensory analysis revealed distinct profiles for each formulation, despite having been inoculated at the end of the same alcoholic fermentation. These MLFS could replace imports, enhancing the region's unique *terroir*.

Keywords: malolactic fermentation; innovative starters; sensory analysis



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1. Introduction

According to the International Organization of Vine and Wine [1], Argentina is a significant player in the global market, ranking 7th in wine production and 2nd in must exports. The top wine-producing regions include the provinces of Mendoza, San Juan, La Rioja, Catamarca, Salta, Río Negro, and, more recently, the southern part of Buenos Aires, with Malbec as the emblematic grape variety.

Winemaking is a complex process that involves various biotransformations, with malolactic fermentation (MLF) being a key step. MLF, driven by lactic acid bacteria (LAB), transforms L-malic acid, which has a harsh taste, into the more pleasant lactic acid, resulting in a decrease in the total acidity of the wine and a slight increase in pH. It also enhances microbiological stability, volume, and roundness, while enriching the complexity of the wine's flavors, depending on the LAB strains used in the MLFS [2–4]. However, spontaneous MLF can be unpredictable and carries several risks since LAB can affect the impact that wine may have on human health. Recent trends in the wine industry emphasize the exploration of microbial resources to enhance quality. While studies initially focused on *Saccharomyces cerevisiae* and *Oenococcus oeni*, others have highlighted non-*Saccharomyces* yeasts and non-traditional LAB linked to spontaneous fermentation [5,6]. One of the primary challenges in oenological research is the production of efficient MLFS [7–9]. LAB strains selected for MLFS must meet specific criteria for optimal wine quality [10], including tolerance to low pH and high ethanol levels, good growth capability, compatibility with yeast, and the absence of off-flavors or biogenic amines production [11,12]. The use of new species/strains of LAB associated with spontaneous MLF can offer technological solutions to specific challenges, leading to improvements in the sensory characteristics of wine [13]. All commercial MLFS used in Argentina are foreign, primarily containing *O. oeni* strains. In previous studies, we showed that, under mesophilic conditions, *Lactiplantibacillus plantarum* and *O. oeni* were the dominant LAB species found in the spontaneous MLF of a Pinot Noir wine from the Province of Río Negro. In the Patagonian region of Argentina, south of latitude 40° S, MLF may occur at low temperatures (4 to 10 °C), and the winery environment or the fermentation tanks must be heated. To address this issue, it was necessary to identify the Lactobacillaceae microbiota related to low-temperature MLF and assess their contribution. Nine psychrotrophic *Lentilactibacillus hilgardii* strains able to consume L-malic acid at 4 and 10 °C in sterile wine were selected. The UNQLh1.1 strain revealed implantation capacity and efficient L-malic acid consumption at 4 and 10 °C in the presence of the native microbial consortium. The prevalence of *Len. hilgardii* under low-temperature conditions was a novelty compared to previous findings of LAB diversity in the MLF of Patagonian wines; thus, these *Len. hilgardii* strains were proposed as new players in fermentations conducted at low temperatures [14].

More recently, we have focused on the use of autochthonous LAB strains as MLFS from a re-emerging wine-growing region (Province of Buenos Aires), conducting winemaking in a pilot-scale trial in a relevant environment. The native strains were able to successfully implant, achieving a faster and more efficient MLF compared to the spontaneous MLF that took place simultaneously at the winery. These findings supported the use of the strains *Lpb. plantarum* UNQLp1001 and *O. oeni* UNQOe1101 as potential MLFS in Malbec wines [15].

The aim of this study was to explore the oenological potential of three native MLFS to modify the sensory profile of a Malbec wine, at a pilot scale. The MFLS were formulated with LAB strains selected from our stock collection, including the psychrotrophic strains *O. oeni* UNQOe19 and *Len. hilgardii* UNQLh1.1 and the fast malic acid consumer *Lpb. plantarum* UNQLp1001. We set out to assess their efficiency at conducting MLF, as well as their influence on the organoleptic profile of the final product, with the ultimate goal of developing a competitive alternative to imported MLFS that could be advantageous to local wineries.

2. Materials and Methods

2.1. Microorganisms and Starter Culture Preparation

A commercial dried *Saccharomyces cerevisiae* preparation (ACTIFLORE® BO213, Laffort, Bordeaux Cedex, France), rehydrated according to the manufacturer's specifications, was used to start the alcoholic fermentation (AF).

For the MLF, three malolactic starters were developed: Starter 1, which included the mesophilic strain *Lpb. plantarum* UNQLp1001, isolated from spontaneous MLF of Malbec wine, 2019 vintage, of the re-emerging wine-producing region Saldungaray, Buenos Aires Province, Argentina; Starter 2, which included in a 1:1 ratio the mesophilic strains *Lpb. plantarum* UNQLp11 (RGPRN-/-M-/-357-/-Semorile-/-626-/-UNQLp11-/-2024) [16] and *O. oeni* UNQOe 73.2 (RGPRN-/-M-/-358-/-Semorile-/-626-/-UNQOe73.2-/-2024) [17], both isolated from spontaneous MLF of Patagonian Pinot Noir wine, 2008 vintage; and Starter 3, elaborated with the psychrotrophic strains, in a 1:1 ratio, *O. oeni* UNQOe19 (RGPRN-/-M-/-358-/-Semorile-/-626-/-UNQOe19-/-2024) [18] and *Lentilactibacillus hilgardii* UNQLh 1.1 (RGPRN-/-M-/-359-/-Semorile-/-626-/-UNQLh1.1-/-2024) [14], both isolated from the spontaneous MLF of Patagonian Pinot Noir wine, 2014 vintage. A fourth MLF batch, which served as control, was inoculated with a commercial MLF starter culture (an *Oenococcus oeni* strain acclimated for direct inoculation, LACTOOENOS® B7 Direct, Laffort). Starters 1 and 3 were prepared in a concentrated wet format and activated in an acclimation medium [18]; Starter 2 was preserved by freeze-drying procedure after acclimation (see Sections 2.2 and 2.3). It is important to point out that these strains are at different stages regarding their technological development, hence the differences in preparation conditions.

Sovereign Rights Over Natural Resources: The indigenous LAB strains mentioned above are genetic resources belonging to the Provinces of Río Negro (UNQLp11, UNQOe73.2, UNQOe19, and UNQLh1.1) and Buenos Aires (UNQLp1001). They were deposited in the strain collection of the Laboratorio de Microbiología Molecular (IMBA-DCyT-UNQ-CIC) and registered with the Environment and Climate Change Secretariat—Province of Río Negro Government, and the Direction of Flora and Fauna—Ministry of Agrarian Development—Province of Buenos Aires Government. Their preservation follows the protocols of the Convention on Biological Diversity and the Nagoya Protocol, which safeguard the ownership and use of genetic resources from the regions where they were isolated (RESOL-2024-626-E-GDERNE-SAYCC#SGG, NO-2024-00628737-GDERNE-SAYCC#SGG, DISPO-2025-16-GDEBA-DFYFMDAGP).

2.2. Acclimation and Inoculation Conditions

Cells were grown at 28 °C for 5 days for *O. oeni* strains and 48 h for *Lentilactibacillus* and *Lactiplantibacillus* strains until the early stationary phase ($\sim 10^9$ cfu mL⁻¹). After this, cells were harvested by centrifugation at 5000 × g for 10 min and suspended in 200 mL of a modified acclimation medium containing 6% (v/v) ethanol, 50 g L⁻¹ MRS, 40 g L⁻¹ D (–) fructose, 20 g L⁻¹ D (–) glucose, 4 g L⁻¹ L-malate, 1 g L⁻¹ Tween 80, and 0.1 mg L⁻¹ pyridoxine, with pH 4.6 [19]. After incubation at 21 °C for 48 h, the acclimated cells were harvested by centrifugation and prepared for freeze-drying in the case of Starter 2, whereas for Starters 1 and 3, cells were resuspended in wine for the wet starter format, both at a concentration of $\sim 10^7$ cfu mL⁻¹. Culturable cell concentration was determined after acclimation treatment by plating onto MRS agar for *Lentilactibacillus* and *Lactiplantibacillus* and on MLO agar for *O. oeni* cell concentration, respectively.

2.3. Sample Preparation for Freeze-Drying

To preserve Starter 2 by freeze-drying, 200 mL of the culture ($\sim 10^7$ cfu mL⁻¹) previously acclimated, was harvested by centrifugation at 5000 × g for 10 min. Pellets were

washed twice with NaCl 0.85% (*w/v*) and resuspended in 40 mL of 20% (*w/w*) aqueous trehalose solution, previously sterilized using 0.2- μm pore sterile filters [20]. Samples were frozen for at least 1 week at $-20\text{ }^{\circ}\text{C}$ in glass vials and were dried by freeze-drying (BIOBASE freeze dryer system/BK-FD10P, Rui'an, China) for 48 h (condenser temperature: $-60\text{ }^{\circ}\text{C}$; chamber pressure: 10 Pa). After freeze-drying, samples were vacuum sealed at $4\text{ }^{\circ}\text{C}$ for 24–48 h until use.

2.4. Winemaking Process

Winemaking was carried out in the Centro de Enólogos de Buenos Aires (CEBA) with Malbec grapes from Caucete, San Juan Province, Argentina. The grapes were manually selected in March 2024 at the CEBA winery, after which they were destemmed, macerated, and crushed. Sulfiting was carried out during vatting by adding potassium metabisulphite at a concentration of 5 g hL^{-1} (Química Palumbo, Mendoza, Argentina). After overnight maceration, the commercial AF starter employed was added at 20 g hL^{-1} .

The progress of AF was monitored three times a day by measuring Baumé degrees, estimating the sugar consumption and temperature, which did not exceed $24\text{ }^{\circ}\text{C}$. Winemaking was performed in stainless steel fermenter tanks containing 250 L of grape must. At the end of AF, the oenologist recommended a second sulfiting, which was carried out for Wine 4 (inoculated with the commercial MLFS), but not for the other three wines.

When AF was completed, the wines were de-vatted and transferred to 50 L tanks, kept at $19\text{ }^{\circ}\text{C}$ ($\pm 2\text{ }^{\circ}\text{C}$), and malolactic fermentation was performed through individual inoculation: Wine 1, inoculated with Starter 1; Wine 2, inoculated with Starter 2; Wine 3, inoculated with Starter 3; and Wine 4, inoculated with the commercial MLF starter culture. Wines 1 and 3 were inoculated with acclimated cells on a wet format of each strain at a final concentration of $\sim 5 \times 10^7\text{ cfu mL}^{-1}$. Wine 2 was inoculated with freeze-dried, previously acclimated cells at a final concentration of $\sim 5 \times 10^7\text{ cfu mL}^{-1}$ (2 g hL^{-1}) rehydrated in sterile physiological solution 1 h prior to inoculation. The commercial MLF starter culture was inoculated in dry format, following the producer's recommendations. The MLF was considered finalized when the residual L-malic acid was less than 0.5 g L^{-1} [15].

2.5. Monitoring of LAB Viability

During the MLF, samples were collected at days 0, 5, 10, and 15. The survival of LAB throughout the fermentation process was assessed by performing viable counts on MRS (to promote growth of *Lactiplantibacillus* sp. and *Lentilactibacillus* sp.) and MLO (to promote growth of *Oenococcus* sp.) media at pH 4.8, with the addition of cycloheximide 100 mg L^{-1} , nystatin 20 mg mL^{-1} , and sodium azide 0.01% (*w/v*) (Sigma-Aldrich Argentina, Buenos Aires, Argentina) to prevent the growth of yeasts and other fungi. Samples were plated using the seeding technique according to Corral et al. [21]. The plates were incubated aerobically at $28\text{ }^{\circ}\text{C}$, with counts conducted after 48 h for MRS medium and after 5 days for MLO medium.

2.6. L-Malic Acid Consumption

The remaining malic acid was measured with an enzymatic kit (L-Malic Acid Enology Enzymatic kit, BioSystems SA, Barcelona, Spain) for one bottle of wine inoculated with each of the starters assayed (natives plus commercial). An exponential one-phase decay equation model was used to fit the Malic Acid Consumption (MAC) kinetics of the different strains tested [14,16]. The equation for this model, obtained by the Graph Pad Prism[®] 6.01 software, is

$$[\text{MA}_t] = ([\text{MA}_0] - [\text{MA}_i]) e^{-kt} + [\text{MA}_i] \quad (1)$$

where $[MA_t]$ is the MA concentration at time = t , $[MA_0]$ is the initial concentration of MA (2.3 g L^{-1} in the wine used), $[MA_i]$ is the MA concentration at infinite time, and K is the rate constant.

2.7. Wine Physicochemical Analysis

Wine physicochemical analyses were carried out on samples of bottled wine for the four wines. The parameters were determined according to the methods proposed by the Instituto Nacional de Vitivinicultura (INV). Ethanol concentration was determined by distillation, Method 920.57, AOAC, 1997, based on densitometry. Volatile acidity was quantified by steam distillation followed by titration and titratable acidity was determined by direct titration with NaHO 0.1N. The total reducing sugars (TRS) were evaluated by the Fehling–Causse–Bonnans method 962.12, AOAC, 1997. Free sulfur dioxide (LSO_2), total sulfur dioxide, and pH determination were determined through Method 960.19, AOAC, 1997, using a pH meter (Orion 420A). The color intensity and nuances of the wines were evaluated using the Glories method [22].

2.8. Implantation Capacity

The implantation capacity of the LAB comprising the native MFLS was assessed by RAPD-PCR with the primer M13 [23]. Samples were randomly collected from MLO agar to evaluate the implantation of *O. oeni* strains, and from MRS agar to assess the implantation of *Lactobacillus* spp. strains, in each wine. The PCR products were separated by electrophoresis using a 2.0% (w/v) agarose gel, along with a 100 bp ladder (PB-L, Productos Bio-Lógicos, Universidad Nacional de Quilmes, Buenos Aires, Argentina). The implantation capacity was determined by comparing the RAPD profiles from pure culture colonies with those of the inoculated strains collected from the different wines.

2.9. Sensorial Analysis

Sensorial analyses were conducted by an evaluation panel composed of 8 red wine consumers, recruited from the Universidad Nacional de Quilmes community, plus 5 expert judges who regularly conduct sensory assessments. Individuals were selected for this panel based on criteria that excluded smoking, taste deficits, oral piercings, and oral lesions [24]. Room temperature was set to 22°C and air conditioning was run before each session to eliminate any residual ambient aromas [25]. A preliminary testing was conducted 1.5 months after the end of MLF, with the aim of ascertaining whether the wines were of basic acceptable quality. In this instance, only the general scoring was performed (see below). This was of particular interest because this is the first time we have carried out a pilot-scale vinification with the strains UNQOe19 and UNQLh 1.1 combined in a MLFS.

Afterwards, the wines were evaluated once they had undergone 7 months of bottle aging. For this analysis, we selected Wine 1, elaborated with Starter 1 (LAB from the re-emerging wine-growing region of Buenos Aires Province); Wine 2, elaborated with Starter 2 (mesophilic LAB from Río Negro Province, Patagonia); and Wine 4 (commercial MLF starter). Wine 3, which was inoculated with a starter formulation containing psychrotrophic LAB, will be sensorially evaluated as part of an ongoing project involving pilot-scale vinification at low temperatures.

The wines were assessed in terms of general scoring plus three sensory phases: visual, olfactory, and taste/mouthfeel [26]. Before the testing, a brief training session was carried out, according to [27], with modifications for descriptors specific to Malbec. For the general score, judges were asked to rate each wine on a 1 to 10 scale in terms of the wine's aspect, aroma, flavor, and balance. For the sensory phases, wines were rated on a 0 to 5 scale (undetectable to extremely intense) regarding the following attributes: visual phase (color, intensity, and opacity); olfactory phase (plum, white pepper, red fruits, green pepper,

and floral aromas); taste/mouthfeel phase (acidity, bitterness, astringency, body, alcohol, mouthfeel, aftertaste, and persistency) [28,29]. Judges were provided with a color chart [30] to homogenize terminology. This color chart was then numerically coded into series for color and subseries for hue, i.e., 1 for series “ruby” and decimals 1 to 3 for hues “pale, medium, and deep”. The color series assessed were ruby (1), garnet (2), purple (3), and tawny (4) [31]. Finally, the judges were asked to give each wine an overall score on a 1 to 10 scale, considering the sum of the qualities assessed and their general satisfaction. To minimize carry-over effects, panelists were instructed to follow a sip-and-spit protocol, rinsing their mouths with mineral water between samples. Each wine sample, consisting of 20 mL, was served in a standard wineglass (ISO 3591:1977 [32]) according to the CATA procedure [33,34]. Each session featured one sample of each wine. The panel members were not informed about the nature of the samples they would be evaluating (blind sampling), to prevent preconception biases.

2.10. Statistical Analysis and Reproducibility Assay

All determinations were the average of three independent replicate assays. Data are shown as mean values. The statistical analyses were carried out using Graph Pad Prism 6.01 software [35]. Means were compared by one-way ANOVA, with a significance level of $p < 0.05$.

Data from the sensory evaluation were analyzed using One Way ANOVA test with the Tukey HSD test for post hoc comparisons. Significance level was set at $p < 0.05$. Analyses were carried out using Statistix 8.0 software [36].

3. Results

In previous research, LAB selected from two wine-growing regions in Argentina were evaluated for their technological properties at laboratory-scale winemaking, indicating that these LAB were strong candidates for formulating native MLF starters. In this study, we evaluated how three different native MLF starter formulations influenced the sensory and physicochemical profile of four wines elaborated from the same must of Malbec grapes from a traditional wine-growing region of Argentina.

3.1. Pilot-Scale Winemaking

The AF progressed well, beginning with average sugar values of 12.3 °Baumé at 25 ± 2 °C; at 6 days after wine fermentation, the average value was 0 °Baumé at 25 ± 2 °C and the initial L-malic acid level was 2.3 g L⁻¹ at the end of AF. Four different MLFS were used to inoculate one Malbec wine at the end of AF, resulting in four distinct wines, thus revealing the influence of native biological resources as innovative tools for controlling MLF.

3.2. Evaluation of MAC and Cell Survival

The LAB used in these starter formulations had previously shown tolerance to the stressful wine environment, which includes high levels of ethanol, phenols, low pH, and competitive microbial activity. The native MLFS were challenged to differentiate the resulting wines based on their oenological properties during a winemaking process that followed a shared AF. The MLF was assessed by controlling the viability of the inoculated LAB and measuring residual L-malic acid levels (Figure 1). At day 15, all the wines inoculated with the native starters and the commercial starter fully consumed the L-malic acid.

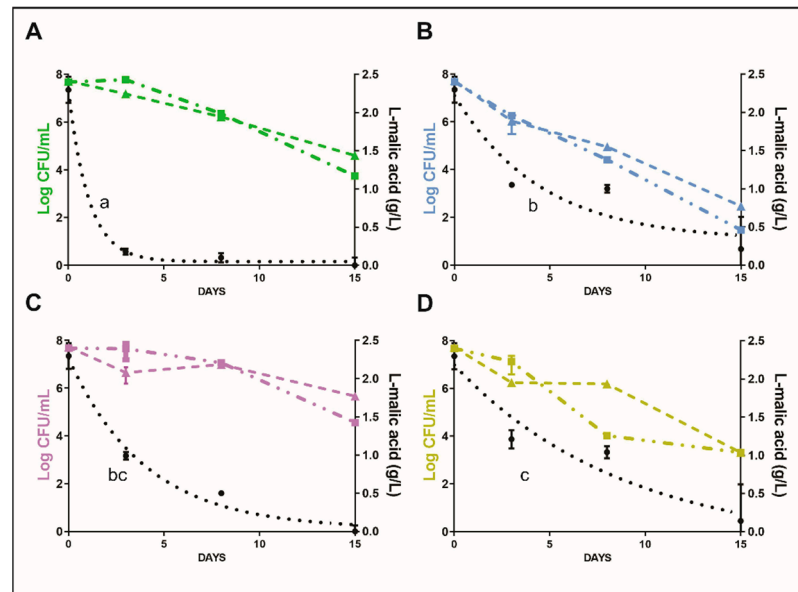


Figure 1. Evaluation of L-malic acid consumption and cell survival. Cell survival in MRS (■) and MLO (▲) medium. The black dashed line represents MAC kinetics (g L^{-1}). Kinetics of L-malic acid consumption and cell viability in (A) Wine 1, (B) Wine 2, (C) Wine 3, and (D) Wine 4. Lowercase letters in curves denote significant differences. See Table S1 in Supplementary Materials.

The kinetics analysis of L-malic acid consumption showed that the two wines inoculated with the native Río Negro Province starters tended to a gradual reduction in residual L-malic acid values to 0 g L^{-1} within 15 days of MLF. On the other hand, Wine 1, inoculated with the native Buenos Aires Province starter, completed the MLF to 0 g L^{-1} by day 5. A statistical comparison between the consumption rates detected in the four wines showed significant differences ($F = 443$, $DF = 3$; 11 , $p < 0.0001$), with Wine 1 exhibiting the fastest consumption rate and Wine 4 the slowest (Figure 1, Table S1 Supplementary Material).

All the analyzed wines showed cell survival in the MRS medium used for the growth of *Lactobacillus* sp., as well as in the MLO medium used for the growth of *O. oeni*. The survival of LAB during MLF was not directly linked to the consumption of L-malic acid. Viable LAB were found in all wines, although their survival varied. Towards the end of MLF (15 days), a trend towards decreased cell viability was observed. In Wine 1, LAB viability remained stable in MRS medium for the first three days of MLF; however, it then decreased by 4 log orders. In contrast, LAB grown in MLO showed a continuous decline in viability throughout MLF, ultimately decreasing by 3 log orders by the end of MLF (Figure 1A).

Wine 2 exhibited a similar decrease in viability through days 1 to 5. After that, LAB grown in MLO decreased by approximately 4 log orders, while those in MRS dropped by 5 log orders. This wine had the lowest LAB survival rates during MLF (Figure 1B). In wine 3, LAB grown in MRS maintained stability for the first three days, but a slight decrease followed by stability was detected in MLO. By the end of MLF, LAB in MLO showed a reduction of 1 log order, while those in MRS decreased by 2 log orders (Figure 1C). It is noteworthy that Wine 3 was inoculated in a dry format because it was preserved through freeze-drying, which adds a stress factor to the viability of LAB. Wine 4 exhibited a decrease of 4 log orders (Figure 1D).

Wine 3 displayed the highest LAB viability and Wine 2 the lowest. All wines showed that in the first 3 to 5 days of MLF, *Lactobacillus* sp. appeared to tolerate the stressful environment of the wine, while *O. oeni* viability decreased. However, towards the end of MLF, a greater degree of tolerance was observed in *O. oeni* compared to *Lactobacillus* sp.

The cell viability in wines also showed a varying tendency to decline. Viability stabilized during the first 8 days of MLF, after which the trend of microbial decline accelerated. Moreover, during the first 8 days of MLF, L-malic acid progressively decreased; at this point, Wine 1 was the only one to fully consume the L-malic acid.

3.3. Implantation of LAB

All native LAB comprising MLFS were implanted in wines according to the similarity of their RAPD-PCR profiles (Figure 2). The native strain UNQLp1001 exhibited 25% implantation in samples from Wine 1. In Wine 2, the UNQLp11 strain exhibited a 75% implantation value, whereas the UNQOe73.2 strain showed 50% (see Figure 2A). In Wine 3, the UNQLh1.1 strain had a 62.5% implantation, while the UNQOe19 strain had 50% (Figure 2B). The implantation percentage was calculated by relating the number of electrophoretic profiles identical to those of the inoculated strains to the total number of different RAPD-PCR profiles analyzed. If the RAPD-PCR profiles of the LAB strains present in the starters were detected in both culture media (MRS and MLO), the total number was 8. If they were detected only in the culture medium expected for them, the total number was 4. Given that we detected electrophoretic profiles of the strains UNQLp11 and UNQLh1.1 in colonies grown in MRS and MLO, the total number of profiles for these strains was 8 for MLFS 2 and 3. These results show the adaptation capacity of native strains to the conditions of the wine and encourage us to explore different combinations of native strains in future formulations.

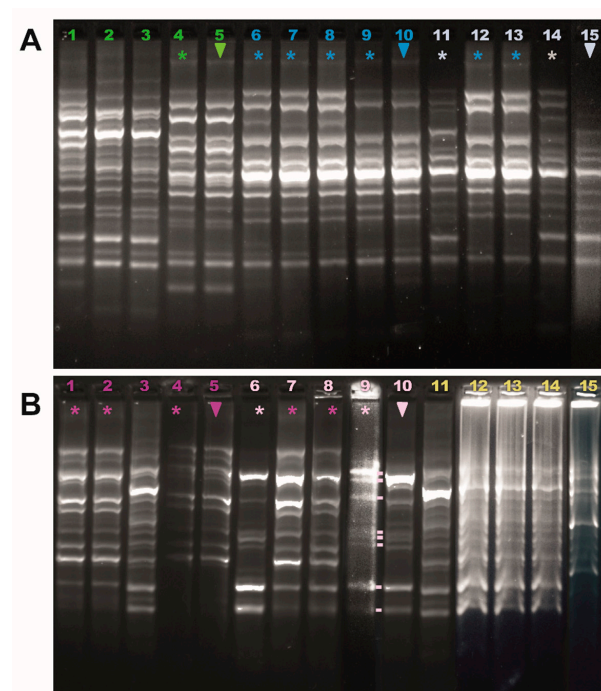


Figure 2. RAPD-PCR profiles of MLFS assessing LAB implantation. (*) indicates the RAPD-PCR profiles that matched with the control strain profile. (▼) indicates the RAPD-PCR profile for each control strain. (A) Wells 1–4 (green): colonies selected from MRS agar inoculated with Wine 1. Well 5: control strain UNQLp1001. Wells 6–9 (blue): colonies selected from MRS agar inoculated with Wine 2. Well 10: control strain UNQLp11. Wells 11–14 (white): colonies selected from MLO agar inoculated with Wine 2. Well 15: control strain UNQOe73.2. (B) Wells 1–4 (violet): colonies selected from MRS agar inoculated with Wine 3. Well 5: strain UNQLh1.1. Wells 6–9 (pink): colonies selected from MLO agar inoculated with Wine 3. Well 10: control strain UNQOe19. Wells 11–15 (yellow): colonies selected from MLO agar inoculated with Wine 4. See Table S2 in Supplementary Materials.

3.4. Physicochemical Analysis of Wines

All evaluated wines complied with the OIV's established physical and chemical parameters (Figure 3). The alcohol content was 12.5% for Wines 1 to 3 and 12% for Wine 4. The reducing sugars varied from 2.34 to 3.12 g L⁻¹, with Wine 3 exhibiting the highest amount. Wines 1 and 2 had similar reducing sugar levels of 2.34 g L⁻¹.

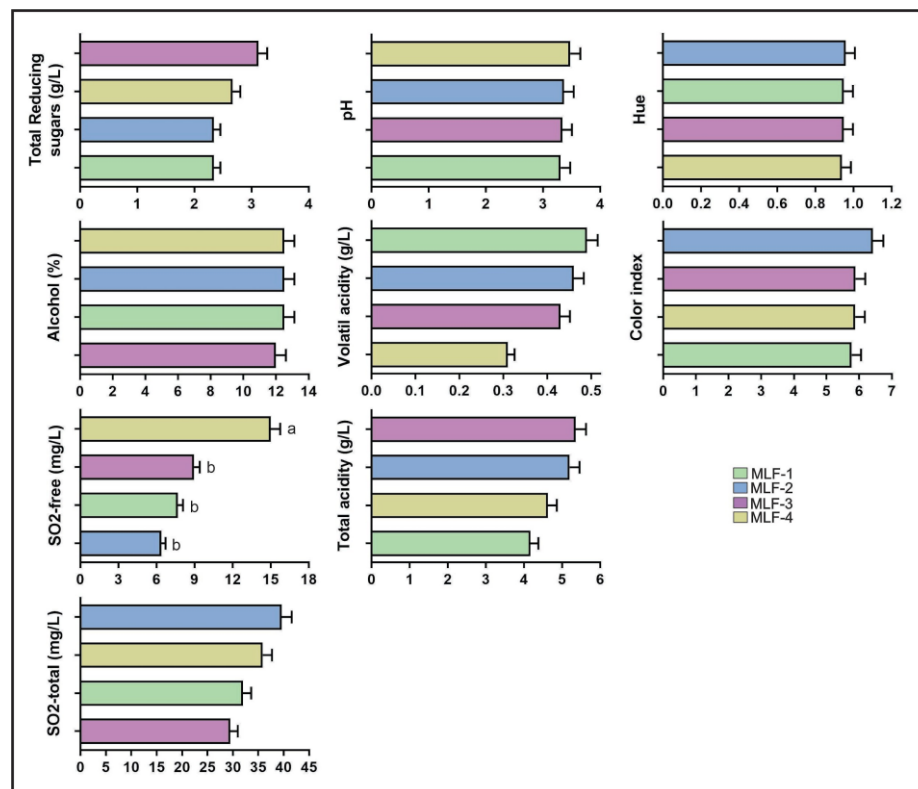


Figure 3. Physicochemical analysis of wines; letters represent significant differences ($p < 0.05$).

Wine 4 had significantly higher free SO₂ levels at 15 mg L⁻¹ compared to the other wines. The wines inoculated with native formulations showed free SO₂ values ranging from 6.4 to 8.97 mg L⁻¹. Total SO₂ levels varied from 29.47 to 39.59 mg L⁻¹, with a tendency for higher values detected in Wine 2 and lower values in Wine 3.

The pH values showed no significant differences, ranging from 3.31 to 3.48. Volatile acidity ranged from 0.31 to 0.49 g L⁻¹, with Wine 4 showing lower values, while Wine 1 had the highest volatile acidity. Total acidity values ranged from 5.39 to 4.17 g L⁻¹, with no significant differences; a trend was noted for higher values in Wine 3 and lower in Wine 1.

Additionally, color intensity and hue did not exhibit significant differences among the wines.

3.5. Sensory Analysis

The four wines evaluated 1.5 months after bottling, in the preliminary tasting test, showed acceptable quality, with average scores ranging from 6.5 to 8. Wines 1 to 3, elaborated with native MLFS, were generally given equal or higher scores than Wine 4, made from a commercial MLFS, particularly regarding aspect, flavor, and balance. However, the differences in scores were not statistically significant (see Figure 4).

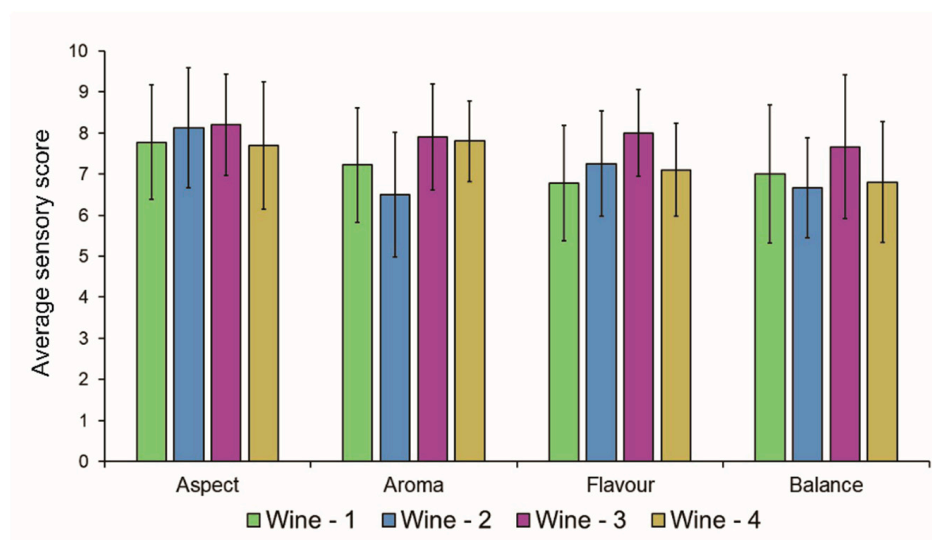


Figure 4. General scoring of the wines 1.5 months after bottling.

On the other hand, the three wines assessed at the 7-month testing performed significantly differently in the sensory analysis. Wine 1 ranked the highest in the general scoring in terms of aspect ($F = 3.33$; $DF = 2$; 38 ; $p = 0.046$), aroma ($F = 11.31$; $DF = 2$; 38 ; $p < 0.001$), flavor ($F = 6.45$; $DF = 2$; 38 ; $p = 0.004$), and balance ($F = 15.12$; $DF = 2$; 38 ; $p < 0.001$) (Figure 5).

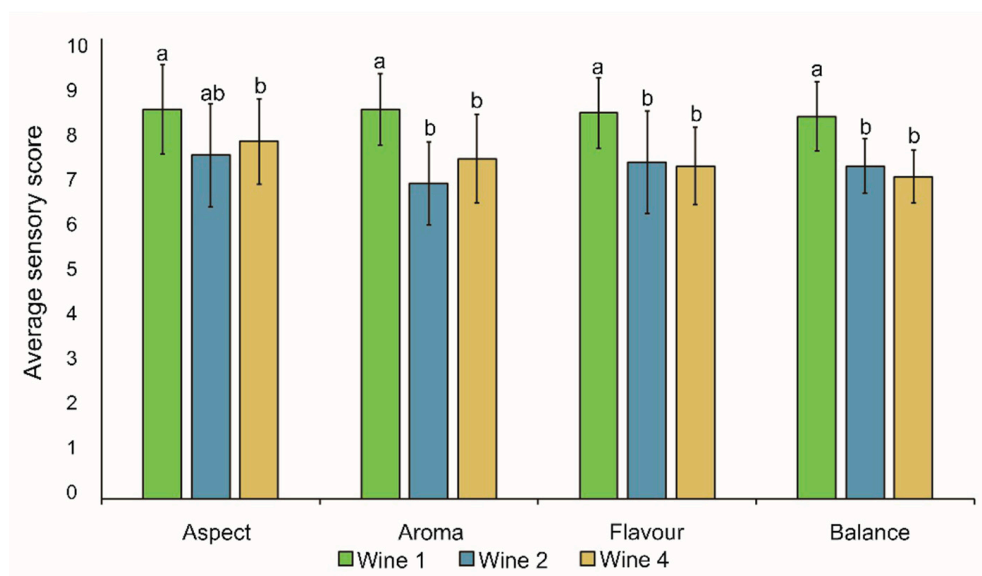


Figure 5. General scoring of the wines analyzed after 7 months of bottle aging in four major attribute categories. Lowercase letters indicate significant differences between wines for each attribute.

Regarding the sensory phases, we found significant differences in the visual phase (Figure 6), in which Wine 4 was the least intense ($F = 5.31$; $DF = 2$; 38 ; $p = 0.009$) and Wine 2 was the most turbid ($F = 10.4$; $DF = 2$; 38 ; $p < 0.001$) (Figure 5). The color of Wine 1 was mostly described as medium to deep purple, Wine 2 varied from pale/deep purple to pale/deep ruby, and Wine 4 was mostly perceived as pale to medium purple to medium ruby. However, we could not find significant differences in colors, probably due to the high dispersion of the data from Wine 2, which had a great variety of hues, and was therefore ascribed to many different colors by the judges.

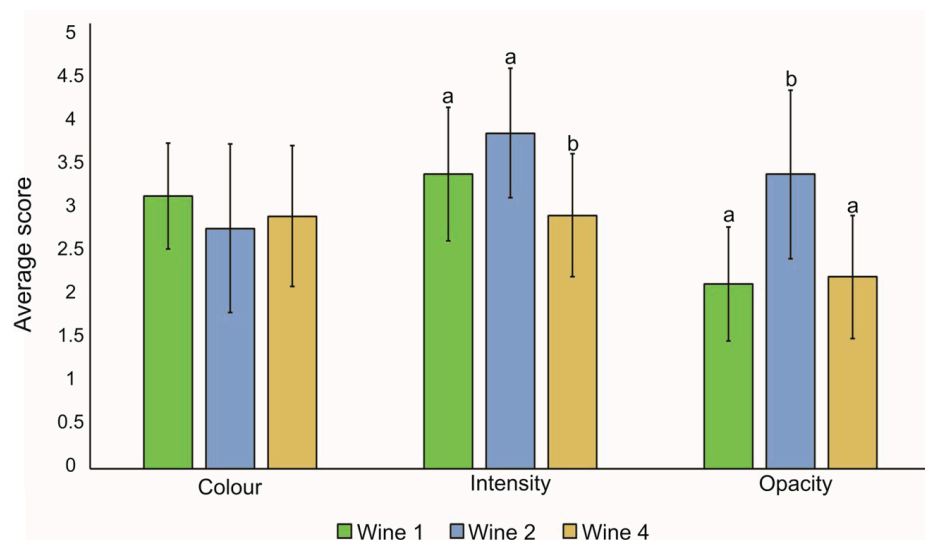


Figure 6. Visual phase scoring of the wines analyzed after 7 months of bottle aging. Lowercase letters indicate significant differences between wines for each attribute.

In the olfactory phase, Wines 1 and 2 showed a trend towards more complex aromas, whereas Wine 4's aromas of white and green pepper were subtler than the other two wines, although the differences were not statistically significant (Figure 7A). The wines were rather similar in terms of flavor/mouthfeel, except for aftertaste, for which the judges found Wine 1 significantly more pleasant than the others ($F = 4.83$; $DF = 2$; 38 ; $p = 0.013$) (Figure 7B).

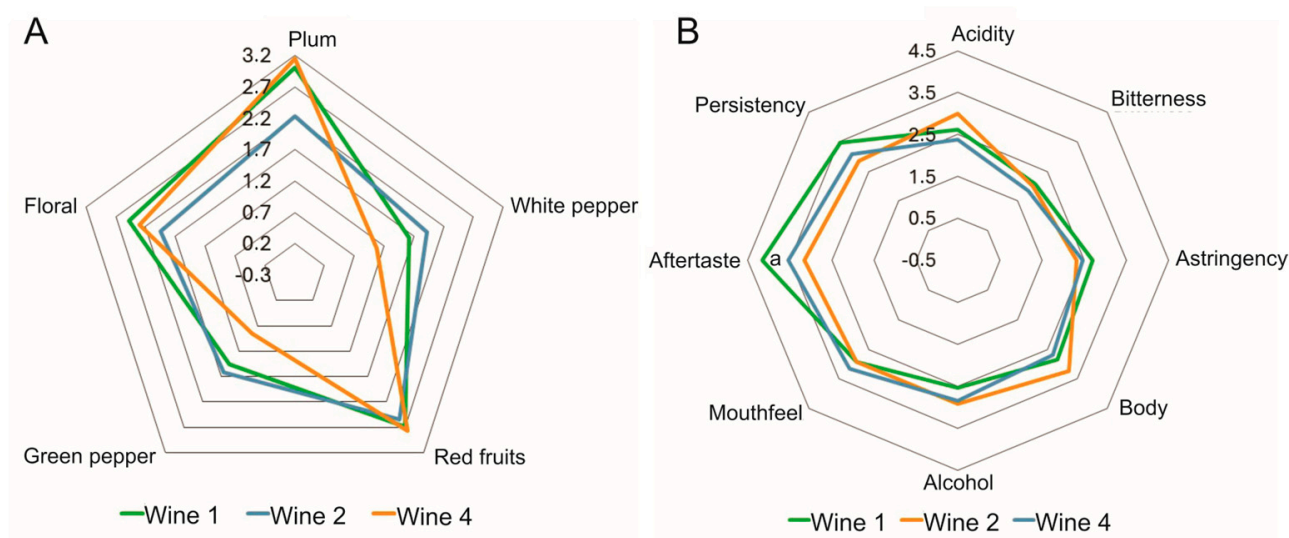


Figure 7. Olfactory (A) and taste/mouthfeel (B) phases scoring of the wines analyzed after 7 months of bottle aging. Lowercase letters indicate significant differences between wines for each attribute.

Finally, the judges deemed Wine 1 to be overall more satisfactory than Wines 2 and 4, as it obtained a significantly higher general score ($F: 12.38$; $DF: 2$; 38 ; $p < 0.001$), whereas there were no significant differences between Wines 2 and 4 (Figure 8).

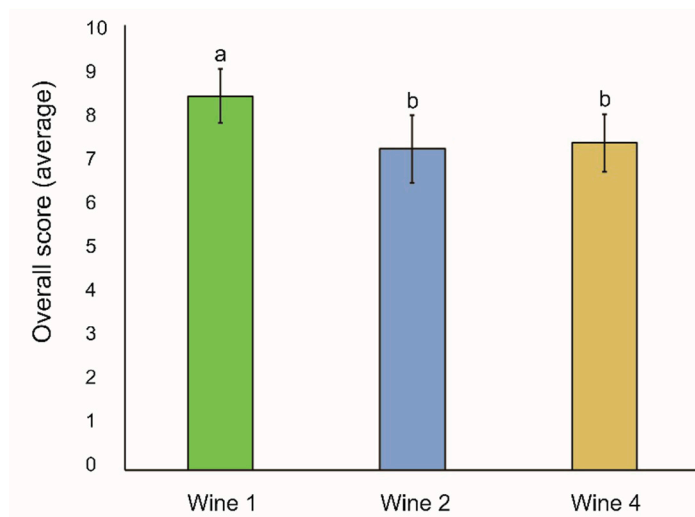


Figure 8. General score (overall quality assessment) of the three wines analyzed. Lowercase letters indicate statistically significant differences.

4. Discussion

4.1. Effect of Native MLFS on Wine Properties

The results obtained here emphasized the distinctive sensory effects produced by the three native MLFS used to inoculate one Malbec wine at the end of AF. The development and use of MLFS depend on the characterization, propagation, and conservation of suitable LAB strains for this purpose. Expanding the availability of candidate strains to formulate native MLFS, which are better adapted to given conditions, is a crucial technological tool for the wine industry that prevents risks such as an increase in volatile acidity, consumption of residual sugars, and formation of undesirable metabolites such as biogenic amines [37–39].

The native MLFS tested in Malbec wine, at a pilot scale, were developed based on a comprehensive assessment of their safety and oenological and technological potential, as outlined in previous research [15–17]. Their innovative aspect lies in its formulation. Starter 1 included a highly efficient MLF time-reducing native LAB strain, Starter 2 consisted of two native mesophilic LAB strains able to produce biomass in alternative media and tolerant to freeze-drying preservation, and Starter 3 included a psychrotrophic *Len. hilgardii* strain able to conduct MLF at low temperatures. To this day, *O. oeni* is still one of the most used MLF starter species [40]. However, with the changes in temperatures during growth and harvest due to global climate change, other LAB, specially *Lactiplantibacillus* strains, have the potential to play a key role in the modification of wine [41–44]. Currently, the commercial starter cultures used to lead the MLF in Argentina are imported from United States and France. Therefore, using locally developed technological tools would decrease reliance on imports and enable better control of MLF, while maintaining the integrity of the regional *terroir*.

The kinetics of L-malic acid consumption and viability of the three MLFS (Figures 1 and 2) revealed strain-dependent behavior, as proven in previous studies [15–18]. Good cell survival in wine was achieved following freeze-drying preservation for Starter 2, and in wet format for Starters 1 and 3, influenced by the pre-acclimated treatment. Previous findings in several LAB strains indicated that pre-acclimation treatment is a key factor in enhancing ethanol tolerance (Figure 3) [19,45]. Furthermore, the performance of the strains used in MLFS 2, in terms of viability and L-malic acid consumption, has been found to be better when inoculated in synthetic wine after freeze-drying [46]. Other authors have shown that LAB, even non-traditional ones, are known to present a population drop (after freeze-drying), but also adapt and show good MLF operational stability [47]. Future studies

will explore the effects of preservation techniques on the LAB strains used in the other MLFS, in order to optimize this process, so that they retain their technological properties. In response to the demands of the winemaking industry regarding strategies to conduct MLF with freeze-dried starters, it would be advantageous to explore the immobilization of freeze-dried LAB in grape lees, in order to avoid introducing off-flavors in the wine.

Sulphitation is another stress factor that impacts the survival of LAB during MLF [48–50]. Free and total SO₂ levels were detected in all wines at acceptable concentrations according to the OIV standards. Starter 2, which was inoculated in a dry format, exhibited an expected decline in viability and showed a tendency towards higher total SO₂ levels; however, L-malic acid was entirely consumed. The results encourage us to further study the freeze-drying preservation of Starters 1 and 3, while also optimizing the preservation of Starter 2.

The three native MLFS were successful in consuming L-malic acid in wine; this finding agrees with results obtained at the laboratory-scale for Starters 2 and 3 and at pilot-scale for Starter 1. The formulations showed flexibility to adapt to the biological and physicochemical environment of another grape varietal. The MLFS assessed here proved their potential as microbial resources for optimizing the management of MLF timing and pH levels to mitigate risky situations.

4.2. Starters Implantation Capacity

The implantation of commercial starters depends on the strains used, as well as several factors that impact the adaptation to wine-specific conditions in each wine-growing area [51]. The successful implantation of LAB in wine is affected by the possible interactions, both positive and negative, operating within the microbial consortium of wine.

The MLFS implantation trials (Figure 2) agree with previous studies [14,15,18], at laboratory scale, in which LAB strains were inoculated into wines equal to those of origin. The *O. oeni* cell survival in Starters 2 and 3, tested in MLO agar, was equal or greater than *Lactobacillus* spp. cell survival detected in MRS agar (Figure 1). However, a greater implantation ability was detected for *Lpb. plantarum* and *Len. hilgardii* species, suggesting that other RAPD-PCR profiles detected from MLO agar correspond to native *O. oeni* strains present in the wine. The resilience of *Len. hilgardii* and *Lpb. plantarum* to stressful wine conditions has also been described for Australian and Albariño wines [43,52]. These results suggest a balanced implantation of the starters and an adaptation to the wine environment, as well as a positive interaction with both the existing microbiota and the added commercial yeast inoculant.

4.3. Sensory Analysis

The relationship between sensory descriptors of a varietal and its *terroir* is complex and influenced by various factors. Recent research in Argentina described a methodology that provides a proof of concept for understanding both *terroir* and vintage effects from a sensorial perspective [53]. Malbec (*Vitis vinifera* L.) is a red grape variety originally from Cahors (France). In Argentina, this grape variety exhibits distinct characteristics from its original *terroir*, influenced by differences in climate, soil, genetic traits of the plants, vineyard management, and winemaking methods. Descriptors such as vegetable, hot (mouthfeel), red fruits, and astringency have been significant in previous studies on Malbec [26,28,30,53,54].

The three MLFS used in this work changed the flavor and sensory complexity of the same wine, through the synthesis of different volatile compounds. The wines assessed at the 7-month testing performed significantly differently in the sensory analysis, showing differences in color and coincidence in certain descriptors, such as red fruits, plum, and

white pepper, typical to Malbec. These results confirm the previous findings from the analysis of volatile compounds in Starters 1 and 2, which identified compounds that reduce bitter and herbaceous notes, while enhancing floral and fruity notes in the final wines [4,15].

The technological resources available nowadays need to be incorporated into the tried-and-true winemaking traditions, especially when the issue in question is the controlled driving of the MLF and consumer safety [51,55,56]. The use of native MLFS allowed us to produce different wines originating from a common must, with final products that had distinctive organoleptic profiles, and therefore could be marketed to different consumers. This could be very attractive to wineries aiming to increase the variety of products offered, considering that most of the establishments in re-emerging wine regions are small-scale productions with an average 20 ha of planted vineyard, and mostly one to three grape varieties [28]. Further advances in preservation techniques have the potential to scale-up the process for these three starters, while alternative culture media for these new starters will positively impact the environment, re-using industrial residues and re-enforcing circular economy.

5. Conclusions

The present study showed the oenological and technological capacity of native MLFS to conduct malolactic fermentation and positively influence the sensory perception of wines. One of those starters also represents the first successful use of freeze-dried native MLFS on a pilot scale in Argentina. Furthermore, our re-search sheds light on the role of LAB in shaping the sensory properties of wine, since we began with a single batch of Malbec grape from one province and obtained four very distinctive wines reminiscent of different viticultural regions.

Our results open several lines of future research, from exploring LAB diversity from other viticultural regions, with the concomitant oenological benefits associated with the exploitation of microbial diversity in winemaking. Other promising research lines involve assaying different LAB combinations for MLF starters, as well as optimizing preservation techniques for the starters already developed so that they can be transferred to the productive sector, thus offering local producers national MLFS that can replace imports, enhancing regional *terroirs*.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fermentation11060337/s1>, Table S1: Number of viable cells of the four Wines at initial time and after 15 days of inoculation in MRS and MLO media, as well as kinetics parameters of L-malic acid consumption (Equation (1)) shown in Figure 1; Table S2: Implantation capacity of the BAL strains present in the MLFS according to their RAPD-PCR profiles.

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Abbreviations

The following abbreviations are used in this manuscript:

AF	Alcoholic Fermentation
MLF	Malolactic Fermentation
LAB	Lactic Acid Bacteria
IMBA	Instituto de Microbiología Básica y Aplicada
DCyT	Departamento de Ciencia y Tecnología
UNQ	Universidad Nacional de Quilmes
CIC	Comisión de Investigaciones Científicas
RGPRN	Registro Provincial de Recursos Naturales

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