

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

## The Use of Indigenous Yeast to Develop High-Quality Patagonian Wines

---

Silvana María Del Mónaco, Yolanda Curilen,  
Ramona Del Carmen Maturano,  
Sebastián Mario Ezequiel Bravo,  
Adriana Beatriz Simes and  
Adriana Catalina Caballero

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64750>

---

### Abstract

In young wines, the compounds responsible for wine *flavor* come from two possible origins: grapes and microorganisms involved in winemaking. Yeasts play the most important role in flavor influence because of their role in conducting the alcoholic fermentation (FA), the key process of winemaking. Ecological studies show that yeast diversity is significantly influenced by geographical and technological features of each particular winegrowing region. Wines from Argentina have achieved high-quality certifications, and particularly, in the Comahue region, wine production is mainly oriented to young red wines varieties, some of which found in this region optimal ecological condition to express all their enological potential. Despite this, the need to satisfy the demands of an increasingly competitive and globalized international market and the consumer demand for new wine styles with the best quality/price ratio imposes the regional productive sector new challenges that require technological innovation. The use of starter cultures developed from indigenous yeast isolated from our region, specially selected for its enological properties, appears as a valuable tool for differentiation, diversification, and quality improvement of wines. In this context, conventional and non-conventional yeasts were isolated and selected over the years and used for vinifications in red grape varieties (Pinot noir and Malbec). Assays were carried out at laboratory and pilot scale, in the 2010–2015 vintages. The experiences developed along the years contribute to a better understanding of the processes involved in the production of improved wines by autochthonous strains, an important practice to develop a more competitive regional wine industry.

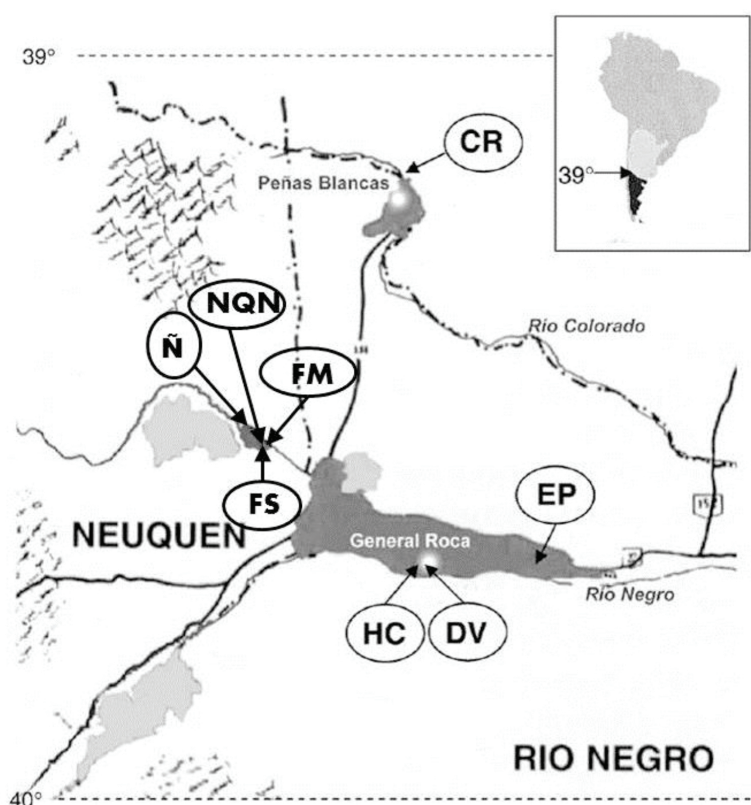
**Keywords:** wine flavor, fermentation, *Saccharomyces cerevisiae*, non-*Saccharomyces* yeasts, mixed starters, microbial interactions, indigenous biota

---

## 1. Introduction

Wine chemical composition is the foundation for its sensorial features, as color, appearance, body, flavor (aroma and taste), as well as its mouth and palate sensations [1]. Among these, wine flavor is a key attribute for quality and choice from consumers. The flavor of wine is a sensory perception that varies with the individual, the context of the consumer experience, and the chemical composition of the product [2, 3]. The wine chemical composition is determined by many factors such as grape variety, the geographical and viticultural conditions of grape cultivation, the microbial ecology of the grape and fermentation processes, and winemaking practices [4]. In young wines (without aging), like the ones mainly produced in the Patagonia Argentina, the compounds responsible for wine flavor come from two possible origins: grapes and microorganisms involved in winemaking, mostly yeast and to a lesser extent lactic acid bacteria (LAB) [5]. Grapes contribute with varietal aroma compounds, such as floral monoterpenes or volatile thiols, among others [1]. Among the microorganisms, yeasts play the most important role in flavor influence because of their role in conducting the alcoholic fermentation (AF), the key process of winemaking. During AF yeasts transform grape sugars and other components to ethanol, carbon dioxide, and different primary metabolites that confer a particular character to wine, but they also contribute with minority volatile compounds involved in determining the fermentative or secondary aroma [1, 2, 6, 7]. These compounds (esters, higher alcohols, carbonyls, short-chain fatty acids and sulfur compounds) arise from the metabolism of sugars and amino acids from the must, and their quality and content depends on the ecology wine yeast associated with the process [1]. Finally, when needed, LAB through malolactic fermentation not only provides wine deacidification, but can also enhance its flavor profile [1, 2, 8].

In Argentina, wine production has historically occupied a place of importance in the agricultural industries. The country is currently the fifth largest producer of wines, the seventh consumer, and the tenth largest exporter. There are two ways of imposing a wine in the market, highlighting its grape quality, that is, *Vitis vinifera* variety or alluding to the region from where they were harvested and vinified (*terroir*). While wines from Argentina are sold taking into account the variety, there are regions and subregions in the country where wines achieved high-quality certifications (of origin or geographical designations) [9]. One of them is the Comahue region, located in the Argentinean North Patagonia at 39–40° southern latitude; it is the southernmost winegrowing region of Argentina and one of the most southern regions in the world (**Figure 1**). This region has optimal agro-ecological conditions for high-quality viticulture and a long winemaking tradition [10] so although the wine industry is still a secondary economic activity, it represents a very interesting alternative to diversify the local production.



**Figure 1.** Comahue region (Argentinean North Patagonia). Location of sampled Patagonian cellars and vineyards (EP, DV, HC, and CR in Río Negro Province and FS, FM, NQN, and Ñ in Neuquén Province). Dark gray: cultured vine areas. In the right top corner: South America (dark gray), Argentina (light gray), and Argentinean Patagonia (black). Source: INV.

North Patagonian wine production is mainly oriented to elaboration of young dry wines from red grape varieties (80%) some of each, as Pinot noir and Merlot, have found in this region the optimal ecological conditions to express all their enological potential [11]. Additionally, important volumes of Malbec grapes, the *Vitis vinifera*, L variety emblematic of Argentina, are also vinified [12]. Despite this, the need to satisfy the demands of an increasingly competitive and globalized international market actually oversupplied, and the consumer demand for new wine styles with the best quality/price ratio, impose the national productive sector, and in particular the regional one, new challenges that require technological innovation.

Actually, regional wine production is based on both spontaneous alcoholic fermentations of the grape musts or conducted fermentations using commercial yeast starters. However, commercial starters for alcoholic fermentation found actually in the market are composed by yeast strains isolated from the most important winegrowing areas in the world, except Argentina. Given the significant influence that the biota of yeast has on the aromatic quality of young wines elaborated from aromatically neutral grapes, wine style produced mostly in Patagonia, development of starter cultures consisting of yeast strains isolated from the own region (indigenous yeasts) appears as a valuable tool to improve the quality of Patagonian vitiviniculture, upgrading its capacity of commercial competition in domestic and international market.

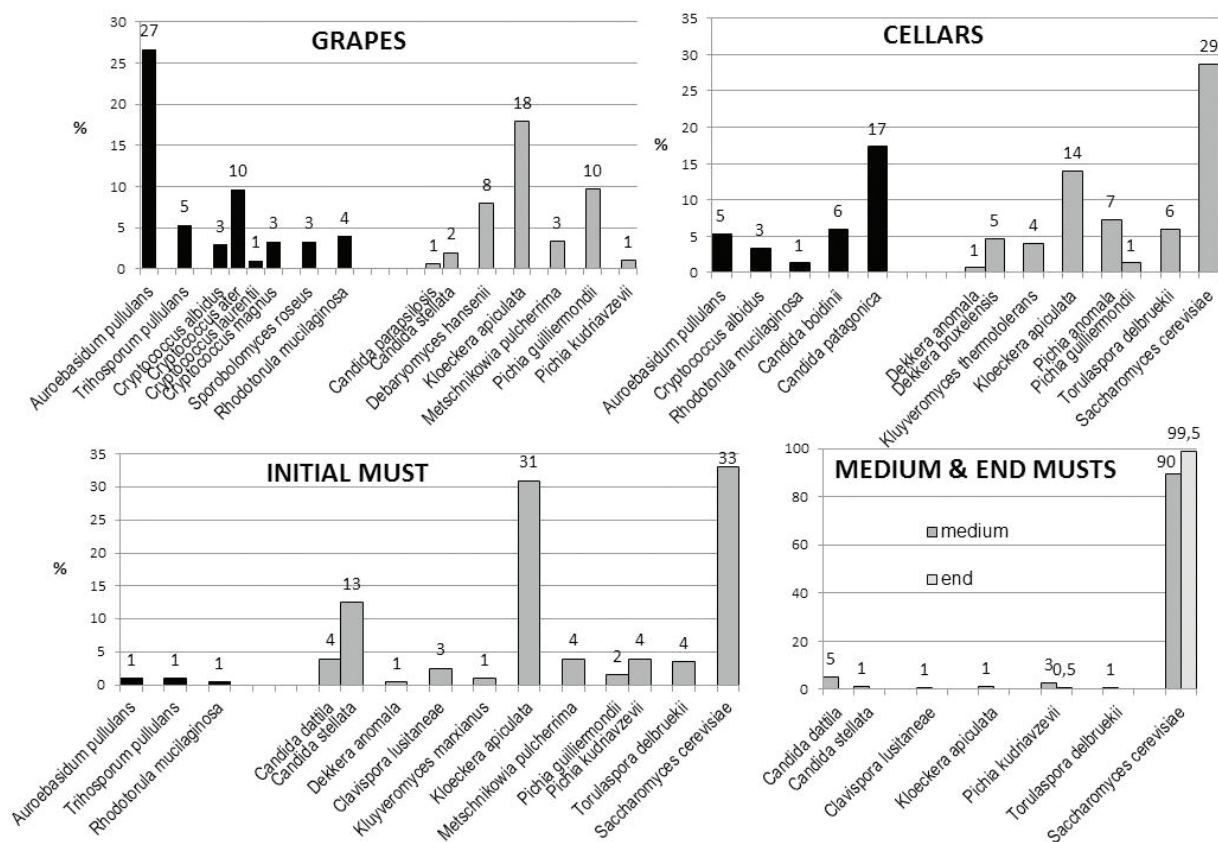
### 1.1. The microbial ecology of Patagonian red winemaking

Winemaking is a complex microbial ecosystem that involves interactions between filamentous fungi, yeasts, and bacteria with different physiological and metabolic characteristics [3, 13, 14]. Ecological studies have shown that this microbial diversity is significantly influenced by geographical and technological features of each particular winegrowing region, which defines the terroir. To understand how this microbial terroir contributes to the natural environment of vineyard and how it imprints differential character of wine required to know all processes associated with winemaking which start at the harvest of grapes and then evolve throughout fermentation process [15–17].

Microbiological studies carried out during several years in the Patagonian region allowed to characterize the biota associated with grapes [18–22], cellars [23, 24], and red vinification environments [19–22, 25, 26], (**Figure 2**). Yeasts associated with spontaneous wine fermentations come from two possible origins: grapes and cellar surfaces. Several factors such as development stage and sanitary state of the berries, the climate (particularly temperature and rainfall), water availability, direct exposure to sunlight, use of agrichemicals, grape vine canopy management system as well as nature, cleaning and sanitization of equipment surfaces, nature, cleaning and sanitization of equipment surfaces, among others, as well as certain enological practices could affect the yeast community composition on grapes and cellar surfaces affecting the kinetics of yeasts growth during fermentation [3, 14, 27]. In this context, in all cases, ripe, whole and healthy grapes from Merlot, Malbec, and Pinot varieties were gathered by random sampling ( $n = 536$ ) from vineyards associated with cellars which is noted in **Figure 1** for 1993–1998 and 2005–2009 vintages at harvest time, and cellar sampling was carried out on the internal walls of the fermentation vats from the same cellars approximately four weeks before harvest. Yeast samples from grape surfaces were obtained by agitation followed sonication of each grape in pure and sterile water. Additionally, samples (1L) of Malbec, Merlot, and Pinot noir fermentation musts samples were taken in duplicates during spontaneous alcoholic fermentation at the initial (14°Bé), middle (6°Bé), and end ( $\geq 2$  g/L TRS) stages in the same cellars described above. All yeasts were isolated on GPY agar (composition in g/l: yeast extract 10, glucose 20, peptone 20, and agar 20; pH 4.5 supplemented with 100 ppm of ampicillin) plates, and they were identified according to the methods and keys proposed by Kurtzman and Fell [28] and by PCR-RFLP analysis of the ITS1-5,8S-ITS2 region from the nuclear rDNA gene complex [29]. Results of these studies evidence that yeast biota associated with grape surfaces are mostly aerobic (57%), while the one associated with cellar surface is mostly facultative (64%). These results of yeast diversity obtained from initial musts are consistent with the hypothesis proposed on origin of yeasts musts (**Figure 2**). On the other hand, this study also evidences that *Saccharomyces cerevisiae* occur in extremely low population on healthy, undamaged grapes (1/536 isolates, <0.2%) and it is the major species (45/150 isolates, 30%) together with species of genera *Candida*, *Kloeckera/Hanseniaspora*, and other sporadic yeasts on winery surface (artificial man-made environmental), hypothesis claimed by Martini school [30–33]. Additionally and in agreement with the reported in bibliography [13, 34–36], even though *S. cerevisiae* is the most important yeast in spontaneous vinifications, other species of yeasts belonging to non-*Saccharomyces* species such as *Kloeckera apiculata/Hanseniaspora*



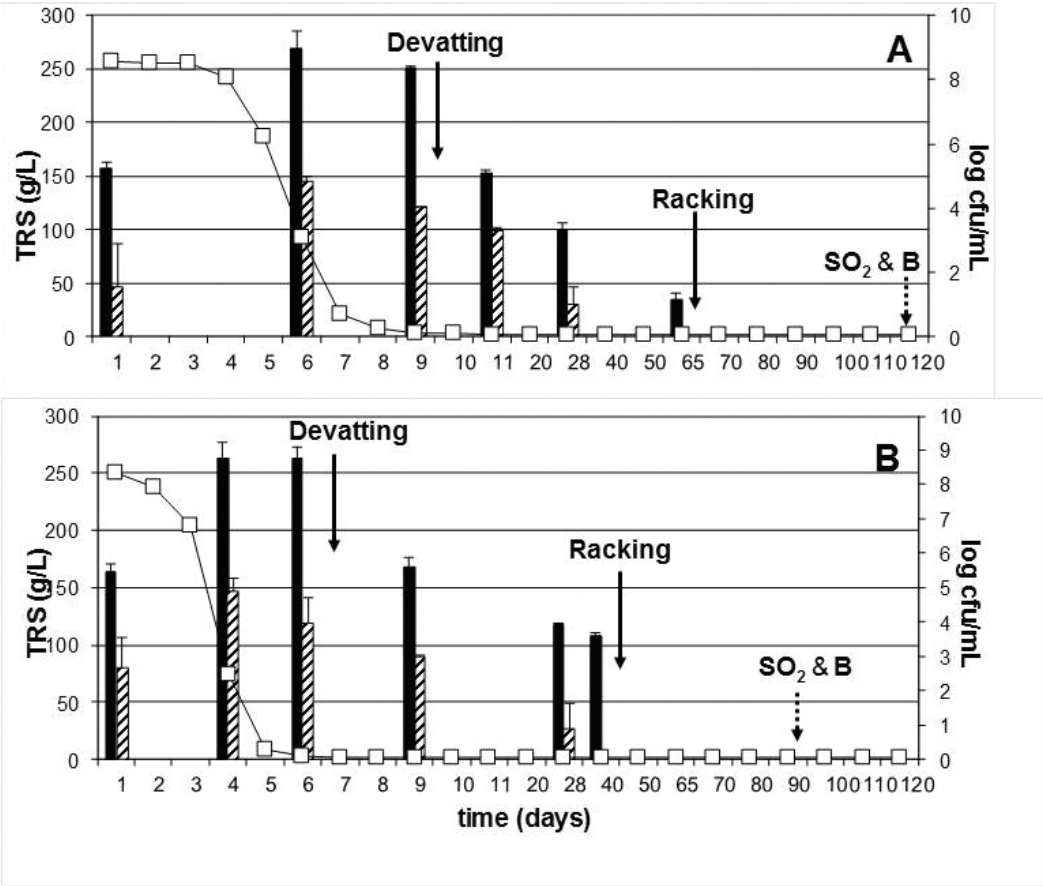
*uvarum*, *Candida stellata* and *dattila*, *Pichia kudriavzevii* and *Torulaspora delbrueckii* are also able to remain in Patagonian musts and during fermentation periods in appropriate concentrations to significantly contribute to the sensory quality of the product (**Figure 2**). Hence, the yeast ecology of wine fermentation has been found to be much more complex than assumed dominance of *S. cerevisiae* species, and the metabolic impact of yeasts on wine character is much more diverse than simple fermentation of grape juice sugars [36, 37]. Additionally, the incorporation of molecular methods (mitADN RFLP using Hinf I) together with killer biotype in these studies demonstrated the existence of a wide variety of individuals (strains) within indigenous populations of *S. cerevisiae* [38] and other different species of yeast such as *K. apiculata*, *Metschnikowia pulcherrima*, and *Pichia guilliermondii* [21], as well as *P. kudriavzevii* [39]. This intraspecific variability was significantly influenced by geographical or/and technological specific factors of productive region and it is very important from the enological point of view. Similar results have been reported from other winegrowing regions of the world [40, 41].



**Figure 2.** Diversity of yeasts associated with Grape,  $n = 536$ ; and cellar surfaces,  $n = 150$  (up) and musts from initial ( $14^{\circ}\text{Bé}$ ,  $n = 322$ ), middle ( $6^{\circ}\text{Bé}$ ,  $n = 320$ ), and end ( $0^{\circ}\text{Bé}$ ,  $n = 397$ ) stages from spontaneous fermentations (down). Black bars, aerobic yeasts (respiratory strict metabolism), and gray bars, facultative yeasts (respiratory and fermentative metabolism).

With this greater knowledge, alcoholic fermentation is now seen as a key process where winemakers can creatively engineer wine character and value through better yeast management and can strategically tailor wines to a changing market [17].

Malolactic fermentation (MLF) is other opportunity to modulate the aroma of wine [1, 42]. MLF, the decarboxylation of L(-)malic acid to L(+)lactic acid, is an important secondary fermentation carried out by lactic acid bacteria (LAB) during the vinification of most red wine styles as those elaborated in Patagonia. Malic acid, together with tartaric acid, is the most important constituents of organic nonvolatile acid fraction in grapes and grape musts, accounting for 90% of the titratable acidity and imbalances in this fraction can affect the physicochemical and sensory properties of wine, mainly mouthfeel [43–48]. In addition to deacidification, MLF can increase microbiological stability [13, 49] and enhance wine flavor and/or complexity [1, 50] but off-flavors as well as dangerous health compounds could also be formed [45]. Although *Oenococcus oeni* is the major species during malolactic fermentation other LAB species belonging to *Lactobacillus*, *Leuconostoc* and *Pediococcus* genera can grow in the wine [8, 22, 34, 45, 51, 52] and their contribution on wine quality should not be underestimated [34, 53]. Recent red wine trials carried out at laboratory scale have shown that strains of *Lactobacillus plantarum* have the potential to conduct an efficient MLF and also produce desirable sensory attributes in red wines [54, 55]. However, at industrial-scale, spontaneous MLF is a very difficult process because different factors associated with winemaking, as yeast-



**Figure 3.** Time course of fermentative processes and growth kinetics of yeasts (black bars) and lactic acid bacteria (stripped bars) in spontaneous (a) and guided (b) Pinot noir vinifications carried out at pilot scale. SO<sub>2</sub> and B: Sulfur dioxide adding and bottling. Extracted from Curilén et al. [22].

LAB antagonistic interactions, inhibit lactic bacteria growth [8, 22, 34, 56, 57] and the use of commercial starters to induce and guide the process is not always effective [58]. Various studies have been done to attempt an understanding of the interaction between yeast and bacteria [59–64]. The degree and type of interactions vary from one pair to another and seem to be closely related to the chosen yeast strain.

Several factors such as grapevine variety, vineyard agricultural practice, temperature, humidity and berry maturity degree, among others, may affect organic nonvolatile acid concentration in grape musts [44, 65, 66]. In particular, L(–)malic acid content, directly related to respiratory quotient of berries, is higher in grape musts from cooler regions than the ones from warmer regions [67]. In the Comahue region, one of the southernmost winegrowing regions of the world, malic acid concentrations account for the 56% of red grape must titratable acidity reaching the 66% in Pinot noir [19] the emblematic regional vine variety [11]. Additionally, to its contribution to wine acidity, malic acid represents a fermentable substrate for other microorganisms which can spoil the wine before and after bottling [68]. Without adjustment of acidity, the wines will be regarded as unbalanced or spoiled [1]. For these reasons, MLF is a routine enological practice in the Patagonian red winemaking and yeast-LAB interactions are a great concern for winemakers and researchers.

Organic acid *	Grapes	Wines				P value*
		GF		NF		
		Running wine	Bottled	Running wine	Bottled	
L(-)malic	2.20 ± 0.66 <sup>a</sup>	2.08 ± 0.02 <sup>ab</sup>	2.01 ± 0.01 <sup>b</sup>	1.16 ± 0.02 <sup>c</sup>	0.97 ± 0.01 <sup>d</sup>	<0.001
DL lactic	Nd	0.84 ± 0.00 <sup>a</sup>	0.58 ± 0.00 <sup>b</sup>	0.97 ± 0.09 <sup>c</sup>	1.12 ± 0.02 <sup>d</sup>	<0.001
L(+)lactic	Nd	0.25 ± 0.03 <sup>a</sup>	0.30 ± 0.01 <sup>a</sup>	0.55 ± 0.04 <sup>b</sup>	0.75 ± 0.04 <sup>c</sup>	<0.001
Citric	0.78 ± 0.11 <sup>a</sup>	1.06 ± 0.04 <sup>a</sup>	nd <sup>b</sup>	0.83 ± 0.14 <sup>a</sup>	nd <sup>b</sup>	<0.001
Succinic	Nd	1.50 ± 0.12 <sup>a</sup>	0.96 ± 0.16 <sup>b</sup>	1.07 ± 0.13 <sup>ab</sup>	0.86 ± 0.05 <sup>b</sup>	<0.001

<sup>a</sup>g L<sup>–1</sup>; <sup>b</sup>mg L<sup>–1</sup>; nd: not detected; \*one-way ANOVA and Tukey's test n = 2.

**Table 1.** Organic acids composition of Patagonian Pinot noir grapes and wines obtained from guided (GF) and spontaneous (NF) winemaking (extracted from Curilén et al. [22]).

In Patagonian winegrowing region, potential yeast-LAB interaction was studied in Pinot noir wine fermentations carried out at pilot [22] and industrial scales [8]. In all cases, malolactic fermentations were carried out spontaneously, whereas alcoholic fermentations were carried out in both, spontaneous (NF, carried out by indigenous yeast biota) and guided (GF, carried out by *S. cerevisiae* F15, Laffort) forms. All microbiological and physicochemical processes were characterized (**Figure 3** and **Table 1**). Musts and wines samples were appropriately diluted, and they were spread in duplicate onto MRS plus tomato agar (total LAB) supplemented with 100 ppm cycloheximide. Organic acid were quantified by HPLC and enzymatically (L and D malic acids). Results evidence that the numerical dominance of commercial starters at the GF beginning affects their fermentation kinetic and yeast diversity. Although AF as well as MLF



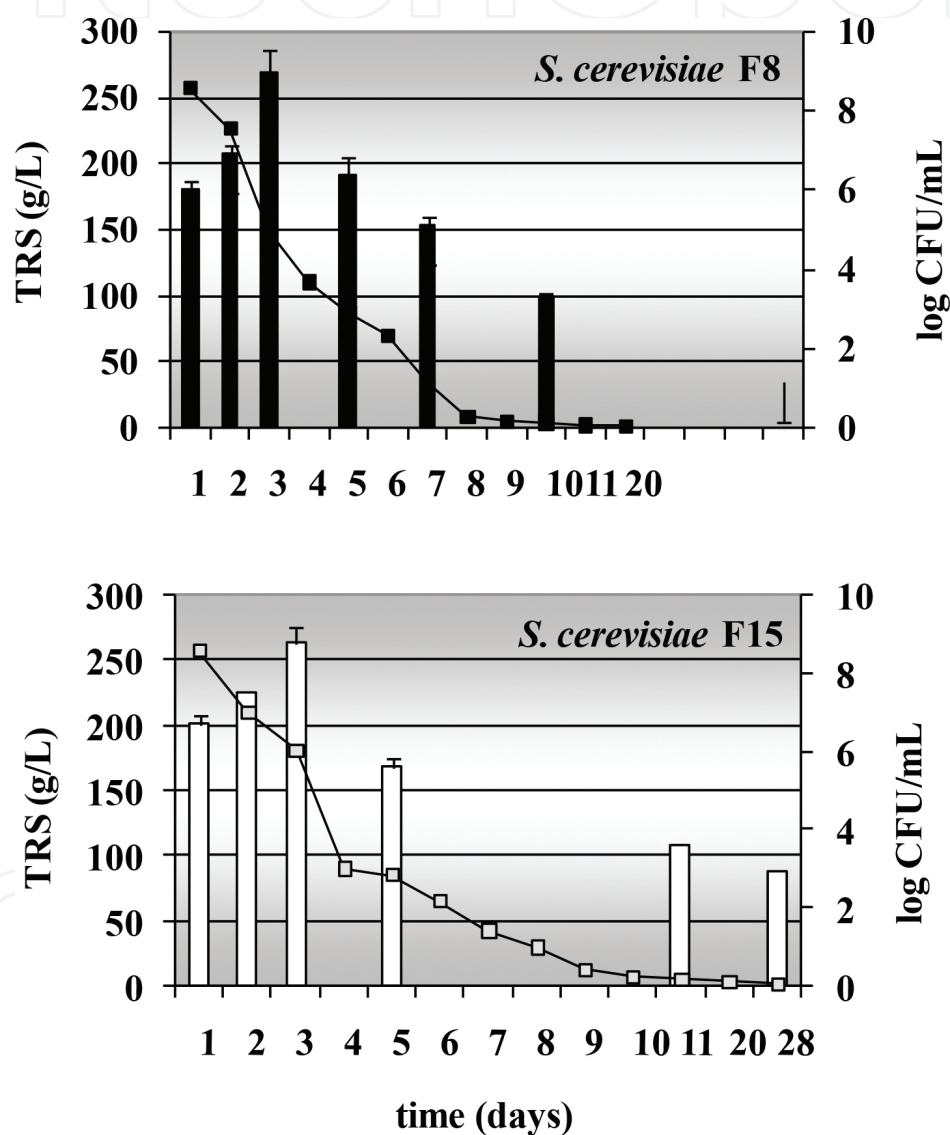
showed normal kinetics for the assayed scale and both were complete fermentations, inoculation with commercial *S. cerevisiae* F15 starter significantly affected the time course of the global fermentative process, which was faster in GF than in NF (**Figure 3**). **Figure 3** also shows no significant changes in total yeast biota extension when similar stages between both fermentations are compared, but their qualities at initial stages were significantly different because all non-*Saccharomyces* yeasts were eliminated by starter inoculation (data not shown). Like for yeasts, no changes were observed in the LAB biota extension (**Figure 3**), but its quality was significantly affected by the inoculation. At the beginning of both fermentations, LAB biota were mostly facultatively homofermentative (62%) with *Lb. plantarum* as the major species (8 of 13 isolates), and minor heterofermentative LAB isolates were presumptively identified as belonging to *Leuconostoc* (1) and *Oenococcus oeni* (1) [22]. However, NF LAB evolved to a mostly heterofermentative biota ( $\geq 66\%$  in average) from middle stage up to racking, whereas the GF LAB one was always mostly facultatively homofermentative ( $\geq 50\%$  in average). These differences in LAB biota between NF and GF were consistent with the data obtained for FML evolution from chemical analysis evidencing antagonist interaction between *S. cerevisiae* F15 and LAB biota. In this context, yeast-LAB interactions should be included in the selection criteria for Patagonian wine yeasts.

## 2. Development of patagonian yeast starters

The practical consequence of studies on microbial ecology in winemaking was the development of starter cultures of AF. The use of commercial starter cultures for the FA in enology, with selected cultures of *S. cerevisiae* for the inoculation of fruit juice, has been applied since the 60s from the last century, and it has been one of the most important technological advances in the wine industry [69, 70].

The inoculation of grape musts with commercial starters was an enological practice strongly resisted by wine producers from Europe. The solidest argument for this resistance referred to the sensory quality standard, where flattened aromatic profiles were produced in each wine fermented with those starters. In modern wineries, the use of commercial starter culture to steer fermentations is being doubted, since they often lack of some advantageous enological traits, which are present when the spontaneous fermentation is ruled by indigenous populations [6]. For that reason, the exploitation of indigenous strains biodiversity has great importance for the characterization and selection of strains with peculiar phenotypes [17, 36]. While the preservation of spontaneous microflora is essential to obtain the typical flavor and aroma of wines deriving from different grape varieties [5, 13], the development of starter from *S. cerevisiae* strains indigenous of each winegrowing region ended this controversy [26]. The advantages to use starters of indigenous *S. cerevisiae* strains, better adapted to the ecological and technological features of each particular winegrowing area preserving its own natural biodiversity, are now recognized by all wine producers, including the European ones. However, the knowledge generated in recent years in the field of enological microbiology and described above, evidence that during the process, certain non-*Saccharomyces* species can also contribute significantly to the sensory characteristics of wines by producing compounds that

impact on the varietal aroma and in its taste. The potential of using them in winemaking as stand-alone, single starter cultures, or together with *Saccharomyces* yeast cultures as mixed starter cultures could be especially interesting in elaboration of wines from *Vitis vinifera* varieties aromatically neutral, such as mostly vinified in Comahue region, whose aroma is developed exclusively during fermentation. For that reason, in the last years, there has been an increasing interest for the selection of strains of non-*Saccharomyces* yeasts for the winemaking industry, mainly due to their ability to enhance the analytical composition of the wines, particularly for their effects on wine composition, flavor, and aroma [39, 71–81].



**Figure 4.** Yeast growth (bars) and total reducing sugars evolution (squares) during pilot scale Pinot noir vinifications guided by indigenous F8 (upper panel, black symbols) and commercial F15 (bottom panel, white symbols) *S. cerevisiae* strains [26].

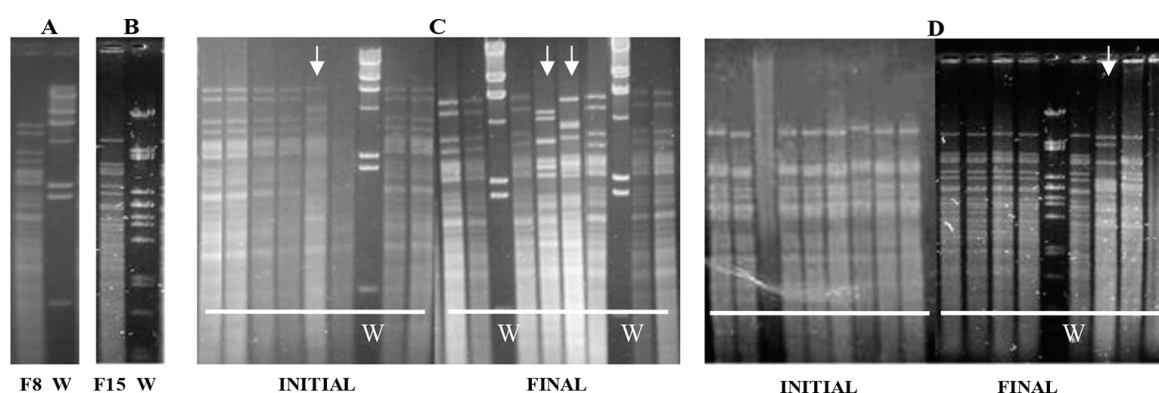
Microbiological studies carried out during several years in the Patagonian region described above allowed constituting an important collection of organisms relevant for enological

application and wine starter elaboration. Our enology applied studies were divided into two basic objectives: isolation of indigenous *S. cerevisiae* strains and isolation of local non-*Saccharomyces* strains with potential use in enology for develop pure and mixed indigenous starters to conduct AF. From those studies two yeast strains were selected as follows: an indigenous *S. cerevisiae* named F8 and a *P. kudriavzevii* strain called ÑNI15 which could metabolize malic acid and control wine acidity. Vinification studies were carried out using Pinot noir, Merlot, and Malbec grape musts as substrates and alcoholic fermentations were guided by with those strains, using a commercial *S. cerevisiae* (F15, Laffort) strain as control for comparison.

### 2.1. *Saccharomyces cerevisiae* indigenous starter

When indigenous F8 and commercial F15 *S. cerevisiae* strains were evaluated, the initial cellular densities and biomass evolution were similar in Pinot noir fermentations (**Figure 4**) as well as in Merlot and Malbec fermentations (data not shown). Additionally, fermentations were mostly completed to dryness ( $\text{TRS} \leq 2 \text{ g/L}$ ), but the sugar consumption rates during dryness stages, such as it is displayed in **Figure 4** for Pinot noir, were ever higher in F8-guided fermentations than in F15 fermentations. As a consequence, fermentative processes guided by the indigenous starter were faster than those guided by the commercial starter.

In order to evaluate the capacity of the indigenous starter to dominate the fermentations, the dynamics of the *S. cerevisiae* populations were determined by means of mtDNA-RFLP analysis. The results obtained from these studies, and partially showed in **Figure 5**, evidence that indigenous F8 and commercial F15 *S. cerevisiae*, were the strains mostly found at the initial and final stages of their respective fermentations proving their very good and similar implantation capabilities.

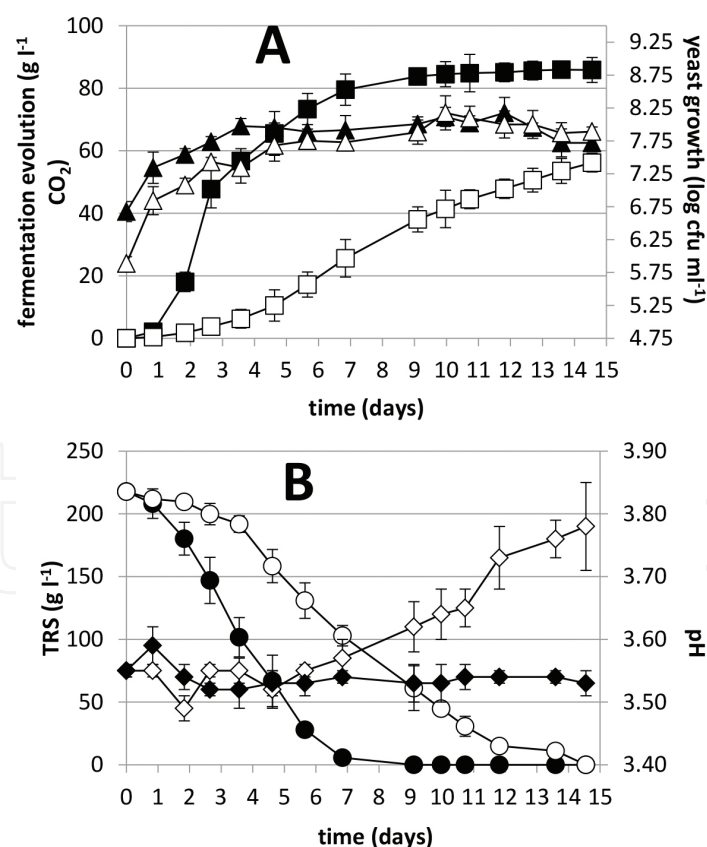


**Figure 5.** mtDNA-RFLP patterns of indigenous (a) and commercial (b) starters and of *S. cerevisiae* isolates obtained from F8 (c) and F15 (d) guided Pinot noir fermentations (2013 Vintage) at the initial and final stages. Arrows indicate isolates with mtDNA-RFLP patterns different from the inoculated starters. W = molecular weight marker [26].

On the other hand, physicochemical analysis of the wines obtained during different years from Pinot noir, Merlot, and Malbec varieties were highly similar between both inoculated strains, where every product was considered acceptable for local young wines [26]. Nonetheless,

sensorial analysis carried out by experts and consumers using qualitative and quantitative tests, respectively, showed significant differences between F8 and F15 wines.

Qualitative analysis was performed by a panel of experts using descriptive tests. As a whole, the global quality scores obtained in this analysis by F8 Pinot noir (68 = good) and Merlot (6.6 = pleasant) wines were higher than those obtained by F15 Pinot noir (52 = correct) and Merlot (5.7 = slightly pleasant) wines. Particular descriptions evidenced that Pinot noir F8 wines had good color intensities (showing a red color typical for the variety) and aromas of red fruits (cherries) with notes of sherry. In mouth, they were described as middling fruity, slightly rusty, sweet alcoholic. Meanwhile, Pinot noir F15 wines showed limpid and bright aspect and an intense reduced aroma that did not disappear with agitation. In mouth, they were perceived as slightly fruity and bitter, astringent, and tannic. On the other hand, both F8 and F15 Merlot wines showed a limpid and bright aspect and an intense brick red color, but the F8 aroma was more intense than the F15 aroma, being both aromas of medium quality. Pepper, red fruits, butter, leather, spice, and vanilla were the aromatic descriptors highlighted in the former and green pepper, cooked red fruits, spices, and pepper were described for the later. In the mouth, both wines showed good acidity and body, and they were persistent. However, F8 wines were described as round and equilibrated while F15 wines showed a tart taste [26].



**Figure 6.** Microvinifications guided by *P. kudriavzevii* (white symbols) and *S. cerevisiae* (black symbols) indigenous strains. (a) Fermentation evolution (squares) and yeast growth (triangles). (b) Total reducing sugars (TRS) (circles) and pH values (diamonds) evolution during the processes [39].

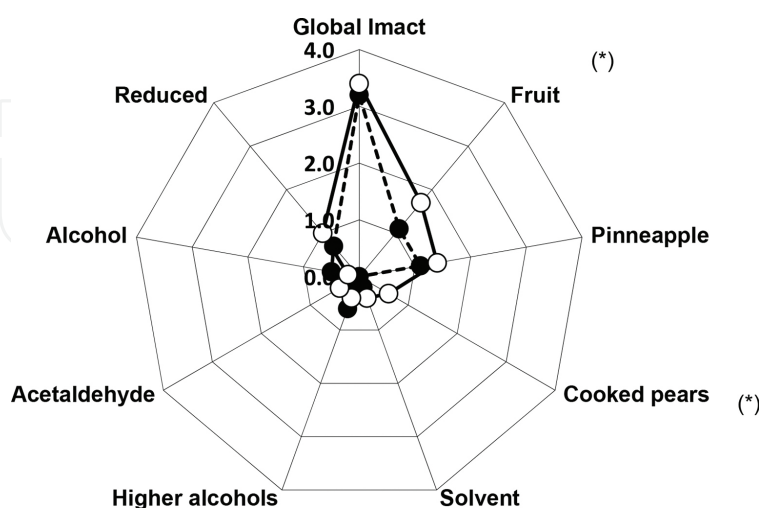


At last, F8 Pinot noir and F8 Merlot wines were the favorite for the consumers ( $p < 0.05$ ) with 72 favorable responses out of 119 questioned and 13 favorable responses out of 17 questioned, respectively, compared with their F15 controls according to the paired-preference test.

## 2.2. Non-Saccharomyces indigenous starter

One of the purposes of the study was to select autochthonous yeasts with metabolic ability to degrade L-malic acid for its potential use in equilibrated young wine elaboration. A total of 57 Patagonian non-*Saccharomyces* yeast of enological origin were identified by conventional molecular methods and tested in their capability to grow at the expense of L-malic acid. An isolate, noted as *P. kudriavzevii* ÑNI15, was able to degrade L-malic acid in microvinifications, increasing the pH 0.2–0.3 units with a minimal effect on the acid structure of wine. Additionally, this isolate was a weak producer of ethanol, an important producer of glycerol ( $10.41 \pm 0.48 \text{ g l}^{-1}$ ), a producer of acceptable amounts of acetic acid ( $0.86 \pm 0.13 \text{ g l}^{-1}$ ), as well as it was able to improve the sensorial attributes of wine increasing its fruity aroma [39].

**Figure 6** shows the results obtained from vinifications guided by pure cultures indigenous *P. kudriavzevii* ÑNI15 and *S. cerevisiae* F8, carried out at laboratory and using synthetic musts with similar amino and organic acids composition to Patagonian Pinot noir juice as substrate. An acceptable yield in biomass (**Figure 6a**) and similar end sugar concentrations (**Figure 6b**) were observed in both microvinifications; however, the fermentative efficiency (**Figure 6a**) as well as the sugar consumption rate (**Figure 6b**) were higher for *S. cerevisiae* than for *P. kudriavzevii*. A noteworthy fact, and in agreement with what was reported in the broth assays, is that *P. kudriavzevii* was again capable of raising significantly the medium pH with a minimal effect on acid structure of the wine, whereas in the *S. cerevisiae*, culture pH was maintained constant along the fermentation (**Figure 6b**).



**Figure 7.** Sensory quality of microvinification wines obtained from *P. kudriavzevii* (white circles) or *S. cerevisiae* (black circles) inoculation. ANOVA and Tukey's test,  $n = 12$ . Asterisks indicate statistic differences ( $p < 0.05$ ) [39].



Finally, sensorial analysis evidenced significantly differences in aromatic perception between *P. kudriavzevii* and *S. cerevisiae* wines. These differences were in favor of the former, which showed a higher fruity and cooked pears aroma than the latter (**Figure 7**).

### 3. Conclusions

The extension of the selection of yeast for enological use among *Saccharomyces* and non-*Saccharomyces* species led to the finding of yeast strains with novel and interesting enological characteristics which could have significant implications in the production quality improved Patagonian young wines. Results presented show that *S. cerevisiae* F8 strain drives red vinifications improving the quality of the local fermented products. On the other hand, the use of *P. kudriavzevii* ÑNI15 as wine starter would eliminate the cultural and cellar operations undertaken to adjust the musts acidity improving wine quality and reducing production costs. The co-inoculation of *S. cerevisiae* F8 and *P. kudriavzevii* ÑNI15 in local musts implies the enological potential of using these strains to formulate a regional starter culture for the production of well-balanced and physicochemical stable Patagonian young red wines.

### Acknowledgements

This work was supported by grants from Universidad Nacional del Comahue (Programa de Investigación 04/L003 Desarrollo de Tecnologías y de Productos de Interés para la Industria Agroalimentaria) and MINCyT (PICT SU 2804/12 Levaduras y Bacterias Lácticas para la diferenciación de vinos Patagónicos).

### Author details

Silvana María Del Mónaco<sup>1\*</sup>, Yolanda Curilen<sup>1,2</sup>, Ramona Del Carmen Maturano<sup>1</sup>, Sebastián Mario Ezequiel Bravo<sup>1,2</sup>, Adriana Beatriz Simes<sup>2</sup> and Adriana Catalina Caballero<sup>1,2</sup>

\*Address all correspondence to: silmdm@yahoo.com

1 Enology Group, Institute of Investigation and Development in Process Engineering, Biotechnology and Alternative Energies (PROBIEN), National Council of Scientific and Technical Investigations (CONICET), Neuquén City, Neuquén Province, Argentina

2 Faculty of Science and Food Technology, National University of Comahue, Villa Regina City, Río Negro Province, Argentina

## References

- [1] Swiegers, J.H., Bartowsky E.J. Henschke, P.A., Pretorius I.S. Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research*. 2005;11:139–173.
- [2] Lambrechts, M.G., Pretorius, I.S. Yeast and its importance. *South African Journal of Enology and Viticulture*. 2000;21:97–129.
- [3] Fleet, G.H. Yeast interactions and wine flavour. *International Journal of Food Microbiology*. 2003;86:11–22.
- [4] Cole, V.C., Noble, A.C. Flavour chemistry and assessment. In: Lea, A.G.H., Piggott, J.R., editors. *Fermented Beverage Production*. London: Blackie Academic and Professional; 1997. p. 361–385.
- [5] Renouf, V., Claisse, O., Lonvaud-Funel, A. Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. *Australian Journal of Grape Wine and Research*. 2005;11:316–327.
- [6] Fleet, G.H., Heard, G. Yeasts-growth during fermentation. In: Fleet, G.H., editors. *Wine Microbiology and Biotechnology*. Chur, Switzerland: Harwood Academic Publishers; 1993. p. 27–54.
- [7] Romano, P., Fiore, C., Paraggio, M., Caruso, M., Capece, A. Function of yeast species and strains in wine flavour. *International Journal of Food Microbiology*. 2003;86:169–180.
- [8] Curilén, Y., Carreño, V., Camacho, E., del Mónaco, S., Semorile, L., Caballero, A. Influence of yeast biota on spontaneous malolactic fermentations associated with Patagonian winemaking. In: *Proceedings of 37th World Congress of Vine and Wine: Southern Vitiviniculture, a Confluence of Knowledge and Nature*; 9–14 November 2014; Mendoza (ARGENTINA). 514:2014. p. 1–6.
- [9] Llorente, A., Casazza, M. Southern Winegrowing Region from Argentina. *El vino y su industria*. 2005;40:7–13.
- [10] Le Guillou, B. Río Negro. Vines in the oases from Argentine Patagonia. *La Vigne*. 2000;116:112–113.
- [11] Weizman, D. The Argentine map of the senses. *Rumbos*. 2009;326:18–24.
- [12] Catania C., Avagnina S. Sensory interpretation of Wine . In: *Argentina's wine terroir*. INTA y Editora Andina Sur.; 2010. p. 111–121.
- [13] Pretorius, I.S. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast*. 2000;16:675–729.

- [14] Barata, A., Malfeito-Ferreira, M., Loureiro, V. The microbial ecology of wine grape berries. *International Journal of Food Microbiology*. 2012;153:243–259.
- [15] Van Leeuwen, C., Seguin, G. The concept of terroir in viticulture. *Journal of Wine Research*. 2006;17:1–10.
- [16] Bokulich, N.A., Ohta, M., Richardson, P.M., Mills, D.A. Monitoring seasonal changes in winery-resident microbiota. *PLoS One*. 2013; 8:e66437.
- [17] Pinto, C., Pinho, D., Cardoso, R., Custódio, V., Fernandes, J., Sousa, S., Pinheiro, M., Egas, C., Gomes, A.C. Wine fermentation microbiome: a landscape from different Portuguese wine appellations. *Frontiers in Microbiology*. 2015;6(905):1–13.
- [18] van Broock, M., Zajonskovsky, I., Assadourian, M., Lavallo L., Caballero de Castro, A. Wine yeasts associated to merlot type grapes from North Patagonian region. An ecological study. In: *Proceedings 10th International Biotechnology Symposium and 9th International Symposium on Yeasts*. Sydney, Australia. 1996. p. 13–5.
- [19] Caballero, A., Sangorrín, M., Lopes, C.A., Rodríguez, M.E., Lavallo, T.L., Zajonskovsky, I.E., Arcucci, G., Mariconda, L., Méndez, P. Diversity of indigenous yeasts of Southern Winegrowing Region. *El vino y su industria*. 2004;19:56–68.
- [20] Caballero, A., Sangorrín, M., Lopes, C., Rodríguez, M.E., Zajonskovsky, I., Barbagelata, R., Lavallo, T.L. Vineyards and cellars from Neuquén, new source of yeast for the development of regional starter cultures. *Proyectos Federales de Innovación productiva*. Agencia Promoción Científica y Técnica. Informe Final: MECyT Resol 8 N°1028/04. Universidad Nacional del Comahue; 2008.
- [21] Rodríguez, M.E., Lopes, C.A., van Broock, M., Vallés, S., Ramón, D., Caballero, A.C. Screening and typing of Patagonian wine yeasts for glycosidase activities. *Journal of Applied Microbiology*. 2004;96:84–95.
- [22] Curilén, Y.L., Barda, N.B., Barbagelata, R.J., Bravo Ferrada, B., Gallina, M., Semorile L.C., Caballero, A.C. Commercial yeast inoculation effect on kinetic and microbiology of fermentative process and its relation to the sensorial quality of wine. A preliminary study in patagonian pinot noir winemaking. In: *Facultad de Ciencias de la Alimentación, UNER, editors. Actas del XII Congreso CYTAL—AATA; October 2009; Entre Ríos, Argentina. Sección 3, Alimentos Fermentados y Bebidas:2009*. p. 6.
- [23] Lopes, C.A., Sangorrín, M.P., Marongiú, A.R., Caballero, A.C. Yeasts killer as potential control agents for contaminants from Patagonian cellars. *Enología*. 2006a;4:44–48.
- [24] Sangorrín, M.P., Lopes, C.A., Guiraud, M.R., Caballero, A.C. Diversity and killer behaviour of indigenous yeasts isolated from the fermentation vat surfaces in four Patagonian wineries. *International Journal of Food Microbiology*. 2007;119:351–357.

- [25] Lopes, C.A., Rodríguez, M.E., Sangorrín, M., Querol, A., Caballero, A.C. Patagonian wines: the selection of an indigenous yeast starter. *Journal of industrial microbiology and biotechnology*. 2007;34:539–546.
- [26] del Mónaco, S.M., Bravo, S.M.E., Curilén, Y.L., Carreño, V.A., Caballero, A.C. A regional starter for high quality wines: an Argentinean Patagonia experience. *Bulletin de L'OIV*. 2014a;87:217–222.
- [27] Fleet, G.H., Prakitchaiwattana, C., Beh, A.L., Heard, G.M. The yeast ecology of wine grapes. In: Ciani M, editor. *Biodiversity and Biotechnology of Wine Yeasts*. Kerala, India: Research Signpost; 2002. p. 1–18.
- [28] Kurtzman, P.C., Fell, J.W., editors. *The Yeasts – A Taxonomic Study*, 4th ed. Amsterdam. Elsevier; 1998.
- [29] Esteve-Zarzoso, B., Belloch, C., Uruburu, F., Querol, A. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and two ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology*. 1999;49:329–337.
- [30] Martini, A. Biotechnology of natural and winery-associated strains of *Saccharomyces cerevisiae*. *International Microbiology*. 2003;6:207–209.
- [31] Schuller, D., Alves, H., Dequin, S., Casal, M. Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde Region of Portugal. *FEMS Microbiology and Ecology*. 2005;51:167–177.
- [32] Santamaría, P., Garijo, P., López, R., Tenorio, C., Gutiérrez, A.R. Analysis of yeast population during spontaneous fermentation. Effect of the age of the cellar and the practice of inoculation. *International Journal of Food Microbiology* 2005;103:49–56.
- [33] Mercado, L., Dalcero, A., Masuelli, R., Combina, M. Diversity of *Saccharomyces* strains on grapes and winery surfaces: analysis of their contribution to fermentative flora of Malbec wine from Mendoza (Argentina) during two consecutive years. *Food Microbiology*. 2007;24:403–412.
- [34] Costantini, A., García-Moruno, E., Moreno-Arribas, M.V. Biochemical Transformations Produced by Malolactic Fermentation. In: Moreno-Arribas, M.V., Polo, M.C., editors. *Wine Chemistry and Biochemistry*. Berlin, Germany. Springer Science+ Business Media; 2009. p. 27–49.
- [35] Jolly, N.P., Varela, C., Pretorius, I.S. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Research*. 2014;14:215–237.
- [36] Capozzi, V., Garofalo, C., Chiriatti, M.A., Grieco, F., Spano, G. Microbial terroir and food innovation: the case of yeast biodiversity. *Microbiological Research*. 2015;181:75–83.
- [37] Fleet, G.H. Wine yeasts for the future. *FEMS Yeast Research*. 2008;8: 979–995.

- [38] Lopes, C.A., Lavalle, T.L., Querol, A., Caballero, A.C. Combined use of killer biotype and mtDNA-RFLP patterns in a Patagonian Wine *Saccharomyces cerevisiae* diversity study. *Antonie van Leeuwenhoek*. 2006b;89:147–156.
- [39] del Mónaco, S.M., Barda, N.B., Rubio, N.C., Caballero, A.C. Selection and characterization of a Patagonian *Pichia kudriavzevii* for wine deacidification. *Journal of Applied Microbiology*. 2014b;117:451–464.
- [40] Vigentini, I., De Lorenzis, G., Fabrizio, V., Valdetara, F., Faccincani, M., Panont, C.A., Picozzi, C., Imazio, S., Failla, O., Foschino, R. The vintage effect overcomes the terroir effect: a three year survey on the wine yeast biodiversity in Franciacorta and Oltrepò Pavese, two northern Italian vine-growing areas. *Microbiology*. 2015;161:362–373.
- [41] Garofalo, C., Russo, P., Beneduce, L., Massa, S., Spano, G., Capozzi, V. Non-*Saccharomyces* biodiversity in wine and the ‘microbial terroir’: a survey on Nero di Troia wine from the Apulian region, Italy. *Annals of Microbiology*. 2016;66:143–150.
- [42] Lonvaud-Funel, A. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie van Leeuwenhoek*. 1999;76:317–331.
- [43] Beelman, R.B., Gallander, J.F. Wine deacidification. *Advances in Food Research*. 1979;25:1–53.
- [44] Ruffner, H.P. Metabolism of tartaric and malic acids. *Vitis*. 1982;21:247–259.
- [45] Henick-Kling, T. Malolactic fermentation. In: Fleet, G.H., editors. *Wine Microbiology and Biotechnology*. Berlin: Springer; 1993. p. 286–326.
- [46] Radler, F. Yeasts-metabolism of organic acids. In: Fleet, G.H., editors. *Wine Microbiology and Biotechnology*. Switzerland: Harwood Academic Publishers; 1993. p. 165–182.
- [47] Gao, C., Fleet, G.H. Degradation of malic and tartaric acids by high density cell suspensions of wine yeasts. *Food Microbiology*. 1995;12:65–71.
- [48] Gawel, R., Francis, L., Waters, E.J. Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. *Journal of Agricultural and Food Chemistry*. 2007;55:2683–2687.
- [49] Delcourt, F., Taillandier, P., Vidal, F., Strehaiano, P. Influence of pH, malic acid and glucose concentrations on malic acid consumption by *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*. 1995;43:321–324.
- [50] Hernandez-Orte P., Cersosimo M., Loscos N., Cacho J., Garcia-Moruno E., Ferreira V. Aroma development from non-floral grape precursors by wine lactic acid bacteria. *Food Research International*. 2009;42:773–781.
- [51] Bravo-ferrada, B.M., Delfederico, L., Hollmann, A., Valdés la hens, D., Curilén, Y., Caballero, A., Semorile, L. *Oenococcus oeni* from patagonian red wines: isolation, characterization and technological properties. *International Journal of Microbiology Research*. 2011;3:48–52.



- [52] Valdés La Hens, D., Bravo-Ferrada, B.M., Delfederico, L., Caballero, A., Semorile, L. Patagonian red wines: PCR-DGGE analysis with two targeted genes revealed the prevalence of *Lactobacillus plantarum* and *Oenococcus oeni* during spontaneous malolactic fermentations. *Australian Journal of Grape and Wines Research*. 2015;21:49–56.
- [53] Henick-Kling, T., Sandine, W.E., Heatherbell, D.A. Evaluation of malolactic bacteria isolated from Oregon Wines. *Applied and Environmental Microbiology*. 1989;55:2010–2016.
- [54] Lerm, E., Engelbrecht, L., du Toit, M. Selection and characterisation of *Oenococcus oeni* and *Lactobacillus plantarum* South African wine isolates for use as malolactic fermentation starter cultures. *South African Journal Enology and Viticulture*. 2011;32:280–295.
- [55] Bravo-Ferrada, B., Hollmann, A., Delfederico, L., Valdés La Hens, D., Caballero, A., Semorile, L. Patagonian red wines: selection of *Lactobacillus plantarum* isolates as potential starter cultures for malolactic fermentation. *World Journal of Microbiology and Biotechnology*. 2013;29:1537–1549.
- [56] Wibowo, D., Eschenbruch, R., Davis, C.R., Fleet, G.H., Lee, T.H. Occurrence and growth of lactic acid bacteria in wine: review. *American Journal of Enology and Viticulture*. 1985;36:302–313.
- [57] Thornton, R.J., Rodriguez, S.B. Deacidification of red and white wines by a mutant of *Schizosaccharomyces malidevorans* under commercial winemaking conditions. *Food Microbiology*. 1996;13:475–482.
- [58] Coucheney, F., Desroche, N., Bou, M., Tourdot-Marèchal, R., Dulau, L., Guzzo, J. A new approach for selection of *Oenococcus oeni* strains in order to produce malolactic starters. *International Journal of Food Microbiology*. 2005;105:463–470.
- [59] Henick-Kling, T., Park, Y.H. Considerations for the use of yeast and bacterial starter cultures: SO<sub>2</sub> and timing of inoculation. *American Journal of Enology and Viticulture*. 1994;45:464–469.
- [60] Rossi, I., Fia, G., Canuti, V. Influence of different pH values and inoculation time on the growth and malolactic activity of a strain of *Oenococcus oeni*. *Australian Journal of Grape Wine Research*. 2003;9:194–199.
- [61] Arnink, K., Henick-Kling, T. Influence of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on successful malolactic conversion in wine. *American Journal of Enology and Viticulture*. 2005;56:228–237.
- [62] Guilloux-Benatier, M., Remize, F., Gal, L., Guzzo, J., Alexandre, H. Effects of yeast proteolytic activity on *Oenococcus oeni* and malolactic fermentation. *FEMS Microbiology Letters*. 2006;263:183–188.
- [63] Jussier, D., Morneau, A.D., Mira de Orduña, R.M. Effect of simultaneous inoculation with yeast and bacteria on fermentation kinetics and key wine

parameters of cool-climate Chardonnay. *Applied and Environmental Microbiology*. 2006;72:221–227.

- [64] Osborne, J.P., Edwards, C.G. Inhibition of malolactic fermentation by *Saccharomyces* during alcoholic fermentation under low- and high nitrogen conditions: a study in synthetic media. *Australian Journal of Grape Wine Research*. 2006;12:69–78.
- [65] Flanzy, C. Mundi Prensa Libros S.A., editor. *Oenology: Scientific and technological foundations*. Madrid, España: AMV; 2000.
- [66] Volschenk, H., Viljoen-Bloom, M., Subden, R.E., Van Vuuren, H.J.J. Malo-ethanolic fermentation in grape must by recombinant strains of *Saccharomyces cerevisiae*. *Yeast*. 2001;18:963–970.
- [67] Ribéreau-Gayon P., Dubourdieu D., Doneche B., Lonvaud A. Wine chemistry. Stabilization and treatments. In: *Tratado de enología*, 1ª ed. Buenos Aires, Argentina: Editorial Hemisferio Sur y Mundi Prensa; 2003. p. 3–49.
- [68] du Toit, M., Pretorius, I.S. Microbial spoilage and preservation of wine: using weapons from nature's own arsenal — a review. *South African Journal of Enology and Viticulture*. 2000;21:74–96.
- [69] Molina, A.M., Guadalupe, V., Varelab, C., Swiegers, J.H., Pretorius, I.S., Agosin E. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chemistry*. 2009;117:189–195.
- [70] Sun, S.Y., Gong, H.S., Jiang, X.M., Zhao, Y.P. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae* on alcoholic fermentation behaviour and wine aroma of cherry wines. *Food Microbiology*. 2014;44:15–23.
- [71] Ciani, M., Maccarelli, F. Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. *World Journal of Microbiology and Biotechnology*. 1998;14:199–203.
- [72] Soden, A., Francis, I.L., Oakey, H., Henschke, P.A. Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. *Australian Journal of Grape and Wine Research*. 2000;6:21–30.
- [73] Ciani, M., Beco, L., Comitini, F. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. *International Journal of Food Microbiology*. 2006;108:239–245.
- [74] Ciani, M., Comitini, F., Mannazzu, I., Domizio, P. Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Research*. 2010;10:123–133.
- [75] Rodríguez, M.E., Lopes, C.A., Barbagelata, R.J., Barda, N.B., Caballero, A.C. Influence of *Candida pulcherrima* Patagonian strain on alcoholic fermentation

behaviour and wine aroma. *International Journal of Food Microbiology*. 2010;138:19–25.

- [76] Benito, S., Morata, A., Palomero, F., Gonzalez, M.C., Suarez-Lepe, J.A. Formation of vinylphenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *Pichia guilliermondii* in red wines produced following different fermentation strategies. *Food Chemistry*. 2011;124:15–23.
- [77] Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiology*. 2011;28:873–882.
- [78] Jolly, N.P., Augustyn, O.P.H. and Pretorius, I.S. The role and use of non-*Saccharomyces* yeasts in wine production. *South African Journal of Enology and Viticulture*. 2006;27:15–39.
- [79] Morata, A., Benito, S., Loira, I., Palomero, F., Gonzalez, M.C., Suarez-Lepe, J.A. Formation of pyranoanthocyanins by *Schizosaccharomyces pombe* during the fermentation of red must. *International Journal of Food Microbiology*. 2012;159:47–53.
- [80] Di Maio, S., Genna, G., Gandolfo, V., Amore, G., Ciaccio, M., Oliva, D. Presence of *Candida zemplinina* in Sicilian musts and selection of a strain for wine mixed fermentations. *South African Journal of Enology and Viticulture*. 2012;33:80–87.
- [81] Contreras, A., Hidalgo, C., Schmidt, S., Henschke, P.A., Curtin, C., Varela, C. The application of non-*Saccharomyces* yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content. *International Journal of Food Microbiology*. 2015;205:7–15.