J Ind Microbiol Biotechnol (2011) 38:1777–1785
DOI 10.1007/s10295-011-0964-1

ORIGINAL PAPER

Characterization of wines produced by mixed culture of autochthonous yeasts and *Oenococcus oeni* from the northwest region of Argentina

Lucía M. Mendoza · María G. Merín · Vilma I. Morata · Marta E. Farías

Received: 27 October 2010 / Accepted: 15 March 2011 / Published online: 2 April 2011
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Abstract Two autochthonous yeasts from the northwest region of Argentina, *Kloeckera apiculata* mc1 and *Saccharomyces cerevisiae* mc2, were used as pure or mixed starter cultures in microvinification trials conducted in Malbec red must. Also, the effect of *Oenococcus oeni* X_2L was evaluated. *S. cerevisiae* mc2 showed adequate growth and fermentative activity in single and composite fermentations, producing standard concentration of ethanol. The amount of esters was higher in fermentations conducted using mixed yeast starters. Independent of the timing of inoculation of *O. oeni*, this malolactic bacterium completely depleted malic acid. Sensory evaluation indicated that young wines fermented with mixed yeast cultures and sequential inoculation of *O. oeni* were preferred, achieving the highest scores for positive descriptors and they allowed better control of the sensory quality. Consequently, this study proposes inclusion of autochthonous *K. apiculata* mc1 as an adjunct culture to *S. cerevisiae* mc2 during Malbec must fermentation to improve the organoleptic properties of red wines. Furthermore, sequential inoculation of *O. oeni* X_2L should be carried out after completion of the alcoholic fermentation to enhance sensory characteristics.

Keywords Non-*Saccharomyces* yeast · Mixed culture · *Oenococcus oeni* · Organoleptic characteristics · Malbec wine

L. M. Mendoza · M. E. Farías
Centro de Referencia para Lactobacilos (CERELA), Chacabuco 145, 4000 Tucumán, Argentina

L. M. Mendoza · M. G. Merín · V. I. Morata · M. E. Farías
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

M. G. Merín · V. I. Morata
Facultad de Ciencias Aplicadas a la Industria, Universidad Nacional de Cuyo, Bernardo de Irigoyen 375, 5600 San Rafael-Mendoza, Argentina

M. E. Farías (✉)
Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000 Tucumán, Argentina
e-mail: mfarias@cerela.org.ar

Introduction

In the winemaking process, alcoholic fermentations (AF) are currently conducted using starters of selected strains of *Saccharomyces cerevisiae* in contrast to traditional spontaneous fermentations carried out by the flora present on the grapes and in the winery. Despite having advantages such as the easy control and homogeneity of fermentations, wines produced with *S. cerevisiae* monocultures have often shown lack of flavor complexity, stylistic distinction, and vintage variability [25, 45]. New fermentation technologies for optimizing wine quality and producing wines with particular flavor profiles is one of the worldwide trends in oenology [45]. The use of mixed starter cultures would permit one to improve wine quality, taking advantage of spontaneous fermentations without the risks of stuck fermentations or wine spoilage [6, 20, 43, 45]. Some non-*Saccharomyces* yeast species can improve the fermentation behavior of yeast starter cultures and lead to a more complex aroma [5, 9, 17, 44].

In a previous report we determined that *K. apiculata* mc1 in mixed cultures with *S. cerevisiae* mc2 in basal medium survive longer than in pure culture [32]. Despite

the consistent production of secondary products by single culture of *K. apiculata*, an adequate production of these compounds was observed in mixed culture. The existence of *K. apiculata* mc1, autochthonous non-*Saccharomyces* yeast might be of technological interest in Argentinean winemaking. However, in wine biotechnology more specific information on the extent of its contribution is required.

In this regard it is important to know the fermentation behavior of these non-*Saccharomyces* yeasts in mixed culture with *S. cerevisiae* in regional musts. Also, in the red wine fermentation it is necessary to consider the inclusion of *Oenococcus oeni*, a bacterium mainly involved in malolactic fermentation (MLF). This fermentation improves the wine quality because acidity decreases, the microbiological stability increases, and the sensory characteristics are enhanced with additional flavors [7, 16]. This step is often difficult to accomplish because of the inadequate physicochemical conditions after the AF [2, 3, 27, 38].

The aim of this study was to carry out a comparative analysis of chemical and sensory characteristics of Argentinean typical red wines fermented by pure or mixed cultures of two indigenous yeasts, *K. apiculata* mc1 and *S. cerevisiae* mc2. Also, the influence of *O. oeni* X_2L on the wine quality was evaluated.

Materials and methods

Microorganisms and growth conditions

K. apiculata mc1 and *S. cerevisiae* mc2, previously isolated from Malbec grape musts (northwest region, Argentina) were used throughout the study. The two yeasts strains were chosen according to their kinetics and metabolic performance previously examined [32]. Yeast strains were subcultured at 6-month intervals in YPD (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, pH 4.0) agar slants and maintained at 4°C. Yeast strains were propagated in YPD broth at 25°C for 24 h at least three times prior to experimental use.

O. oeni strain X_2L, isolated from Malbec red wine (Cafayate, Argentina) was utilized [47]. This bacterium was kept frozen at −20°C in MRS (Man Rogosa Sharpe) broth containing 52 g/l MRS broth (Merck, Darmstadt, Germany) added with 20% glycerol (v/v). *O. oeni* was grown in MRS broth supplemented with 150 ml/l of natural tomato juice (pH 4.0) at 30°C for 24 h at least three times prior to experimental use.

All microorganisms were obtained from the wine yeasts and lactic acid bacteria (LAB) collection at the Microbiology Institute of the Biochemistry, Chemistry and Pharmacy Faculty (Tucumán National University, Argentina).

Preparation of starter cultures

Erlenmeyer flasks (100 ml) containing 80 ml of pasteurized grape juice, previously diluted 1:2 with sterile water and adjusted to pH 4.0, were inoculated with 2 ml of young cultures of *K. apiculata* mc1, *S. cerevisiae* mc2, or *O. oeni* X_2L. Cultures were incubated at 28°C during 24 or 48 h for yeasts and bacterium, respectively. Subsequently, cells were collected by centrifugation at 8,000*g* for 10 min at 4°C and resuspended in 5 ml of sterile grape juice.

Fermentation conditions

The Malbec variety was utilized to conduct microvinification trials because it is the most typical red grape of Argentina. Grapes were harvested during the 2009 vintage.

Fermentations were carried out in 1-l Erlenmeyer flasks containing 800 ml of Malbec red must in the presence of skins (sugars 245 g/l, assimilable nitrogen 125 mg/l, titratable acidity 4.7 g/l, L-malic acid 1.9 g/l, pH 4.1) at 25°C. The grape must was exposed to heat treatment (80°C for 10 min) and subsequently the must was treated with 50 mg/l sulfur dioxide (as sodium metabisulfite). Concentration of the preservative was chosen because of its lack inhibitory effect on the yeasts and lactic acid bacterium employed in the fermentation trials.

The flasks were aseptically inoculated with different starter cultures to obtain an initial cell density of 5×10^6 cfu/ml for yeasts and/or malolactic bacterium.

Five inoculation strategies were applied: (1) pure culture of *K. apiculata* mc1; (2) pure culture of *S. cerevisiae* mc2; (3) mixed culture of both yeasts; (4) mixed culture of yeasts and simultaneous inoculation of *O. oeni* X_2L; and (5) mixed culture of yeasts and sequential inoculation of bacterium after completion of the AF. Fermentations inoculated with pure cultures were carried out as controls.

After aseptic stoppering of the flasks with a special valve containing sulfuric acid to allow only CO_2 to escape from the system [4], weight loss was monitored for several days until the end of the fermentation (constant weight for two consecutive days). Fermentations were carried out under static conditions.

Enumeration of microbiological populations

Populations of yeasts and bacterium were followed by counting viable cells. Differential enumeration of wine yeasts was performed by plating on the following selective media: (1) Malt agar medium (20 g/l glucose, 1 g/l peptone, 20 g/l yeast extract, 3 g/l malt extract, and 20 g/l agar, pH 6.8) that allows morphologically non-*Saccharomyces* yeast to be distinguished from *Saccharomyces* colonies. (2) Modified malt agar medium by addition of 40 mg/l bromophenol,

pH 4.6; non-*Saccharomyces* yeast appears as blue colonies while *Saccharomyces* forms yellow ones. (3) Malt agar medium with the addition of cycloheximide at a concentration (1 mg/l) that is only inhibitory for the growth of *Saccharomyces* yeast [23, 32]. The plates were incubated at 28°C for 3–5 days.

O. oeni was enumerated by inoculating adequate dilutions onto the plates of MRS agar supplemented with cycloheximide at a concentration (100 mg/l) to inhibit the growth of both yeast genera. The plates were incubated at 28°C for 5 days.

Analytical determinations

Glucose, fructose, ethanol, glycerol, acetic acid, acetaldehyde, and malic acid were analyzed with enzymatic test kits using standard solution as assay control (R-Biopharm AG, Darmstadt, Germany). Titratable acidity was measured by acid–base titration with standardized 0.1 M NaOH.

The analysis of main esters related to wine aroma was conducted using gas chromatography (GC). The extraction of volatile compounds from the wine samples was done by addition of 5 ml diethyl ether to 3 ml of each wine, followed by vigorous shaking for about 2 min, and centrifugation (1,300*g* for 5 min). The top solvent layer was then transferred to a vial [29].

A gas chromatograph (Agilent 6890N, Agilent Technologies, CA, USA) equipped with a flame ionization detector (FID) was used. An HP-5 column (length 30 m, i.d. 0.32 mm, thickness 0.25 μm) (Hewlett-Packard, CA, USA) was utilized to analyze the following esters: ethyl acetate, ethyl caproate, ethyl caprylate, isoamyl acetate, 2-phenylethyl acetate. The operating conditions were as follows: the oven temperature programmed at 30°C for 5 min, followed by increasing the temperature to 250°C at 10°C/min and held at 250°C for 2 min. Sample injections of 1 μl were performed in splitless mode. The injector and FID detector temperatures were 270 and 300°C, respectively. Column flow rate was 1 ml/min using nitrogen as carrier gas. The FID output signal was recorded and processed using appropriate software (Agilent ChemStation Software, Agilent, CA, USA). Esters were quantified from the regression curve ($R^2 > 98\%$) of the corresponding standard (Sigma, MO, USA), using external standard calibration and the GC conditions described above. In order to check the recovery efficiency of each ester from the wine sample, a known concentration of standard solution was added to each sample. Correction factors were calculated and used to adjust ester concentration values accordingly as described by Abeijón Mukdsi et al. [1].

The color of different wines was measured on samples diluted 1:10 with buffer (0.1 M citric acid with 0.2 M Na_2HPO_4, pH 4.1). The absorbance (A) of the samples was read at 420, 520, and 700 nm. The color intensity and hue were calculated as described by Wrolstad [51] using the equations $[(A_{520} - A_{700}) + (A_{420} - A_{700})]$ and $[(A_{520} - A_{700})/(A_{420} - A_{700})]$, respectively.

Sensory analysis

Sensory descriptive analysis of the young wines (1 month after bottling) was carried out by a tasting panel that consisted of six trained judges (Bianchi Winery and National Institute of Viticulture, Mendoza, Argentina). Wines were equilibrated at room temperature (22°C) and 50 ml-samples were poured into randomly numbered wineglasses. Two consecutive sessions were performed on different days. The selection of sensory descriptors was done by the panelists during the first session, taking into account those that allow discrimination among treatments [18]. At the second session the intensity of each descriptor was rated on a scale from 0 (not perceivable) to 5 (very strong). The violet color, astringency, bitterness, floral aroma, fruity aroma, phenolic aroma, and equilibrium-harmony were tested.

Statistical analysis

Experiments were performed in duplicate. One-way analysis of variance (ANOVA) was applied to the experimental data and the Tukey test was used for multiple mean comparisons. All statistical analyses were performed with Statistica software version 7.

Results and discussion

Evolution of yeast microbial populations and fermentation kinetics

Because most of the non-*Saccharomyces* wine-related species show limited fermentation aptitudes, they may be only employed as starter cultures in conjunction with *S. cerevisiae* strains, in this way ensuring the completion of the AF [6, 34, 41].

Figure 1 shows the evolution of microbial populations during microvinification trials conducted in Malbec must by pure and mixed cultures of *S. cerevisiae* mc2 and *K. apiculata* mc1. Pure cultures of *S. cerevisiae* reached a maximal cell density of 1.6×10^8 cfu/ml after 3 days of fermentation. Under co-culture conditions, the population of this yeast was lower than in single fermentations. After 6 days of incubation *S. cerevisiae* started to decline with a loss of 1 log cycle at the end of the AF. *K. apiculata* in pure and mixed cultures showed the maximal cell density after 1 day and immediately afterwards, the apiculate yeast started the phase of death, with viable cells decreasing to

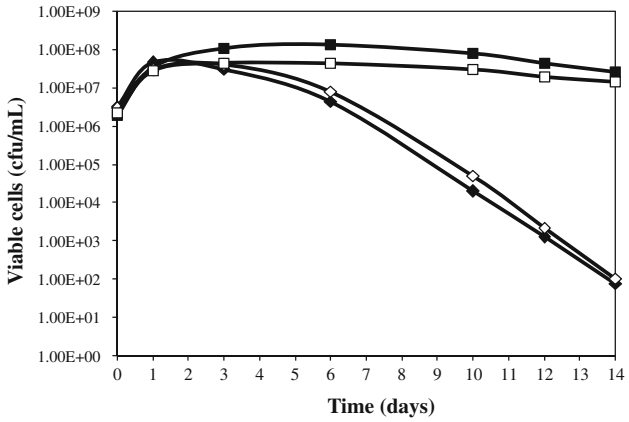

Fig. 1 Viable cell counts during microfermentations of non-*Saccharomyces* (*filled diamonds*, *open diamonds*) and *Saccharomyces* yeasts (*filled squares*, *open squares*) in pure and mixed cultures, respectively

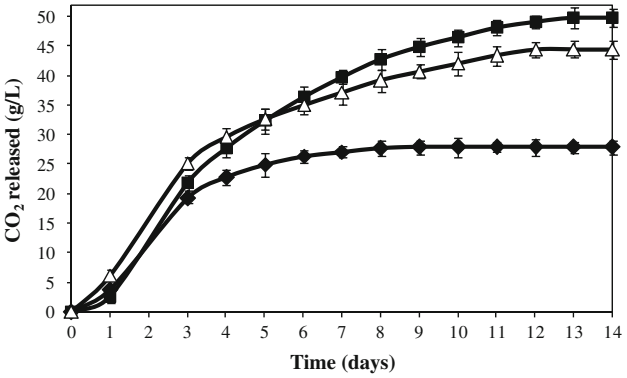

Fig. 2 Kinetics of alcoholic fermentations carried out by pure *K. apiculata* mc1 culture (*filled diamonds*), pure *S. cerevisiae* mc2 culture (*filled squares*), mixed *K. apiculata*/*S. cerevisiae* culture (*open triangles*) in microvinifications conditions

approximately 10^2 cfu/ml at the end of the process. This behavior is commonly due to the fact that these yeasts only grow and predominate during the early stages of wine fermentation [5, 11, 12]. Ciani et al. [6] examined cell population development in multistarter trials with non-*Saccharomyces*/*S. cerevisiae* cultures and reported that in mixed trials, non-*Saccharomyces* yeasts persisted during the first stages of fermentation. Although *S. cerevisiae* kept its viability in composite cultures for a longer period than non-*Saccharomyces* strains, *Saccharomyces* cell populations did not reach those of pure cultures [6, 34, 43].

Figure 2 shows the fermentation kinetics of pure and mixed starter cultures of wine yeasts during the AF. In the early fermentation stages, microvinifications carried out by single cultures of *K. apiculata* or *S. cerevisiae* exhibited similar fermentation rates. As expected, pure cultures of the apiculate yeast showed a stuck fermentation after 3 days of incubation. Fermentations carried out by mixed cultures of non-*Saccharomyces* and *Saccharomyces* yeasts exhibited similar kinetics but the final CO_2 production was slightly lower when compared with *S. cerevisiae* fermentations. These results are in agreement with those reported by Ciani et al. [6]. These authors found that inoculation of non-*Saccharomyces* yeasts can influence the kinetics of the AF conducted by *S. cerevisiae* and a reduction in fermentation rate is often observed.

Influence of *O. oeni* X_2L on microbial populations and malic acid evolution

Most red and some white wines undergo MLF, which is often encouraged during the final phases of AF or after its conclusion to avoid stuck AF by LAB strains or increased concentrations of acetic acid that render the wines unacceptable for consumption [7, 8]. However, differences between sequential and simultaneous AF and MLF are still controversial, specifically with regard to the effect on wine composition.

Figure 3 shows the influence of inoculation of *O. oeni* X_2L on the growth kinetics and malic acid concentration during microvinifications in Malbec red must. Yeast cell populations were not modified by simultaneous bacterial inoculation compared with fermentations conducted without the malolactic bacterium (Fig. 3a). Also, *O. oeni* inoculation did not modify the kinetics of the AF conducted by yeasts (data not shown). Previous studies demonstrated similar results for yeast growth, indicating that the viable yeast population was not negatively affected by the presence of the bacteria [30, 35]. Under the current assay conditions, *O. oeni* exhibited a maximal cell density of 1.2×10^7 cfu/ml after 3 days of incubation and at this stage malic acid was completely removed. Subsequently, the bacterial density decreased and at the end of the process it was undetectable. Our results are in disagreement with those reported by Massera et al. [30] who found that the viable LAB population did not decline after simultaneous inoculation. *O. oeni* stayed constant or increased and reached peak populations of 10^6 cfu/ml.

Sequential inoculation of *O. oeni* after completion of the AF led to a decrease in bacterial cell population and growth rate compared with microvinifications with simultaneous inoculation (Fig. 3b). However, after 5 days of incubation complete depletion of malic acid was observed.

The influence of the time of *O. oeni* inoculation on malolactic activity seems to be strain-specific for yeasts and bacteria used in wine fermentations. Jussier et al. [21] reported that simultaneous inoculation of yeasts and bacteria led to faster malic acid degradation, whereas Massera et al. [30] found that utilization of malic acid was similar for both inoculation strategies.

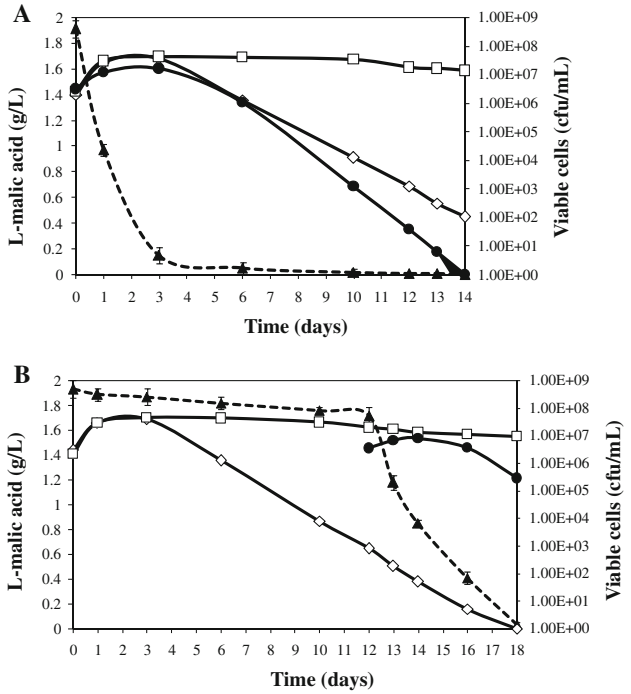

Fig. 3 Malic acid consumption (*filled triangles*) and evolution of cell population of *K. apiculata* mc1 (*open diamonds*), *S. cerevisiae* mc2 (*open squares*), and *O. oeni* X_2L (*filled circles*) in simultaneous (**a**) and sequential (**b**) inoculations

Chemical and sensorial analysis of young wines

Table 1 shows the chemical characteristics of wines fermented by different starter cultures. Sugars in microvinifications conducted by *S. cerevisiae* in pure or mixed cultures were completely consumed and the dryness of the must was achieved at the end of the AF. Ethanol concentrations reached standard values (12–13%). Similar results have been observed by Rodríguez et al. [41] who found that wines obtained with pure and mixed cultures of *S. cerevisiae* MMf9 showed physical–chemical characteristics like most regular wines. However, products fermented by single cultures of *K. apiculata* showed high residual sugar and low ethanol contents. The stuck fermentation was due to the growth kinetics exhibited by this apiculate yeast. Rodríguez et al. [41] indicated that the general composition of wine obtained with a single culture of *C. pulcherrima* V6 was consistent with its stuck fermentation kinetics.

Glycerol contents were similar in all products (8–8.5 g/l) and these values are in accordance with those usually found in wines, between 5 and 20 g/l [40].

Acetic acid becomes unpleasant at concentrations near its sensory threshold (0.7–1.0 g/l) and values between 0.2 and 0.7 g/l are usually considered adequate [25]. Levels of volatile acidity were significantly higher in fermentations with pure cultures of the non-*Saccharomyces* yeast or mixed starter cultures of yeasts and bacterium inoculated simultaneously. Under the latter conditions, the acetic acid concentration in the fermented product was the highest (1.23 g/l) and could affect the wine organoleptic characteristics. It has been reported that at elevated sugar concentrations this mostly heterofermentative wine LAB can produce high acetic acid concentrations through its sugar metabolism [24, 28]. However, other authors claim that simultaneous fermentations did not affect acetic acid levels [22, 46].

Acetaldehyde is another important flavor-active fermentation compound with average values of about 40 and

Table 1 General characteristics of wines fermented by different starter cultures

Analytical determinations*	Pure starter cultures		Composite starter cultures		
	K	S	K + S	K + S + O (simultaneous)	K + S + O (sequential)
Residual sugars (g/l)	20.84 ± 0.97^{a}	0.72 ± 0.08^{b}	0.81 ± 0.06^{b}	0.86 ± 0.04^{b}	0.75 ± 0.05^{b}
Ethanol (% v/v)	8.82 ± 0.56^{a}	13.91 ± 0.27^{b}	13.56 ± 0.19^{b}	12.97 ± 0.44^{c}	13.38 ± 0.29^{bc}
Glycerol (g/l)	7.98 ± 0.23^{a}	8.55 ± 0.31^{b}	8.43 ± 0.22^{b}	8.27 ± 0.24^{ab}	8.16 ± 0.30^{ab}
Volatile acidity (g/l)	0.79 ± 0.04^{a}	0.45 ± 0.03^{b}	0.58 ± 0.03^{c}	1.23 ± 0.05^{d}	0.61 ± 0.02^{c}
Acetaldehyde (mg/l)	37.83 ± 1.37^{a}	56.54 ± 2.32^{b}	52.35 ± 1.95^{b}	17.76 ± 1.13^{c}	48.14 ± 2.11^{d}
Titratable acidity (g/l)	5.27 ± 0.20^{a}	5.91 ± 0.34^{b}	6.22 ± 0.32^{b}	6.79 ± 0.21^{c}	4.90 ± 0.37^{d}
L-Malic acid (g/l)	1.89 ± 0.03^{a}	1.76 ± 0.02^{b}	1.81 ± 0.03^{b}	0.04 ± 0.01^{c}	0.02 ± 0.01^{c}
pH	3.81 ± 0.02^{a}	3.83 ± 0.02^{a}	3.81 ± 0.02^{a}	3.92 ± 0.03^{b}	4.05 ± 0.03^{c}
Color intensity	1.60 ± 0.006^{a}	1.62 ± 0.036^{a}	1.59 ± 0.028^{a}	1.52 ± 0.009^{b}	1.70 ± 0.018^{c}
Color hue	1.24 ± 0.008^{a}	1.19 ± 0.003^{b}	1.22 ± 0.001^{c}	1.20 ± 0.004^{b}	1.15 ± 0.001^{d}

Mean values with different superscript letters within the same row are significantly different according to the Tukey test ($P \leq 0.05$)

Data are mean values of two experiments ± standard deviation

K, *K. apiculata* mc1; S, *S. cerevisiae* mc2; O, *O. oeni* X_2L

*Evaluated in the finished wines

Table 2 Esters concentrations of wines fermented by pure and mixed starter cultures

	Pure starter cultures		Composite starter cultures		
	K	S	K + S	K + S + O (simultaneous)	K + S + O (sequential)
Ethyl acetate (mg/l)	178.84 ± 5.67^{a}	29.21 ± 0.92^{b}	79.73 ± 1.31^{c}	76.92 ± 1.63^{c}	68.33 ± 1.21^{d}
Ethyl caproate (mg/l)	0.65 ± 0.06^{a}	1.23 ± 0.08^{b}	1.31 ± 0.04^{c}	1.12 ± 0.05^{b}	1.41 ± 0.07^{d}
Ethyl caprylate (mg/l)	0.41 ± 0.05^{a}	0.92 ± 0.04^{b}	1.12 ± 0.03^{c}	0.83 ± 0.06^{b}	1.02 ± 0.05^{c}
Isoamyl acetate (mg/l)	3.73 ± 0.22^{a}	0.94 ± 0.06^{b}	2.32 ± 0.13^{c}	2.81 ± 0.15^{d}	2.13 ± 0.11^{c}
2-Phenylethyl acetate (mg/l)	29.42 ± 1.21^{a}	0.94 ± 0.03^{b}	12.81 ± 0.93^{c}	9.95 ± 0.52^{d}	11.51 ± 0.73^{c}

Mean values with different superscript letters within the same row are significantly different according to the Tukey test ($P \leq 0.05$)

Data are mean values of two experiments ± standard deviation

K, *K. apiculata* mc1; S, *S. cerevisiae* mc2; O, *O. oeni* X_2L

80 mg/l for red and white wines, respectively [26]. Acetaldehyde concentrations in the different wines assayed depended on the starter culture utilized in the microfermentation. An important decrease in this metabolite was observed in wines with simultaneous fermentation of yeasts and *O. oeni*. This would indicate that the bacterium was able to metabolize acetaldehyde under the given assay conditions, which is in agreement with previous studies that showed that simultaneous fermentations displayed the lowest overall acetaldehyde concentrations during the AF, likely because of the degradation of this compound by bacteria [21]. Furthermore, Osborne et al. [37] reported degradation of acetaldehyde by homofermentative and heterofermentative LAB and the two major catabolic products were identified as ethanol and acetic acid. However, those authors believe that the impact of both products on the chemical and sensory composition of wine is limited, because the increase in ethanol and acetic acid from acetaldehyde degradation would be insignificant. Wines obtained with simultaneous inoculation of yeasts and *O. oeni* showed an increase in acetic acid and this behavior could in part be due to acetaldehyde degradation (Table 1). Other studies found that LAB also degraded citric acid with acetic acid as the end product [21, 36].

Wines with MLF induction after the AF showed a titratable acidity significantly lower than in other products fermented by mixed starter cultures. In addition, inoculation of *O. oeni* increased the wine pH because of bacterial consumption of L-malic acid.

Fermentation-derived esters are largely responsible for wine fruitiness, and therefore they play an important role in the sensory composition of young red and white wines [10, 33]. The final concentration of esters compounds in wine is the result of the balance between alcohol acetyltransferase enzymes promoting their synthesis, and esterase enzymes promoting their hydrolysis [13, 39]. The two main groups of esters that have long been associated with wine fruitiness are acetate and ethyl esters that are recognized as important flavor compounds [42, 43]. Ethyl acetate, the major ester in wine can produce a solvent-like odor at concentrations beyond the threshold of 150–200 mg/l [25], whereas at levels of 80 mg/l it contributes to fruity notes and adds to a general complexity [15, 19]. Despite the high production of ethyl acetate in single cultures of *K. apiculata* mc1, in trials conducted using mixed culture a reduction of this ester was observed (Table 2).

The next highest ester concentration corresponded to 2-phenylethyl acetate, which contributes to fruity and flowery notes [25]. Our results demonstrate that products fermented by composite cultures of *K. apiculata* and *S. cerevisiae* showed statistically higher concentrations of acetate esters such as isoamyl acetate and 2-phenylethyl acetate compared to wines fermented with *S. cerevisiae* monocultures (Table 2). This characteristic could be due to inoculation with the non-*Saccharomyces* strain, showing that this yeast could improve the wine aroma. Previous studies have already reported that non-*Saccharomyces* yeasts are good producers of several esters. *Hanseniaspora/Kloeckera* species were shown to be strong producers of ethyl acetate and 2-phenylethyl acetate [43, 49, 50].

With respect to ethyl esters the genus *Saccharomyces* is the best producer of ethyl caprylate and ethyl caproate [25, 43]. Our assays revealed that ethyl ester levels in mixed fermentations were similar to those produced by a pure culture of *S. cerevisiae*. Positive interactions between wild yeasts and *S. cerevisiae* cultures regarding volatile aroma compounds have been described by several authors who observed an increase in ester concentrations in wines produced by composite starters in comparison with pure fermentations [14, 34, 48].

All products fermented with a mixture of yeasts and *O. oeni*, independently of the timing of bacterial inoculation, showed a similar composition of the analyzed aroma compounds and only a slight decrease in the ester concentrations was observed. Matthews et al. [31] showed that LAB possess esterase activities that could potentially alter the

ester profile of wine. Also the authors reported that *O. oeni* has higher esterase activity than lactobacilli and pediococci.

Sensory analysis of young wines was carried out to evaluate the influence of each starter culture on the organoleptic quality of the fermented products. General sensory descriptors like color intensity, flavor, mouth-feel attributes, and equilibrium-harmony of the wine were considered (Fig. 4). Independently of the type of culture utilized to carry out microvinification trials, floral aroma and bitterness descriptors exhibited similar scores. Also, wines fermented by different starter cultures showed slight modifications of violet color in accordance with those observed by spectral analysis of color intensity and hue (Table 1). Products obtained with mixed cultures of non-*Saccharomyces* and *Saccharomyces* yeasts scored higher for fruity aroma and equilibrium-harmony evaluation than wines fermented with a monoculture of *S. cerevisiae* mc2. Wines fermented with a monoculture of *K. apiculata* mc1 were evaluated as the most intense of all regarding their fruity character, but their astringency and equilibrium-harmony scored the lowest values, which made them sensorily faulty. Other authors have also reported on wine production using mixed starter cultures. Rodríguez et al. [41] studied mixed fermentations of *S. cerevisiae* MMf9, isolated in the Patagonia region, and *C. pulcherrima* V6, a β-glucosidase producer, at laboratory scale. The results evidenced a positive impact on the wine when *C. pulcherrima* V6 was adequately combined with *S. cerevisiae* MMf9, enhancing its fruity and floral aroma. Similar results have been obtained with foreign wines produced by *S. cerevisiae* in co-culture with non-*Saccharomyces* yeasts mainly belonging to *Hanseniaspora* and *Candida* genera. These wines presented the highest total concentration of higher alcohols, esters and terpenols as well as the strongest aroma [17, 20, 45].

Products simultaneously fermented by yeasts and *O. oeni* scored the highest for phenolic aroma and consequently obtained the lowest equilibrium-harmony ratings (Fig. 4). This is contrary to wines sequentially inoculated with malolactic bacterium, which had the highest acceptance, with better fruity and floral aromas and high scores for the equilibrium-harmony descriptor. These results are in disagreement with those reported by Massera et al. [30] who, working with Malbec must from another region of Argentina, found that wines with simultaneous treatment showed enhanced sensorial attributes related to high-quality wine like color and fruity flavor. Perhaps, this behavior was correlated with the different fermentation kinetics of the microorganisms employed in both studies. Whereas in our fermentation trials, *O. oeni* X_2L represents an autochthonous microorganism from the northwest region of Argentina, Massera et al. [30] used a freeze-dried commercial *O. oeni* strain to inoculate regional musts.

In conclusion, red wines obtained with simultaneous inoculation of malolactic bacterium, non-*Saccharomyces*, and *Saccharomyces* yeasts were not accepted by our judges because of their strong phenolic aroma and high level of volatile acidity. However, wines fermented by mixed starter cultures of yeasts and sequential inoculation of *O. oeni* scored the highest for positive descriptors and allowed better control of the sensory quality of red wines from vineyards of Argentina.

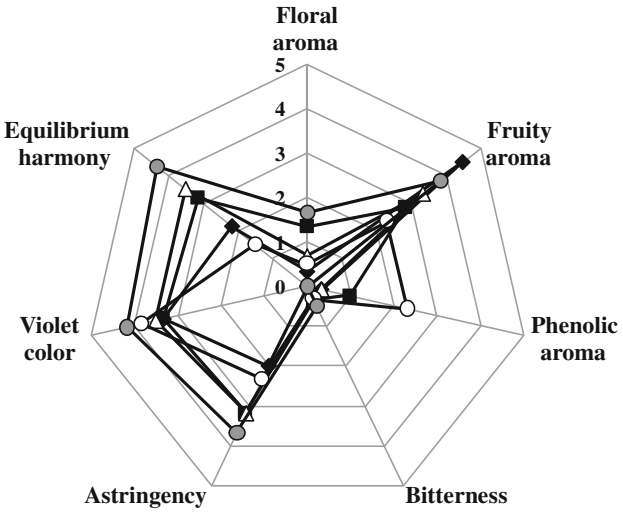

Fig. 4 Cobweb graph of scores obtained from sensory analysis for wines fermented by pure *K. apiculata* mc1 culture (*filled diamonds*), pure *S. cerevisiae* mc2 culture (*filled squares*), mixed *K. apiculata*/*S. cerevisiae* culture (*open triangles*), and composite culture of yeasts and malobacterium with simultaneous (*open circles*) and sequential (*filled circles*) inoculations

The results of the present study allow us to propose the inclusion of *K. apiculata* mc1 as an adjunct culture to *S. cerevisiae* mc2 during must fermentation to improve the organoleptic properties of Malbec wines. In addition, sequential inoculation of *O. oeni* X_2L after the AF enhanced sensory characteristics. Nevertheless, more specific information is required about the contribution of these strains. Large-scale experiments should be carried out to confirm the behavior of the mixed starter cultures proposed in this work.

To our knowledge, this is the first study on red wine obtained with indigenous strains of yeasts and bacterium from the northwest of Argentina, a region with growing oenological relevance.

Acknowledgments This work was financially supported by Consejo Nacional de Investigaciones Científicas y Técnicas and Consejo de Investigaciones de la Universidad Nacional de Tucumán, Argentina. The authors are grateful to Elena Bru for her assistance in statistical analysis.

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