



Effect of pH on malolactic fermentation in southern Italian wines

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Abstract

This study investigated the influence of pH on malolactic fermentation (MLF) in white wines (adjusted to pH 3.2, 3.4 and 3.8, respectively) from Falanghina grapes and red wines (adjusted to pH 3.4 and 3.8, respectively) from Tintilia grapes. The wines were inoculated with *Oenococcus oeni* and *Lactobacillus plantarum* strains, and a mix of them (50:50), in red Tintilia only. The time required to complete MLF in wines from white Falanghina grapes at pH 3.4 and 3.8 was lower with *O. oeni*, while MLF did not occur at pH 3.2. In red Tintilia, MLF was always completed within 35 days; at high pH (3.8) a significant increase in histamine was detected, while the decrease in citric acid concentration caused an increase in volatile acidity. Sensorial analysis showed an enhancement of red berry and spicy notes in red Tintilia at pH 3.8. PCA on white Falanghina showed that wines at pH 3.2 were located on the negative side of PC1 with higher scores for dry vegetable, sulphide, violet and toasted attributes. Wines at pH 3.4 and 3.8 were located on the positive side of the PC1 with butter and apple attributes. *L. plantarum* enhanced floral notes in white Falanghina wines and showed a good organoleptic impact on red Tintilia wines, which sensorial intensity was improved by a commercial mix (50:50) of *O. oeni* and *L. plantarum*.

Keywords Malolactic fermentation · pH · Citric acid · Histamine · Sensorial analysis

Introduction

An efficient control of malolactic fermentation (MLF) requires an increase in knowledge of lactic acid bacteria (LAB) behaviour under stress conditions, such as low pH and high ethanol content [1–3]. *Oenococcus oeni* is the predominant species in spontaneous fermentation [4] and is well adapted to harsh wine conditions; it has an optimum pH for growth ranging from pH 4.3 to 4.8, but it is capable of growing even at pH 3.2. This last characteristic was also found in *Lactobacillus plantarum* [5], which showed further technological and stress tolerance features, useful for the selection and design of strains suitable for MLF [6, 7]. During MLF, LAB produce glycosidases, which cleaves the sugars from the aromatic compounds and release these, increasing a wine's overall flavour and enhancing varietal

aromas [8]. Nevertheless, some glycoside compound was lost by enzymatic activity [9].

L. plantarum have a more diverse array of β -glucosidase and esterase whose level of activity is strongly strain dependent [10]. Due to these characteristics, selected strains of *L. plantarum* are currently being commercialized to induce MLF in wine [11], such as *L. plantarum* 22 strain, which possess a gene pool capable of increasing the aromatic complexity [12].

Moreover, although classified as a facultative heterofermentator, *L. plantarum* is considered homo-fermentative for hexoses, with lower acetic acid production [13]. However, LAB are also capable of metabolising residual sugar and citric acid in wine, during and after MLF, thereby, playing an important role in the sensory profiles of wine [14]. Playing an important role in the sensory profiles of wine [14], for instance, with the formation of excess diacetyl. Furthermore, biogenic amines, such as histamine, are harmful to human health [15, 16], and may be formed by the action of LAB during alcoholic and MLF, mainly at high pH [17, 18].

This paper aims to assess the influence of pH on MLF in southern Italian wines made from Falanghina white grapes and Tintilia red grapes, both of indigenous grapevine varieties. The tests were conducted on wines at various adjusted

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pH, inoculated with two different commercial strains of LAB: *O. oeni* and *L. plantarum* and a mix (50:50) of them, only in red Tintilia. The wines were evaluated by analysing the evolution of L-malic, L-lactic, citric and acetic acids during MLF. Moreover, on the different wines, after the MLF, the histamine content was detected and sensorial analysis was performed.

Materials and methods

Wines and inoculation

Samples of white Falanghina wine were collected at the cellar *Palummo* at Galluccio (CE) in Campania region, Italy, before starting sequential MLF. The tests of MLF were conducted at 20 °C in 5-L carboys, after adjusting the pH (equal to 3.4 in the wine), with tartaric acid and calcium carbonate, at pH values of 3.2 and 3.8, respectively. The two different commercial preparations used for this study were *L. plantarum* V22, homo-fermentative LAB, and *Oenococcus oeni* Lalvin31 (MBR), generously donated by Lallemant Italy (Castel d’Azzano, VR, Italy). In brief, the inoculation (about 10^6 cfu/mL) was performed according to the method of rehydrating active dry bacteria Lallemant protocol. The Tintilia red wine was drawn from the cellar *D’Uva* at Larino (CB) in Molise region, Italy, before starting MLF. The trials were carried out at the pH of the wine (pH 3.8) and after acidification (pH 3.4). On the samples, both strains previously used for white Falanghina, *O. oeni* and *L. plantarum*, and a commercial mix (50:50) of them (mix) have been used (about 10^6 cfu/mL). In both wines, the inoculation of different bacteria was carried out at the end of the alcoholic fermentation after racking. Fermentations were conducted in triplicate. To the wine with complete MLF was added 50 mg/L of SO₂, before bottling. Moreover, to evaluate the effect of wine sterilisation on MLF completion times, 1 L of red Tintilia at pH 3.8, divided into three sub-samples, was sterile filtered, through 0.45-µm Vitapore II Plus membranes (Millipore, Bedford, MA, USA), before inoculation with *O. oeni*. During MLF of such samples, L-malic and L-lactic acids were daily monitored.

Analyses

The alcohol content, pH, free and total sulphur dioxide, reducing sugar, total and volatile acidity of the wine quantification were performed according to the methods of the Office International de la Vigne et du Vin [19]. The course of MLF was monitored by verifying L-malic acid consumption and L-lactic formation. Enzymatic assays (Boehringer Mannheim, Germany) were used to determine the content of L-malic, L-lactic and citric acids. For detecting histamine,

a competitive enzyme immunoassay analysis was carried out (Histamine ELISA Kit, Techna®, Trieste, Italy). Histamine was detected before and at the end of MLF (malic acid levels < 50 mg/L), which lasted for about 56 and 35 days in white (except for samples at pH 3.2, see below) and red wines, respectively. Colour of red wine samples was measured before and after MLF, with Cielab colour space (L^* , a^* , b^*), using a tri-stimulus colorimeter (CR-200 Chromometer Minolta, Osaka, Japan) having an aperture size of 10 mm [20]. All analyses were performed in triplicate.

Sensory analysis

A committee of 12 expert trained judges did the sensory assessment of wines after MLF. The participants were officially approved tasters for the quality assessment of Italian wines. Wine samples were codified and served in certified tasting glasses of 200 cm³ filled with 50 cm³ of wine at 18 °C. The sensory evaluation of the wines was performed using a questionnaire consisting of 14 aroma terms for white wines (floral, spicy, rose, violet, apple, orange blossom, smoked, fresh vegetable, dry vegetable, oxidised, butter, alcohol, toasted, sulphide), and 11 for red wines (floral, spicy, vanilla, rose, violet, fruits, red berries, vegetable, oxidised, butter, alcohol). An unstructured 7-unit scale, in which 1 was “attribute not perceptible” and 7 was “attribute highly perceptible”, was used. Data from all judges for all samples were used, and the average values of three tasting sessions were shown using the so-called “spider web diagrams”. In this diagram, the centre of the figure represented the lowest average intensity, with the intensity of each attribute increasing to an intensity of seven at the perimeter [21].

Statistical analysis

The data reported are means and standard deviations calculated from three replicates. The analysis of variance (ANOVA) and principal component analysis (PCA) were performed using SPSS version 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). The scores from chemical and descriptive sensory analysis were used to construct a PCA biplot. The least significant differences were obtained using Fisher’s least significant difference (LSD) test ($p \leq 0.05$).

Results

Evolution of malic acid content

In white Falanghina at pH 3.8, the initial content of malic acid (Table 1) was metabolised after about 35 days from inoculation with *O. oeni*, whilst about 56 days were needed to complete MLF with *L. plantarum* at the same pH (Fig. 1).

Table 1 Enological parameters before malolactic fermentation

	Falanghina	Tintilia
Alcohol level (% v/v)	15.0±0.3	13.0±0.2
pH	3.4±0.1	3.8±0.1
Total acidity ^a (g/L)	7.6±0.2	7.2±0.3
Malic acid (g/L)	3.2±0.1	3.5±0.1
Citric acid (g/L)	0.6±0.1	0.6±0.1
Reducing sugar (g/L)	3.1±0.3	1.8±0.2
Volatile acidity ^b (g/L)	0.2±0.01	0.4±0.02
Free SO ₂ (mg/L)	12.8±0.4	11.2±0.5
Total SO ₂ (mg/L)	43.8±0.8	45.2±0.9
Histamine (mg/L)	0.6±0.03	4.2±0.1

^aExpressed as tartaric acid^bExpressed as acetic acid

In pH 3.4 samples, it took approximately 49 days to complete MLF with *O. oeni*, while with *L. plantarum*, MLF has just begun after 35 days. Instead, at pH 3.2, MLF did not take place with either LAB strains. These results were confirmed by the parallel formation of lactic acid, in which the samples at pH 3.8 reached almost the same final value of 2.1 g/L, after 35 days from inoculation with *O. oeni*, and 56 days with *L. plantarum* (Table 2). In white wines at pH 3.4, after 56 days from inoculation with *L. plantarum*, only 0.35 g/L of lactic acid was produced.

In red Tintilia wines, the malic acid was completely degraded in all samples after about 35 days, slightly faster in samples at pH 3.8 with the mix (Fig. 2). The lactic acid increase was almost parallel to the decrease of malic acid

(Table 2). The decrease of citric acid in white Falanghina was up to 68% after 56 days in samples at pH 3.8 inoculated with *O. oeni* (Table 2). White Falanghina with *O. oeni* at pH 3.4 roughly halved the acid citric concentration after 56 days of MLF. In red Tintilia, citric acid reduced mainly at pH 3.8, up to about 32% of the initial content after 35 days from the inoculum with LAB (Table 2). During the same period, the citric acid reduction in all samples at pH 3.4 was up to 43%. The volatile acidity in white wines increased at a constant trend during the MLF (data not reported): higher values were recorded at pH 3.8 in samples inoculated with *O. oeni* (Table 2). The volatile acidity increased significantly ($p \leq 0.05$) in all red Tintilia samples at pH 3.8, with respect to those at pH 3.4 (Table 2).

The MLF was completed in only 8 days (data not reported) in red Tintilia at pH 3.8, inoculated with *O. oeni*, and previously submitted to a sterile-filtered treatment.

Histamine production

The production of histamine in white Falanghina was maximum (4.4 mg/L) at pH 3.4 with *O. oeni* (Table 2). In red Tintilia, there was a great production of histamine (Table 2), especially at pH 3.8 with the mix inoculum (18.3 mg/L).

Changes in colour

The colour changes owing to the MLF were analysed in red wine. The lightness (L^*) did not undergo significant changes in all samples examined, while there was a significant

Fig. 1 Degradation of malic acid (g/L) in white Falanghina wine at different pH inoculated with *O. oeni* and *L. plantarum*. Open triangle: pH 3.2 *L.pl*; filled triangle: pH 3.2 *O. oeni*; open square: pH 3.4 *L.pl*; filled square: pH 3.4 *O. oeni*; open circle: pH 3.8 *L.pl*; filled circle: pH 3.8 *O. oeni*

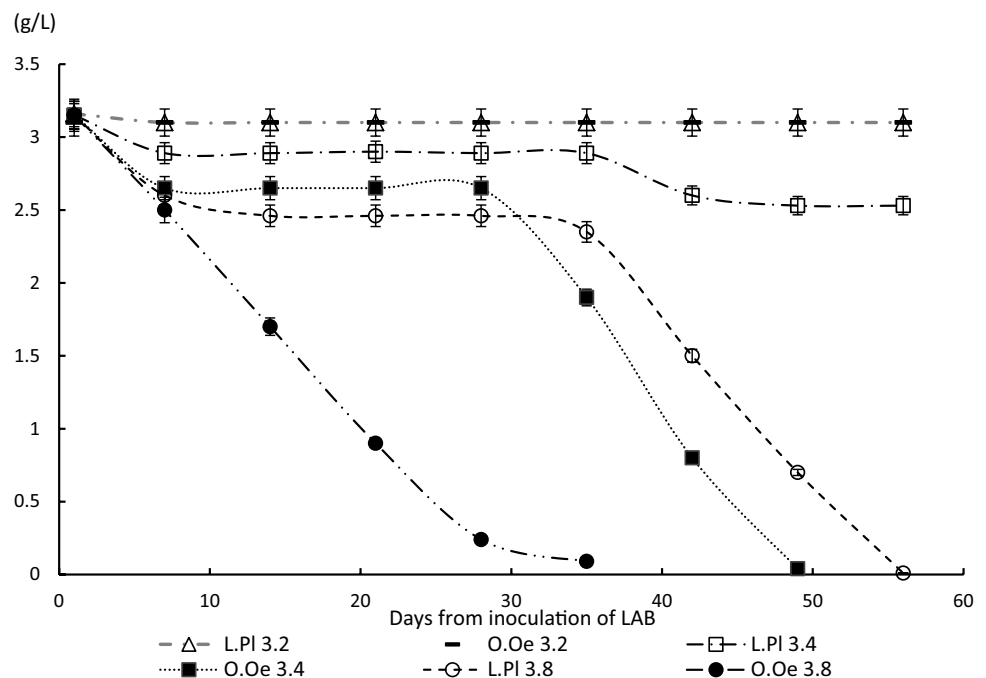


Table 2 Lactic acid, citric acid, volatile acidity and histamine content in white Falanghina and red Tintilia wines at different pH inoculated with *O. oeni* (O.o), *L. plantarum* (L.p) and a mix of O.o and L.p (mix) after MLF

Samples	Falanghina				Tintilia			
	L-Lactic acid (g/L)	Citric acid (mg/L)	Volatile acidity ^a	Histamine (mg/L)	L-Lactic acid (g/L)	Citric acid (mg/L)	Volatile acidity ^a	Histamine (mg/L)
pH 3.2 <i>O. oeni</i>	0.15 ± 0.02a	0.44 ± 0.02a	0.31 ± 0.02b	0.69 ± 0.07a	–	–	–	–
pH 3.2 <i>L. plant.</i>	0.23 ± 0.03a	0.41 ± 0.02a	0.24 ± 0.01a	0.66 ± 0.06a	–	–	–	–
pH 3.4 <i>O. oeni</i>	2.15 ± 0.11c	0.29 ± 0.02b	0.36 ± 0.03b	4.43 ± 0.31c	2.31 ± 0.11a	0.25 ± 0.01a	0.52 ± 0.02a	4.51 ± 0.31a
pH 3.4 <i>L. plant.</i>	0.35 ± 0.05b	0.37 ± 0.02a	0.30 ± 0.02a	2.76 ± 0.18b	2.33 ± 0.18a	0.26 ± 0.02a	0.50 ± 0.03a	4.08 ± 0.52a
pH 3.8 <i>O. oeni</i>	2.14 ± 0.11c	0.22 ± 0.01b	0.39 ± 0.03b	2.51 ± 0.27b	2.28 ± 0.21a	0.20 ± 0.02b	0.63 ± 0.01b	15.45 ± 0.91c
pH 3.8 <i>L. plant.</i>	1.95 ± 0.10c	0.19 ± 0.01b	0.42 ± 0.02b	3.09 ± 0.39b	2.41 ± 0.15a	0.19 ± 0.02b	0.63 ± 0.03b	7.23 ± 0.65b
pH 3.4 mix	–	–	–	–	2.34 ± 0.22a	0.25 ± 0.01a	0.52 ± 0.01a	5.99 ± 0.41a
pH 3.8 mix	–	–	–	–	2.42 ± 0.16a	0.21 ± 0.01b	0.65 ± 0.04b	18.26 ± 1.27c

Different letters in the same column indicate significant differences ($p \leq 0.05$) among samples

^aAs acetic acid (g/L)

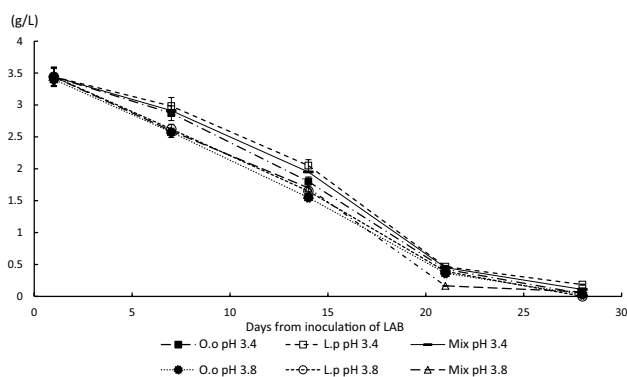


Fig. 2 Degradation of malic acid (g/L) in red Tintilia wine at different pH inoculated with *O. oeni*, *L. plantarum* and mix (50:50%) of them. Open square: pH 3.4 *L.pl*; filled square: pH 3.4 *O. oeni*; open circle: pH 3.8 *L.pl*; filled circle: pH 3.8 *O. oeni*; open triangle: pH 3.8 mix; filled triangle: pH 3.4 mix

decrease in the redness (a^*) and yellowness (b^*) indexes at the end of MLF (Table 3).

Sensorial analysis

The tasting conducted on white Falanghina samples showed a similar pattern in spider web diagrams (Fig. 3). Notes of butter and apple, in samples that completed the MLF with *O. oeni*, were perceived with an average rating (Fig. 3a). The resulting floral notes prevailed in samples at pH 3.4 inoculated with *L. plantarum* (Fig. 3b). In red Tintilia wines, the sensorial olfactory values were lower at pH 3.4 (Fig. 4a), than at pH 3.8. Moreover, the intensity increased with the mix at pH 3.8, by a development in olfactory notes of red berries and spicy (Fig. 4b). Principal component analysis (PCA) was used to elucidate differences in chemical and

Table 3 Colour changes in Tintilia wines at different pH inoculated with *O. oeni* (O.o), *L. plantarum* (L.p) and a mix of O.o and L.p (mix) before and after MLF

Samples	Before MLF			After MLF		
	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)
pH 3.4 O.o	32.29 ± 0.81a	4.85 ± 0.06b	2.35 ± 0.05b	35.57 ± 0.91a	2.50 ± 0.08d	1.99 ± 0.02c
pH 3.4 L.p	31.88 ± 0.62a	4.78 ± 0.05b	2.71 ± 0.05b	35.47 ± 0.82a	0.99 ± 0.02b	1.35 ± 0.03a
pH 3.8 O.o	35.38 ± 0.83b	1.53 ± 0.06a	1.46 ± 0.04a	35.86 ± 0.88a	0.76 ± 0.02a	1.21 ± 0.03a
pH 3.8 L.p	32.57 ± 0.77a	1.53 ± 0.06a	1.41 ± 0.04a	35.53 ± 0.93a	0.98 ± 0.02b	1.31 ± 0.03a
pH 3.4 mix	33.24 ± 0.64a	6.45 ± 0.11c	2.93 ± 0.05b	35.26 ± 0.80a	1.84 ± 0.03c	1.61 ± 0.04b
pH 3.8 mix	35.04 ± 0.81b	2.45 ± 0.05a	1.19 ± 0.03a	35.26 ± 0.94a	0.75 ± 0.03a	1.26 ± 0.03a

Different letters indicate significant differences ($p \leq 0.05$) among samples

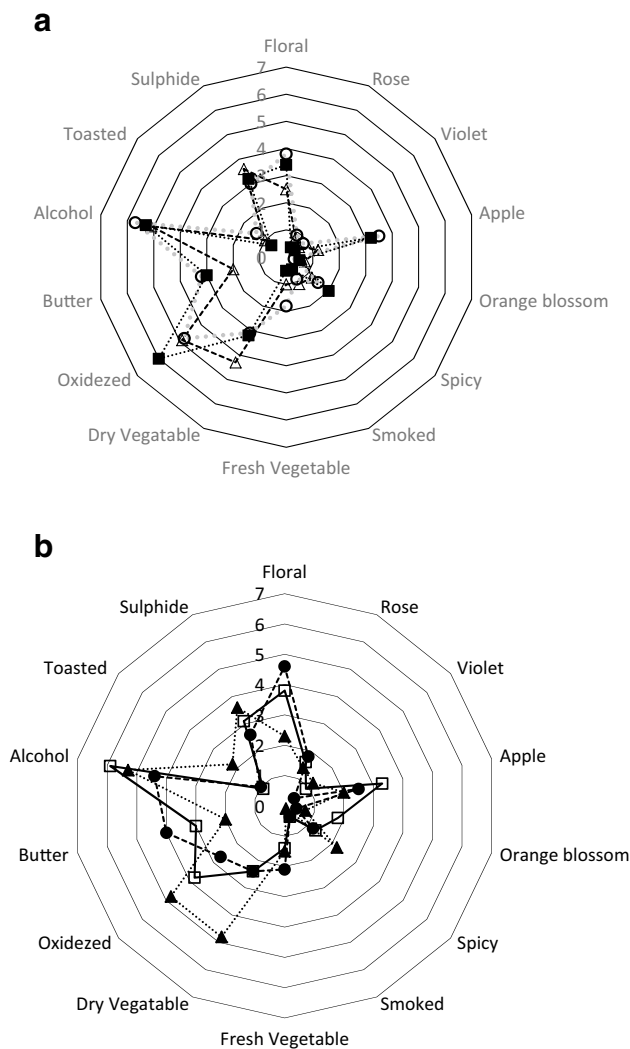


Fig. 3 Sensory profiles of white Falanghina wine at different pH inoculated with *O. oeni*. **a** Open triangle: pH 3.2; open circle: pH 3.4; filled square: pH 3.8 and *L. plantarum*. **b** Filled triangle: pH 3.2; filled circle: pH 3.4; open square: pH 3.8

sensorial descriptors produced by different LAB at various pH. In white Falanghina, the first component (PC1) accounted for 44% of total variance and correlated with floral, apple and butter attributes (Fig. 5), while the resulting PC2 (26% of total variance) was defined by rose and fresh vegetables. Wines at pH 3.2 were located on the negative side of PC1 showing higher scores for dry vegetable, sulphide, violet and toasted attributes.

Wines at pH 3.4 and 3.8 were located on the positive side of the PC1 with butter and apple attributes, these notes were present in the samples that completed MLF, together with higher scores for histamine content. The two LAB at different pH were localised in well-defined areas, in particular, *O. oeni* was situated on the positive side of PC2, and instead, *L. plantarum* was located on the

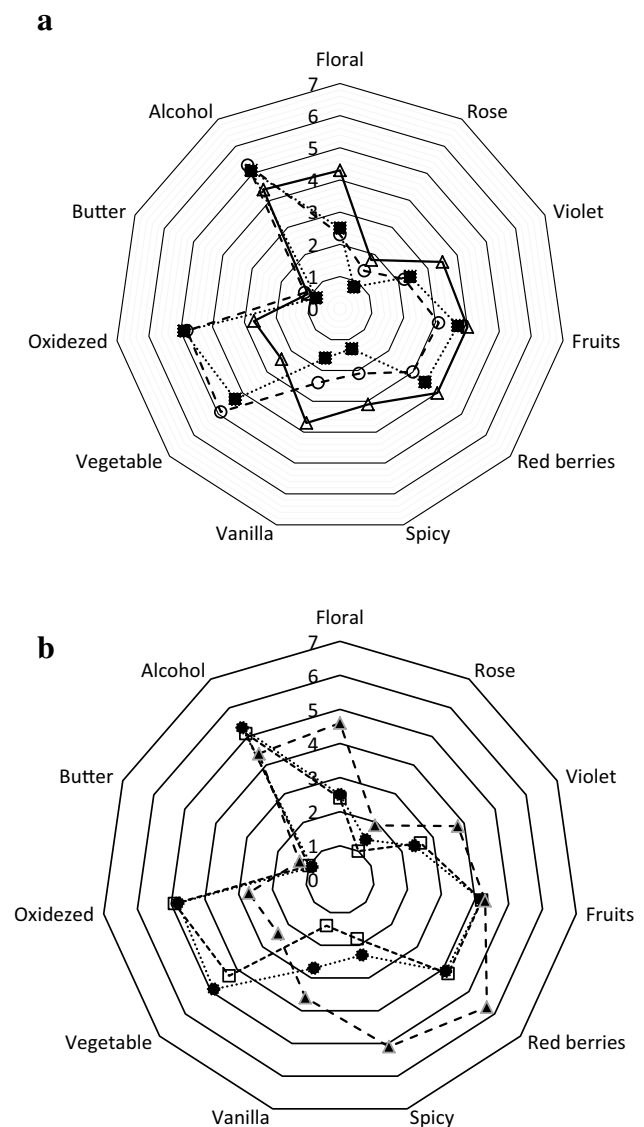


Fig. 4 Sensory profiles of red Tintilia wines inoculated with different LAB at pH 3.4: **a** Open circle: *O. oeni*; filled square: *L. pl.*; open triangle: mix and pH 3.8. **b** Filled circle: *O. oeni*; open square: *L. pl.*; filled triangle: mix

negative side. In red Tintilia, PC1 accounted for 57% of total variance and correlated with floral, apple and butter attributes (Fig. 6). Samples showed a clear separation based on the LAB used. In particular, all the samples inoculated with mix LAB were placed on the positive side of PC1, characterised by a greater number of sensory attributes. Moreover, wines at pH 3.8 showed the best sensory characteristics: red berry, spicy and violet (Fig. 6). The samples inoculated with mix appeared well differentiated from those inoculated with *O. oeni* and *L. plantarum*, which in turn seemed to be also strongly influenced by pH [22].

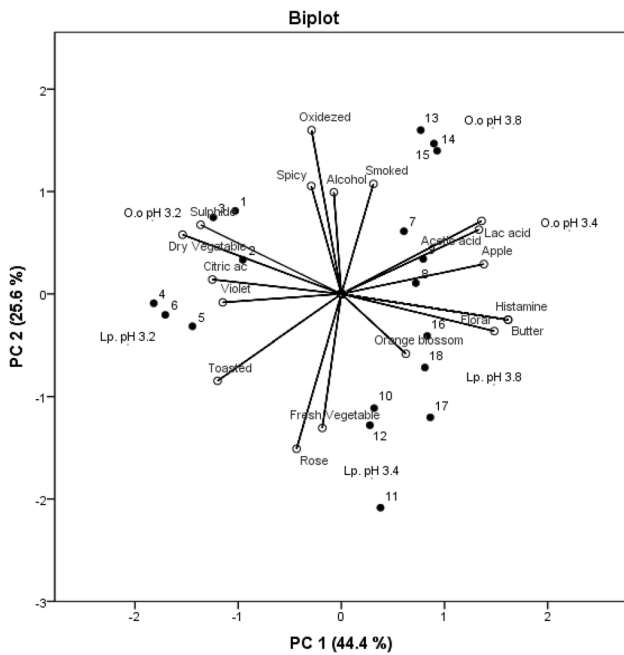


Fig. 5 PCA biplot of white Falanghina wines at different pH (3.2; 3.4 and 3.8) inoculated with *O. oeni* and *L. plantarum*. Wine samples: (1–2–3) *O. oeni* at pH 3.2; (4–5–6) *L.pl.* at pH 3.2; (7–8–9) *O. oeni* at pH 3.4; (10–11–12) *L.pl.* at pH 3.4; (13–14–15) *O. oeni* at pH 3.8; (16–17–18) *L.pl.* at pH 3.8

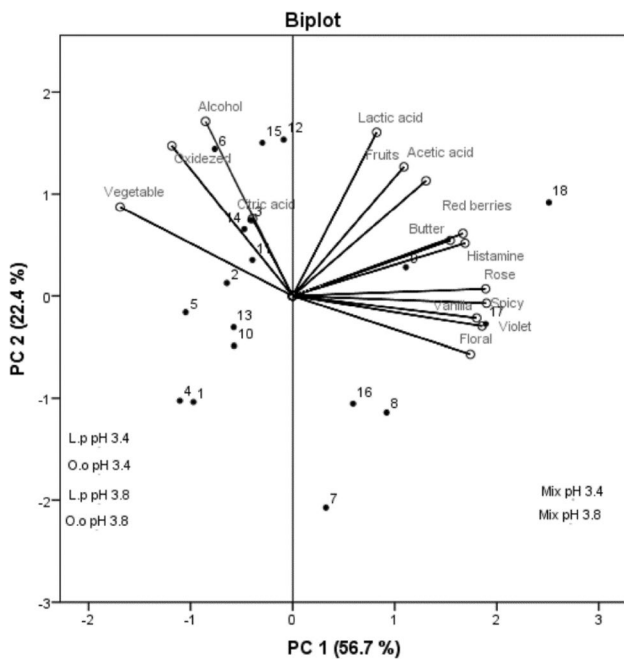


Fig. 6 PCA biplot of red Tintilia wines at pH 3.4 and pH 3.8 inoculated with *O. oeni*, *L. plantarum* and a mix (50:50%) of them. Wine samples: (1–2–3) *O. oeni* at pH 3.4; (4–5–6) *L.pl.* at pH 3.4; (7–8–9) mix at pH 3.4; (10–11–12) *O. oeni* at pH 3.8; (13–14–15) *L.pl.* at pH 3.8; (16–17–18) mix at pH 3.8

Discussion

In white Falanghina at pH 3.8, *O. oeni* employed about two-third of the time required by *L. plantarum*, to complete MLF, such better performance was also achieved at pH 3.4 [17]. Conversely, there was difficulty in conducting MLF by both LAB at pH 3.2, owing also to the harsh wine conditions for high-ethanol levels (15%), which affects LAB growth ability [23, 24]. It should also be pointed out that SO_2 is more inhibitory to LAB at low pH, as a greater percentage of the SO_2 are in the molecular form, which has the greatest antimicrobial activity. In red wine (13% alcohol) at pH 3.4 and 3.8, all the inoculants completed MLF within 35 days. As a rule, the reduction of time in completing MLF in red wines with respect to white ones depends on skin contact, which has an effect on the extraction of nitrogenous and other macromolecules capable of stimulating MLF [25]. Moreover, the marked reduction in time (77%) for completion of MLF in previously sterile-filtered red Tintilia samples, confirmed a robust competitive effect exerted by the different microorganisms present in wine.

The citric acid decrease has followed the degradation of malic acid, with an increase in volatile acidity, more marked in wines with high pH, which completed MLF and where SO_2 was less inhibitory to LAB [25].

The histamine content was higher in red wines, where maceration was prolonged, because more substrate was most likely available from yeast autolysis [17]. Histamine-producing strains belong to species of both genera *Oenococcus* and *Lactobacillus*, all carrying an *hdc* which is a gene coding for a histidine decarboxylase that converts histidine into histamine [26]. Moreover, in red wines, the greater the pH value was (3.8), the higher (> 15 mg/L) the histamine content (Table 2), as low pH prevented biogenic amine formation [27], because pH acts as a selective factor of microorganisms in wine and SO_2 is more active. At high pH, biogenic amines are always produced in high amounts as a consequence of an easier total growth and of the greater bacterial diversity, since the decarboxylating capacity of bacteria is very variable according to strain [18]. Therefore, a monitoring of malic and citric acid levels would allow that, once malic acid is completely degraded, the metabolic activity could be interrupted in such a way as to control the amount of citric acid [28] and amino acid degradation.

MLF decreases acidity and can influence additional wine quality parameters, like the colour in red wines, independently of pH change [29]. A decrease in colour intensity after MLF [30] affected red Tintilia samples (Table 3), owing to the pH increase and the degradation of acetaldehyde caused by LAB metabolism, with a consequent decrease in stable polymeric pigments [31]. During MLF,

LAB influenced aroma and flavour of wines by the production of volatile metabolites and the modification of aroma compounds already present in enhancing a wine's fruit aroma [8]. *L. plantarum* enhanced floral notes in white wines (Fig. 5), probably due to the release of monoterpenes by β -glycosidase activity [32]. In white Falanghina, where LAB completed the MLF, also the descriptor "butter" was perceived as a pleasant aroma. Generally, the higher the pH, the higher is the aroma intensity. The wines at pH 3.2 did not carry out MLF from LAB, which are potential sources of β -glucosidase activity, needed in non-aromatic grapes, such as Falanghina and Tintilia, because of their potential for liberation of grape-derived aroma compounds from their natural glycosylated state. It should be emphasized that also *Saccharomyces* yeast strains are able to express β -glucosidase activity during the alcoholic fermentation favouring the aroma expression of wines [33]. However, the acidic wine conditions (i.e. pH 3.2) might cause denaturation of these enzymes and inhibition of their activity [34].

In red Tintilia, the mix bacteria seemed to improve the sensorial intensity. The reduction in vegetative aroma may be due to the catabolism of aldehydes by LAB [35], while the enhanced fruitiness resulted from the formation of esters [36, 37]. Moreover, *L. plantarum* also showed a good organoleptic impact on red wines. Generally, the higher the pH, the higher is the aroma intensity, probably owing to the more favourable conditions for the LAB enzymatic activities (i.e. glycosidases) [38].

Conclusion

MLF tests on indigenous varieties of southern Italy, white Falanghina and red Tintilia grapes, were carried out by evaluating the effect of different pH and LAB on sensory and qualitative features of wines. In particular, the effect of a mix (50:50) of *O. oeni* and *L. plantarum* was compared to the single commercial strains. The duration of MLF was influenced by pH and LAB. In white Falanghina at pH 3.8, about 35 days was needed to complete the MLF using *O. oeni*, with a time reduction of about a third, with respect to *L. plantarum*. Such better performance of *O. oeni* was also achieved at pH 3.4. Conversely, at pH 3.2 neither LAB were capable of performing MLF, owing also to the harsh white wine conditions (15% alcohol degree). In red Tintilia wines at pH 3.4 and 3.8, MLF was completed in about 35 days with all inoculants. Higher pH led to an increase in histamine production, mainly in red wines at pH 3.8. Generally, the higher the pH, the higher is the aroma intensity. Sensorial analysis showed a positive impact of *L. plantarum* in the enhancement of floral notes in white wines. In red Tintilia, positive sensory characteristics were associated with pH 3.8.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethical requirements This article does not contain any studies with human or animal subject.

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