PAPER

MANAGEMENT OF MALOLACTIC FERMENTATION AND INFLUENCE ON CHEMICAL COMPOSITION OF AGLIANICO RED WINES

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ABSTRACT

A study has been carried out to determine the effects of lactic acid bacteria inoculation time on the fundamental components, procyanidins and biogenic amines content of Aglianico wines produced in Apulia region. Three different malolactic fermentation (MLF) techniques were compared: the co-inoculation, the sequential inoculation, and the traditional technique (spontaneous MLF). In the co-inoculation technique there was a delayed start and a late finish of the alcoholic fermentation. The colour intensity of the wine obtained with a spontaneous MLF was higher both at racking and after 12 months. Significant changes in content of flavan-3-ols were found in wines made with different MLF managements. The levels of catechin monomers ((+)-catechin, (-)-epicatechin, (-)-epicatechin-O-gallate) and procyanidin oligomers (B1-B4, and trimer C1) were lower in the co-inoculation wine. In the wine produced with a spontaneous MLF, the content of biogenic amines was significantly higher compared to the other two wines.

- Keywords: anthocyanins, biogenic amines, co-inoculation, procyanidin, sequential inoculation, wine -

INTRODUCTION

The most important microbial activity that is responsible for the conversion of must into wine is the alcoholic fermentation (AF), which is carried out by Saccharomyces yeasts. The malolactic fermentation (MLF) typically follows alcoholic fermentation and is carried out by indigenous lactic acid bacteria (LAB) or induced by inoculation with selected bacterial starters. It is a decarboxylation of L-malic acid, a dicarboxylic acid, with formation of a monocarboxylic acid, the L-lactic acid and carbon dioxide, which is catalyzed by malolactic enzymes which are NADP dependent and require divalent cations such as manganese or magnesium ions (VINCENZINI *et al.*, 2005). The MLF causes a significant evolution of wine and produces remarkable changes in its phenolic composition and sensorial characteristics (COSTELLO *et al.*, 2012; CABRITA *et al.*, 2008; LÒPEZ *et al.*, 2011; SURIANO *et al.*, 2012). In addition to the reduction of the acidity of the wine, MLF increases the aromatic complexity and smoothness (VERSARI *et al.*, 1999; COSTANTINI *et al.*, 2009; LOPEZ *et al.*, 2011). Generally the MLF is favoured in red wines, in *novello* wines, in white wines aged in barrique, or in some sparkling base wines (CAVAZZA *et al.*, 2003). On the other hand, this fermentation produces a small amount of acetic acid and sometimes may also generate unpleasant odours, bitter-tasting compounds or substances that may be dangerous to consumers' health, such as biogenic amines or precursors of ethyl carbamate (LONVAUD-FUNEL, 1999). It has been verified by analysis that the concentration of biogenic amines in wine at the end AF is always quite low, while increases after MLF (GAFNER, 2005). Moreover, it was found that wines which undergo spontaneous MLF often have higher biogenic amine concentration than those in which the MLF is conducted by select malolactic bacteria (CERRUTI *et al.*, 1987; MASQUÈ *et al.*, 2008). Biogenic amines are synthesized by microorganism through decarboxylation of amino acids. Between the main biogenic amines in wines there are tyramine, histamine, putrescine, cadaverine and phenylethylamine, synthesized by the decarboxylation of the amino acids tyrosine, histidine, ornithine, lysine and phenylalanine. These compound can cause adverse physiological reactions in susceptible individuals. Histamine can cause headaches, allergies, diarrhoea, palpitations and vomiting (STOCKLEY, 2004; BODMER *et al.*, 1999), while tyramine is strongly vasoconstrictive (SILLA-SANTOS, 1996). These effects may be enhanced by alcohol, which prevents the organism's detoxifying mechanisms from working properly and by the presence of other amines such as putrescine and cadaverine (LANDETE *et al.*, 2005), both associated with poor sanitary quality of grapes (LEITAO *et al.*, 2005) and responsible for major sensory defects in wines (LEHTONEN, 1996). Usually, the LAB used for MLF belong to the Oenococcus oeni species, anyway, it is possible to also find other bacteria of the Lactobacillus, Leuconostoc and Pediococcus species (DICKS *et al.*, 1995). However, even for the most resistant bacteria the conditions found in wine are close to the limits of survival, so that the transformation of 4-5 g/L of malic acid may requires even 15-20 days (CAVAZZA *et al.*, 2003). Several times, this process may take several months, may occur in some barrels and tanks but not in others and may be responsible for the occurrence of problems related to indigenous LAB species carrying out the MLF (LONVAUD-FUNEL, 2001) which may cause a range of undesirable changes to wine sensory properties, altered wine colour, and may even lead to the generation of biogenic amines (DAVIS *et al.*, 1985). Such a long time can be especially critical for those wines (such as *novello* wines) that must be processed and placed on the market in a short time, and moreover could be a risk since in the season in which the MLF takes place there may be sudden temperature drops which may determine an arrest of the process until the next spring. There are advantages subsequent to an early and fast MLF such as: a more efficient utilization of fermentation tank in the busy postharvest period, thus a decrease of energetic costs resulting in optimization of the winemaking process; moreover it is possible a decrease of the microbiological risks reducing the growth of undesired microorganism and also allows an early commercialization of wines (JUSSIER *et al.* 2006). It is therefore of fundamental importance a correct management of MLF. In this paper the influence of inoculation of lactic bacteria on changes occurring on the polyphenolic characteristics, colour, biogenic amines and proanthocyanidin in Aglianico red wines was investigated by comparing the techniques of co-inoculation and sequential inoculation to a spontaneous MLF.

MATERIALS AND METHODS

Experimental design and winemaking

This research was conducted during the 2012 harvest on Aglianico grape variety, grown in a vineyard trained on espalier training system with Guyot pruning and cultivated according to the principles of organic viticulture. Concerning the different possibilities of MLF management, in the Le.Vin.Sud Company of Cerignola (Foggia, Southern Italy) were carried out three experimental tests in order to evaluate the influence of the timing of lactic bacteria inoculation, comparing the technique of co-inoculation (inoculation of bacteria 24 hours after the yeast

inoculation), sequential inoculation (at the end of the AF) and the traditional technique without inoculation of any LAB, i.e. a spontaneous MLF, which was favoured by acting on certain oenological practices, as further explained. The Aglianico grapes were first destemmed and crushed, subsequently the mass of must and pomace was mixed, homogeneized and introduced in three different steel tank. From each steel tank, 3 x 100 Kg (in triplicate) of must with pomace was utilized for each of the three winemaking techniques adopted, with the aim to determine the repeatability of the differences among the compared treatments. The different batches of must and pomace were subjected to the following winemaking protocols:

- Co-inoculation or simultaneous inoculation of LAB (SIM). After crushing and destemming of grapes 40 mg/L of SO_2 was added. After two hours, *Saccharomyces cerevisiae* strain Lalvin R7 (Lallemand Inc, Castel D'Avezzano-Verona – Italia) previously hydrated in water for 15 min at 38 °C was inoculated in the must (20 g/hL, about 6 x 10^6 cfu/mL.). After 24 hours a lactic bacterial culture of *Lactobacillus plantarum* V22™ (Lallemand Inc, Verona-Italy) was inoculated. The inoculation rate was 1g/hL (2 x 10^7 cfu/mL) must/wine prior re-hydrated in chlorine free water at 20°C for 15 min. The alcoholic fermentation took place under controlled temperature by cooling the mass if the temperature exceeded the threshold of 26°C.

- Sequential inoculation post alcoholic fermentation of LAB (PAF). The only difference from the previous protocol was the time of addition of the bacteria. The lactic bacteria were added at racking, which was performed at the end of the alcoholic fermentation (10 day pomace contact). The doses of yeast and bacteria employed were the same. After the inoculation of bacteria, at a dosage of 20 g/hL Opti'Malo Plus bacterial nutrient (Lallemand Inc, Verona –Italy) were added at wine in according to the manufacturers instructions.

- Spontaneous MLF (Control). This MLF process was used as a comparison test for the others processes. After crushing and destemming of grapes were added about 40 mg/L of SO2, and then 20 g/L of previously hydrated *Saccharomyces cerevisiae* (Lalvin R7) yeast were inoculated. Also for this thesis, at racking/post alcoholic fermentation were added 20 g/hL of Opti'Malo Plus bacterial nutrient.

All the vinification were carried out at 26°C ± 1. During the fermentative pomace contact period (10 days in all vinifications) the cap was pumping over three times a day and the temperature and must density were recorded. At the end of this period, all wines were pressed at 2 bars, racked with no added sulphur dioxide for encourage MLF (in Control and PAF) and stored at 25°C. After MLF, the wines were racked again and 20 mg/L sulphur dioxide was added. The wines were cold stabilised (-4°C) for 1 month and then bottled without filtration. All analyses were made in triplicate at racking and after 6 months in the bottle (12 months after racking).

AF was monitored by ethanol production and sugar depletion. MLF was monitored by l-malic acid degradation and l-lactic acid production. AF and MLF were considered complete when residual sugars were less than 2.5 g/L and l-malic acid was less than 0.12 g/L.

Wine composition

Total acidity, volatile acidity, reducing sugars, pH, total SO_2, alcohol and total dry extract were all determined on wine according to EEC regulation 2676/90.

Chemicals and reference compounds

Standards, including trans-caffeoyl-tartaric acid, trans-p-coumaroyl-tartaric acid, caffeic acid, ferulic acid, p-coumaric acid, quercetin, myricetin, kaempferol, were supplied by Sigma Aldrich. While standard of (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, epigallocatechin, epigallocatechin gallate were supplied by Extrasynthese. The purities of the standards were all over 95%. All the solvents (methanol, acetonitrile, ethyl acetate) were HPLC grade. All the solutions were obtained with distilled deionised water using Carlo Erba reagents.

Spectrophotometric analysis

Phenolic compounds were determined by spectrophotometric methods (DI STEFANO *et al.*, 1989; Di Stefano *et al.*, 1997) using a UV/VIS Mod Lambda 25 double beam Spectrophotometer (Perkin Elmer S.p.A.). The total anthocyanins index was expressed as malvidin 3-glucoside and calculate by the following expression: E_{maxvis} x 16.17 x d (d=dilutions). The monomeric anthocyanins after separation and absorption on a C18 Set Pak cartridge were eluted with 5 ml of acetonitrile and then diluted with hydrochloric ethanol and calculated by: E_{maxvis} x 16.17 x d (d=dilutions). The total polyphenols index expressed as (+)-catechin was measured by: E1cm, 75 0nm x 186.5 x d (d=dilutions). The total flavonoids index was expressed as (+)-catechin and calculate with the graphic method of DI STEFANO (1989). The flavanols reactive to vanillin (flavonols vanillin assay) were expressed as (+)-catechin = ΔE x 290.8 x d (ΔE=absorbance difference between tests with and without vanillin; d=dilution). The proanthocyanidin content was determined after hot acid hydrolysis (Bate-Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst and expressed as cyanidin chloride. Colour intensity and hue were estimated by measuring absorbance at 420, 520 and 620 nm according to EU Regulation 1990.

HPLC analysis

The fixed acids of wine (tartaric, malic, lactic, citric and shikimic acids) were determined by an HPLC isocratic elution (HPLC 1100 series Agilent technologies) with a Phenomenex Synergi 4u Hydro-RP 80A (250x4.60 mm, 4 micron) with guard column, a mobile phase of phosphoric acid 10^{-3} M, 0.7 mL/min flow rate, 25°C and a UV detector set at 210 nm (CANE, 1990).

For flavans determination, the wine was separated into two fractions containing, respectively, individual catechins and oligomeric proanthocyanidins, using a C18 1g Sep-Pak cartridge as described by SUN *et al.* (1999). About 5 ml of wine was adjusted to pH 7 and then filtered through a Sep-Pak cartridge preconditioned with H_2O. Elution was carried on with 10 mL of H_2O to eliminate phenolic acids. After drying the cartridges with N_2, elution was carried out with 15mL of ethyl acetate to elute catechins and oligomeric proanthocyanidins (F I + F II). Each fraction was evaporated to dryness and dissolved in methanol, followed by HPLC analysis. A Thermo ODS RP-C18 Hypersil 200x2.1 (5 μm) column with a guard column was used for flavans analysis. Two ml of each extracted fraction were filtered on a 0.45 μm nylon membrane and immediately inject according to Squadrito's method (2007). Separation was carried out at 30°C, the flow rate was 0.25 mL/min and the injection volume 10 μL. The detection was set at 280 nm, using phosphoric acid 10^{-3} M (solvent A) and acetonitrile (solvent B). The gradient elution program was: from 91 to 86% A in ten minutes; from 86 to 82% A in ten minutes; from 82 to 60% A in ten minutes; from 60 to 40% A in five minutes; from 40 to 91% A in five minutes; equilibration time of five minutes. The peaks identification was performed comparing the retention times and absorption spectra of pure compounds (supplied from Extrasynthese) and were found analogues to values reported in the literature (BAOSHAN *et al.*, 1998; RICARDO *et al.*, 1991).

The determination of biogenic amines (BA) in wine was carried out by HPLC/FLD. A Hewlett-Packard (Agilent Technologies Palo Alto, CA, USA) 1100 series HPLC instrument was used, with a fluorescence detector set at excitation and emission wavelengths of 340 and 450 nm, respectively. The samples were subjected to an automatic pre-column derivatization procedure using o-phthalaldehyde (OPA Reagent, Agilent Technologies, Palo Alto, CA, USA). All separations were performed on a 200 x 4.6 mm, 5-μm Alltima C18 column (Alltech, Deerfield, IL, USA), protected by a 7.5x4.6 mm guard cartridge of the same type. Samples were injected into the column after being filtered through a 0.2 mm RC filter (Schleicher and Schuell, Keen, NH, USA). The two eluents used as mobile phases were sodium acetate 50 mM (pH 7.2)/THF (96:4) v/v (eluent A) and methanol (eluent B). The elution gradient programme followed the method described by NICOLINI (2003). From a stock solution of 200 mg/L containing agmatine, cadaverine, phenylethylamine, histamine, putrescine, and tyramine (standards purchased by Sigma-Aldrich) in methanol, four diluted solution were prepared and injected: 2.5, 5.0, 10.0, 20.0 mg/L. Quantification of the BA was performed with an internal standard of 10mM of norvaline solution.

Statistical analysis

Multivariate statistical analysis was performed using R Statistical Software (R Core Team (2013), R Foundation for Statistical Computing, Vienna, Austria). Chemical analyses were repeated three times for each sample and the data are presented as mean ± SD. The one way analysis of variance (ANOVA), and Duncan multiple comparison test to measure variation between treatments at a probability level of $p<0.05$ were performed.

RESULTS AND DISCUSSION

Wines composition

The musts collected from the steel tanks had the following chemical/physical characteristics: Control (spontaneous MLF) 210 g/L of reducing sugars, pH 3.30 and total acidity 6.40 g/L; SIM: 205 g/L of reducing sugars, pH 3.27 and total acidity 6.52 g/L; PAF: 214 g/L of reducing sugars, pH 3.35 and total acidity 6.24 g/L. The winemaking process began on the 12th of October with the crushing and destemming of grapes and the yeasts inoculation for all the three experimental processes. The kinetics of AF and malic acid degradation are reported in Figs. 1 and 2 respectively. In Table 1 it is reported the time required for the AF and the MFL for each winemaking. The duration of the fermentation process was identical for the PAF and the Control (both 8 days), while it was longer for the SIM (about 10 days). However, all alcoholic fermentations were regular and complete. LAB in the SIM were able to perform MLF in 23-24 days from the beginning of winemaking. The wine obtained by sequential inoculation (PAF) carried out the degradation of malic acid in 40-41 days from the beginning of the winemaking. Instead, the wine underwent a spontaneous MLF, despite the absence of added LAB, has finished the MLF after 57 days from the beginning of the vinification. Therefore, the wine obtained by the SIM technique has finished the MLF 33-34 days before of Control wine. This data is important since time is a key factor from an economic, techni-

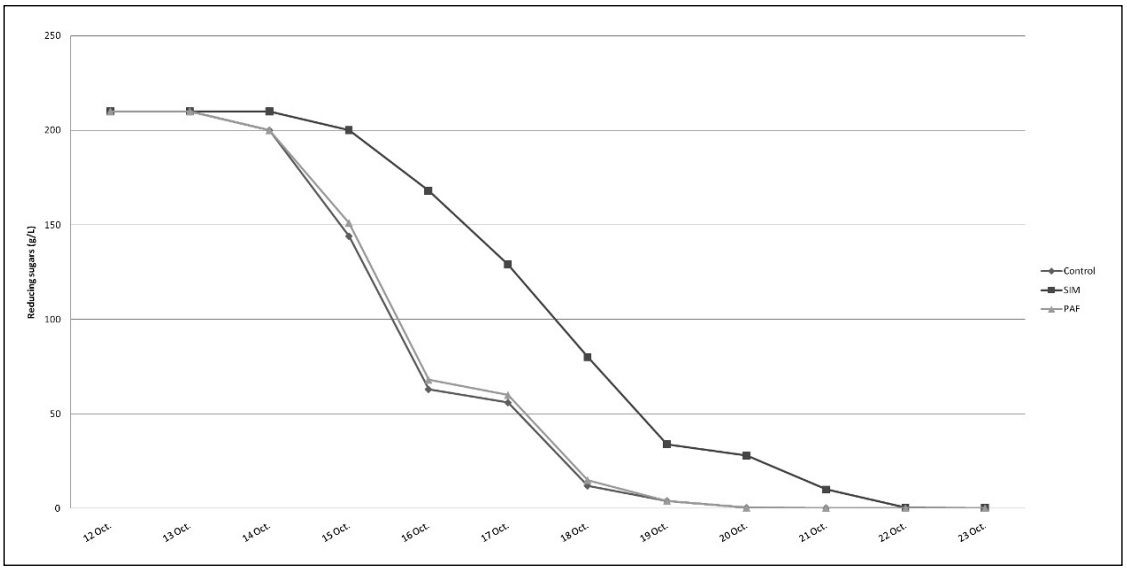

Fig. 1 - Kinetics of alcoholic fermentation.

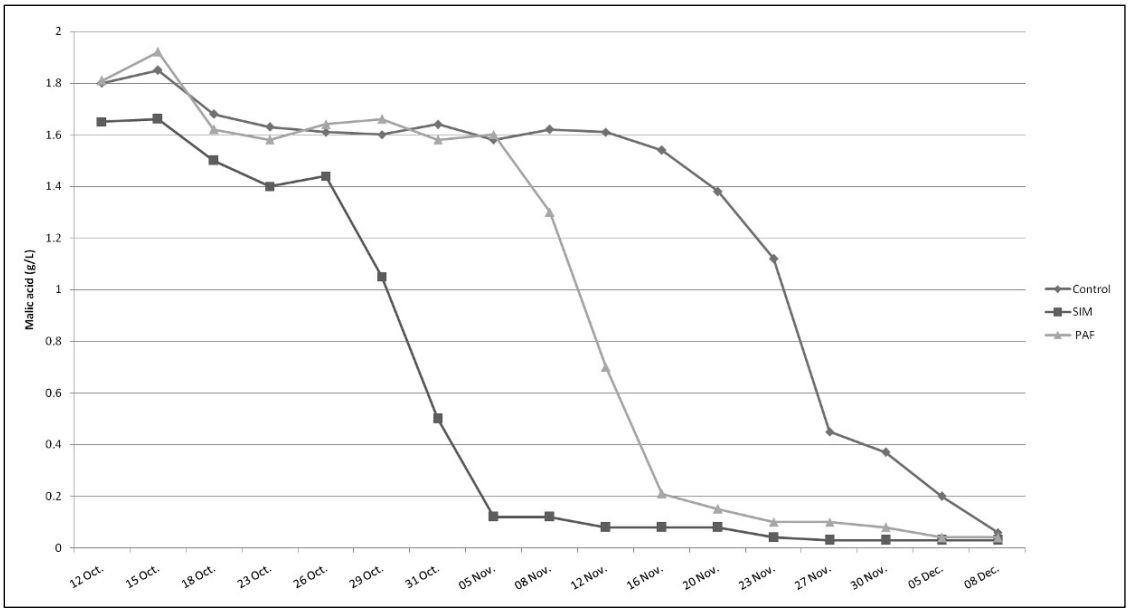

Fig. 2 - Time course of malic acid degradation from the start of alcoholic fermentation.

cal and practical point of view, for a good organizational management of the winery. Table 2 shows the results of the chemical/physical analysis of wines at the end of alcoholic fermentation (racking) and 12 months after racking. Differences were observed in the alcohol content, acidic profile, pH and total acidity. The SIM wine after alcoholic fermentation showed obvious signs of the beginning of MLF. Indeed, the malic acid content (1.44

Table 1 - Time required to complete AF and MLF.

Treatment	Time for AF (days)	Time for malic acid degradation (days after bacterial inoculation) †	Vinification time AF + MLF (days) ‡
Co-inoculation (SIM)	10±0	23±1	24±1
Sequential inoculation (PAF)	8±0	33±4	41±4
Spontaneous MLF (Control)	8±0	NA	57±4

† MLF was considered complete when malic acid concentration was below 0.12 g/L.
‡Vinification time is the time from destemming/crushing to completion of AF and MLF.

Table 2 - Wines composition after AF (racking) and after MLF (12 months after racking).

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Alcohol (Vol. %)	x	12.39 ab	12.06 b	12.48 a	12.40 a	12.10 b	12.42 a
	s	0.08	0.07	0.08	0.12	0.08	0.09
Residual sugars (g/L)	x	2.40 a	2.40 a	2.40 a	2.35 b	2.42 a	2.45 a
	s	0.18	0.15	0.20	0.22	0.25	0.20
Total dry extract (g/L)	x	30.50 a	29.40 b	30.50 a	30.20 a	29.80 b	30.40 a
	s	2.50	1.80	2.10	2.08	2.18	2.10
pH	x	3.35 b	3.53 a	3.36 b	3.45 b	3.61 a	3.44 b
	s	0.02	0.03	0.02	0.03	0.02	0.02
Total acidity (g/L)	x	7.65 a	6.15 b	7.50 ab	5.63 a	5.03 c	5.10 b
	s	0.38	0.42	0.45	0.35	0.40	0.39
Volatile acidity (mg/L)	x	0.54 b	0.60 a	0.56 b	0.55 b	0.60 a	0.50 c
	s	0.03	0.02	0.03	0.07	0.08	0.06
Total SO2 (mg/L	x	24.05 a	22.10 b	22.04 b	32.10 b	58.10 a	30.10 c
	s	4.10	3.80	3.50	2.90	3.50	2.80
Tartaric acid	x	3.04 b	2.90 c	3.20 a	2.91 a	2.86 ab	2.62 b
	s	0.28	0.22	0.18	0.4	0.38	0.25
L-malic acid (g/L)	x	1.61 ab	1.44 b	1.64 a	0.12 a	0.05 b	0.06 b
	s	0.09	0.10	0.12	0.01	0.00	0.00
L-lactic acid (g/L)	x	0.10 b	0.60 a	0.12 b	1.45 b	1.52 a	1.41 b
	s	0.00	0.01	0.00	0.08	0.06	0.05
Citric acid (g/L)	x	0.25 b	0.27 ab	0.28 a	0.23 b	0.30 a	0.25 ab
	s	0.02	0.02	0.04	0.01	0.02	0.02

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

g/L) was less than Control (1.61 g/L) and PAF (1.64 g/L) wines. In SIM wine the partial transformation of malic acid has produced a certain amount of lactic acid already at racking. Moreover, SIM wine showed a lower total acidity and a higher pH compared to Control and PAF wines. This was mainly due to the transformation of a diprotic acid (malic acid) with two acidic functional groups into a monoprotic acid (lactic acid) with only one acidic functional group, with a corresponding decrease in acidity and an increase of pH. It was observed a difference in the alcohol content of wines, in particular the SIM wine showed the lowest alcohol content. Probably, since the sugar content of the must subjected to the SIM process was slightly smaller thus less alcohol was produced. Another possible explanation is linked to the lactic acid bacteria that could have used part of the reducing sugars, in addition to malic acid, as nutrients for their metabolism. This not only may have influenced the alcohol content of wine, but furthermore had furnished an increased energy for the cellular development of the bacteria resulting in the production of more volatile compounds and greater amounts of acetic acid, as it was found in SIM wine. Indeed, the volatile acidity expressed as acetic acid was slightly higher in the SIM wine than in the other two wines. Both possibilities may have contributed to the lower alcoholic content, as it is confirmed by the findings of some other authors which have observed a delay of alcoholic fermentation and a use of the sugars of must by LAB (LAFON-LAFOURCADE *et al.*, 1983). After one year of storage, the SIM wine still has a lower acid strength, represented by a higher pH and a lower total acidity compared to the other wines. The content of sulfur dioxide, in order to favour the MLF especially in the Control, has been deliberately kept low. There were no significant differences in respect of tartaric acid and citric acid.

Polyphenolic composition and wine colour

Table 3 shows the polyphenolic composition and chromatic characteristics of wines after alcoholic fermentation and 12 months after racking. The effects of different MLF starts showed a marked change in the content of polyphenols in SIM wine already at the end of the AF. Indeed, the index of total polyphenols, the total flavonoids, the total and monomeric anthocyanins contents are higher in the SIM wine, with variations ranging from 5 to 17%, than in the other wines. The differences in tannins (proanthocyanidins and flavans reacting with vanillin) content between wines were not significant. After 12 months from racking, all the wines had finished the MLF thus a natural reduction of the polyphenolic compounds (total flavonoids, flavans, and an-

Table 3 - Phenolic composition and chromatic characteristics of Aglianico wines after AF (racking) and after MLF (12 months after racking).

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Total phenols (mg/L)	x	1812 b	2062 a	1831 b	1674 b	1793 a	1652 ab
	s	66	66	37	49	52	41
Total flavonoids (mg/L)	x	1982 c	2388 a	2008 b	1466 b	1585 a	1583 a
	s	73	74	45	42	44	36
Vanillin index (mg/L) V	x	1026 a	1013 b	1017 b	829 b	935 a	659 c
	s	38	39	23	19	33	21
Proanthocyanidins (mg/L) L	x	2489 a	2490 a	2398 b	2135 a	1693 b	1533 c
	s	71	77	59	67	55	38
Total anthocyanins (mg/L)	x	321 ab	338 a	315 b	223 b	242 a	169 c
	s	15	16	19	12	12	15
Monomeric anthocyanins (mg/L)	x	232 ab	251 a	227 b	90 b	110 a	92 ab
	s	12	9	12	4	6	8
D.O. 420 nm (P.O. 1cm)	x	3.49 a	2.82 c	3.06 b	2.84 a	2.54 b	2.47 ab
	s	0.07	0.07	0.06	0.06	0.05	0.05
D.O. 520 nm (P.O. 1cm)	x	7.16 a	5.17 c	6.23 b	4.03 a	3.69 b	3.39 c
	s	0.20	0.16	0.18	0.10	0.01	0.01
D.O. 620 nm (P.O. 1cm)	x	1.06 a	0.85 c	0.94 b	0.75 c	0.78 b	0.99 a
	s	0.03	0.02	0.03	0.02	0.03	0.04
Colour intensity (P.O. 1cm)	x	11.71 a	8.84 c	10.23 b	7.62 a	7.01 b	6.85 c
	s	0.20	0.10	0.14	0.10	0.12	0.08
Tint (E420/E520)	x	0.49	0.54	0.49	0.70	0.69	0.73
V/L index	x	0.41	0.41	0.44	0.39	0.55	0.43

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

thocyanins) was observed. Anyway, the total anthocyanins were still slightly higher in the SIM wine compared to Control and considerably higher compared to PAF wine. From the other side, the content of proanthocyanidins (high molecular weight tannins) was significantly higher in the Control than in the other wines. Therefore, an early MLF as for the SIM and PAF processes, causes greater losses over time of higher molecular weight tannins (proanthocyanidins). The increase in pH observed at racking for SIM wine has promoted the polymerization processes of high molecular weight tannins leading to a partial precipitation and thus their removal by pouring operations. Some authors (CAROLINE and EVELINE, 2011; COSTELLO *et al.*, 2012) in tests of management of MLF have observed similar effects in respect of these compounds. It is well known that MLF can reduce the colour intensity in red wines due to numerous factors associated with the MLF (BURNS *et al.*, 2013). Indeed, the Aglianico wines obtained with the SIM process, already at the end of alcoholic fermentation (Table 3), showed a colour intensity of 8.84 which was significantly lower than PAF and Control wines. Usually, this index is positively correlated to the anthocyanin content, in this case, considered that in SIM wine there was an increase in pH mostly due to the partial MLF, despite a slightly higher anthocyanin content respect to the other wines, a change in the balance of the pH-dependent anthocyanin pigments has determined a certain loss of the red colour. The colour reduction may also be due to the precipitation of the free anthocyanins molecules with polysaccharides and potassium bitartrate. Twelve months after racking, the differences in colour intensity, although reduced, remained quite significant and the Control wine still showed the highest value. Also the 420 nm and 520 nm absorbances were higher in the Control wine, while the hue did not show significant differences between wines.

The composition of monomeric catechins and oligomeric procyanidins is shown in Table 4. A common feature to all wines is the predominance of the (+)-catechin among all monomeric flavanols. Among dimeric procyanidins B2 and B4 are present in greater quantities. The trimeric procyanidins were detected in all wines but in small quantities. At racking, the SIM wine differed from the other two wines because of the lowest content of almost all the flavan-3-ols, with the exception of a few gallic acid esters such as epicatechin gallate, epigallocatechin gallate and procyanidin B2 gallate. Less difference were found between the Control and PAF. After one year from racking, all wines showed a reduction in the content of (+)-catechin, (-)-epicatechin, epigallocatechin, procyanidin B1, B3 and B4, while there was a general increase of procyanidin B2, gallic acid esters and trimeric procy-

Table 4 - Concentration (mg/L) of monomeric catechins and oligomeric procyanidins in Aglianico wines.

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
(+)-Catechin	x	40.36 ab	36.7 c	41.34 a	35.06 b	28.73 c	39.68 a
	s	1.74	1.83	1.88	1.25	1.44	1.32
(–)-Epicatechin	x	25.61 b	19.24 c	27.22 a	21.98 b	16.96 c	24.59 a
	s	1.21	0.71	1.29	1.12	1.09	1.15
Procyanidin B1	x	17.60 a	13.30 b	17.42 ab	12.09 a	7.76 c	10.39 b
	s	0.45	0.80	0.73	0.71	0.74	0.83
Procyanidin B2	x	37.40 a	29.60 b	36.40 ab	39.06 b	33.18 c	40.95 a
	s	1.75	1.67	1.98	1.44	1.56	1.22
Procyanidin B3	x	10.80 a	5.81 b	10.30 ab	7.52 b	4.95 c	8.84 a
	s	0.78	0.46	1.04	0.34	0.46	0.27
Procyanidin B4	x	30.73 b	27.55 c	32.60 a	28.26 b	24.77 c	32.59 a
	s	1.75	1.85	1.78	1.88	1.36	1.33
Procyanidin B2 gallate	x	22.28 c	23.10 b	25.20 a	32.36 a	26.33 c	27.39 b
	s	0.97	0.85	1.04	0.98	1.44	1.65
Epicatechin gallate	x	3.74 b	4.30 a	3.31 c	5.73 b	4.55 c	7.12 a
	s	0.24	0.88	0.37	0.54	0.74	0.67
Gallocatechin	x	5.21 a	3.40 c	4.30 b	5.62 a	2.48 c	5.04 b
	s	0.24	0.23	0.27	0.38	0.55	0.29
Epigallocatechin	x	4.33 ab	2.41 b	4.73 a	3.99 a	3.08 b	3.48 ab
	s	0.37	0.46	0.63	0.74	0.74	0.74
Epigallocatechin gallate	x	1.35 c	3.30 a	2.37 b	1.24 c	4.45 a	4.01 b
	s	0.08	0.08	0.04	0.04	0.03	0.04
Trimer T2	x	6.53 c	6.95 b	8.64 a	7.67 b	8.44 a	8.22 ab
	s	0.72	0.83	6.78	0.34	0.46	0.48
Trimer C1	x	7.33 a	5.39 b	7.11 a	8.17 b	6.70 c	9.28 a
	s	0.77	0.71	0.84	0.66	0.53	0.79

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

anidins C1 and T2. The SIM wine confirmed a lower content of almost all forms of monomeric catechins and oligomeric procyanidins, while the PAF showed concentrations that were even higher than Control wine. Also in this case, a faster MLF in SIM and PAF from the early stages of racking had caused a lower acidic strength, resulting in a loss of these compounds.

Biogenic amines composition

Table 5 shows the concentrations of biogenic amines in Aglianico wines. The average concentration of total amines at racking differs slightly between thesis submitted at different management of MLF, ranging from 9.73 mg/L in SIM wine to 10.38 mg/L in Control wine. Af-

Table 5 - Concentration (mg/L) of biogenic amines in Aglianico wines.

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Histamine	x	2.78 a	2.44 c	2.65 b	3.53 a	0.24 ab	0.20 b
	s	0.74	0.22	0.39	0.92	0.02	0.02
Agmatine	x	1.45 b	1.54 a	1.57 a	1.56 c	2.41 b	2.93 a
	s	0.46	0.38	0.40	0.55	0.63	0.74
Putrescine	x	3.74 a	3.32 b	3.66 ab	10.51 a	8.48 c	9.54 b
	s	0.82	0.67	0.74	1.86	1.59	1.48
Tyramine	x	0.70 b	0.72 ab	0.74 a	0.76 b	1.40 a	0.60 c
	s	0.08	0.09	0.06	0.09	0.10	0.03
Cadaverine	x	1.42 ab	1.35 b	1.44 a	1.60 a	1.59 a	1.16 b
	s	0.36	0.42	0.40	0.39	0.28	0.29
Phenylethylamine	x	0.29 ab	0.36 a	0.27 b	0.42 b	0.60 a	0.58 ab
	s	0.05	0.06	0.06	0.06	0.07	0.06
Total biogenic amines	x	10.38	9.73	10.33	18.38	14.72	15.01

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

ter 12 months of storage, when wines had already reached a chemical/physical and biological stabilization, it was observed an increase in the BA content. The differences found between the different winemaking protocols were significant. The average content of total biogenic amines in the wine obtained from spontaneous MLF (18.38 mg/L) was higher than co-inoculation (14.72 mg/L) and sequential inoculation (15.01 mg/L) wines. In percentage the increase was 77.0% in Control, 51.3% in SIM and 45.3% in PAF wine. This higher percentage in the Control wine could be ascribed to a release of amino acids as a consequence of yeast lysis during AF and to the proliferation of LABs with carboxylase activity during spontaneous MLF. Putrescine was the most abundant amine, with Control showing the highest amount at racking (3.74 mg/L before MFL) and also 12 months after racking (10.51 mg/L). Histamine, which is thought to be the cause of various adverse reactions to wines (TAYLOR *et al.*, 1989; WANTKE *et al.*, 1996), after 12 months increased its concentration in the wine produced with spontaneous MLF (Control) wine and vice versa decreased in SIM and PAF wines. Indeed, after 12 months histamine was almost absent in wines subjected to inoculation of LAB (0.24 mg/L in SIM and 0.20 mg/L in PAF), while the Control wine showed an higher value (3.53 mg/L) which was anyway very similar to values reported by other authors (IZQUIERDO-CAÑAS *et al.*, 2008; PRAMATEFTAKI *et al.*, 2006). Probably the histidine decarboxylase activity was present and active in wine produced with spontaneous MLF for the whole period of aging, instead was absent or not active in wines subjected to inoculation of LAB.

CONCLUSION

The simultaneous inoculation with yeast and bacteria (SIM) has reduced the duration of MLF of about 33 days compared to the wine obtained without the addition of any LAB (Control). The simultaneous inoculation already at the end of the AF showed evident signs of the onset of MLF. After 12 months from racking, there was a weakening of differences in phenolic compounds content, but the wine underwent a spontaneous MLF (Control) remained more colourful and more rich in high molecular weight tannins. Concerning the content of monomeric catechins and oligomeric procyanidins it was observed the predominance of (+)-catechin among the monomeric flavanols and the procyanidin B2 among the dimeric procyanidins. These compounds were lower in the co-inoculation wine compared to the other two wines. The different MLF managements led to a different evolution in the content of biogenic amines. The simultaneous (SIM) and the sequential (PAF) inoculation of lactic acid bacteria for the MLF led to a significant reduction in almost all the amines investigated with respect to the Control (spontaneous MLF). After 12 months from racking, the average total content of biogenic amines was lower in the wine underwent sequential inoculation compared to the co-inoculation.

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REFERENCES

Abrahamse C.E. and Bartowsky E.J. 2012. Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. World J. Microbiol. Biotechnol (2012) 28:255-265.

Bodmer S., Imark C. and Kneubuhl M. 1999. Biogenic amines in foods: histamine and food processing. Inflamm. Res. 48:296-300.

Burns T.R., and Osborne J.P. 2013. Impact of Malolactic Fermentation on the Color and Color Stability of Pinot noir and Merlot Wine. Am. J. Enol. Vitic. 64(3):370-377.

Cabrita M.J., Torres M., Palma V., Alves E., Patão R. and Costa Freitas A.M. 2008. Impact of malolactic fermentation on low molecular weight phenolic compounds. Talanta 74:1281-1286.

Cane P. 1990. Il controllo della qualità dei vini mediante HPLC: Determinazione degli acidi organici. L'Enotecnico, 26(1-3):69-72.

Capozzi V., Russo P., Ladero V., Fernández M., Fiocco F., Alvarez M., Grieco F. and Spano G. 2012. Biogenicamines degradation by Lactobacillus plantarum: toward a potential application in wine. Frontiers in Microbiology - Food Microbiology. April 2012, Volume 3, Article 122, 6

Cavazza A., Poznanski E., Chiodini A. and Zini C. 2003. Batteri per la fermentazione Malolattica: una selezione da vini Trentini. Terra Trentina, 24-28.

Cerutti G., Margheri G. and Bongini F. 1987. Sulla correlazione tra fermentazione malolattica ed ammine biogene vasoattive. Vigne vini n. 14(11):39-42.

Costantini A., Garcìa Moruno E. and Moreno-Arribas M.V. 2009. Biochemical transformations produced by malolactic fermentation. In: Wine Chemistry and biochemistry. Eds. M.V. Moreno-Arribas and M.C. Polo (Springer Sience Business Media: New York), pp. 25-57.

Costello P.J., Francis I.L. and Bartowsky E.J. 2012. Variations in the effect of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of Oenococcusoeni strain and wine matrix composition. Aust. J. Grape Wine Res. 18:287-301.

Coton, M., Romano, A., Spano, G., Ziegler, K., Vetrana, C., Desmarais, C., Lonvaud-Funel, A., Lucas P. and Coton E., 2010. Occurrence of biogenic amine-forming lactic acid bacteria in wine and cider. Food Microbiol. 27, 1078-1085.

Gazzetta Ufficiale CE L 272 del 03/10/1990 (EEC Regulation 2676/90).

Davis C.R.D., Wibowo D., Eschenbruch R., Lee T.H. and Fleet G.H. 1985. Practical implications of malolactic fermentation: A review. Am. J. Enol. Vitic. 36:290-301.

Jussier D., Morneau A.D. and De Ordunal R. M. 2006. Effect of Simultaneous Inoculation with Yeast and Bacteria on Fermentation Kinetics and Key Wine Parameters of Cool-Climate Chardonnay. Applied and Enviromental Microbiology, p. 221-227 Vol. 72, No. 1.

Dicks L.M.T., Dellaglio F. and Collins M.D. 1995. Proposal to

reclassify *Leuconostoc oenos* as *Oenococcus oeni* [corrig.] gen. nov., comb. nov.. Int. J. Syst. Bacteriol. 45:395-397.

Di Stefano R., Cravero M.C. and Gentilini N. 1989. Metodi per lo studio dei polifenoli dei vini. L'Enotecnico, 25(5):83-89.

Di Stefano R., Ummarino I. and Gentilini N. 1997. Alcuni aspetti del controllo di qualità nel campo enologico. Lo stato di combinazione degli antociani. Ann. Istit. Sperim. Enol. Asti: 105-121.

Gafner J. 2005. Stabilità biologica e amine biogene. Vinidea. net, Rivista Internet Tecnica del Vino. N. 2/1.

Izquierdo-Cañas P.M., García-Romero E., Gómez-Alonso S., González M.F. and Palop-Herreros M.L. 2008. Amino acids and biogenic amines during spontaneous malolactic fermentation in Tempranillo red wines. J. Food Compos. Anal. 21(8):731-735.

Lafon-Lafourcade S., Carre E. and Ribéreau-Gayon P. 1983. Occurrence of lactic acid bacteria during the different stages of vinification and conservation of wines. Appl. Environ. Microbiol. 46:874-880.

Landete J.M., Ferrer S., Polo L. and Pardo I. 2005. Biogenic amines in wines from three Spanish regions. *J. Agric. Food Chem.* 53:1119-1124.

Leitao M., Marques A. P. and San Romao M. V. 2005. A survey of biogenic amines in commercial Portuguese wines. Food Control 16:199-204.

Lonvaud-Funel A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Anton, Leeuw, 67:317-331.

Lonvaud-Funel A. 2001. Biogenic amines in wines: role of lactic acid bacteria. FEMS Microbiol. Lett. 199:9-13.

Lòpez R., Lòpez-Alfaro I., Gutièrrez A. R., Tenorio C., Carijo P., Gonzàles-Arenzana L. and Santamaria P. 2011. Malolactic fermentation of Tempranillo wine: contribution of the lactic acid bacteria inoculation to sensory quality and chemical composition. Int. J. Food Science Tech. 46:166-174.

Masqué M.C., Romero S.V., Rico S., Elórduy X., Puig A., Capdevila F., Suárez C., Heras J.M. and Palacios A.T. 2008. Coinoculo di lieviti e batteri: effetti sulla qualità e sulle amine biogene**.** www.infowine.com, Rivista internet di viticoltura ed enologia, N. 8/2.

Nicolini G., Larcher R., and Bertoldi D. 2003. Indagine sul tenore di ammine libere in mosti d'uve di varietà autoctone. Riv.Viti. Enol. 1:15-27.

Pramateftaki P.V., Metafa M., Kallithraka S. and Lanaridis P. 2006. Evolution of malolactic bacteria and biogenic amines during spontaneous malolactic fermentations in a Greek winery. Lett. in Appl. Microbiol. 43:155-160.

Ricardo da Silva J.M., Bourzeix M., Cheynie V. and Moutounet M. 1991. Procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes. Vitis 30:245-252.

Silla-Santos M.H. 1996. Biogenic amines: their importance in foods. Int. J. Food Microbiol. 29:213-231.

Squadrito M., Corona O., Ansaldi G. and Di Stefano R. 2007. Relazione fra i percorsi biosintetici degli HCTA, dei flavonoli e degli antociani nella buccia dell'uva. Riv. Vit. Enol. 3:59-70.

Sun B.S., Pinto T., Leandro M.C., Ricardo-Da-Silva J.M. and Spranger I. 1999. Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine. Am. J. Enol. Vitic. 50(2):179-183.

Suriano S., Ceci G. and Tamborra P. 2012. Impact of different winemaking techniques on polyphenolic compounds of Nero di Troia wine. Italian Food & Beverage Technology 70:5-15.

Stockley C.S. 2004. Can histamine in wine cause adverse reactions for consume. Australian and New Zealand Grapegrower and Winemaker 485(77):79-82.

Taylor S.L., Stratton J.E. and Nordlee J.A. 1989. Histamine Poisoning (Scombroid Fish Poisoning): An Allergy-Like Intoxication. Clinical Intoxication. 27(4-5):225-240.

Versari A., Parpinello G.P. and Cattaneo M. 1999. *Leuconostoc Oenos* and malolactic fermentation in wine: a review. J. Ind. Microbiol. Biotechnol. 23:447-455.

Wantke F., Hemmer W., Haglmüller T., Götz M.,·and Jarisch R. 1996. Histamine in Wine. Int. Arch. Allergy Immunol. 110:397-400.

Zùniga M., Pardo I. and Ferrer S. 1993. An improved medium for distinguishing between homofermentative and heterofermentative lactic acid bacteria. International Journal of Food Microbiology, 18: 37-42.

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