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Effect of Antioxidant Protection of Must on Volatile Compounds and Aroma Shelf Life of Falanghina (*Vitis vinifera* L.) Wine

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Two vinification methods involving different degrees of antioxidant protection of Falanghina must during prefermentative steps, and referred as HAMP (high antioxidant must protection) and LAMP (low antioxidant must protection), were compared in terms of fermentation performances of four different yeast strains, composition of the volatile fraction of wines at the end of alcoholic fermentation, and shelf life of wines during storage. The use of HAMP technology resulted in wines with lower volatile acidity and higher concentrations of medium-chain fatty acid ethyl esters, acetates, and volatile fatty acids. For two of the four strains a lower concentration of isoamyl alcohol was also observed. HAMP wines also revealed increased shelf life because of the higher concentration of odor active esters at the end of storage and better preservation of varietal aromas.

KEYWORDS: Antioxidant protection; oxygen; fermentation performances; aroma compounds; shelf life

INTRODUCTION

Traditionally, winemaking procedures for the production of white wine involve careful protection of must against oxidation. During prefermentative steps, oxygen is known to promote several chemical and enzymatic reactions that may cause a decrease of the overall wine aroma quality, such as the production of C6 alcohols and aldehydes responsible for herbaceous off-flavor (*1*, *2*) and the oxidation of volatile compounds involved in the expression of wine varietal character (*3*). Moreover, the amount of molecular oxygen dissolved in must is also known to influence the metabolism of yeast cells (*4*), and reduced oxygen availability has been reported to enhance the production of attractive flavor compounds such as ethyl fatty acid and acetate esters during alcoholic fermentation (*5*, *6*). By contrast, some studies reported that controlled oxidation of must before alcoholic fermentation, a vinification technique generally referred to as hyperoxidation, resulted in increased aroma quality and shelf life of wines obtained from Chardonnay, Parellada, and Muscat grapes (*7*, *8*), although in many other cases significant losses in aroma intensity and varietal aroma characteristics were reported (*3*, *9*, *10*).

Vitis vinifera L. cv. Falanghina is a white berry grape widely grown in several DOC areas of Campania, as well as in other growing regions of southern Italy. The wines obtained with this grape are characterized by intense fruit and balsamic scents (*11*), which make them easily recognizable during tasting. However, anecdotal evidence suggests that the aroma shelf life of Falanghina wine is usually short, as 8−10 months after bottling the typical flavor of this wine disappears, leaving a flat, uncharacteristic aroma. To date, the few studies concerning Falanghina wines are related to their chemical composition (*11*, *12*), whereas the influence of different winemaking practices on the composition of the volatile fraction and on the aroma shelf life of these wines is for the most part unknown.

In this study we report the effects of two distinct vinification processes involving different degrees of must antioxidant protection on the volatile composition and aroma shelf life of wines obtained from Falanghina grapes. Vinifications were carried out with four different yeast strains to investigate the effect of different oxygen availabilities on their fermentation performances and production of volatile compounds during alcoholic fermentation.

MATERIALS AND METHODS

Vinification. Wines were produced with *V. vinifera* L. cv. Falanghina grapes from vineyards surrounding the city of Benevento. Grapes were harvested in mid-October and were transferred to the winery in 20 L food grade plastic boxes. Vinifications were carried out following the two distinct processes described in **Figure 1**. Low antioxidant must protection (LAMP) technology consisted basically of a traditional vinification process, wherein the only antioxidant effect is owed to the SO_2 added before must clarification. On the contrary, high antioxidant must protection (HAMP) was achieved by large use of antioxidants such as SO_2 and ascorbic acid, whereas contact between must and oxygen during the prefermentative steps of vinification was avoided by means of nitrogen-saturated environments. For this purpose, uncrushed grapes were directly pressed in a pneumatic press previously sparged with nitrogen, and the juice obtained was transferred into a nitrogen-saturated stainless steel tank through a custom-made tubing system equipped with stainless steel nozzles allowing continuous sparging of nitrogen into the system. Both musts were allowed to settle

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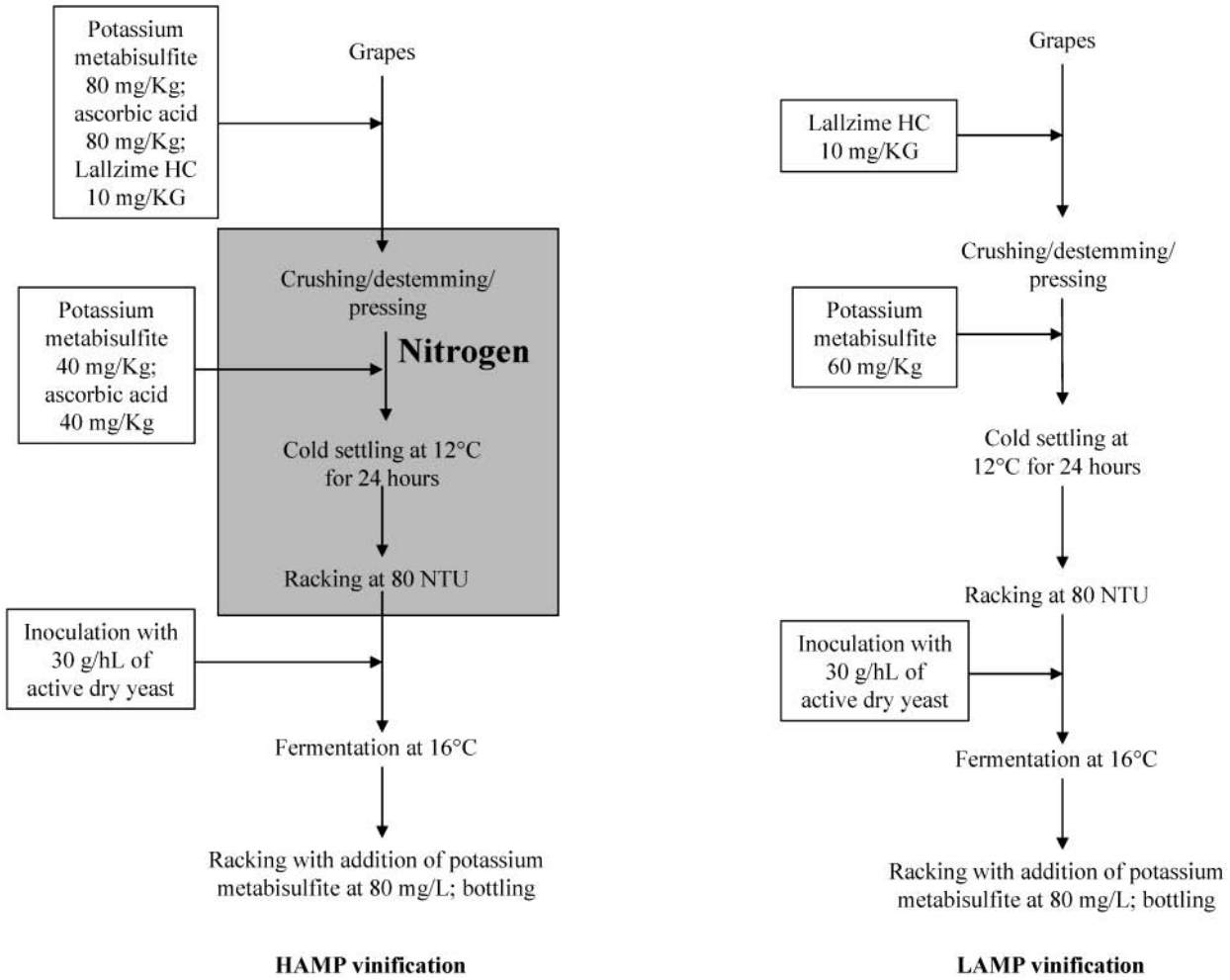

Figure 1. Vinification protocol.

spontaneously for 24 h at 12 °C and were racked at the same degree of turbidity (80 NTU), to avoid differences in higher alcohols production commonly associated with different levels of particulate material in must (*13*). Chemical parameters of must at inoculation were as follows: °Brix, 22; pH, 3.2; titratable acidity, 9.37 g/L; malic acid, 3.9 g/L; total nitrogen, 165 mg/L. Inoculation was carried out with one of the four different yeast strains Lalvin CY 3079, Lalvin R-HST, Lalvin SV 02, and Lalvin T 306, provided by Lallemand Inc. Italy (Castel d'Azzano, VR). After bottling, wines were stored in a cellar, at a constant temperature of 15 °C, for the aging experiment.

Standard Chemical Analysis. Degrees Brix, titratable acidity, volatile acidity, pH, and ethanol were measured according to Official European Regulation 2676/90. Malic acid was determined enzymatically (Boehringer Mannheim, Indianapolis, IN).

Extraction and Analysis of Volatile Compounds. For gas chromatographic analysis, 200 mL of wine obtained after the whole contents of three equal bottles had been mixed was submitted to continuous liquid−liquid extraction for 3 h with 20 mL of dichloromethane. As internal standard, 2-methyl-1-pentanol at a final concentration of 1 mg/L was added. The organic layer was recovered in a separating funnel. Residual water was removed by means of the addition of Na_2SO_4, and the solvent was concentrated first in a Kuderna−Danish concentrator to 1 mL and finally under a stream of nitrogen (1.5 L/min). Extraction of each sample was performed in triplicate.

Gas chromatography was performed using a Hewlett-Packard 5890 chromatograph equipped with a split/splitless injector (Hewlett-Packard, Avondale, PA), a J&W DB-Wax column (30 m length × 0.25 i.d. × 0.25 film thickness; J&W Scientific, Folsom, CA), and a flame ionization detector (FID). The temperature program used was 40 °C for 3 min, raised at 4 °C/min to 220 °C, and held for 20 min at maximum temperature. Carrier gas (He) velocity was 37 cm/s. Both detector and injector temperatures were maintained at 250 °C. Identification of compounds was performed by comparison of their linear retention indices with those of pure reference standards. Comparison of mass spectra stored in the NIST database with those obtained for each compound on an HP 5972 quadrupole mass spectrometer coupled with an HP5890 gas chromatograph was also carried out. Compounds for which pure reference standards were not available were tentatively identified only on the basis of mass spectra comparison. The same column of HRGC was employed during this analysis. Electron impact mass spectra were recorded with an ion source energy of 70 eV.

Statistical Analysis. Analysis of variance was carried out on standard chemical and volatile compound data, and means were compared by Tukey's test. Elaborations were carried out using Statgraphics Plus-PC.

RESULTS AND DISCUSSION

Fermentation Performances. For all yeast strains, HAMP technology resulted in a significant decrease in the fermentation rate (**Table 1**). Days required for completion of alcoholic fermentation were approximately 19 for HAMP and 15 for LAMP vinifications, respectively. Within each treatment, differences between strains were minor. The largest variations observed between treatments with regard to the fermentation performances were relative to the rate between 5 and 50% of sugar consumption. This behavior suggests that, during the proliferation phase, HAMP fermentations were characterized by a slower yeast growth, probably owing to higher amounts of SO_2 and poor oxygen availability (*4*). Minor differences were

Table 1. Effect of Different Strains and Treatments on Fermentation Rate (°Brix/Day) within Two Sugar Concentrations

	HAMP				LAMP			
sugar range (%)	CY 3079	R-HST	SV 02	T 306	CY 3079	R-HST	SV 02	T 306
5–50	2.42	2.4	2.48	2.39	3.96	3.9	3.94	3.9
0–99	1.22	1.19	1.16	1.18	1.49	1.42	1.44	1.44

Table 2. Effect of Different Treatments and Yeast Strains on the Composition of Falanghina Wines[a]

	HAMP				LAMP			
parameter	CY 3079	R-HST	SV 02	T 306	CY 3079	R-HST	SV 02	T 306
sugar (g/L)	1.9 a	1.9 a	1.6 a	1.6 a	2.0 a	1.6 a	1.7 a	1.5 a
alcohol %	12.0 a	12.3 a	12.2 a	12.3 a	12.5 a	12.2 a	12.5 a	12.6 a
SO_2 free (mg/L)	44.8 b	38.4 a	38.4 a	44.8 b	6.4 a	6.4 a	6.4 a	9.6 b
SO_2 total (mg/L)	89.6 b	88.9 b	83.2 a	89.6 b	19.2 a	22.4 b	19.2 a	19.2 a
pH	3.20 a	3.20 a	3.25 a	3.30 a	3.30 a	3.25 a	3.23 a	3.30 a
titratable acidity (g/L)	8.8 a	9.1 a	9.4 a	8.6 a	8.9 a	9.1 a	9.4 a	8.6 a
volatile acidity (g/L)	0.3 a	0.26 a	0.2 a	0.3 a	0.3 a	0.5 b	0.3 a	0.6 b
malic acid (g/L)	3.7 a	3.7 a	3.7 a	3.6 a	3.3 a	3.5 a	3.6 a	3.3 a

[a] Different letters within each vinification treatment denote significant differences between yeasts at $p < 0.05$ (Tukey's test).

observed between different treatments with regard to the fermentation rates between 0 and 99% of sugar utilization.

Standard Chemical Analysis. Ethanol and acetic acid are among the major volatile compounds resulting from alcoholic fermentation. No significant difference was evident between yeast strains or treatments with regard to the concentration of ethanol at the end of alcoholic fermentation (**Table 2**). The concentration of acetic acid was quite low and did not vary among strains within HAMP treatment, whereas for T 306 and R-HST strains higher levels of this metabolite were observed in the LAMP treatment, probably owing to the stronger oxidation of acetaldehyde to acetic acid associated with increased must oxygenation. Although large differences between yeast strains in the ability to metabolize malic acid have been reported (*14*), no significant difference between yeasts or treatment was observed.

Volatile Compounds. A total of 45 compounds were identified and measured by GC-MS and GC-FID, including 19 alcohols, 13 esters, 5 acids, 2 aldehydes, 2 ketones, 2 phenols, 1 furan, and 2 sulfur compounds (**Table 3**). Medium-chain fatty acid ethyl esters (MCFA ethyl esters) and acetates (mainly 3-methylbutyl acetate and 2-phenylethyl acetate) are generally considered to be responsible for the fruity character of white wine. These compounds were found at higher concentrations in wines obtained by means of HAMP treatment (**Figure 2**). The use of larger amounts of SO_2 during HAMP vinification may be a significant factor in explaining these findings. Previous studies reported the occurrence of significant differences in the concentration of esters of wines fermented with and without SO_2. For example, Herraiz et al. (*15*) found that wines fermented with SO_2 were characterized by higher levels of ethyl octanoate, although differences in the concentrations of ethyl hexanoate and 3-methylbutyl acetate were not significant. Similar findings were reported by Margheri and Versini (*16*). However, the effect of SO_2 on the production of esters during fermentation seems to be not systematic and may depend on several factors. Shinohara and Watanabe (*17*) found that differences in the concentration of MCFA ethyl esters and acetates were significant only when SO_2 was added at 100 mg/L, whereas Daudt and Ough (*18*) reported that the amount of acetate esters was increased or decreased by the presence of SO_2, according to the type of yeast strain employed for fermentation. In partial contrast with these findings, during our experiment an increase in the concentration of esters associated with higher amounts of SO_2 was observed regardless of the type of yeast strain employed for fermentation, and the differences occurring between treatments were larger than previously reported. Such behavior is probably not due only to the influence of SO_2, but may be the result of the combined action of higher SO_2 concentrations and low oxygen availability associated with HAMP treatment. While examining the influence of different factors on the biosynthesis of esters, Nykänen (*6*) reported that reduced oxygen concentration increased the production of MCFA ethyl esters. More recently, the release of MCFA ethyl esters by yeast has been linked to the availability of oxygen for the biosynthesis of unsaturated long-chain fatty acids. During alcoholic fermentation, unsaturated fatty acids, which are important constituents of cell membrane, can be assumed by yeast directly from must or can be synthesized by oxidation of free saturated fatty acids present in medium, with a process that involves the presence of free oxygen. Lacking this element, unsaturated fatty acid synthesis is impossible and the whole process is stopped, with corresponding accumulation of medium-chain acyl-CoA. To recover free coenzyme A, ester formation is promoted by yeast, and the wine obtained in these conditions is richer in esters containing the corresponding acylic group (*19*). With regard to the increased concentrations of acetates detected in wines obtained with HAMP technology, it has been reported that the production of these compounds in *S. cerevisiae* is the result of the balance of activities of two enzymes: alcohol acetyltransferase, which promotes esterification of the corresponding alcohol, and specific cell esterase, which is responsible for ester hydrolysis (*20*). Because a reduction in the production of alcohol acetyltransferase has been observed in *S. cerevisiae* as a consequence of aeration (*21*), it may be supposed that the use of enhanced anaerobic conditions may favor the production of acetates. This behavior was particularly evident for CY 3079 and R-HST yeast strains, for which a larger increase in the production of acetates, especially isoamyl acetate, was observed in HAMP wines. This observation was confirmed by the fact that, for these two strains, a lower concentration of isoamyl alcohols was detected (**Table 3**), suggesting increased alcohol acetyltransferase activity.

A positive correlation was also observed between the use of strong antioxidant conditions and the concentration of MCFA (**Figure 3**), the positive contribution of which to the quality of

Table 3. Concentration (Milligrams per Liter)[a] of Volatile Compounds of Falanghina Wines

	HAMP				LAMP			
compound	CY 3079	R-HST	SV 02	T 306	CY 3079	R-HST	SV 02	T 306
alcohols								
1-propanol	0.809 a	0.985 b	0.875 ab	1.077 c	1.099 b	1.079 b	1.018 a	1.488 c
2-methyl-1-propanol	2.368 a	3.618 c	2.763 ab	2.823 b	4.780 c	5.329 d	3.128 a	3.800 b
1-butanol	0.077 b	0.060 a	0.073 b	0.093 c	0.068 a	0.066 a	0.080 b	0.071 a
2+3-methyl-1-butanol	80.321 b	82.677 b	80.133 b	75.946 a	98.121 d	96.330 c	85.320 b	79.029 a
3-methyl-3-buten-1-ol	0.012 a	0.015 b	0.011 a	0.017 b	0.020 b	0.014 a	0.013 a	0.016 b
1-pentanol	0.019 a	0.019 a	0.018 a	0.021 b	0.022 a	0.022 a	0.020 a	0.022 a
3-methyl-1-pentanol	0.075 b	0.050 a	0.075 b	0.097 c	0.043 b	0.024 a	0.071 d	0.052 c
1-hexanol	0.353 c	0.332 b	0.342 bc	0.322 a	0.357 a	0.434 c	0.400 b	0.351 a
(*E*)-3-hexen-1-ol	0.019 a	0.023 b	0.022 b	0.022 b	0.011 a	0.010 a	0.010 a	0.010 a
3-ethoxy-1-propanol	0.011 b	0.018 c	0.008 a	0.007 a	0.040 b	0.041 b	0.020 a	0.039 b
(*Z*)-3-hexen-1-ol	0.020 a	0.021 a	0.022 a	0.019 a	0.016 a	0.015 a	0.018 a	0.014 a
(*Z*)-2-hexen-1-ol	0.005 a	0.004 a	0.004 a	0.005 a	0.006 a	0.005 a	0.007 a	0.006 a
2-ethylhexanol	0.025 b	0.027 b	0.022 ab	0.019 a	0.016 b	0.026 c	0.012 a	0.014 a
linalool	0.055 a	0.067 a	0.071 b	0.057 a	0.061 a	0.071 b	0.062 a	0.066 a
1-octanol	0.032 a	0.033 a	0.031 a	0.036 a	0.033 b	0.034 b	0.012 a	0.009 a
α-terpineol	0.062 a	0.052 a	0.051 a	0.051 a	0.049 a	0.056 b	0.056 b	0.044 a
nerol	0.005 a	0.009 a	0.007 a	0.008 a	0.008 a	0.008 a	0.009 a	0.008 a
geraniol	nd	0.008 a	0.005 a	nd	nd	0.007 a	nd	0.011 a
2-phenylethanol	35.377 b	27.892 a	32.381 b	26.628 a	39.496 d	23.704 b	35.320 c	21.624 a
esters								
2-methylpropyl acetate*	0.082 a	0.128 b	0.064 a	0.072 a	0.067 b	0.069 b	0.047 a	0.053 a
3-ethylmethyl butanoate	0.011 a	0.009 a	0.010 a	0.011 a	0.017 b	0.012 a	0.018 b	0.017 b
3-methylbutyl acetate	5.270 c	5.823 d	3.678 a	4.690 b	3.022 c	2.406 a	2.365 a	2.800 b
(*Z*)-ethyl 2-butenoate	0.015 a	0.020 b	0.015 a	0.020 b	0.015 a	nd	0.016 a	0.016 a
ethyl hexanoate	1.099 a	1.153 b	1.156 b	1.301 c	0.777 b	0.715 a	0.874 c	0.884 c
hexyl acetate	0.090 c	0.081 b	0.066 a	0.072 a	0.052 bc	0.040 a	0.047 ab	0.054 c
(*E*)-3-hexen-1-ol acetate*	0.025 a	0.023 a	0.026 a	0.029 a	0.025 b	0.018 a	0.032 c	0.024 b
ethyl octanoate	2.111 a	2.139 a	2.193 a	2.427 b	1.520 b	1.318 a	1.704 c	1.757 c
ethyl 3-hydroxybutanoate	0.110 b	0.108 b	0.073 a	0.202 c	0.157 c	0.090 a	0.120 b	0.198 d
ethyl decanoate	0.965 c	0.814 a	0.840 b	1.016 c	0.588 a	0.530 a	0.624 a	0.661 a
ethyl succinate	0.969 b	0.756 a	1.227 c	0.757 a	1.474 c	0.852 a	1.667 d	1.071 b
2-phenylethyl acetate	0.948 c	0.705 ab	0.612 a	0.811 b	0.626 c	0.350 a	0.471 b	0.431 ab
hydroxyethyl butanoate	1.585 b	1.237 a	1.815 c	1.721 c	0.714 ab	0.648 a	1.154 c	0.784 b
acids								
acetic acid	0.993 a	1.440 b	0.877 a	0.828 a	2.513 b	2.458 b	1.254 a	3.099 c
2-methylpropanoic acid	0.031 a	0.036 a	0.028 a	0.038 a	0.033 b	0.045 c	0.017 a	0.022 a
hexanoic acid	4.290 a	4.514 a	4.585 a	5.563 b	3.270 b	3.030 a	3.470 c	3.800 d
octanoic acid	10.053 a	11.239 b	11.084 b	12.585 c	8.129 b	7.193 a	8.291 b	9.616 c
decanoic acid	3.843 a	4.291 b	3.738 a	4.496 b	3.078 b	2.400 a	2.813 ab	3.282 b
aldehydes and ketones								
acetoin	0.245 b	1.306 c	0.043 a	0.096a	3.005 d	1.062 a	2.338 b	2.515 c
furfural	0.149 c	0.074 a	0.120 b	0.109 b	0.059 a	0.050 a	0.044 a	0.026 a
ethylphenyl acetaldehyde*	0.018 a	0.022 a	0.024 a	nd	0.022 a	0.022 a	0.023 a	nd
β-damascenone*	0.024 a	0.030 a	0.027 a	0.020 a	0.020 ab	0.025 b	0.016 a	0.020 ab
phenols								
4-propylphenol	0.031 b	0.022 a	0.039 c	0.028 ab	0.023 a	0.025 a	0.028 a	0.022 a
4-vinylguaiacol	0.170 a	0.208 b	0.169 a	0.170 a	0.124 a	0.106 a	0.108 a	0.152 b
furans								
2,3-dihydrobenzofuran*	0.360 a	0.529 a	0.413 a	1.454 b	0.295 a	0.262 a	0.237 a	0.227 a
sulfur compounds								
2-(methylthio)ethanol	0.007 a	0.007 a	0.008 a	0.008 a	0.007 b	0.008 b	0.008 b	0.003 a
3-(methylthio)-1-propanol	0.192 c	0.134 b	0.142 b	0.088 a	0.213 b	0.128 a	0.153 a	0.123 a

[a] Values are means of triplicate analysis; different letters within a row denote significant differences at $p < 0.05$ (Tukey's test). nd, not detected; *, tentatively identified by comparing experimental mass spectrum with the one contained in NIST database.

white wine has been previously described (*22*−*24*). With regard to the possible factors influencing their formation during alcoholic fermentation, Herraiz et al. (*25*) reported a minor influence of different SO_2 concentration on the release of fatty acids by yeast cells. On the other and, as in the case of MCFA esters, MCFA are intermediate products of the biosynthetic pathway that leads to the production of long-chain unsaturated fatty acid (*5*). The higher concentration observed for these compounds in HAMP wines may be therefore the consequence of reduced oxygen availability, with a mechanism similar to the one discussed for esters.

Changes in Concentration of Volatile Compounds during Storage. Composition and sensory modifications occurring in wine during bottle aging are mainly related to a relatively small group of chemical reactions. In the case of white wine, the evolution of esters and terpenols has been reported to have a major influence on the formation of typical aging bouquet (*26*, *27*). The behavior of ethyl and acetates, both associated with the fresh and fruity character of white wine, is shown in **Figure 4**. The hydrolysis of acetates was generally more rapid than that of MCFA ethyl esters, consistent with previous findings (*23*, *28*). Although the kinetics of degradation of both ester classes was similar between HAMP and LAMP treatments, it was interesting to observe that, after 14 months, the concentration of acetates of HAMP wines was almost equal to the one measured in LAMP wines at the beginning of the experiment,

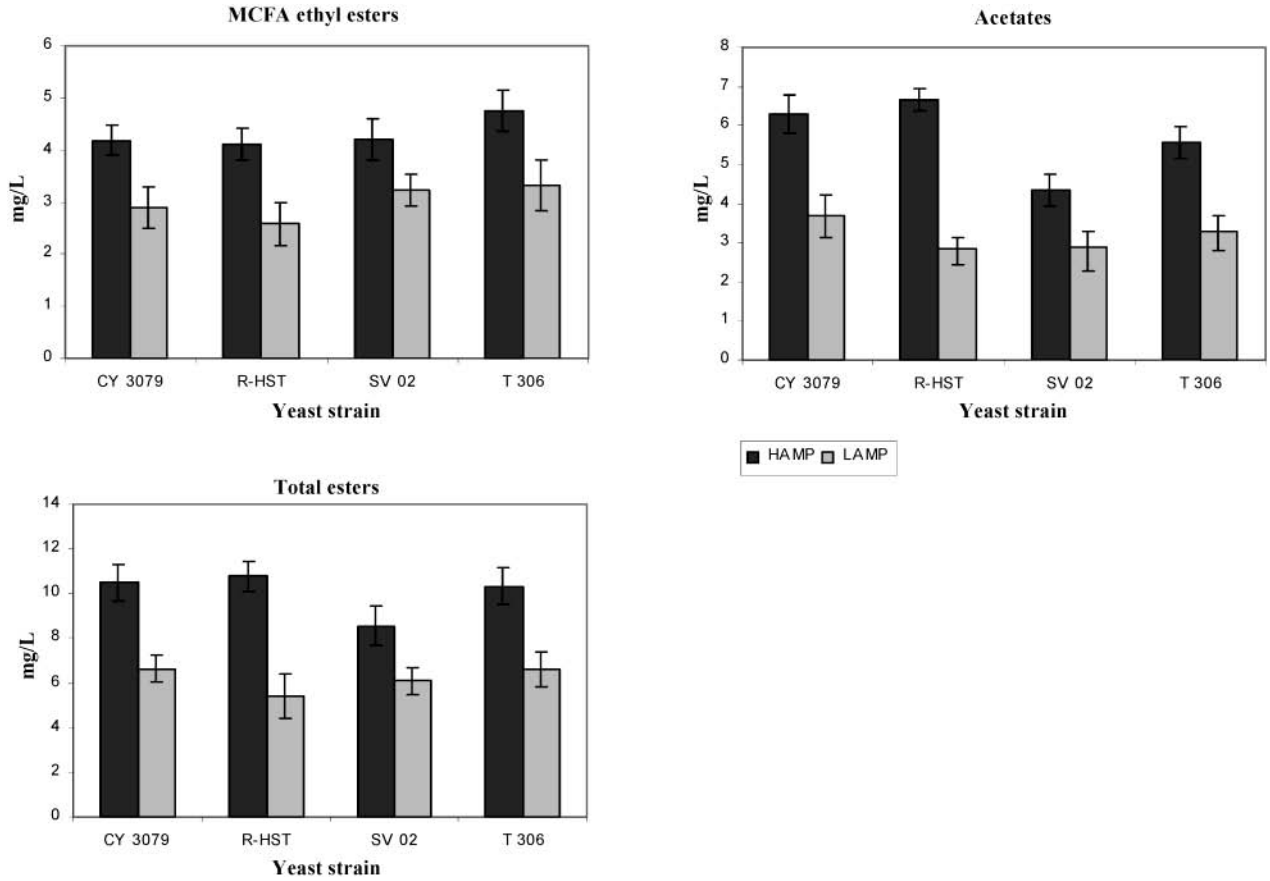

Figure 2. Influence of vinification treatment on the concentration of volatile esters in Falanghina wines obtained with different yeast strains. For each strain differences between treatments were in all cases significant at $p < 0.05$. MCFA ethyl esters represent the sum of ethyl hexanoate, ethyl octanoate, and ethyl decanoate. Acetates are the sum of 3-methylbutyl acetate and 2-phenylethyl acetate. Total esters represent the sum of all the aforementioned.

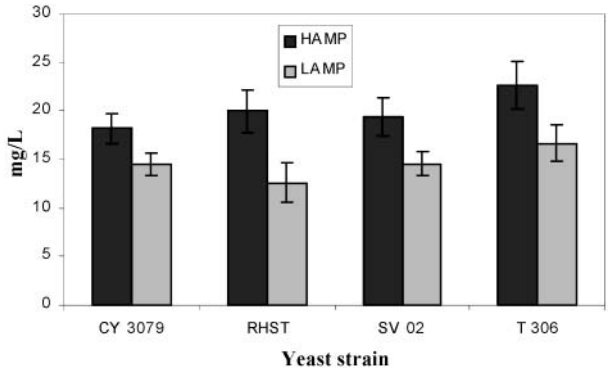

Figure 3. Influence of vinification treatment on the concentration of MCFA (sum of hexanoic, octanoic, and decanoic acid) in Falanghina wines obtained with different yeast strains. Differences between treatments were in all cases significant at $p < 0.05$.

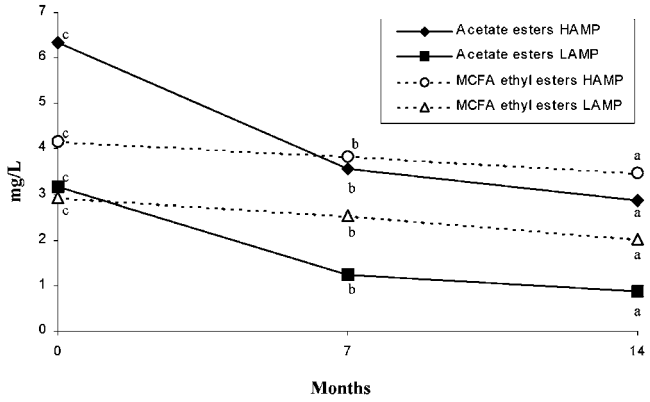

Figure 4. Changes in the concentration of volatile fermentation esters during aging of Falanghina wines obtained with different treatments. Values are means of wines obtained with different yeast strains. Different letters within a row denote significant differences at $p < 0.05$. For each point, differences between treatments were always significant at $p < 0.05$.

whereas that of MCFA ethyl esters was still significantly higher, attesting the strong increase of wine aroma shelf life obtained with the use of HAMP technology. Changes in concentration of linalool, the most powerful odorant within the chemical class of terpenols, are shown in **Figure 5**. After 14 months of bottle storage, HAMP wines exhibited a higher concentration of linalool, mainly owing to a less intense decline of this compound during the first 7 months. Linalool degradation is the consequence of the wide array of acid-catalyzed cyclization and/or oxidation reactions occurring during wine storage (*28*). In acid aqueous solution, these reactions yield α-terpineol as the main product, whereas minor byproducts are linalool oxides and hydroxylinalool (*27*, *29*). In our case, the higher decline observed in LAMP wines for linalool during the first 7 months of storage coincided with a more significant increase of α-terpineol (**Figure 5**), although the difference in the concentration of α-terpineol after 14 months was not significant. In any case, as linalool degradation products have a higher odor threshold, weakening of the flowery−fruity character of LAMP wines has to be expected.

In summary, the level of antioxidant protection of must had a significant influence on the aroma composition of young Falanghina wines produced with different yeast strains, probably owing to the combined effect of reduced oxygen availability

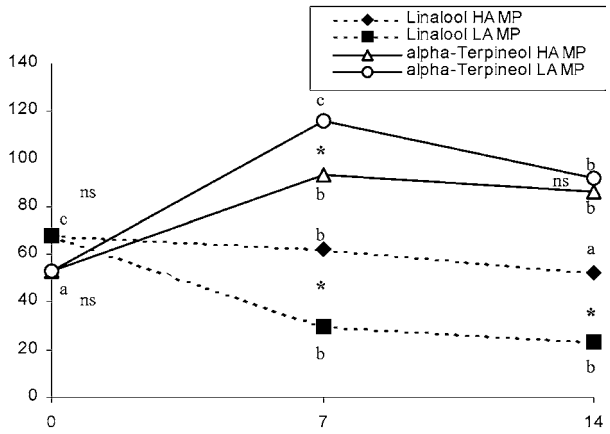

Figure 5. Changes in the concentration of selected terpenols during aging of Falanghina wines obtained with different treatments. Values are means of wines obtained with different yeast strains. Different letters within a row denote significant differences at $p < 0.05$. Asterisks denote significant differences between treatments ($p < 0.05$). ns, not significant.

and higher SO_2 concentration on yeast metabolism. High antioxidant protection of must yielded wines with lower volatile acidity and higher content of esters responsible for the fruity character of young wines, reducing at the same time the production of fusel alcohols. As a consequence, during aging, wines obtained with this technology retained most of their fruitiness, owing to the high initial concentration of odor active esters. Moreover, antioxidant protection of must seems to have a positive effect on the preservation of linalool during aging. On the basis of these results it may be deduced that winemaking practices involving an extensive reduction of the contact between must and oxygen during prefermentative steps can be considered an interesting option for the improvement of aroma quality and aging potential of Falanghina wines.

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