

# Trebbiano wine produced by using *Saccharomyces cerevisiae* strains endowed with $\beta$ -glucosidase activity

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**Abstract** The purpose of this research was to evaluate the ability of four strains of *Saccharomyces cerevisiae* endowed with  $\beta$ -glucosidase activity to improve the Trebbiano wine aroma profiles and quality. In particular, the strain effects on fermentation kinetics, sugar and acid concentrations, volatile aroma compound profiles, colour, and catechins parameters were evaluated. The four strains of *S. cerevisiae* were tested in comparison with a commercial strain. The results showed that the four wild strains had high fermentation rates associated with an efficient conversion of grape sugars to alcohol. In addition, each strain imparted specific features to the wine. In fact, significant differences in relation to the inoculated strain were observed mainly in residual sugar content, succinic and tartaric acid concentrations, and volatile aroma compound profiles as esters, higher alcohols, and terpenes. In particular, the products obtained with *S. cerevisiae* BV12 and BV14 were characterized by lowest residual sugar contents. Moreover, BV14 and AS15 produced the wines with the highest concentrations of succinic and tartaric acids. Also, the wine obtained with the strains AS11 and AS15 showed high concentrations of monoterpenes and particularly linalool

and geraniol having a strong sensorial impact. Furthermore, the wine obtained with the strain AS11 showed a minor decrease in colour intensity, probably due to the highest content of total SO<sub>2</sub>. These results outlined the importance of the strain selection, tailor made based on the wine feature and the wine making process adopted.

**Keywords** *Saccharomyces cerevisiae* · Trebbiano wine ·  $\beta$ -glucosidase activity · Volatile molecule profile · Terpenes

## Introduction

The new tendency of the market for white wines is presently directed to full-bodied and aromatic products. To fulfill this goal, technological and or biotechnological tools to enhance extract and flavor of white wines are requested to enological research (Antonelli et al. 2010). The influence of yeast strain on wine composition and quality is well-known. In fact, besides ethanol and CO<sub>2</sub>, the metabolism of yeasts yields a great number of by-products, for example, glycerol, acetic acid, succinic acid, and lactic acid. Moreover, the aromatic wine properties can be deeply affected by production of higher alcohols and other volatile substances including terpenic volatile compounds (Ugliano and Henschke 2009). The main role of yeast fermentation in the wine aroma formation is recognized particularly for products like Chardonnay, Semillon, Pinot Gris, most of the red varieties, and Trebbiano (Ugliano and Henschke 2009).

Trebbiano is an Italian white wine of widespread consumption in Italy and Europe (Spagna et al. 2000). This white wine is obtained from Trebbiano Romagnolo classified as a neutral variety not dependent upon monoterpenes for its flavor (Mateo and Jimenez 2000). It is well known that the grape contributes to wine aroma through free volatile terpenes (i.e. linalool, geraniol, nerol, citronellol,  $\alpha$ -terpineol, linalool

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oxide, etc.) and several odourless glycoconjugate precursors (Maicas and Mateo 2005; Ribereau-Gayon et al. 2006; Vernocchi et al. 2011). These precursors can contribute to the wine aroma profiles after their hydrolysis by naturally occurring grape enzymes or enzymes produced by the microorganisms involved in the process (Sanchez-Torres et al. 1998; Manzanares et al. 2000). Although the greatest  $\beta$ -glucosidase activity is recognized as belonging to the non-*Saccharomyces* strains involved in wine making,  $\beta$ -glucosidase activity is reported also in some *S. cerevisiae* strains (Hernandez et al. 2003; Vernocchi et al. 2011). In fact,  $\beta$ -glucosidase activity of some *S. cerevisiae* strains has been detected during winemaking resulting less sensible to low pH, high sugar and ethanol contents indicating the potential of the starter culture to improve the sensorial profile of wine adding terpenic notes indicating the potential of the starter culture to improve the sensorial profile of wine (Hernandez et al. 2003; Tosi et al. 2009). Vernocchi et al. (2011) studied suitability of four *S. cerevisiae* strains endowed with in vitro  $\beta$ -glucosidase to improve the Sangiovese wine quality demonstrating that all the strains were able to impart a specific volatile profile increasing the concentration of volatile terpenes. However, the best performances were detected in two of the four.

In this perspective, the main goal of this work was to evaluate the suitability of the same four strains of *S. cerevisiae* to improve the varietal aroma of Trebbiano obtained from the variety Trebbiano Romagnolo. This grape variety is classified as a neutral variety not dependent upon monoterpenes for its flavor (Mateo and Jimenez 2000). In particular, the effects of the strains on fermentation kinetics, wine sugar and acid concentrations, GC-MS/SPME profiles, and catechin content index were evaluated. The four wild strains of *S. cerevisiae* were tested in comparison with a commercial *S. cerevisiae* strain.

## Material and methods

### Strains

The *Saccharomyces cerevisiae* strains AS11, AS15, BV12, and BV14, belonging to the collection of Department of Food Science, University of Teramo (Italy), were used. They were previously characterized for their in vitro  $\beta$ -D-glucosidase activity according to Vernocchi et al. (2011). All strains were grown on YPD medium composed of 1 % (w/v) yeast extract, 2 % peptone, and 2 % glucose (all chemicals provided by Oxoid, Milan, Italy). In addition, a commercial strain of *S. cerevisiae* (“Fermol complet killer”, AEB Group, Brescia, Italy) was used as a control.

### Micro-vinification

Grape must of Trebbiano variety of 2008 vintage was used to test the effect of different strains of *S. cerevisiae* on wine characteristics. The fermentations were carried out in 130-mL Erlenmeyer flasks filled with 100 mL of 0.2  $\mu$ m filtered Trebbiano must inoculated with the different yeast strains at levels of about 6 Log CFU mL<sup>-1</sup>. The inoculations were performed using 48-h pre-cultures in the same must. The temperature was kept at 22 °C during alcoholic fermentation. The weight lost, as parameters to follow the fermentation process, was used. After the completion of alcoholic fermentation, the different wine samples were clarified with bentonite (1 g L<sup>-1</sup>) by stirring them very slightly for about 30 min at room temperature. The wine was then separated by filtration. Three samples for each condition tested were prepared.

### Microbiological analysis

Viable cell counts were evaluated at sampling time by plate counting using YPD agar (Oxoid) incubated at 28 °C for 48 h. Three repetitions for each sample were considered.

### Chemical-physical analysis

Sugars (glucose and fructose) and organic acids (tartaric, malic, succinic, citric, lactic, and acetic acids) were detected by HPLC using a Jasco HPLC system with a pump (PU980) (Jasco Inc, Tokyo, Japan) equipped with a variable wavelength detector (UV970) or a refractive index detector (RI830) for the determination of organic acids and sugars, respectively (Vernocchi et al. 2011). Furthermore, SAX cartridges were used in order to eliminate organic acids from grape juices and wines, according to Castellari et al. (2000). Peak identification and quantification were based on retention times (Rt) and spiking technique, and relied on the external standard method. Three repetitions for each condition were analysed.

Determination of ethanol, percent decrease of sugars, volatile acidity, and pH were performed on the wines according to the official EU methods (EC 2000). SO<sub>2</sub> was determined using iodine by colorimetric titration according to Lowinsohn and Bertotti (2001).

### Evaluation of colour intensity and total catechins

To evaluate colour intensity, three repetitions for each sample were used according to the spectrophotometric methods such as absorbance of a wine directly measured at 420, 520, and 620 nm as proposed by Glories (1984).

The concentration of total catechins was determined using the *p*-(dimethylamino) cinamaldehyde (*p*-DMACA) method.

Briefly, 1 mL of an appropriately diluted sample was added to a 10 mL volumetric flask followed by the addition of three drops of glycerol and 5 mL *p*-DMACA reagent. The total volume was made up to 10 mL with methanol and after 7 min, the absorbance was read at 640 nm against a methanol blank. The DMACA reagent was prepared immediately before use, and contained 1 % (w/v) DMACA in a cold mixture of methanol and HCl (4:1) as proposed by Ivanova et al. (2010).

#### GC–SPME analysis and mass spectrometry

In this study a polyacrylate coated fiber (85  $\mu\text{m}$ ) and a manual SPME holder (Supelco Inc., Bellefonte, PA, USA) were used after a preconditioning according to the manufacturer's instruction manual. Before each head space sampling, the fiber was exposed to the GC inlet for 5 min for thermal desorption at 250 °C in a blank sample. Five-milliliter wine samples were placed in 10-mL glass vials, to which 1 g NaCl and 10  $\mu\text{L}$  of 4-methyl-2-pentanol were added (final concentration 4  $\text{mg L}^{-1}$ ) as internal standard. The samples were then equilibrated for 10 min at 45 °C. The SPME fiber was exposed to each sample for 40 min. Both the equilibration and absorption phases were carried out under stirring condition. The fiber was then inserted into the injection port of the GC for a 5 min sample desorption. The instrumental conditions were performed according to Vernocchi et al. (2011).

Molecule identification was based on comparison of their retention times with those of pure compounds (Sigma–Aldrich, Milan, Italy) analyzed in the same conditions. The identification was further confirmed by comparing mass spectra of compounds with those contained in the available database (NIST version 2005) and those of pure standards. The concentration of volatile compounds, without pure references, was obtained by using the same calibration graphs as one of

the compounds with the most similar chemical structure (Li et al. 2008). Quantitative data of the identified compounds were obtained by interpolation of the relative areas versus the internal standard area, in calibration curves built with pure reference compounds.

#### Statistical analysis of data

The data reported are the means of three repetitions. The data were analyzed by Student's *t* test independent for groups using the statistical package Statistica for Windows 8.0 (Statsoft Inc. Tulsa, OK, USA). Evaluations were based on significance level  $P \leq 0.05$ .

## Results

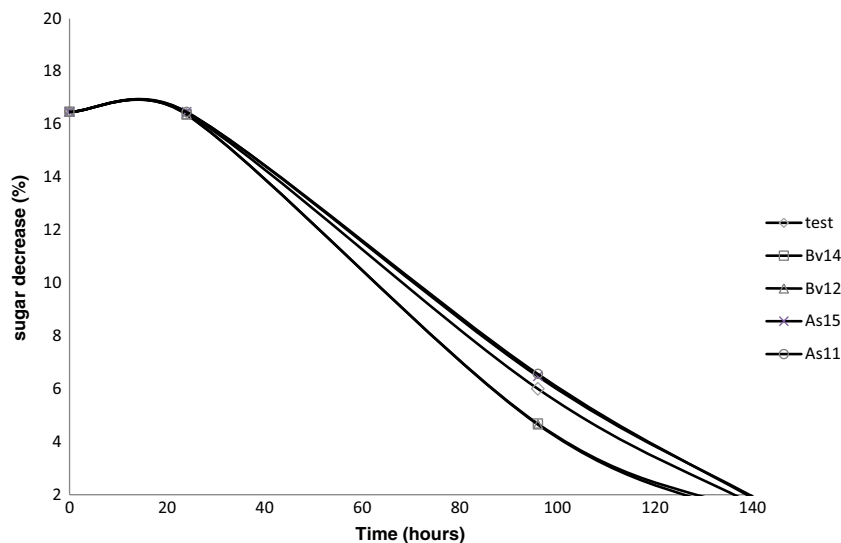
In order to evaluate the effects of yeast strain on the physico-chemical wine characteristics, Trebbiano musts were inoculated with about 6 Log CFU  $\text{mL}^{-1}$  of the four wild yeasts and with the commercial strain. During fermentation, the inoculated samples were maintained at 22 °C and periodically analyzed to assess yeast growth and fermentation kinetics.

At the end of fermentation, ethanol, sugars, organic acids, volatile molecule profiles, colour parameters, and catechin concentrations were measured.

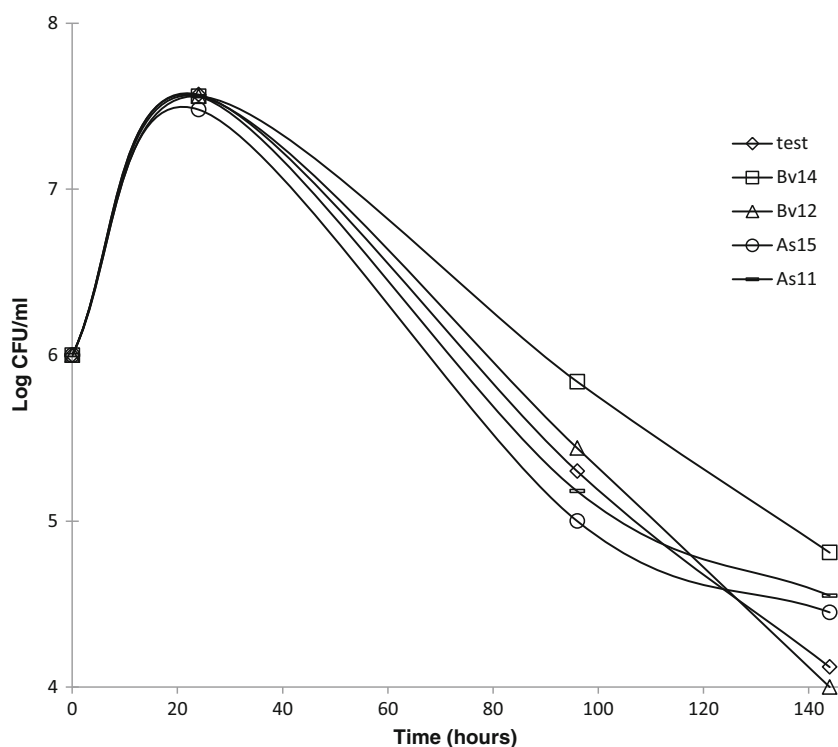
#### Course of fermentation and yeast cell load evolution

The fermentation kinetics were evaluated measuring the percent decrease of sugars, and, as shown by Fig. 1, significant differences ( $P > 0.05$ ) were not observed for the considered

**Fig. 1** Sugar percent decrease (%) during fermentation in relation to the used strain. The results are reported as mean of three repetitions. The coefficient of variability was  $\leq 5$  %



**Fig. 2** Evolution of the cell loads (Log CFU mL<sup>-1</sup>) of yeasts during fermentation at 25 °C. The results are reported as mean of three repetitions. The coefficient of variability was ≤8 %



strains. Also, the total yeast cell loads, recorded during the incubation at 22 °C (Fig. 2), supported the fermentation behaviors of the different samples. After 24 h of incubation at 22 °C, all samples inoculated with *S.cerevisiae* strains gave rise to cell loads higher than 7 Log CFU mL<sup>-1</sup> without significant differences ( $P>0.05$ ). The cell loads of all the considered strains began to decrease after 36 h of incubation reaching levels ranging between 4 and 5 Log CFU mL<sup>-1</sup>. Significant differences ( $P<0.05$ ) in the death kinetics were detected for BV12 (4 Log CFU mL<sup>-1</sup>) and BV14 (5 Log CFU mL<sup>-1</sup>).

#### Wine analytical profile

Table 1 reports the concentration of glucose, fructose, acetic, succinic, citric, malic, and tartaric acids of must before alcoholic fermentation and wines obtained with the different strains. In addition, it includes the ethanol concentration and the wine pH at the end of fermentation in relation to the used strain.

The concentrations of glucose and fructose detected at the end of fermentation showed that the residual sugar content did not exceed 2.8 g L<sup>-1</sup>, independently of the employed strain.

**Table 1** Sugars, organic acids, pH values, and ethanol concentration of different Trebbiano wine samples obtained by the fermentation of *Saccharomyces cerevisiae* commercial strain (Test) and BV14, BV12, AS15,

AS11 wild strains. The results are reported as mean of three repetitions with the relative standard deviations

	Test	BV14	BV12	AS15	AS11
Sugars (g L <sup>-1</sup> )					
Glucose	0.44±0.02 <sup>b</sup>	0.32±0.02	0.56±0.04	0.49±0.03	0.6±0.05
Fructose	2.33±0.21	0.65±0.04	0.80±0.07	1.75±0.13	2.08±0.17
Organic acids (g L <sup>-1</sup> )					
Acetic	0.013±0.001	0.005±0.0001	0.002±0.0001	0.004±0.0001	0.003±0.0001
Citric	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.04±0.00
Malic	0.26±0.01	0.22±0.02	0.23±0.01	0.27±0.01	0.42±0.03
Succinic	0.1±0.01	0.89±0.09	0.09±0.007	0.56±0.06	0.14±0.09
Tartaric	0.22±0.01	1.63±0.09	0.20±0.01	1.92±0.16	0.34±0.02
pH	2.89±0.23	2.86±0.23	2.89±0.23	2.89±0.23	2.90±0.23
Ethanol (% of alcohol)	9.32±0.89	9.36±0.90	9.35±0.87	9.24±0.87	9.34±0.89

**Table 2** Colour intensity, expressed as optical density (OD) at 420 nm, and catechins (mg L<sup>-1</sup>) in Trebbiano wine samples obtained by the fermentation of *Saccharomyces cerevisiae* commercial strain (Test) and

BV14, BV12, AS15, AS11 wild strains. The results are reported as mean of three repetitions with the relative standard deviations

	Test	BV14	BV12	AS15	AS11
Colour intensity	0.12±0.02	0.11±0.01	0.11±0.02	0.09±0.01	0.12±0.02
Catechins	17.15±1.63	14.68±1.36	15.2±1.41	17.98±1.54	19.88±1.67

However, the strain B14, followed by the strains BV12, gave rise to the wine with the lowest glucose and fructose concentrations ( $P<0.05$ ) with respect to the other strains. No significant differences in residual sugar concentrations were detected among the strains AS11, AS14, and the commercial one ( $P>0.05$ ). As expected, fructose was always the main residual sugar and different ratios of glucose and fructose were observed in relation to the starter employed. No significant differences ( $P>0.05$ ) in ethanol, citric, and malic acid concentrations were observed. On the contrary, significant differences ( $P<0.05$ ) were observed for succinic and tartaric concentrations. In fact, the strains BV14 and AS15 produced the wines with the highest concentrations of the two acids. AS 15 produced also the wine with the highest content of total SO<sub>2</sub> (34 mg L<sup>-1</sup>), while no difference in total SO<sub>2</sub> content were observed in the other wines (26±2 mg L<sup>-1</sup>). No significant differences ( $P>0.05$ ) in pH values were observed in relation to the strain used.

The highest levels (0.013 g L<sup>-1</sup>) of acetic acid recovered in this study were detected in the samples obtained with the

commercial starter. On the contrary, BV12 (0.002 g L<sup>-1</sup>) and AS 11 (0.003 g L<sup>-1</sup>) strains gave rise to wine having the lower acetic acid levels ( $P<0.05$ ). The strains AS15 and BV14 produced wines with acetic acid concentration of 0.004 and 0.005 g L<sup>-1</sup>, respectively.

#### Colour intensity and catechin content

Table 2 shows the colour intensity and the catechin contents of the wines in relation to the used yeast strain. The data clearly indicated the stability of catechins, independently of strain ( $P>0.05$ ) and the suitability of the strains AS15 to improve the wine colour. In fact, the catechin contents remained quite stable in the wines obtained with the strains BV12 and BV14, while it weakly increased in those obtained with the remaining strains ( $P<0.05$ ). The increase observed can be attributed to the low sensitiveness of the method used and to the release of catechins from dispersed solids due to the incomplete clarification of wine.

**Table 3** Volatile molecules (mg L<sup>-1</sup>) identified by GC-MS/SPME in Trebbiano wine samples obtained by the fermentation of *Saccharomyces cerevisiae* commercial strain (Test) and BV14, BV12, AS15, AS11 wild

strains. The results are reported as mean of three repetitions with the relative standard deviations

	Molecules	Test	BV14	BV12	AS15	AS11
Alcohols	N-propanol	1.14±0.09	1.69±0.14	2.01±0.19	2.52±0.22	2.16±0.19
	Isobutanol	6.27±0.59	5.45±0.49	6.50±0.61	11.81±1.02	6.99±0.67
	Isoamyl alcohol	72.41±6.98	69.75±6.77	83.10±8.11	123.38±11.78	105.33±11.00
	Hexanol	1.97±0.17	1.52±0.12	1.88±0.16	2.63±0.24	2.12±0.18
	Phenyl ethyl alcohol	38.60±2.98	38.47±3.13	45.15±4.23	53.01±5.12	57.99±5.51
Esters	Ethyl acetate	2.86±0.26	0.74±0.71	1.73±0.15	4.20±0.41	1.80±0.14
	Isoamyl acetate	0.99±0.08	0.62±0.06	1.05±0.10	1.45±0.13	1.16±0.09
	Ethyl hexanoate	0.98±0.08	0.80±0.07	1.14±0.10	1.37±0.12	2.18±0.22
	Ethyl 9 decanoate	1.75±0.13	0.78±0.06	0.98±0.08	5.86±0.52	1.19±0.11
	Ethyl decanoate	2.22±0.20	0.77±0.06	2.08±0.21	5.22±0.51	2.30±0.19
Acids	Acetic acid	1.91±0.17	0.70±0.06	1.74±0.16	2.65±0.25	2.20±0.20
Terpenes	Linalol	0.54±0.05	0.86±0.08	1.01±0.09	1.62±0.13	1.29±0.18
	Alfa terpineol	0.13±0.01	0.29±0.02	0.27±0.02	0.13±0.08	0.13±0.08
	Geraniol	0.12±0.01	0.08±0.01	0.05±0.01	0.38±0.03	0.08±0.01
	Nerolidol	0.06±0.001	0.14±0.02	0.20±0.02	0.12±0.07	0.15±0.09
	Farnesol	0.25±0.01	0.83±0.07	1.19±0.16	0.65±0.05	0.84±0.07
Aldehydes	Acetaldehyde	1.41±0.12	0.78±0.07	0.84±0.08	0.72±0.07	0.52±0.05

## GC–MS–SPME analysis and mass spectrometry

The gas-chromatographic method used allowed the identification of 30 molecules belonging to different chemical classes such as aldehydes, lactones, higher alcohols, esters, short chain fatty acids, and terpenes. The concentrations of the main molecules indicated the marked effects of strains on volatile molecule profiles of wines (Table 3). More specifically, the wine obtained with the inoculum of the commercial strain had highest concentrations of acetic acid, acetaldehyde, and esters (mainly ethyl acetate) ( $P < 0.05$ ). In contrast, the wines obtained with BV12 and BV14 had high concentrations of higher alcohols associated to low levels of ethyl acetate. These strains gave rise also to wines with interesting concentrations of molecules such as  $\alpha$ -terpineol ( $P < 0.05$ ) having a great sensorial impact. Moreover, AS11 and AS15 produce the highest levels of isoamylol and phenyl ethyl alcohol ( $P < 0.05$ ) and interesting concentrations of volatile terpenes. In particular, the strain AS15 produced the wine with the highest content ( $P < 0.05$ ) of linalool ( $1.62 \text{ mg L}^{-1}$ ) and geraniol ( $0.38 \text{ mg L}^{-1}$ ).

## Discussion

The results of the present work showed that the yeast strain used influenced and drove wine features.

All the wild strains showed high fermentation and growth rates. These were absolutely comparable with those of the commercial strain generally employed for Trebbiano wine production. Moreover, they were able to convert grape sugars to alcohol efficiently, fitting the primary selection criteria of yeast strain selection (Henschke 1997; Ubeda Iranzo et al. 1998). In our experimental conditions, the inoculation with *S. cerevisiae* BV12 and BV14 resulted in wine characterized by lowest residual sugar contents. However, the other strains also left low concentrations of unfermented sugar, but with different ratios of fructose and glucose. The preference of *S. cerevisiae* for glucose and the wide variability in fructose utilization among *S. cerevisiae* strains are well documented (Berthels et al. 2004). However, the highest concentration of fructose was detected in the wine obtained with the commercial *S. cerevisiae* strain. The strains BV14 and AS15 produced the wines with the highest concentrations of succinic and tartaric acids. On the other hand, the strain dependence of succinic acid production is widely recognized. Moreover, the ability of *S. cerevisiae* strain to influence the tartaric stability throughout the release on mannoproteins during fermentation has been recognized (Rosi et al. 2000). The entity of the mannoprotein release, although influenced by physico-chemical conditions, is strain dependent (Rosi et al. 2000; Escot et al. 2001). In addition, each strain imparted specific features in volatile molecule profiles to the wine obtained.

In our experimental conditions, the inoculation with *S. cerevisiae* BV12 and BV14 resulted in wines characterized by lowest volatile acidity and the highest level of  $\alpha$ -terpineol and higher alcohols without detrimental effects on colour intensity and catechin content. On the other hand, these strains proved to be suitable to improve the quality of Sangiovese wine (Vernocchi et al. 2011). Also, the wines obtained with the strains AS11 and AS15 showed high concentrations of monoterpenes and particularly of linalool and geraniol having a strong sensorial impact. Moreover, the wine obtained with the strain AS11 showed a minor decrease in color intensity, probably due to the high content of total  $\text{SO}_2$ .

The colour instability and browning is a serious problem during white wine storage, shortening its commercial life. A comprehensive research proved that the choice of a yeast strain is an important factor to maintain the stability of wine colour since these microorganisms have different capacity to retain or adsorb phenolic compounds depending on the outer cell structure composition (Morata et al. 2003). Moreover, yeasts are reported to contribute to wine colour stabilization, as result of participating in the formation of vitisins during fermentation or liberating mannoproteins that have the capacity to bind to anthocyanins and tannins, protecting them from precipitation (Bautista-Ortín et al. 2007). Yeast can affect the colour of white wine, also competing with phenols for oxygen dissolved in the wine (Salomon et al. 2002). The strains AS11 showed great potentiality to improve Trebbiano wine colour. On the contrary, this strain, analogously to AS15, decreased colour intensity of Sangiovese wine (Vernocchi et al. 2011) probably due to its ability to adsorb anthocyanins or to an anthocyanin- $\beta$ -glucosidase. Both mechanisms to decrease color parameters of red wine are documented in *S. cerevisiae* strains (Rodríguez et al. 2004; Caridi 2007; Todaro et al. 2008; Tosi et al. 2009). These results outlined the importance of a strain selection tailor made on the wine feature and the wine making process adopted.

In conclusion, the data obtained showed that some *S. cerevisiae* wild strains endowed with high in vitro  $\beta$ -glucosidase activity can increase the volatile terpene concentrations and the colour stability of Trebbiano wine without detrimental effects on fermentation rates and conversion of grape sugars to alcohol.

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