Use of grape racemes from Grillo cultivar to increase the acidity level of Sicilian sparkling wines produced with different Saccharomyces cerevisiae strains

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Complete List of Authors:	Alfonzo, Antonio; University of Palermo Department of Agricultural and Forestry Sciences, Francesca, Nicola ; Universita degli Studi di Palermo, Scienze Agrarie e Forestali Mercurio, Vincenzo; Le Ali di Mercurio s.r.l. Prestianni, Rosario; University of Palermo, Department of Agricultural, Food and Forestry Science Settanni, Luca; Universita degli Studi di Palermo, Scienze Agrarie, Alimentari e Forestali Spanò, Giacomo ; Cantine Europa Naselli, Vincenzo; Cantine Europa Moschetti, Giancarlo; University of Palermo, Department of Agricultural, Food and Forestry Science
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Running Title: Use of racemes to increase acidity of sparkling base

Authors: Antonio Alfonzo[1], Nicola Francesca[1], Vincenzo Mercurio[2], Rosario Prestianni[1], Luca Settanni[1], Giacomo Spanò[3], Vincenzo Naselli[3], Giancarlo Moschetti[1]

Contact Information: [1]Department of Agricultural, Food and Forestry Science, University of Palermo, Viale delle Scienze 4, 90128 Palermo; [2]Le Ali di Mercurio s.r.l., Vincenzo Mercurio Wine Consultant, Castellammare di Stabia, Napoli, Italy; [3]Cantine Europa Società Cooperativa Agricola SS 115 Km 42, 400 – 91020 Petrosino (TP);

Correspondence: Nicola Francesca, Department of Agricultural, Food and Forestry Science, University of Palermo, Viale delle Scienze 4, 90128 Palermo. Email: nicola.francesca@unipa.it

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Abstract

The production of sparkling wines in Sicily is increasing considerably. The most important oenological characteristics of high quality sparkling wines are high levels of acidity and low pHs. Due to hot climate and reduced rainfall that characterize Sicily region, white grape varieties such as Grillo cultivar cultivated in this area are characterized by very low concentrations of malic and tartaric acids. Interestingly, Grillo cultivar is characterized by an intense production of raceme grapes with low pH and high content of tartaric and malic acids. Thus, these fruits possess the chemical properties useful to increase the amounts of acids in the final wines. With this in mind, the present research was carried out to test the ability of four *Saccharomyces cerevisiae* strains (CS182, GR1 , MSE13 and MSE41) to ferment a raceme must with a pH of 2.9 at two concentrations (14 and 16° Babo degree) of total sugars. The inoculation of the strains was performed after a pre-adaptation at pH 2.5. The chemical parameters were monitored from must to bottled wines and novel insights on fermentation kinetics were provided. The experimental sparkling base wines were characterized by a very high total acidity with 16-17 g/l of tartaric acid and 9-10 gl^{-1} of malic acids. On the other hand, ethanol was detected at low values in the range 9 – 10% (vv^{-1}). The experimental wines produced in this study represent an innovative strategy for "blending wines" to produce sparkling wines in dry-Mediterranean climate.

1. INTRODUCTION

Grapes to be transformed into sparkling wine production are generally harvested at a relatively low pH, higher titratable acidity, and lower soluble sugar content than those for table wine production. The desired maturity of fruits at harvest is represented by a potential 9% (v/v) alcohol production, high tartaric acid content (about 9 gl^{-1}) and pH in the range 2.9 – 3.2 (Coppolani, 1994). Grape variety has been described as one of the three major factors, including vineyard location and yeast autolysis, influencing the characteristics of bottle-fermented sparkling wines (de La Presa-Owens *et al.*, 1998). On the basis of the geographical area, different grape varieties are used to produce sparkling wines. In Italy, the main varieties used to this purpose are Pinot Noir, Chardonnay and Pinot Meunier.

Due to the warm temperature climate characterizing Sicily island, only limited areas can be dedicated to production of grapes with low pH and high acidity. The majority of Sicilian sparkling wines is currently characterized by pHs higher than 3.2 and a reduced shelf life is expected. In recent years, Grillo variety became one the most cultivated grape in Sicily acquiring an increasing enological importance.

Grape racemes are the bunch that are formed by the secondary shoots of the vine deriving from the ready buds (Pastena, 1990). Some varieties produce a lot of racemes. Among the Sicilian varieties, Grillo is characterized by the highest production of racemes. The high amount of total acidity and malic acid content as well as the low pH make Grillo racemes important to improve the quality of sparkling wines. In general, the grape racemes might represent a novel raw material to produce high quality base wines. However, the consistent presence of organic acids in raceme grape must might inhibit the growth of fermenting yeasts and an *ad hoc* selection of starter strains is necessary.

The yeasts to be applied in sparkling base wine production belong to the species *Saccharomyces cerevisiae* commonly used in wine production. From this perspective they have to show a high fermentation strength, resistance to sulfur dioxide and alcohol, fermentative purity and development

as dispersed cells and the ability to ferment grape must at very low pH and high total acidity. Furthermore, starter strains for sparkling wines might be selected to enhance certain aromatic notes. To our knowledge no data have been published on the microbiological and chemical kinetics of grape raceme fermentation. For these reasons, the present study was carried out to monitor the microbiological, physico-chemical and sensory parameters of fermentation process during the vinification of raceme grape must under different oenological conditions. Four indigenous strains of *S. cerevisiae* capable of initiating alcoholic fermentation in high acidity musts were tested at two different concentrations of total sugars.

2. MATERIAL AND METHODS

2.1. Strains, media and culture conditions

Saccharomyces cerevisiae strains CS182, GR1, MSE13 and MSE41 belonging to the yeast culture collection of Food Microbiology section of the Department of Agricultural, Food and Forestry Sciences were used in this study. Yeast cells were cultivated for 24 h at 25 °C in yeast potato dextrose (YPD) broth (Difco™, Italy). All strains were then adapted to low pHs through cultivation into YPD broth modified for the final pH at 2.5 for 48 h at 25 °C under aerobic conditions. The cells were centrifuged at 5,000 rpm for 15 min at 4 °C, washed with sterile physiological solution (0.9% NaCl) and stored at 4 °C until inoculation

2.2. Experimental winemaking with grape racemes

White grape racemes of *Vitis vinifera* cv Grillo cultivated in Sicily were used to carry out the experimental vinification. Grape racemes were harvested during the vintage 2018 and 2019 and transported to an experimental cellar (G. Dalmasso, IRVOS) located in Marsala (Trapani province – Sicily). All results are reported as an average of the two years of winemaking.

Before yeast inoculation, grape raceme must was clarified at 4 °C for 24 h through the addition of 0.2 gl^{-1} ClearSpeed Enoenzymes (Perdomini-IOC Spa, S. Martino B.A. Varese) and aliquoted into 18 stainless steel vats (2.5 hl each) to perform, in duplicate, eight experimental trials (TV1 – TV8), at two total reducing sugar concentrations (14 and 16° Babo degrees), inoculated with the selected strains and the control trial S represented by the spontaneous fermentation. The experimental design is reported in Figure 1.

All trials were added with 10 mgl^{-1} of potassium metabisulphite (Vebi Istituto Biochimico s.r.l., Padova, Italy). Grape raceme must showed the following chemical composition: pH 2.9; total reducing sugars 142 gl^{-1}; total acidity 17.52 gl^{-1} (tartaric acid); malic acid 11.08 gl^{-1}; total SO_2 92 mgl^{-1}; free SO_2 32 mgl^{-1}, and yeast assimilable nitrogen (YAN) 182 mgl^{-1}.

In order to improve the alcoholic fermentation (AF) following the winemaking procedures largely applied at industrial scale vinification for Grillo grape must, all experimental trials received the addition of Nutristart® (0.2 gl^{-1}, Laffort, Bordeaux Cedex, France) at the beginning of winemaking (just after the inoculum of starter) and the addition of Thiazote®PH (0.1 gl^{-1}, Laffort, Bordeaux Cedex, France) at day 3 and day 7 of AF. The Nutristart® is complete nutrient combining, organic nitrogen, mineral nitrogen and thiamine. Thiazote®PH is a nutrient of ammonium salts and hydrochlorate thiamine (vitamin B1).

Trial S was not added with RCM, Nutristart® or Thiazote®PH. The AF of all trials took place at 15 °C for 27 days.

2.3. Microbiological analysis

Microbiological analyses were carried out on the must before and after clarification, after starter inoculation (0 days) and at 3, 7, 16 and 27 days of AF. All samples were transported at 4 °C in a portable fridge and analysed within 24 h from collection.

Samples collected during wine production were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). All samples were analysed in duplicate for total yeasts (TY) on Wallerstein Laboratory (WL) nutrient agar; presumptive *Saccharomyces* spp. (PS) on modified ethanol sulphite agar (MESA), prepared as reported by Francesca *et al.* (2010); mesophilic rod lactic acid bacteria (LAB) on de Man-Rogosa-Sharpe (MRS) agar, coccus LAB on glucose M17 agar, acidophilic LAB on medium for *Leuconostoc oenos* agar (MLO), and acetic acid bacteria (AAB) on Kneifel agar medium as reported by Francesca et al. (2014). All media and the supplements used were purchased from Oxoid (Thermofisher, Basingstoke, England).

In order to analyse the dominant cultivable yeast microbiota, colonies showing different morphologies and colour on MESA plates at the highest cell suspension dilutions were purified on WL-nutrient agar.

2.4. Isolation and genotypic identification of yeasts

After growth, all isolates were picked up from agar plates and purified to homogeneity after several sub-culturing steps onto WL agar. At least 30% of isolates characterized by the morphological traits typical of *Saccharomyces* were subjected to genotypic investigation. Yeast isolates were identified by molecular methods. DNA was extracted by cell lysis using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. To perform a first discrimination of yeasts, all isolates were analysed by restriction fragment length polymorphism (RFLP) of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene. The DNA fragments were amplified with the primer pair ITS1/ITS4 according to Esteve-Zarzoso et al. (1999). The generated amplicons were then digested with the endonucleases *Cfo*I, *Hae*III and *Hinf*I (MBI Fermentas, St. Leon-Rot, Germany) at 37 °C for 8 h. The ITS amplicons, as well as the respectively restriction fragments, were analysed on agarose gel using 1.5% and 3% (w/v) agarose in 1× TBE (89 mM Tris-borate, 2 mM EDTA pH 8) buffer, stained with SYBR safe DNA gel stain (Invitrogen, Milan, Italy), visualized by UV transillumination and acquired by Gel Doc 1000 Video Gel Documentation System (BioRad, Richmond, CA). Standard DNA ladders were 1 kb Plus and 50 pb (Invitrogen).

2.5. Genotypic monitoring of *S. cerevisiae* strains

Genetic diversity within *Saccharomyces* isolates was assessed by Interdelta analysis (Legras & Karst, 2003). Interdelta patterns were analysed using the GelCompar II software (v. 6.1, Applied Maths NV, Sint-Martens-Latem, Belgium) and similarities among patterns were assessed. Profiles showing more than 95% of similarity were considered identical.

2.6. Chemical analysis

The pH, total acidity (TA; gl^{-1} as tartaric acid), volatile acidity (VA; gl^{-1} as acetic acid), reducing sugars (gl^{-1}), ethanol (% vol), glycerol (gl^{-1}), malic acid (gl^{-1}), lactic acid (gl^{-1}) were determined by

means of a Winescan (FOSS, Hillerød, Denmark) calibrated following UNI CEI EN ISO/IEC 17025:2018 (European Commission, 2018; Sannino et al., 2013). Total and free SO_2 were measured in accordance with the official methods described by the European Commission (2018). All chemical analyses were carried out in duplicate.

2.7. Sensory analysis

Descriptive sensory analysis was carried out by a panel of ten judges (five women and five men, ranging from 28 to 56 years old) with an extensive experience in wine tasting, from the National Organization of Wine Taster (ONAV, Italy). The sensory assessments were performed in blind tasting conditions at the tasting room of Regional Institute of wine and oil (Marsala, Italy). Wine samples (50 ml) were served in standard ISO 3591'XL5-type'tasting glasses, labelled with three-digit random codes and evaluated independently.

The panelists selected descriptive attributes regarding appearance, odour, taste and flavour on the basis of the frequency (%) of the terms used by the judges in several sessions. Reference standards were available to define descriptors (Noble et al., 1987). The sensory attributes scored were: one attribute referring to appearance (colour intensity), six to aroma (citrus, apple, floral, herbaceous/vegetative, exotic fruit and pungent) and two to taste (acid and bitter). The different descriptors were quantified using a nine-point intensity scale (ISO 13299:2016) by assigning a score between 1.00 (absence of sensation) and 9.00 (extremely intense). All evaluations were made between 10.00 and 12.00 a.m. in individual booths (ISO 8589:2007). The final score was obtained as a mean of three evaluations with the irrespective statistical analysis.

2.8. Statistical and multivariate analysis

The analysis of variance (ANOVA) test was applied to identify significant differences among data. The post-hoc Tukey's method was applied for pairwise comparison in case of microbial counts and chemical parameters. Statistical significance was $P < 0.05$. An explorative multivariate approach

was also applied to investigate the relationships among data obtained from the different trials. The different trials were grouped by principal component analysis performed with data obtained from AF. The number of principal factors was selected according to the Kaiser's criterion and only factors with eigen-values higher than 1.00 were retained. All data were preliminary evaluated by the Barlett's sphericity test in order to check the statistically significant differences among samples within each data set (Dillon & Goldstein, 1984). The correspondence analysis (CA) was employed to investigate relationships among trials. The input matrix used for CA consisted of results from sensorial profiles.

All statistical analyses were achieved by using XLStat software version 2014.5.03 (Addinsoft, New York, USA) for excel.

3. RESULTS

3.1. Microbiological analysis

Viable cell count of TY and PS throughout the fermentation is shown in Figure 2. TY (Figure 2a) in must before and after clarification were at 4.38 and 4.46 Log CFUml^{-1}, respectively. After inoculation of the selected strains, TY increased up to 7.50 Log CFUml^{-1} in all experimental trials. TY in control trial S was 5.11 Log CFUml^{-1}. After 3 d of AF, the trials TV1, TV2, TV6, TV7 and TV8 reached TY levels higher than 8.0 Log CFUml^{-1}. These values were also observed after 7 d. At the 16th day of AF, all trials showed TY values lower than 8.0 Log CFU/ml with the exception of TV7. At 27 d, a decrease in TY cell density (6.59 - 7.77 Log CFUml^{-1}) was observed for all trials. Trial S was characterised by the lowest TY levels throughout the entire FA and the highest value (7.08 Log CFUml^{-1}) was observed at 7 d of FA. No significant differences were found between the trials added with RCM (TV2, TV4, TV6 and TV8) and those not added (TV1, TV3, TV5 and TV7). The viable counts of PS during the entire winemaking process were lower than those registered for TY (Figure 2b). PS cell densities were around three Log cycles both in must and clarified must, but soon after inoculation of the starters, PS populations increased above 7.0 Log CFUml^{-1}in all

experimental trials. During AF, at 3 and 7 d, PS values showed an increase in all trials. The highest values were observed in TV8 at 3 d (8.10 Log $CFUml^{-1}$) and 7 d (8.28 Log $CFUml^{-1}$). Trial S showed the lowest PS values, between 6.52 (3 d) and 6.78 (7 d) Log $CFUml^{-1}$. At 16 and 27 d of AF, a progressive decline of PS level was registered, reaching 6.28 - 7.82 Log $CFUml^{-1}$ in experimental trials and 5.02 - 5.17 Log $CFUml^{-1}$ in trial S.

LAB and AAB populations were below the detection levels during the entire period of winemaking.

3.2. Determination of the dominance of the starter strains

All isolates identified as PS by morphological inspection were subjected to genotypic identification. The restriction analysis of ITS1-5.8S-ITS2 allowed the identification of *S. cerevisiae* isolates by comparison of restriction profiles with those reported in the literature (Esteve Zarzoso, et al., 1999). In order to verify the dominance of the starters CS182, GR1, MSE13 and MSE41 during AF, all isolates were characterized by Interdelta analysis. Fifteen different Interdelta profiles indicated the presence of 15 *S. cerevisiae* strains isolated at high levels from height trials (TV1 – TV8). In particular, the direct comparison of the Interdelta profiles showed that *S. cerevisiae* CS182, GR1, MSE13 and MSE41 were the strains most frequently (>95%) isolated from the inoculated trials, but was not identified among the isolates from the control S.

3.3. Chemical analysis of conventional parameters during winemaking

The results of the chemical analysis of the samples collected during the winemaking process of grape racemes are reported in Tables 1 and 2. Before the beginning of AF, the grape raceme must showed pH values lower than 2.9. Total acidity and malic acid were inversely correlated and were estimated at high concentrations: 17.52 gl^{-1} of tartaric acid and 11.08 gl^{-1} of malic acid.

After must clarification, the values of the conventional parameters did not change significantly. During winemaking the values of pH increased up to 3.20 for trial TV6.

At the third day of AF, the highest ethanol concentration was reached in trials TV7 (3.07 % vv^{-1}). This trend was also observed at 7 d (8.27 % vv^{-1}). In contrast, trials TV4 and TV6 showed the lowest values of ethanol (0.06, 1.19 and 0.18% vv^{-1}, respectively) at 3 d of AF. After 7 d, all trials showed ethanol in the range 3.14 – 8.27 % (vv^{-1}), while trial S showed the lowest ethanol value (2.07 % vv^{-1}). At 16 d, trials TV2, TV4, TV6 and TV8 showed ethanol concentrations around 9 % (vv^{-1}), while TV1, TV3, TV5 and TV7 displayed lower values (8.03-9.11 % vv^{-1}). This trend was observed until the end of AF for all trials. At 27 d trials S was characterised by a lower ethanol concentration (4.89 % vv^{-1}).

VA of all trials (TV1, TV3, TV5 and TV7) carried out with grape must at 14 % of total sugars showed values lower than 0.3 (gl^{-1} of acetic acid) during the entire period of AF; on the contrary, trials TV2, TV6 and TV8, showed an increase of VA up to 0.40 (gl^{-1} of acetic acid) after 27 d.

The amount of glycerol significantly increased within 7 d of AF reaching the highest values of 4.76 and 5.61 (gl^{-1}) in trials TV2 and TV8, respectively. From 16 d onwards, glycerol concentration also increased up to the highest value of 8.91 (gl^{-1}) registered for trial TV4.

Malic acid amount decreased within 7 d of AF and its concentration was almost constant during the entire period of monitoring. The presence of lactic acid was not detected from grape must to the end of AF in any trial.

In order to better evaluate the differences among the experimental wines, data set based on chemical parameters was subjected to PCA. The biplot illustrated in Figure 3 highlights the distribution of the different trials in relation to their chemical parameters. The trials TV1, TV7 and TV8 was correlated with GL, TY, PS, free SO_2, pH and EtOH. TV5 were associated to EtOH, Total S02 and MA. TV2 were statistically correlated to TA and VA. The trials S with RS, BG.

After bottling, the values of the physico-chemical parameters did not show significant differences compared to those observed at the end of AF.

3.4. Sensory analysis

Scores of the attributes of the sensory profiles of each sample are reported in Figure 4a. The all trials The sparkling base wines did not show off-odours and off-flavours at significant values. The highest values of sensory attributes were registered for acid in all experimental wines. The wines TV1 and TV2, carried out with strain GR1, showed the highest values for aroma (floral and herbaceous/vegetative) in contrast to the experimental wines TV7 and TV8, fermented by the strain CS182, reached the highest scores for odour intensity and exotic fruit. The wines TV3, TV4, TV5 and TV6 showed intermediate values similar to the control trial S. The results of the sensory evaluation were subjected to a statistical multivariate analysis (Figure 4b). A symmetric plot of the correspondence analysis (CA) explained 90.34% of inertia. All trials were mainly separated along factor F1 that explained more than 71.70% of total variability. Trials TV1 and TV2 were grouped for floral and herbaceous/vegetative descriptors, while trials TV7 and TV8 were mainly correlated to the exotic fruit descriptor. The other experimental productions including control showed a close correlation with acid, apple, bitter, citrus, color intensity and pungent.

These results clearly indicated that the four strains evaluated clearly impacted the sensory profiles of the resulting wines.

4. DISCUSSION

The modern trend of wine market is going towards sparkling wines produced in novel area of Italy such as Sicily, Campania, Lazio as well as in Countries that are not traditionally producers such as Spain, Portugal, New Zeland, California, etc.. The recent advances in agricultural research allowed the production of grapes to be used for sparkling wines also in areas that did not produce this kind of wines, like Sicily (Chironi & Ingrassia, 2010).

Grillo grape is an indigenous white Mediterranean variety cultivated in Western Sicily on more than 4,000 ha (Scafidi et al., 2013). Grillo grape is a cultivar with a high tendency to produce racemes until 18-20% of total grape yield per hectare (Pastena, 1990). Thus, raceme grapes might represent a significant natural source of organic acids to improve wine quality. On large scale production, the

raceme utilization might represent a sustainable source for wine economics and policy. However, the quality of sparkling wines does not account only on the amount of acids. Aroma and flavour of bottled products is significantly affected by the fermentation process making the role of the starter strains of paramount importance.

In the recent years, the interest toward autochthonous yeasts to be used as starters in winemaking processes increased consistently and it is still on the increase (Çelik et al., 2019; Grieco et al., 2019). Several researchers reported that yeasts and LAB harboured on grapes and acting during the spontaneous fermentations possess a relevant economic potential (Di Maio et al., 2012; Francesca et al., 2011; Mazzei et al., 2010). A wine produced with indigenous yeast starters enjoys a status of tradition and typicality and is requested by expert wine consumers. Furthermore, the use of yeasts selected in a given geographical area represents a valuable technological alternative to the application of commercial starter cultures responsible for wine flavor standardization, as well as to the spontaneous fermentation that may lead to undesirable aroma developments (Lambrechts & Pretorius, 2000). To this purpose, it has been demonstrated that wine quality can be affected by the growth of different yeasts originating from the microbial communities hosted on grapes (Fleet, 2008; Settanni et al., 2012).

Here, we focused on the application of *S. cerevisiae* strains and we monitored the yeast concentration during alcoholic fermentation. Yeast counts monitored during experimental winemaking with racemes did not show differences from data published on conventional winemaking performed with grape. In fact, the scope of the present work was also to apply a new methodology for the preparation of sparkling base wine by using grape racemes, in order to increase total acidity. The results of conventional chemical parameters clearly showed that a substantial concentration of tartaric acid and malic acid from raceme grape must to raceme wines. Interestingly, the experimental wines showed values of total acidity at least three times higher than the amount reported by Scacco et al. (2012) where the wines were produced with Grillo grapes at 19 BD. Malic acid values, on the other hand, were about 15 times higher. The values of pH, from must to wine,

were significantly lower than those commonly reported for sparkling base wine in Sicily (Scacco et al., 2012).

The ethanol values obtained are slightly lower than the Grillo grapes reported in the literature (Fracassetti et al., 2018). This is due to the lack of physiological maturation of the racemes. The ethanol levels, albeit low, do not seem to affect the shelf life of the base wines, since the high acidity and the pH create difficult conditions for the proliferation of the spoilage microrganisms. The production of base wines with high acidity in terms of malic and tartaric acids represents a source of organic acids of considerable interest in sparkling wines. Sapidity and minerality are two aspects sought after in sparkling wine (Canonico et al., 2018; Charters & Pettigrew, 2007). Consequently base wines obtained from racemes represent wines that can be used for the assembly of base wines to be destined for quality sparkling wine.

It is worth of note the ability of the autochthonous strains tested in this study to ferment raceme grape must starting by very low pH and in presence of high amount of organic acids. Specifically, the strains GR1 and CS182 were able to convert total sugars to ethanol within 27 d of AF, a behaviour observed in other studies. The results of the sensory analyses clearly distinguished the base wines obtained. Herbaceous/vegetative and floral descriptors were associated with base wines fermented by GR1, while exotic fruit was a descriptor perceived by base wines obtained by CS182. The wines fermented by MSE13 and MSE41 showed intermediate characteristics and absence of off-odors.

Our work provided, for the first time, physical-chemical and microbiological information on alcoholic fermentation of raceme grape must to produce sparkling base wines from Grillo cultivar in southern of Sicily. The use of selected indigenous strains of *S. cerevisiae* and the growth of these strains at low pH before the inoculum, allowed to perform a regular alcoholic fermentation of raceme grape must characterized by very low pH and high amount of malic and tartaric acids. All aspects of the physico-chemical and microbiological composition of the experimental raceme wines

were in agreement with those reported for the production regulations of commercial wines, and undesired off-odours and off-flavours were not detected.

The base wines obtained could be used as "blending wines" for the production of sparkling base wines associated with dry-Mediterranean climate typical of Sicily.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Antonio Alfonzo https://orcid.org/0000-0002-7905-9480

Nicola Francesca https://orcid.org/0000-0002-6063-0130

Vincenzo Mercurio https://orcid.org/0000-0002-2673-3371

Rosario Prestianni https://orcid.org/0000-0002-9982-838X

Luca Settanni https://orcid.org/0000-0001-7019-3598

Giancarlo Moschetti http://orcid.org/0000-0003-0218-5769

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Table 1 Chemical parameters during the winemaking process with grape must at 12 Babo degree.

Parameters	Must	Vinification											
		Alcoholic fermentation											
		3 days						7 days					
		TV1	TV3	TV5	TV7	S	Statistical significance	TV1	TV3	TV5	TV7	S	Statistical significance
		Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	Spontaneus		Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	Spontaneus	
pH	2.90±0.07^{a}	2.88±0.01^{ab}	2.91±0.03^{a}	2.91±0.02^{a}	2.78±0.04^{b}	2.87±0.03^{ab}	*	2.86±0.05^{a}	2.92±0.08^{a}	2.91±0.05^{a}	2.78±0.07^{a}	2.88±0.02^{a}	N.S.
TA	17.52±0.04^{a}	17.35±0.05^{a}	17.41±0.17^{a}	17.19±0.15^{a}	17.33±0.21^{a}	17.48±0.19^{a}	N.S.	17.10±0.04^{a}	17.21±0.07^{a}	17.11±0.04^{a}	17.21±0.03^{a}	17.40±0.07^{a}	N.S.
VA	n.d.^{d}	0.22±0.02^{a}	0.11±0.01^{b}	0.09±0.01^{bc}	0.10±0.03^{bc}	0.06±0.01^{c}	***	0.28±0.01^{a}	0.16±0.07^{a}	0.18±0.01^{a}	0.30±0.07^{a}	0.16±0.07^{a}	N.S.
RS‡	142.00±0.34^{a}	120.00±0.22^{b}	118.18±1.24^{b}	116.24±1.66^{b}	97.14±0.04^{c}	139.14±0.00^{a}	***	71.14±0.05^{b}	70.15±0.08^{b}	70.01±0.26^{b}	21.02±0.04^{c}	110.17±0.12^{a}	***
BD	14.00±0.00^{a}	12.00±0.50^{b}	12.00±0.00^{b}	12.00±0.00^{b}	10.00±0.50^{c}	14.00±0.00^{a}	***	7.00±0.50^{b}	7.00±0.00^{b}	7.00±0.00^{b}	2.00±0.50^{c}	11.00±0.00^{a}	***
EtOH	n.d.^{d}	1.38±0.04^{b}	1.55±0.02^{b}	1.50±0.00^{b}	3.07±0.08^{a}	0.19±0.00^{c}	***	4.82±0.14^{b}	4.45±0.01^{b}	4.60±0.07^{b}	8.27±0.01^{a}	2.07±0.00^{c}	***
GL	n.d.^{f}	2.14±0.02^{b}	1.22±0.04^{d}	0.67±0.03^{e}	4.64±0.05^{a}	1.33±0.02^{c}	***	3.57±0.06^{b}	2.27±0.03^{c}	2.00±0.11^{d}	4.64±0.01^{a}	2.27±0.06^{c}	**
MA	11.08±0.14^{a}	10.97±0.11^{b}	10.97±0.05^{e}	11.05±0.14^{bc}	11.00±0.08^{d}	11.07±0.08^{c}	***	10.80±0.07^{a}	10.99±0.21^{a}	11.05±0.03^{a}	11.00±0.07^{a}	11.07±0.02^{a}	N.S.
LA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	96.00±1.00^{a}	58.00±1.00^{b}	58.00±1.0^{b}	51.00±0.00^{c}	33.00±0.00^{d}	49.00±1.00^{c}	***	34.00±1.00^{a}	31.00±3.00^{a}	36.00±2.00^{a}	33.00±3.00^{a}	37.00±2.00^{a}	N.S.
Free SO_2	32.00±1.00^{a}	22.00±0.00^{b}	20.00±1.00^{c}	21.00±1.00^{bc}	16.00±0.00^{d}	22.00±1.00^{b}	***	18.00±1.00^{a}	17.00±1.00^{a}	19.00±1.00^{a}	16.00±2.00^{a}	17.00±1.00^{a}	N.S.

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Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). †The letter reported between brackets refers to the code of each experimental trial. ‡Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).
Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

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Parameters	Vinification											
	Alcoholic fermentation											
	16 days						27 days					
	TV1	TV3	TV5	TV7	S		TV1	TV3	TV5	TV7	S	
	Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	Spontaneus	Statistical significance	Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	Spontaneus	Statistical significance
pH	2.98±0.01^{b}	2.84±0.04^{c}	3.01±0.04^{b}	3.18±0.02^{a}	2.97±0.01^{b}	*	3.08±0.04^{a}	3.04±0.02^{a}	3.04±0.01^{a}	3.09±0.03^{a}	3.10±0.02^{a}	N.S.
TA	17.20±0.01^{a}	17.18±0.07^{a}	17.08±0.03^{a}	17.14±0.10^{b}	17.22±0.07^{a}	N.S.	17.07±0.14^{a}	16.88±0.31^{a}	17.11±0.01^{a}	16.97±0.01^{a}	17.10±0.01^{a}	N.S.
VA	0.29±0.07^{b}	0.26±0.07^{b}	0.22±0.02^{b}	0.33±0.01^{b}	0.49±0.01^{a}	*	0.30±0.05^{b}	0.30±0.01^{b}	0.35±0.01^{b}	0.33±0.01^{b}	0.62±0.01^{a}	*
RS‡	3.01±0.07^{d}	13.80±0.06^{c}	15.08±0.19^{b}	15.15±0.07^{b}	88.04±0.12^{a}	***	1.05±0.07^{d}	1.68±0.00^{b}	0.03±0.01^{e}	1.22±0.04^{c}	66.05±0.07^{a}	***
BD	1.00±0.00^{c}	1.00±0.00^{c}	2.00±0.00^{b}	1.00±0.00^{c}	9.00±0.50^{a}	***	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	7.00±0.50^{a}	*
EtOH	9.11±0.07^{a}	8.21±0.07^{b}	8.03±0.01^{b}	8.68±0.07^{b}	3.46±0.09^{c}	**	9.23±0.07^{a}	8.95±0.01^{b}	8.95±0.14^{b}	9.59±0.01^{a}	4.89±0.07^{c}	*
GL	4.45±0.04^{a}	2.74±0.03^{c}	2.37±0.07^{d}	4.28±0.03^{b}	4.21±0.05^{b}	***	4.31±0.04^{bc}	4.79±0.05^{a}	4.67±0.07^{a}	4.09±0.04^{c}	4.43±0.02^{bc}	*
MA	10.64±0.02^{a}	10.87±0.11^{a}	10.82±0.17^{a}	10.87±0.07^{a}	10.64±0.08^{a}	N.S.	9.87±0.06^{a}	9.74±0.07^{a}	10.01±0.04^{a}	10.11±0.09^{a}	9.97±0.09^{a}	N.S.
LA	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	35.00±3.00^{a}	35.00±1.00^{a}	37.00±2.00^{a}	31.00±2.00^{a}	31.00±3.00^{a}	N.S.	36.00±1.00^{a}	37.00±1.00^{a}	36.00±4.00^{a}	34.00±2.00^{a}	31.00±0.00^{a}	N.S.
Free SO_2	15.00±1.00^{a}	14.00±2.00^{a}	13.00±1.00^{a}	15.00±1.00^{a}	12.00±1.00^{a}	N.S.	12.00±1.00^{a}	10.00±1.00^{a}	11.00±1.00^{a}	10.00±1.70^{a}	11.00±1.00^{a}	N.S.

Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). †The letter reported between brackets refers to the code of each experimental trial. ‡Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).
Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

continued→

Parameters	Wine bottled 2 months					Statistical significance
	TV1	TV3	TV5	TV7	S	
	Strain GR1[†]	Strain MSE13	Strain MSE41	Strain CS182	Spontaneus	
pH	3.03±0.01[a]	3.02±0.02[a]	3.01±0.01[a]	3.06±0.02[a]	3.07±0.01[a]	N.S.
TA	17.00±0.10[a]	16.85±0.15[a]	17.02±0.04[a]	16.86±0.11[a]	17.03±0.03[a]	N.S.
VA	0.29±0.02[b]	0.31±0.02[b]	0.33±0.02[b]	0.31±0.00[b]	0.65±0.09[a]	*
RS[‡]	0.39±0.02[b]	0.58±0.03[b]	0.00±0.00[c]	0.44±0.03[b]	51.85±0.19[a]	***
BD	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	5.00±0.50[a]	*
EtOH	9.21±0.03[a]	8.93±0.03[b]	8.91±0.09[b]	9.43±0.04[a]	4.85±0.02[c]	*
GL	4.28±0.02[b]	4.59±0.06[a]	4.61±0.01[a]	4.00±0.02[c]	4.34±0.03[b]	*
MA	9.82±0.03[a]	9.77±0.03[a]	10.00±0.02[a]	10.08±0.02[a]	9.89±0.03[a]	N.S.
LA	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	71.00±1.00[a]	74.00±1.00[a]	68.00±4.00[a]	66.00±2.00[a]	69.00±0.00[a]	N.S.
Free SO_2	36.00±1.00[a]	38.00±2.00[a]	35.00±1.00[a]	34.00±0.00[a]	35.00±1.00[a]	N.S.

Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). [†]The letter reported between brackets refers to the code of each experimental trial. [‡]Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).
Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

Table 2 Chemical parameters during the winemaking process with grape must at 16 Babo degree.

→ continued

Parameters	Must	Vinification									
		Alcoholic fermentation									
		3 days					7 days				
		TV2	TV4	TV6	TV8	Statistical significance	TV2	TV4	TV6	TV8	Statistical significance
		Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182		Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	
pH	2.92±0.04^{a}	2.92±0.03^{a}	2.91±0.01^{a}	2.91±0.01^{a}	2.92±0.00^{a}	N.S.	2.89±0.01^{a}	2.88±0.04^{a}	2.85±0.04^{a}	2.86±0.02^{a}	N.S.
TA	17.52±0.05^{a}	17.21±0.18^{a}	17.20±0.08^{a}	17.17±0.05^{a}	17.40±0.11^{a}	N.S.	17.23±0.07^{a}	17.14±0.07^{a}	17.20±0.03^{a}	17.19±0.02^{a}	N.S.
VA	n.d.^{d}	0.07±0.02^{a}	0.03±0.02^{a}	0.07±0.01^{a}	0.11±0.01^{a}	N.S.	0.35±0.05^{a}	0.11±0.02^{c}	0.21±0.01^{b}	0.33±0.01^{a}	*
RS‡	163.00±0.34^{b}	146.32±0.18^{c}	162.02±1.14^{b}	168.05±0.17^{a}	130.55±0.14^{d}	***	104.14±0.05^{b}	110.04±1.09^{a}	110.05±2.21^{b}	66.72±0.03^{c}	***
BD	16.00±0.00^{a}	15.00±0.00^{b}	16.00±0.50^{a}	16.00±0.50^{a}	13.00±0.50^{c}	*	10.00±0.00^{b}	11.00±0.50^{a}	11.00±0.00^{a}	7.00±0.50^{c}	**
EtOH	n.d.^{d}	1.17±0.07^{b}	0.06±0.02^{c}	0.18±0.02^{c}	2.35±0.03^{a}	***	3.79±0.14^{c}	3.44±0.01^{bc}	3.14±0.02^{b}	6.41±0.12^{a}	***
GL	n.d.^{e}	2.89±0.09^{b}	1.41±0.13^{d}	2.09±0.02^{c}	4.29±0.04^{a}	***	4.76±0.08^{b}	3.64±0.11^{c}	2.43±0.15^{d}	5.61±0.01^{a}	**
MA	11.08±0.14^{a}	11.02±0.08^{a}	11.00±0.13^{a}	10.87±0.02^{a}	11.02±0.02^{a}	N.S.	10.97±0.04^{a}	11.05±0.02^{a}	10.96±0.04^{a}	10.90±0.07^{a}	N.S.
LA	n.d.	n.d.	n.d.	n.d.	n.d.	N.S.	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	92.00±1.00^{a}	40.00±2.00^{e}	55.00±2.00^{d}	59.00±1.00^{c}	72.00±1.00^{b}	***	36.00±4.00^{a}	30.00±4.00^{a}	32.00±4.00^{a}	34.00±4.00^{a}	N.S.
Free SO_2	36.00±1.00^{a}	24.00±1.00^{c}	24.00±1.00^{c}	28.00±2.00^{b}	27.00±0.00^{bc}	***	19.00±1.00^{a}	18.00±1.00^{a}	17.00±1.00^{a}	16.00±1.00^{a}	N.S.

Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). †The letter reported between brackets refers to the code of each experimental trial. ‡Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).
Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

→ continued

Parameters	Vinification									
	Alcoholic fermentation									
	16 days					27 days				
	TV2	TV4	TV6	TV8	Statistical significance	TV2	TV4	TV6	TV8	Statistical significance
	Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182		Strain GR1†	Strain MSE13	Strain MSE41	Strain CS182	
pH	2.94±0.02^{b}	2.94±0.02^{b}	3.00±0.01^{a}	3.00±0.03^{a}	*	3.07±0.04^{b}	3.11±0.04^{ab}	3.20±0.06^{a}	3.08±0.05^{ab}	*
TA	17.13±0.16^{a}	17.09±0.17^{a}	17.16±0.14^{a}	17.15±0.07^{a}	N.S.	17.03±0.07^{a}	16.92±0.01^{a}	16.78±0.07^{a}	17.07±0. 01^{a}	N.S.
VA	0.36±0.02^{a}	0.23±0.01^{b}	0.35±0.07^{a}	0.38±0.05^{a}	*	0.39±0.05^{a}	0.36±0.07^{a}	0.38±0.01^{a}	0.40±0.07^{a}	N.S.
RS‡	14.05±0.09^{d}	22.90±0.15^{c}	29.02±0.07^{a}	25.02±0.07^{b}	***	1.75±0.04^{a}	1.38±0.05^{bc}	1.09±0.18^{c}	1.45±0.14^{ab}	***
BD	2.00±0.50^{c}	2.00±0.00^{b}	3.00±0.50^{a}	3.00±0.00^{a}	***	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	N.S.
EtOH	9.29±0.07^{a}	8.75±0.14^{b}	8.44±0.07^{b}	9.27±0.07^{a}	**	10.05±0.08^{a}	10.05±0.07^{a}	10.28±0.01^{a}	10.84±0.14^{c}	*
GL	5.62±0.08^{b}	5.73±0.15^{b}	3.64±0.04^{c}	6.07±0.06^{a}	**	5.58±0.06^{c}	8.91±0.11^{a}	7.06±0.08^{b}	5.45±0.02^{c}	**
MA	10.55±0.13^{a}	10.71±0.07^{a}	10.80±0.08^{a}	10.87±0.17^{a}	N.S.	9.90±0.04^{a}	9.99±0.07^{a}	10.02±0.02^{a}	10.12±0.14^{a}	N.S.
LA	n.d.	n.d.	n.d.	n.d.	N.S.	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	33.00±1.00^{a}	28.00±5.00^{a}	28.00±2.00^{a}	34.00±1.00^{a}	N.S.	42.00±2.00^{a}	36.00±1.50^{b}	41.00±2.00^{a}	30.00±0.00^{c}	**
Free SO_2	17.00±1.00^{a}	15.00±1.00^{ab}	13.00±1.00^{b}	14.00±1.00^{b}	N.S.	14.00±1.00^{a}	11.00±1.00^{b}	12.00±1.00^{ab}	11.00±1.00^{b}	*

Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). †The letter reported between brackets refers to the code of each experimental trial. ‡Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).
Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.
Data within a line followed by the same letter are not significantly different according to Tukey's test.

continued→

Parameters	Wine bottled 2 months				
	TV2 Strain GR1†	TV4 Strain MSE13	TV6 Strain MSE41	TV8 Strain CS182	Statistical significance
pH	3.02±0.02[b]	3.01±0.01[b]	3.18±0.02[a]	3.03±0.01[b]	*
TA	17.00±0.10[a]	16.85±0.05[a]	16.76±0.02[a]	17.04±0. 05[a]	N.S.
VA	0.40±0.03[a]	0.38±0.02[a]	0.39±0.09[a]	0.38±0.08[a]	N.S.
RS‡	0.84±0.07[a]	0.83±0.03[a]	0.69±0.09[b]	0.45±0.04[c]	***
BD	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	N.S.
EtOH	10.01±0.03[a]	10.02±0.02[a]	10.18±0.05[a]	10.63±0.04[b]	*
GL	5.43±0.04[c]	8.75±0.01[a]	7.00±0.02[b]	5.36±0.01[c]	**
MA	9.63±0.02[a]	9.89±0.09[a]	10.01±0.03[a]	10.04±0.04[a]	N.S.
LA	n.d.	n.d.	n.d.	n.d.	N.S.
Total SO_2	72.00±3.00[a]	68.00±2.00[a]	71.00±1.00[a]	71.00±1.00[a]	N.S.
Free SO_2	36.00±2.00[a]	34.00±1.00[a]	38.00±3.00[a]	35.00±1.00[a]	N.S.

Results indicate average values ± standard deviation of three replicates. n.d., not detected (values < detection limit). †The letter reported between brackets refers to the code of each experimental trial. ‡Values higher than 100 are reported as a whole number; BG, babo degree (% of total sugars); EtOH, ethanol (% wv^{-1}); GL, glycerol (gl^{-1}); LA, lactic acid (gl^{-1}); MA, malic acid (gl^{-1}); RS, reducing sugar (gl^{-1}); TA, total titratable acidity (tartaric acid gl^{-1}); Total SO_2 and Free SO_2 (mgl^{-1}); VA, volatile acidity (acetic acid gl^{-1}).

Results indicate mean values ± SD of two measurements. P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.

Data within a line followed by the same letter are not significantly different according to Tukey's test.

Figure legends

Figure 1 Experimental design for winemaking with grape racemes. Abbreviations: S, spontaneous alcoholic fermentation (control trial); TV1, must 14 BD + *S. cerevisiae* GR1; TV2, must 16BD + *S. cerevisiae* GR1; TV3, must 14 BD + *S. cerevisiae* MSE13; TV4, must 16 BD + *S. cerevisiae* MSE13; TV5, must 14 BD + *S. cerevisiae* MSE41; TV6, must 16 BD + *S. cerevisiae* MSE41; TV7, must 14 BD + *S. cerevisiae* CS182; TV8, must 14 BD + *S. cerevisiae* CS182.
All experimental trials were set up in duplicate in steel vats (2.5 hl each).
Trials TV2, TV4, TV6 and TV8 received an addition of rectified concentrated must (RCM; 65% of total sugar) to a final sugar content of the raceme grape must of 163 gl^{-1}.

Figure 2 Microbiological concentration (Log $CFUml^{-1}$) of samples during alcoholic fermentation: (a) Total Yeast; (b) Presumptive *Saccharomyces*.

Figure 3 Correspondence analysis of wine produced with different starter in function of sensory descriptors. Abbreviations: S, spontaneous alcoholic fermentation (control trial); TV1, must 12 BD + *S. cerevisiae* GR1; TV2, must 16BD + *S. cerevisiae* GR1; TV3, must 12 BD + *S. cerevisiae* MSE13; TV4, must 16 BD + *S. cerevisiae* MSE13; TV5, must 12 BD + *S. cerevisiae* MSE41; TV6, must 16 BD + *S. cerevisiae* MSE41; TV7, must 12 BD + *S. cerevisiae* CS182; TV8, must 12 BD + *S. cerevisiae* CS182.

Figure 4 (a) Mean ratings for aroma attributes for the Grillo base wine produced using the GR1 (TV1 and TV2), MSE13 (TV3 and TV4), MSE41 (TV5 and TV6) and CS182 (TV7 and TV8) strains (n = two fermentation replicates × 10 judges × three presentation replicates). S, spontaneous alcoholic fermentation (control trial). Ns, not significant; P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; **(b)** Correspondence analysis of wine produced with different starter in function of sensory descriptors. Abbreviations: S, spontaneous alcoholic fermentation (control trial); TV1, must 12 BD + *S. cerevisiae* GR1; TV2, must 16BD + *S. cerevisiae* GR1; TV3, must 12 BD + *S. cerevisiae* MSE13; TV4, must 16 BD + *S. cerevisiae* MSE13; TV5, must 12 BD + *S. cerevisiae* MSE41; TV6, must 16 BD + *S. cerevisiae* MSE41; TV7, must 12 BD + *S. cerevisiae* CS182; TV8, must 12 BD + *S. cerevisiae* CS182.

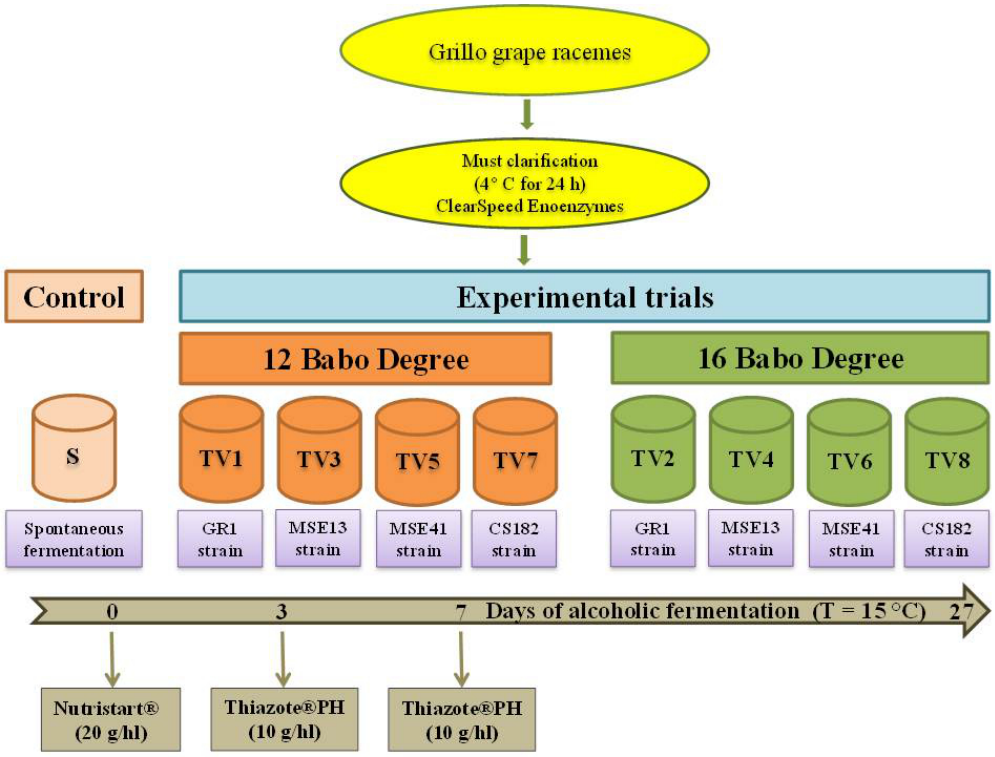

Figure 1 Experimental design for winemaking with grape racemes. Abbreviations: S, spontaneous alcoholic fermentation (control trial); TV1, must 14 BD + S. cerevisiae GR1; TV2, must 16BD + S. cerevisiae GR1; TV3, must 14 BD + S. cerevisiae MSE13; TV4, must 16 BD + S. cerevisiae MSE13; TV5, must 14 BD + S. cerevisiae MSE41; TV6, must 16 BD + S. cerevisiae MSE41; TV7, must 14 BD + S. cerevisiae CS182; TV8, must 14 BD + S. cerevisiae CS182.
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Trials TV2, TV4, TV6 and TV8 received an addition of rectified concentrated must (RCM; 65% of total sugar) to a final sugar content of the raceme grape must of 163 gl-1.

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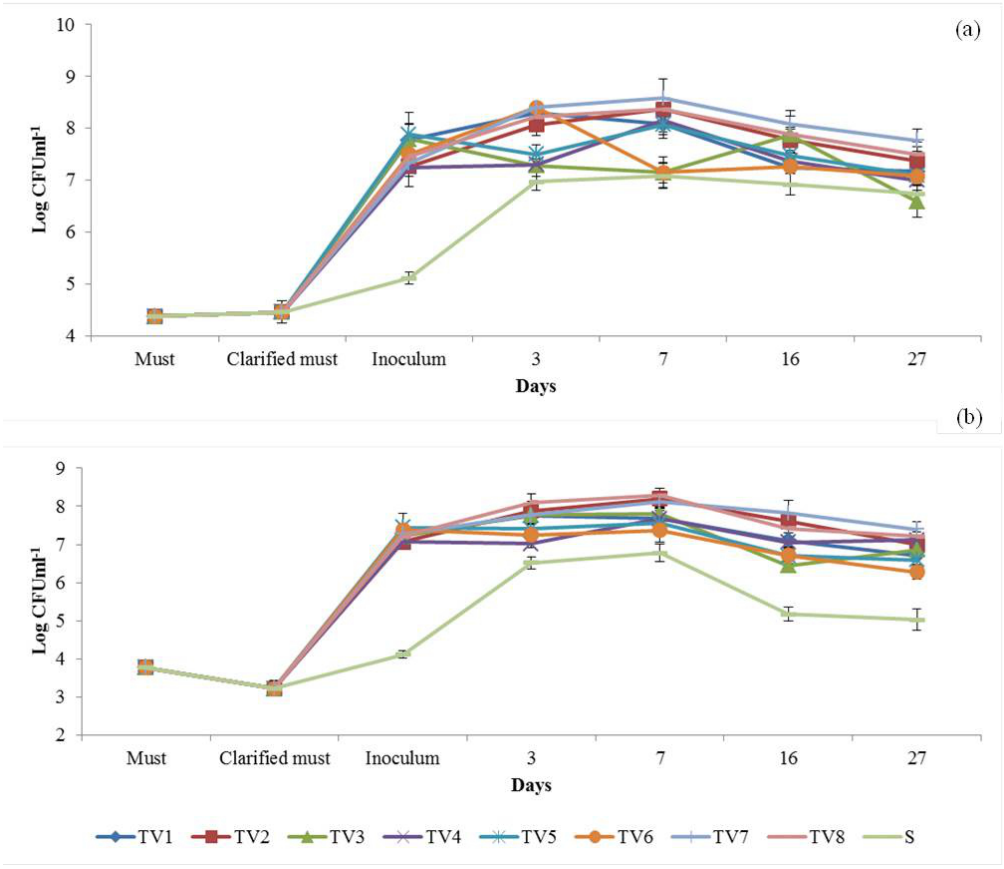

Figure 2 Microbiological concentration (Log CFUml-1) of samples during alcoholic fermentation: (a) Total Yeast; (b) Presumptive Saccharomyces.

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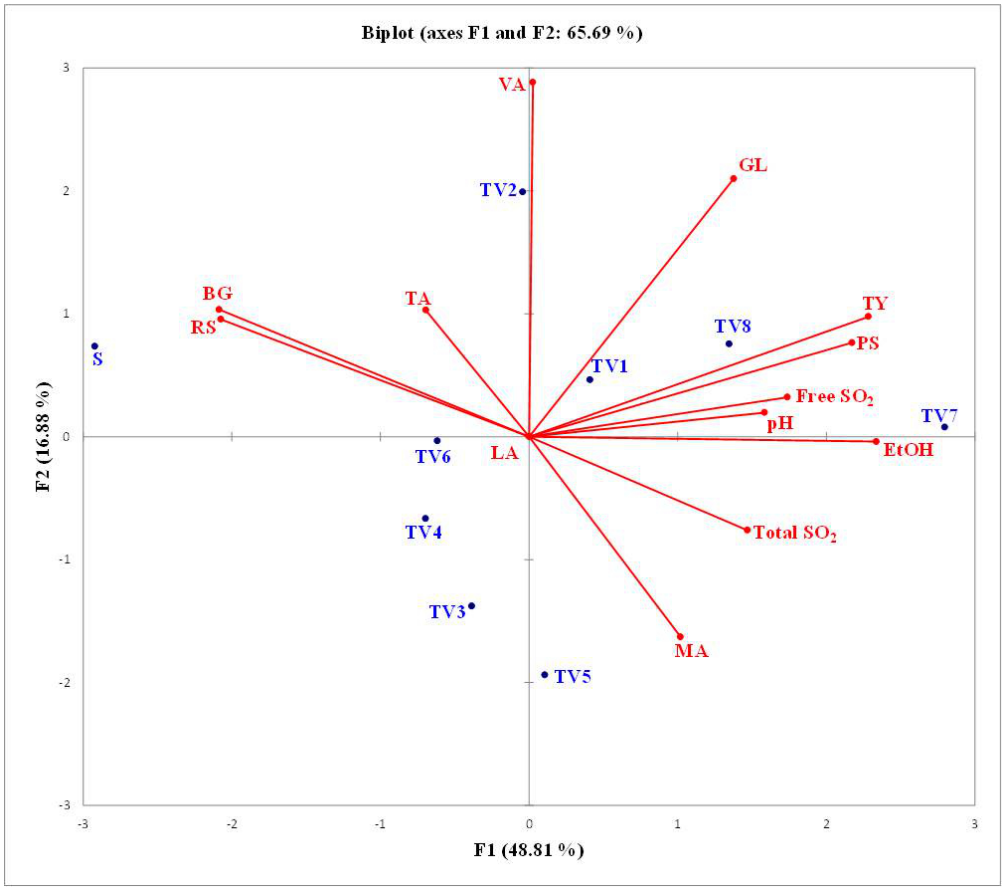

Figure 3 Correspondence analysis of wine produced with different starter in function of sensory descriptors. Abbreviations: S, spontaneous alcoholic fermentation (control trial); TV1, must 12 BD + S. cerevisiae GR1; TV2, must 16BD + S. cerevisiae GR1; TV3, must 12 BD + S. cerevisiae MSE13; TV4, must 16 BD + S. cerevisiae MSE13; TV5, must 12 BD + S. cerevisiae MSE41; TV6, must 16 BD + S. cerevisiae MSE41; TV7, must 12 BD + S. cerevisiae CS182; TV8, must 12 BD + S. cerevisiae CS182.

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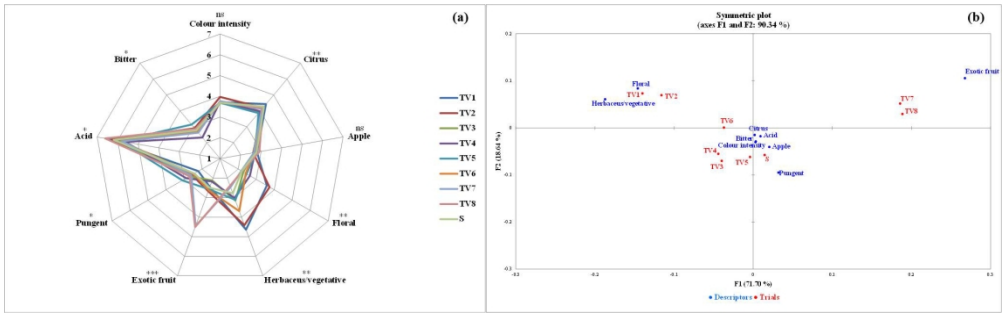

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