Effect of refermentation conditions and MO on the reduction of volatile acidity by commercial S. cerevisiae strains and their impact on the aromatic profile of wines

Vilela-Moura A. (1), Schuller D. (2), Falco V (3), Mendes-Faia A. (1) and Côrte-Real M. (2)∗

(1) Institute for Biotechnology and Bioengineering, Centre of Genetics and Biotechnology, (IBB/CGB-UTAD), Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal
(2) Centre of Molecular and Environmental Biology (CBMA) / Department of Biology / University of Minho, Campus de Gualtar, 4710-057 Braga Portugal
(3) Department of Food Science, Universidade de Trás-os-Montes e Alto Douro, Portugal

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*For correspondence:

Manuela Côrte-Real

e-mail: mcorteral@bio.uminho.pt

Tel.: (+351) 253 604 314
Fax: (+351) 253 678 980.
ABSTRACT

Herein, we evaluate the applicability of previously characterized commercial and indigenous *Saccharomyces cerevisiae* strains and non-*S. cerevisiae* species for the deacidification of white and red wines at a pilot scale. The effect of the refermentation process (mixture of acidic wine with musts from freshly crushed grapes or with residual marc) as well as MO (MO) on acetic acid removal efficiency and wine aromatic composition was also assessed in a red wine. The commercial strains S26 and S29 efficiently reduced both acetic acid (43 and 47 %, respectively) and sugar (100 %) after 264 hours of refermentation of an acidic white wine that was supplemented with grape must. Similar results (60-66 % of acetic acid removal) were observed for red wine deacidification using grape must, independently of MO. When residual marc was used for deacidification, strain S26 removed 40% of acetic acid, whereas strain S29 did not initiate refermentation with or without MO. Wines obtained by refermentation with the must had significantly lower acetic acid and a higher total SO$_2$ concentration in comparison to the wines deacidified by the grape marcs. The volatile aroma compound’s composition of deacidified red wines was dependent on the refermentation process used, rather than on MO. The marc-deacidified wine obtained by the use of strain S26 and without MO achieved the best sensory classification. When data from all analytical and sensory evaluation were combined, Principal Component Analysis (PCA) separated the wines into three distinct groups according to the strain and the refermentation process independently of MO. We successfully established an efficient and cheap enological solution for the rectification of volatile acidity of wines.

Keywords: Biological deacidification of wines, volatile acidity, MO
INTRODUCTION

Volatile acidity corresponds essentially to acetic acid and is an important factor for wine quality. Consequently, its production is carefully monitored and controlled throughout the wine production process. Currently, few processing options are available to winemakers for the removal of sensorial objectionable levels of volatile acidity. Nanofiltration and reverse osmosis are complex and expensive physical methods that may be applied presently (Han and Cheryan 1995; Massot et al. 2008). Bioreduction methods using yeasts have been known for a long time but have not been sufficiently well characterized for commercial application. Actually, winemakers have been using an empirical biological deacidification process to lower acetic acid contents of wines with volatile acidity above 0.8 g/L that consists in a refermentation associated with acetic acid consumption by yeasts. This enological practice is performed by mixing the acidic wine with freshly crushed grapes, musts or marc (remaining pulp, after draining the newly made wine) from finished fermentations, in a proportion of no more than 20-30% (v/v) (Ribéreau-Gayon et al., 2000a). The added wine should be microbiologically stable before incorporation to avoid bacterial growth. In our previous studies, we found that the S. cerevisiae autochthonous strains 43C and 45C and the commercial strains S26, S29 and S30, as well as the non-Saccharomyces strains (Lachancea thermotolerans 44C and Zygosaccharomyces bailii ISA 1307) have distinctive capacity to consume acetic acid from a mixture containing two-thirds of a synthetic medium and one third of an acidic white wine. However, the reduction of acetic acid by these strains was shown to require low amounts of oxygen as observed under limited-aerobic conditions (Vilela-Moura et al., 2008). This constraint might compromise the application of the above mentioned strains in refermention processes for the deacidification of acidic wines.

Oxygen is known to play an important role in the winemaking process (Sablayrolles et al., 1996; Salmon, 2006). Before fermentation the grape juice may be saturated with oxygen,
causing browning of the juice due to enzymatic and non-enzymatic reactions (Traverso-Rueda and Kunkee, 1982). At the beginning of fermentation, a fine balance between oxygen concentration and sulfur dioxide (SO₂) addition must be taken into account due to the possibility of reductive flavors (rotten eggs) formation (Mendes-Ferreira et al., 2002). Close to the end of fermentation, the presence of ethanol, oxygen, and acetic acid bacteria can promote spoilage and wine oxidation to vinegar (Bartowsky and Henschke, 2008; Du Toit et al., 2006; Traverso-Rueda and Kunkee, 1982). Moreover, oxygen can alter significantly the wine’s chemical composition, causing loss of organoleptical fruitiness and the appearance of sherry-like and aldehydic flaws (Ribéreau-Gayon et al., 2000b). The oxidation of phenolic compounds leads to H₂O₂ formation, which oxidizes ethanol to acetaldehyde (Shadyro et al., 2008), with a grass- or apple-like aroma (Henschke and Jiranek, 1993).

However, yeast performance improves when oxygen is delivered in a controlled manner during fermentation (Zoecklein et al., 1995). Yeast require oxygen for the synthesis of lipids such as sterols and unsaturated fatty acids, which are indispensable for plasma membrane integrity (Andreasen and Stier, 1953; Andreasen and Stier, 1954; Traverso-Rueda and Kunkee, 1982; Zoecklein et al., 1995). Ergosterol represents about 50% of the total sterol content in yeast (Bourot, 1995). A recent study showed that lipid synthesis and optimal growth of S. cerevisiae during alcoholic fermentation requires about 5.0 – 7.5 mg of oxygen/L (Rosenfeld et al., 2003). The absorption rate of the oxygen in the must is variable and has an average of 2 mg/L/min. (Macheix et al., 1991).

Controlled wine oxygenation is currently achieved through MO. By this technique small amounts of oxygen are delivered along fermentation. Oxygen is usually added by a stainless steel sparger that produces small bubbles, promoting the dissolution of oxygen. The aim of MO is to provide oxygen at a rate equal to or slightly less than the wine’s oxygen consumption rate to avoid too much oxygen build up in the wine (Llaudy et al., 2006; Parish...
This procedure has an impact on multiple aspects of wine production such as: (i) increased production of sterols and other fatty acids by yeast (Traverso-Rueda and Kunkee, 1982; Zoecklein et al., 1995), (ii) enhanced color stabilization in red wines (Sánchez-Iglesias et al., 2009; Zironi et al., 2010), (iii) removal of unwanted reductive flavors (Paul, 2002) and reduced vegetative aromas (McCord, 2003) (iv) accelerated aging process (McCord, 2003; Llaudy et al., 2006; Zironi et al., 2010). However, MO can promote the growth of acetic acid bacteria (Bartowsky and Henschke, 2008; Du Toit et al., 2006) and the formation of unwanted off-flavors by Brettanomyces sp., depending on the SO$_2$ concentrations (Snowdon, 2006).

To evaluate the applicability of previously characterized commercial strains S26 and S29 (Vilela-Moura et al., 2008; Vilela-Moura et al., in press) in refermentation processes for the removal of volatile acidity from too acidic wines, we herein assess acetic acid reduction of an white wine by refermentation with grape must at a pilot scale (10 L). We also evaluate the effect of refermentation conditions (mixtures of acidic wines with must or residual marc) and of MO at a pilot scale (30 L) on the volatile acidity reduction of an acidic red wine. The influence of MO on the aromatic composition of wines, and other enological parameters was also determined.

This study adds new information on the applicability of two commercial S. cerevisiae strains on the biological reduction of volatile acidity of acidic wines, and on the effect of refermentation conditions and MO on the removal efficiency of acetic acid from a red wine.
MATERIALS AND METHODS

Microorganisms

The strains used for deacidification of wines were previously selected and described. S. cerevisiae strains 43C, 45C and Lachancea thermotolerans 44C were natural isolates (Vilela-Moura et al. 2008); Zygosaccharomyces bailii ISA 1307 was obtained from the Instituto Superior de Agronomia (Lisbon, Portugal); strains S26, S29 and S30 were kindly provided by Lallement and Laffort Oenologie, respectively. Strains used were kept at -80°C in microtubes containing YPD broth (glucose 2%, w/v; peptone 1%, w/v; yeast extract 0.5%, w/v) supplemented with glycerol (30%, v/v).

Refermentation conditions

Fresh grape must from V. vinifera cv. Viosinho was pasteurized (60°C during 20 min.) and used for the deacidification assays of an acidic white wine. Refermentations were performed in vapor-sterilized 10 L vessels, and consisted of 6.6 L of must and 3.3 L of acidic wine. The physico-chemical characteristics of the must, acidic wines and the respective mixtures are summarized in Table 1. Aliquots of the frozen strains were streaked onto YPD plates (glucose 2%, w/v; peptone 1%, w/v; yeast extract 0.5%, w/v and agar 2%, w/v) and incubated for 48 h at 25°C. An overnight culture was then prepared by inoculation of 500 ml of the grape juice used in the mixture (white must plus acidic white wine) and incubated at 25 °C, 100 rpm, until attaining a sufficiently high cell density to achieve \( \approx 10^6 \) CFU/mL after transfer to 10 L vessels, as referred above. Refermentations were carried at 20-23 °C for 264 hours.

Deacidification assays of red wines were performed by refermentation with fresh must or by using marcs (remaining pulp, after draining the newly made wine) from V. vinifera cv Touriga Nacional. The must used for the refermentation process included the grape skins and was...
supplemented with 40 mg/L of sulfur dioxide (SO₂). Ten L of acidic red wine was then added to 20 L of must and refermentations were performed in stainless steel tanks (30 L capacity). The physico-chemical characteristics of the must, acidic red wine and the mixture are mentioned in Table 1. The inoculation of the commercial S. cerevisiae strains S26 and S29 was performed as described above.

The remaining pulp (residual marc), was obtained after draining the newly made wine at the end (96 hours) of fermentation. At this stage, the marc, prepared from V. vinifera cv Touriga Nacional contained 30 - 35 g/L of sugar. Ten L of acidic red wine were then added to 20 L of residual marcs and refermentation was performed in 30 L stainless steel tanks at a temperature between 18 and 20ºC.

Refermentation assays with acidic red wine were conducted with or without MO during one hour per day (20 mg/L/h of oxygen applied with a MicroSafeO₂ - AEB device). Two daily pump overs, of one minute each, were performed in each tank to homogenize the refermenting wine. Yeast cell concentration was evaluated by spreading diluted must samples (10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶) onto YPD plates (glucose 2%, w/v; peptone 1%, w/v; yeast extract 0.5%, w/v and agar 2%, w/v). At the end of refermentations, (264 and 192 hours for the musts and the marcs, respectively), the wines were analyzed and SO₂ – supplemented to a final concentration of 50 mgL⁻¹. After two months, the wines were bottled and organoleptical evaluation was performed. All experiments were performed in triplicate.

### Analytical determinations

Sugar consumption was monitored daily by the DNS method (Miller, 1959). Acetic acid consumption was monitored by the Cazenave-Ferré method, followed by titration with phenolphthalein. Analysis of the density, pH, alcohol concentration, volatile acidity, sulfur dioxide, titratable acidity, estimated alcohol content and residual sugar were performed...
according to standard methods (Office International de la Vigne et du Vin, 1990), as outlined in Table 1.

A solid-phase microextraction (SPME) methodology described by Mendes-Ferreira et al. (2009) was used for the isolation of aroma compounds determined by gas chromatography and mass spectroscopy (GC-MS) analysis. Compounds were adsorbed onto a fiber (100 µm polydimethylsiloxane – PDMS -, 85 µm Carboxen – polydimethylsiloxane – CAR/PDMS - and 50/30 µm Divinylbenzene/Carboxen/PDMS -DVB/CAR/PDMS) by solid-phase microextraction (SPME). Ten ml of sample, 10 ml of internal standard solution and 4 g NaCl were transferred to 40 ml vials (Supelco P/N 27181), containing a 10 mm magnetic stirring bar and then capped with PTFE-faced silicone seals. Extractions in headspace mode were carried out at 20±1°C with magnetic stirring (1300 rpm). The sample solution was equilibrated for 10 minutes; the fiber was then introduced into the vial headspace and held for 60 minutes at constant temperature.

Chromatographic analysis was performed using an Agilent 6890 N gas chromatograph equipped with a 5973N mass spectrometer. The volatile compounds were thermally desorbed in the GC injector port for 10 minutes, where a 0.75 mm liner was used. Analysis was performed in the splitless mode. Volatile compounds were then separated using an Innovax capillary column, 30 m x 0.25 mm, with 0.5 µm film thickness (Agilent, Santa Clara, CA, USA). The desorption temperature was 270°C during 10 min. The column was maintained at 40°C for 5 minutes after desorption, ramped at 4°C per minute up to 200°C, and then ramped at 10°C per minute up to 240°C, where it was held for 15 minutes. Helium was used as the carrier gas at 34 cm/s average linear velocity. All mass spectra were acquired in electron impact (EI) mode at 70 eV, using full scan with a scan range of 26–250 atomic mass units, at a rate of 6.12 scans/s. The Wiley database (Wiley/NBS Registry of Mass Spectral Data 1989 – McLafferty and Stauffer 1989) was used for compounds spectra identification. Whenever
possible, identification was confirmed by comparing mass spectra and retention indices with those of authentic standards.

The compounds were quantified in selected ion monitoring (SIM) mode. 2-octanol was used as the internal standard to eliminate variations in extraction efficiency caused by some divergences in the sample matrix such as ethanol.

**Wine sensory analysis**

The sensory analysis was performed by a trained panel of 5 judges that have an extensive wine tasting experience and participate on a regular basis in Wine Awards. Fourteen attributes were selected: appearance (limpidity, tone and intensity), aroma (limpidity, intensity, vinegar, acetaldehyde, ethyl acetate), oral perception (mouth intensity, body, harmony, persistence, mouth feel, and acidic taste) according to reference standards (Noble et al., 1987). The attributes were quantified using a six-point intensity scale (ISO 4121, 2003). A total sensory score was calculated for each wine as the sum of average scores of appearance, aroma, taste and mouth feel attributes. The judges were also requested to describe the global impression of each wine. Each judge evaluated six wines in one session. All evaluations were conducted from 10:00 to 12.00 A.M. in individual booths (ISO 8589, 2007) and according standardized procedures (ISO 3591, 1977).

**Statistical analysis**

Both sensory and chemical data were submitted to variance analysis (ANOVA) using the STATISTICA 7.0 software (StatSoft Inc., 2004). Tukey honestly significant difference (HSD) test was applied to chemical and sensory data to determine significant differences between the samples; the model was statistically significant with a $P$ value less than 0.05.
A Principal Component Analysis (PCA) of the combined data from chemical and sensory analysis was performed using the STATISTICA 7.0 software (StatSoft Inc., 2004).
RESULTS

Deacidification of an acidic white wine

In our previous studies, the *S. cerevisiae* autochthonous strains 43C and 45C and the commercial strains S26, S29 and S30, as well as the non-*Saccharomyces* strains (*L. thermotolerans* 44C and *Z. bailii* ISA 1307) have demonstrated capacity to remove acetic acid during a refermentation process using synthetic media, under aerobic and limited-aerobic conditions. The commercial strains S26 and S29 had the best acetic acid removal efficiency. We further showed that strain S26 had a higher tolerance to the combined stress factors imposed by acidic wines (Vilela-Moura et al., 2008, Vilela-Moura et al., in press).

Within this study, we aimed to evaluate the capacity of the above mentioned strains to remove acetic acid from an acidic white wine using a refermentation process with grape must of the Viosinho variety at a pilot scale (10 L). The sugar concentration of the mixture of the acidic white wine with the must was 157 g/L, whereas the concentration of ethanol and acetic acid were 4.3 % (v/v) and 1.15 g/L, respectively (Table 1). The chemical characterization of the deacidified wines obtained after 264 h of incubation is shown in Table 2. With the exception of strain *L. thermotolerans* 44C, all strains produced refermented wines with similar ethanol concentration, pH, acetic acid, titrable acidity, total SO$_2$, and free SO$_2$ ($P<0.001$). The *S. cerevisiae* strains (S26, S29, S30, 43C and 45C) were more efficient acetic-acid consuming strains compared to the non-*Saccharomyces* strains *Z. bailii* ISA1307 and *L. thermotolerans* 44C. Acidic white wine that was refermented with the latter strain had a lower pH and a much reduced total SO$_2$ content, about six to eight times lower than the remaining strains (Table 2). This strain also showed an increase in volatile acidity, probably due to the oxidation of ethanol to acetaldehyde and acetic acid. The commercial strains S26 and S29 initiated sugar consumption most rapidly (18 and 16%, respectively, after 48 h) under the unfavourable conditions imposed by the acidic environment, whereas the other strains did not even start to
consume sugar at this time point (data not shown). After 264 hours, both commercial strains exhausted the sugars (Table 2, statistical class “a”).

Taking all data in consideration, strains S26 and S29 revealed as the most promising strains and were used for subsequent refermentation experiments with acidic red grape must or residual marc prepared from the Touriga Nacional variety.

Deacidification of an acidic red wine

We evaluated the capacity of strains S26 and S29 to remove acetic acid from an acidic red wine at a pilot scale (30 L) using two alternative refermentation processes involving (i) grape must (fresh grape juice with grape skins) (ii) a residual marc from a finished fermentation (residual sugars 30-35 g/L), obtained from Touriga Nacional grapes.

In the first process, involving must addition, the initial sugar, ethanol and acetic acid concentrations were 160 g/L, 4.2 % (v/v) and 1.12 g/L, respectively (Table 1). As shown in Table 2, both strains produced wines with a similar final concentration of ethanol, acetic acid and total SO2, independent of MO. Wines obtained by refermentation with the must had significantly lower acetic acid and a higher total SO2 concentration in comparison to the wines deacidified by the grape marcs.

Besides, both strains consumed simultaneously sugar and acetic acid, independent of MO (Table 3). The highest acetic acid consumption of 66% was achieved at the end of refermentation by the strains S29 and S26 with and without MO, respectively. There were no statistical significant differences detected between strains or MO conditions. Oxygen availability in this process has, however, increased the biomass of both strains during refermentation (from 10^7 cells/mL without MO to 10^8 cells/mL with MO).

When the refermentation was carried out with the residual marc from an almost finished fermentation of Touriga Nacional grape variety, the initial sugar concentration in the marc
was 30-35 g/L and dropped to 10-15 g/L after wine addition (Table 1). The ethanol and acetic acid concentrations of the wine-marc mixture were of 9.5-10 % (v/v) and 1.14 g/L, respectively. These experiments were only performed with strain S26 since strain S29 did not initiate fermentation under the experimental conditions used. The acidic red wine was added to a residual marc obtained after 96 hours of fermentation, when the marc had a volatile acidity of 0.4 g/L, increasing its concentration to 1.14 g/L. After 96 hours, the consumption of the sugars (10-15 g/L) was accompanied by a decrease of 40.4% and 39.5% of the volatile acidity, with or without MO conditions, respectively. By the use of marc for refermentation, we observed complete sugar depletion after 72 hours, significantly higher than the concentrations determined after 48 hours of refermentation with grape must.

As shown in Table 3, there were no significant differences regarding acetic acid consumption at early refermentation stages (48 and 72 hours) for both methods. However, strain S26 decreased acetic acid more efficiently in a longer refermentation process with grape must (62 – 66 %, 264 hours), than in a shorter process involving the marc (40 %, 96 hours). It seems that the consumption of the sugars was faster in refermentations conducted with marcs (96 h), possibly due to its lower initial sugar concentration (10-15 g/L). Oxygen availability also increased the biomass of this strain from $10^7$ to $10^8$ cells/mL, similar to the refermentation with grape must.

**Aromatic characterization of the deacidified wines**

The volatile aroma compound’s concentration of the six wines, deacidified by strains S26 and S29, obtained through the use of must or marc of the Touriga Nacional variety, and using or not MO, were determined by GC-MS analysis. Table 4 shows the concentrations of 22 aromatic compounds of the deacidified wines under the different conditions tested. The wines obtained from the refermentation processes with must and using strains S26 and S29 showed...
very similar patterns of aromatic compounds. The MO conditions had no significant impact on the volatile compounds in the respective deacidified wines. Contrarily, when residual marc from fermentation and strain S26 was used, a significant change occurred in the aromatic profile, affecting mainly the concentration of esters, which are well-known for their positive contribution to the wine`s bouquet with strong, fruity aromatic notes. Independently of MO, esters such as ethyl propionate (rum-like), ethyl isobutyrate (strawberry, ethereal, buttery, ripe), ethyl 2-methylbutyrate (sweet, floral, fruity, apple) and ethyl isovalerate (fruity) were found in significantly higher concentrations. Other esters such as ethyl butyrate (buttery, ripe fruit), isoamyl acetate (banana) and 2-phenylethyl acetate (rose-like) decreased significantly in comparison to the must-deacidified wines.

The composition of the fatty acid fraction was also analyzed. Small amounts (depending on the odour threshold) of these volatile compounds contribute positively to the wine quality, while excessive concentrations have detrimental effects. Octanoic acid (grass, acid like odour) occurred in high concentrations in all wines. Isovaleric and hexanoic acids have rancid and cheese sensory descriptors and were the ones occurring at significantly higher concentration in the wines made with marcs, whereas decanoic acid (natural soap odor) was present in higher concentrations in must-deacidified wines; however, the differences were not significant.

Acetaldehyde confers a grass or apple-like aroma when found in concentrations higher than 100 mg/L (Carlton et al., 2007). The concentrations of this compound were rather low in acidic red wine/must and red wine/marc mixtures (14.3 and 20.0 µg/L, respectively, Table 1) and increased during deacidification, but not above the detection limit. When the wine/must mixture was used, strain S29 showed a lower acetaldehyde concentration than strain S26. Interestingly, strain S26 showed a lower concentration when the wine/marc mixture was used.
Under these conditions, and in combination with MO, the acetaldehyde concentration did not change during refermentation.

The concentration of fusel alcohols such as 2-phenylethanol and isoamyl alcohol were similar for all deacidified wines and remained below the detection threshold of 10 and 30 mg/L, respectively. Linalool, the only terpene determined, which has pleasant lavender notes, appeared in very similar concentrations in all deacidified wines.

Wine sensory analysis

The sensory analysis was performed by a trained panel of 5 judges. As mentioned in the Materials and Methods section, fourteen attributes were quantified using a six-point intensity scale. A total score was calculated for each wine and was expressed as the sum of average scores of appearance, aroma, taste and mouth feel attributes. As shown in Table 2, strain S26 was better classified than strain S29 when refermentations were performed with acidic red wine / must mixtures, whereas the MO had no influence. Interestingly, when the acidic wine / marc mixture was used for refermentation, strain S26 achieved the highest and second lowest quotes (statistically most different, \( P<0.05 \)), without and with MO, respectively. Aroma and oral perception were the attributes that mostly distinguished the six wines, while the attributes grouped under the appearance criterion had no contribution for their distinction (not shown). Oxygen availability neither improved nor worsened the wine sensory characteristics in these kinds of fermentations.

All deacidified wines were analyzed by PCA, by combining data from chemical analysis and sensorial evaluation (Table 2), as well as volatile compounds concentration (Table 4). Figure 1A represents the bi-dimensional projection of the data according to the used parameters and shows that the first (factor 1) principal component (PC) explained 55.47% of the total variability between the wines. This factor was mainly associated with analytical parameters.
such as volatile acidity, pH, SO$_2$, ethyl acetate, linalool, but also other chemical compounds. The second PC (factor 2) explained 26.04% of the total variability and was more associated with aromatic compounds such as acetaldehyde, octanoic acid, ethyl octanoate, 2-phenylethanol, diethyl succinate and others. Both principal component explained 81.51% of the variability between the six wines.

PCA analysis also showed that the global characteristics of the six wines could separate them into three well-defined groups according to the strains and the deacidification process (Figure 1B). These results confirm the previously described score values showing that MO had no influence on the formation of these groups. Wines deacidified with strain S26 using must or marc were more characterized by factor 2 and 1, respectively. Contrarily, wines obtained with strain S29 were equally characterized by both factor 1 and 2. There was no clear correlation between the sensorial evaluation by the panel of judges and the global PCA analysis. The most preferred wine (sensory score 59.0, fermentation of acidic red wine using marc and strain S26) was apart from the least preferred wine (sensory score 43.6, fermentation of acidic red wine using must, MO and strain S29). On the other hand, the most preferred wine was close to the second least preferred wine (sensory score 44.4, fermentation of acidic red wine using marc, MO and strain S26).

**DISCUSSION**

This study shows the applicability of *S. cerevisiae* commercial strains S26 and S29 to remove volatile acidity from acidic white and red wines through refermentation processes with grape must or residual marc at pilot scale. Besides, data are provided and discussed regarding the effect of the refermentation process and the application of MO on acetic acid removal and the aromatic composition of the resulting wines. Among the different yeast species tested, the
commercial *S. cerevisiae* strains S26 and S29 were most interesting because they efficiently reduced both acetic acid (43 and 47 %, respectively) and sugar (100 %), after 264 hours of refermentation of an acidic white wine that was supplemented with grape must. Moreover, they initiated sugar consumption much earlier than the other strains, and were therefore used for subsequent experiments with acidic red wines. Their better tolerance to the combined stressful conditions caused by sugar, ethanol and acetic acid is not surprising because commercial strains are selected and improved for a very high robustness. The degree of acetic acid reduction in the refermentations carried out with grape must was not dependent on the yeast strain, but rather on the wine style. With red wine and after 264 h, the decrease was more pronounced than with white wine (2/3 and 1/3 of the initial acetic acid value, respectively). This might be due to a better adaptation of both strains to red wine or a more favourable composition in the red wine / must mixture for acetic acid consumption. Another explanation might be the vinification process itself - red wines are usually produced with some aeration of the grape must during pump overs, that can transfer an amount of 5 mg/L of oxygen (Silva and Lambri, 2006), stimulating yeast growth, and leading also to the formation of tannin-anthocyanin bonds and color stabilization (McCord, 2003; Sánchez-Iglesias et al., 2009; Zironi et al., 2010). However, 40% of removal in white wine was sufficient to attain acetic acid concentrations that correspond to the usual concentration in wines (0.6 – 0.8 g/L).

We then assessed the deacidification performance of both strains using a red acidic wine applying or not MO. Oxygen supplementation improves synthesis of sterols and other unsaturated fatty acids that are necessary for plasma membrane integrity (Rosenfeld et al., 2003; Traverso-Rueda and Kunkee, 1982) and increases cell biomass (Sablayrolles and Barre, 1987). Consistently, we could observe that additional oxygen supplementation increased the final cell number. However, acetic acid consumption during must-mediated refermentation was not affected by MO. This behaviour indicates that the oxygen availability provided
during pumpovers is sufficient for acetic acid removal by strain S26 from a red acidic wine.

Strain S26 conducted both refermentation processes, with grape must or with residual marc, showing higher tolerance than strain S29 to the combined effects of various stress factors, such as high concentration of acetic acid and ethanol.

Both deacidification processes of the acidic red wine mixtures conferred distinct aromatic characteristics to the final wines. This was most notable for the fraction of ester compounds, (e.g. ethyl propionate, ethyl isobutyrate, ethyl-2-methylbutyrate, ethyl isovalerate), but also for the isovaleric and hexanoic fatty acids, that were significantly higher in wines prepared from the marc/wine mixture than from must/wine mixture. Slight and (in most cases) statistically not significant differences were observed between micro-oxygenated or not micro-oxygenated wines, independently of the deacidification process and strain. Nitrogen source limitation (Mendes-Ferreira et al., 2004), high ethanol concentrations (Boulton et al., 1996), or a combination of both may have favored the expression of enzymes like ATF1- and ATF2-encoded alcohol acetyltransferases of S. cerevisiae, responsible for the synthesis of ethyl acetate and isoamyl acetate esters, that improve the floral and fruity aroma of wine (Lilly et al., 2006). In fact, these conditions occur in the deacidification process with the marcs and are most probably the cause of the aromatic characteristics achieved by the wine at the end of refermentation. The aromatic profiles of wines prepared from must/wine mixtures tend to be richer in esters like isoamyl acetate and 2-phenylethyl acetate. The lack of MO conferred more floral notes to the respective wines. González-Sanjosé et al. (2009) found significant lower intensity of vegetal and reduction odor notes, and slightly more intense fruity notes in micro-oxygenated wines.

PCA clearly showed that MO had no significant impact on the final aromatic composition of the wines that are grouped according to the strain and deacidification process used. These findings were, partially, confirmed by a panel of 5 judges. Their order of preference did not
distinguish wines that were micro-oxygenated or not and that were prepared from acidic 
wine/must mixtures. PCA analysis (and the judges’ scores, to some degree) distinguished the 
wines obtained with either strain. This is in agreement with a plethora of publications 
showing the effect of different *S. cerevisiae* strains on the concentration of aromatic volatile 
compounds (Mauriello et al., 2009, Callejon et al., 2010, and references cited therein). Wines 
obtained from marc/wine mixture that were refermented by strain S26 without MO were most 
pREFERRED and obtained clearly the highest rating by the evaluation panel. In general, neither 
the projection of a wine on the PCA factor plane was correlated with the judge’s order of 
preference, nor with single compounds, such as acetic acid or acetaldehyde. These results are 
expected and explained by the very high complexity of hundreds of compounds that occur in 
a wine. Their relative concentrations and interactions that are perceived by a trained wine 
taster is certainly not reflected by the comparatively very low number of 27 compounds that 
were evaluated within the present study.

In summary, we successfully established an efficient enological solution for the biological 
reduction of volatile acidity of acidic wines based on the refermentation with residual marcs 
and the use of the commercial yeast strain S26. About 40% of acetic acid reduction was 
achieved after 72 hours. Besides, MO had no impact on both the acetic acid removal 
efficiency and the global composition of volatile compounds. The judges clearly preferred the 
wine produced without MO, using marc and strain S26. The proposed procedure was 
achieved by a very careful evaluation of both the yeast physiology and the process used for 
refermentation. We propose our approach can be an efficient and cheap alternative for the 
biological rectification of too acidic wines, using marc as a fermentation end product.
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Figure 1.

Bi-dimensional PCA by the combination of data from chemical analysis and sensorial evaluation (Table 2), and volatile compounds concentration (Table 4). (A) projection of the data according to the chemical parameters (B) projection of the data according to the strains and the deacidification process.
Oenological parameters of the acidic wines, musts and mixtures of acidic wines with musts or marcs used in the deacidification assays carried out at a pilot scale.

<table>
<thead>
<tr>
<th>Chemical characteristics</th>
<th>Acidic white wine</th>
<th>Viosinho must</th>
<th>Viosinho must plus acidic white wine</th>
<th>Acidic red wine</th>
<th>T. Nacional must</th>
<th>T. Nacional must plus acidic red wine</th>
<th>T. Nacional marc plus acidic red wine</th>
<th>Analytical Methods (CEE N.º 2676/90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density at 20ºC</td>
<td>0.9906</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.9908</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Densitometry</td>
</tr>
<tr>
<td>Free SO₂ (mg/L)</td>
<td>0.0</td>
<td>n.d.</td>
<td>0.0</td>
<td>0.0</td>
<td>n.d.</td>
<td>40.0</td>
<td>0.0</td>
<td>Ripper Method</td>
</tr>
<tr>
<td>Total SO₂ (mg/L)</td>
<td>23.0</td>
<td>n.d.</td>
<td>7.7</td>
<td>25.0</td>
<td>n.d.</td>
<td>49.0</td>
<td>41.6</td>
<td>Ripper Method</td>
</tr>
<tr>
<td>Volatile acidity (g/L acetic acid)</td>
<td>3.30</td>
<td>0.13</td>
<td>1.15</td>
<td>2.80</td>
<td>0.21</td>
<td>1.12</td>
<td>1.14</td>
<td>Destillation (Cazenave-Ferré, followed by titration with phenolphthalein)</td>
</tr>
<tr>
<td>Sugar (g/L)</td>
<td>1.00</td>
<td>224</td>
<td>157</td>
<td>1.12</td>
<td>230</td>
<td>160</td>
<td>10-15</td>
<td>Lane-Eynon Method</td>
</tr>
<tr>
<td>Titratable acidity (g/L tartaric acid)</td>
<td>7.10</td>
<td>9.83</td>
<td>8.90</td>
<td>7.05</td>
<td>10.73</td>
<td>8.90</td>
<td>8.03</td>
<td>Titration with bromothymol blue</td>
</tr>
<tr>
<td>pH</td>
<td>2.88</td>
<td>3.23</td>
<td>3.00</td>
<td>2.98</td>
<td>3.25</td>
<td>3.02</td>
<td>3.15</td>
<td>Potentiometer</td>
</tr>
<tr>
<td>Alcoholic degree %, Ethanol (v/v)</td>
<td>12.0</td>
<td>n.d.</td>
<td>4.3</td>
<td>12.5</td>
<td>n.d.</td>
<td>4.2</td>
<td>9.5 - 10</td>
<td>Distillation</td>
</tr>
</tbody>
</table>

| Acetaldehyde (µg/L)                            | n.d.              | n.d.          | n.d.                                | 42.9           | n.d.            | 14.3                                 | 20.0                                 | SPME/GC-MS (2)                      |

(1) CEE N.º 2676/90 – Official Journal of the European Communities, 33, 3.10.1990. (ISSN 0257 – 7771)
(2) SPME/GC-MS – As described in Mendes-Ferreira et al. (2009))

n.d. – not determined.
Table 2

Chemical characterization of the mixtures of white and red acidic wines with must or marcs before and after refermentation with grape must of Viosinho grapes (without MO, after 264 h) and with grape must or marc of Touriga Nacional grapes (with or without MO, after 96 h).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Yeast Strains</th>
<th>Ethanol % (v/v)*</th>
<th>pH*</th>
<th>Acetic acid (g/L)*</th>
<th>Titratable acidity (g/L)*</th>
<th>Total SO₂ (mg/L)*</th>
<th>Free SO₂ (mg/L)*</th>
<th>Sugars (g/L)*</th>
<th>Sensory analysis (total score)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidic white wine/must mixture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S26</td>
<td>12.1±0.04 a</td>
<td>3.19±0.01 b</td>
<td>0.61±0.02 a,b</td>
<td>6.62±0.19 a</td>
<td>33.3±1.08 b</td>
<td>0.48±0.23 a</td>
<td>0.0±0.0 a</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>S29</td>
<td>11.9±0.04 a</td>
<td>3.19±0.01 b</td>
<td>0.66±0.08 a,b</td>
<td>6.62±0.04 a</td>
<td>34.3±2.54 b</td>
<td>0.33±0.23 a</td>
<td>0.0±0.0 a</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>S30</td>
<td>11.8±0.04 a</td>
<td>3.16±0.01 b</td>
<td>0.73±0.02 a,b</td>
<td>7.11±0.18 a</td>
<td>37.9±3.26 b</td>
<td>0.64±0.45 a</td>
<td>3.13±1.03 a</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>43C</td>
<td>11.9±0.14 a</td>
<td>3.16±0.01 b</td>
<td>0.72±0.08 a,b</td>
<td>7.13±0.95 a</td>
<td>36.1±3.61 b</td>
<td>0.80±0.68 a</td>
<td>0.79±1.30 a</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>45C</td>
<td>11.9±0.11 a</td>
<td>3.14±0.01 a,b</td>
<td>0.67±0.02 a,b</td>
<td>6.73±0.24 a</td>
<td>39.4±2.54 b</td>
<td>0.91±0.98 a</td>
<td>0.0±0.0 a</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>44C</td>
<td>8.0±5.59 a</td>
<td>3.08±0.03 a</td>
<td>1.40±0.20 b</td>
<td>8.63±1.59 a</td>
<td>4.6±3.53 a</td>
<td>1.14±1.56 a</td>
<td>101.89±2.15 b</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>ISA 1307</td>
<td>11.4±0.11 a</td>
<td>3.17±0.01 b</td>
<td>0.83±0.02 a,b</td>
<td>6.75±0.08 a</td>
<td>38.4±3.26 b</td>
<td>0.32±0.98 a</td>
<td>32.97±2.51 b</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
| * Results obtained for strains/wines marked with the same superscript letters are not significantly different (P<0.001) for the same analytical parameter.
| **Acidic red wine/must mixture** |               |                  |       |                    |                            |                   |                 |               |                             |
|                                 | S26           | 11.8±0.20 a      | 3.29±0.02 c,d | 0.42±0.06 a        | 8.35±0.61 a                | 115.01±4.86 c     | 0.0±0.0 a      | 0.0±0.0 a    | 50.2±5.5 a,b,c             |
|                                 | S29           | 11.3±0.10 a      | 3.31±0.03 c,d | 0.38±0.11 a        | 7.80±0.27 a                | 130.71±17.05 d    | 0.0±0.0 a      | 0.0±0.0 a    | 43.6±9.3 a                |
| **Acidic red wine/marc mixture** |               |                  |       |                    |                            |                   |                 |               |                             |
|                                 | S26           | 11.1±0.30 a      | 3.29±0.05 c  | 0.38±0.07 a        | 8.35±0.17 a                | 105.22±15.57 c    | 0.0±0.0 a      | 0.0±0.0 a    | 55.4±7.9 a,b,c             |
|                                 | S29           | 11.0±0.30 a      | 3.32±0.05 d  | 0.45±0.08 a        | 8.03±0.40 a                | 102.57±2.24 c     | 0.0±0.0 a      | 0.0±0.0 a    | 45.2±10.3 a,b               |
| **Deacidified red wines with MO** |               |                  |       |                    |                            |                   |                 |               |                             |
|                                 | S26           | 12.1±0.60 a      | 3.44±0.03 c  | 0.69±0.09 a,b      | 7.46±0.16 a                | 46.93±2.96 b      | 0.0±0.0 a      | 0.0±0.0 a    | 44.4±8.4 a,b,c             |
|                                 | S29           | 11.9±0.30 a      | 3.50±0.03 c  | 0.68±0.05 a,b      | 7.56±0.49 a                | 56.32±7.13 b      | 0.0±0.0 a      | 0.0±0.0 a    | 59.0±7.2 a                |
| * Results obtained for strains/wines marked with the same superscript letters are not significantly different (P<0.05).
| Total score (sum of average scores for appearance, aroma, taste and mouth feel attributes)
| **Deacidified red wines without MO** |               |                  |       |                    |                            |                   |                 |               |                             |
|                                 | S26           | 12.1±0.60 a      | 3.44±0.03 c  | 0.69±0.09 a,b      | 7.46±0.16 a                | 46.93±2.96 b      | 0.0±0.0 a      | 0.0±0.0 a    | 44.4±8.4 a,b,c             |
|                                 | S29           | 11.9±0.30 a      | 3.50±0.03 c  | 0.68±0.05 a,b      | 7.56±0.49 a                | 56.32±7.13 b      | 0.0±0.0 a      | 0.0±0.0 a    | 59.0±7.2 a                |
| **Deacidified red wines with MO** |               |                  |       |                    |                            |                   |                 |               |                             |
|                                 | S26           | 12.1±0.60 a      | 3.44±0.03 c  | 0.69±0.09 a,b      | 7.46±0.16 a                | 46.93±2.96 b      | 0.0±0.0 a      | 0.0±0.0 a    | 44.4±8.4 a,b,c             |
|                                 | S29           | 11.9±0.30 a      | 3.50±0.03 c  | 0.68±0.05 a,b      | 7.56±0.49 a                | 56.32±7.13 b      | 0.0±0.0 a      | 0.0±0.0 a    | 59.0±7.2 a                |
Table 3

Percentage of acetic acid (bold letters) and sugar consumption after refermentation of red wine with must or marc by *S. cerevisiae* strains S26 and S29, after 48 and 264 hours (refermentation with the must) or 72 and 96 hours (refermentation with the marcs) with and without MO. Results obtained for strains and culture conditions with the same superscript letter are not significantly different (*P*<0.001).

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>Red wine refermentation with grape must</th>
<th>Red wine refermentation with marc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 h</td>
<td>264 h</td>
</tr>
<tr>
<td></td>
<td>With MO</td>
<td>Without MO</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>35.7 ± 1.57 a</td>
<td>37.5 ± 4.72 a</td>
</tr>
<tr>
<td>Sugar</td>
<td>20.6 ± 4.33 a</td>
<td>14.6 ± 6.51 a</td>
</tr>
<tr>
<td>S26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>42.9 ± 5.68 a</td>
<td>44.6 ± 12.30 a</td>
</tr>
<tr>
<td>Sugar</td>
<td>25.8 ± 10.10 a</td>
<td>23.8 ± 4.10 a</td>
</tr>
<tr>
<td>S29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Yeast strains and deacidification processes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>S26 must</th>
<th>S26 must</th>
<th>S29 must</th>
<th>S29 must</th>
<th>S26 marc</th>
<th>S26 marc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MO</td>
<td>MO</td>
<td>MO</td>
<td>MO</td>
<td>MO</td>
<td>MO</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>59.90 ± 0.98 a</td>
<td>47.50 ± 2.28 a</td>
<td>58.16 ± 8.26 a</td>
<td>60.14 ± 0.26 a</td>
<td>54.07 ± 6.19 a</td>
<td>47.95 ± 3.85 a</td>
</tr>
<tr>
<td>Ethyl propionate</td>
<td>90.26 ± 1.57 a,b,c</td>
<td>80.76 ± 7.57 a,b,c</td>
<td>64.77 ± 10.66 a</td>
<td>68.07 ± 1.91 a</td>
<td>117.96 ± 12.14 c</td>
<td>107.48 ± 4.96 b,c</td>
</tr>
<tr>
<td>Ethyl isobutyrate</td>
<td>58.36 ± 0.72 a</td>
<td>52.71 ± 7.06 a</td>
<td>53.01 ± 2.96 a</td>
<td>52.74 ± 4.50 a</td>
<td>93.51 ± 12.35 c</td>
<td>77.19 ± 2.68 b,c</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>179.50 ± 7.04 a</td>
<td>153.60 ± 6.41 c</td>
<td>188.23 ± 2.91 a</td>
<td>191.30 ± 1.63 a</td>
<td>139.46 ± 5.44 b</td>
<td>122.56 ± 7.05 a,b,c</td>
</tr>
<tr>
<td>Ethyl 2-methylbutyrate</td>
<td>10.23 ± 0.37 a,b,c</td>
<td>10.09 ± 1.14 a,b,c</td>
<td>8.96 ± 0.62 a,b,c</td>
<td>8.16 ± 0.40 a</td>
<td>15.22 ± 1.69 f</td>
<td>12.60 ± 0.71 b,c</td>
</tr>
<tr>
<td>Ethyl isovalerate</td>
<td>10.23 ± 0.45 a,b,c</td>
<td>9.82 ± 0.54 a,b,c</td>
<td>9.19 ± 0.16 a</td>
<td>8.91 ± 0.43 a</td>
<td>14.37 ± 0.93 f</td>
<td>11.98 ± 0.71 b</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>4.30 ± 0.22 a</td>
<td>3.59 ± 0.50 a</td>
<td>4.65 ± 0.22 a</td>
<td>4.37 ± 0.56 a</td>
<td>0.57 ± 0.05 b</td>
<td>0.42 ± 0.04 b</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>128.96 ± 33.59 a</td>
<td>132.93 ± 5.42 a</td>
<td>110.41 ± 3.89 a</td>
<td>109.73 ± 2.38 a</td>
<td>149.80 ± 7.46 a</td>
<td>129.38 ± 35.07 a,b,c</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>56.60 ± 23.26 a,b,c</td>
<td>54.26 ± 2.83 a,b,c</td>
<td>44.14 ± 7.26 a</td>
<td>47.34 ± 8.95 a</td>
<td>45.45 ± 10.13 a</td>
<td>46.74 ± 9.02 a,b,c</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>39.33 ± 31.76 a</td>
<td>49.43 ± 12.95 a</td>
<td>25.90 ± 24.04 a</td>
<td>20.29 ± 2.49 a</td>
<td>13.12 ± 8.22 a</td>
<td>18.39 ± 5.06 a,b,c</td>
</tr>
<tr>
<td>Diethyl succinate</td>
<td>2.22 ± 0.19 a,b,c</td>
<td>1.94 ± 0.12 a,b,c</td>
<td>3.23 ± 1.81 a</td>
<td>4.91 ± 0.76 a</td>
<td>3.41 ± 0.40 a</td>
<td>3.04 ± 0.09 a,b,c</td>
</tr>
<tr>
<td>2-Phenylethyl acetate</td>
<td>192.63 ± 9.33 a</td>
<td>155.43 ± 8.56 a</td>
<td>176.12 ± 34.82 a</td>
<td>183.17 ± 23.53 a</td>
<td>15.66 ± 0.36 b</td>
<td>12.09 ± 0.63 b</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>129.21 ± 20.72 a</td>
<td>103.84 ± 7.31 a</td>
<td>121.81 ± 5.57 a</td>
<td>118.85 ± 24.32 a</td>
<td>166.69 ± 33.91 a</td>
<td>139.82 ± 0.79 a,b,c</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>232.50 ± 19.20 a,b,c</td>
<td>210.00 ± 8.85 a,b,c</td>
<td>191.18 ± 16.89 a</td>
<td>215.68 ± 1.43 a</td>
<td>336.11 ± 15.57 c</td>
<td>278.22 ± 17.99 b,c</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>758.49 ± 27.45 a,b,c</td>
<td>660.63 ± 16.35 a,b,c</td>
<td>650.38 ± 36.62 a</td>
<td>653.00 ± 16.05 a</td>
<td>918.75 ± 59.92 b</td>
<td>734.80 ± 121.04 b,c</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>1551.41 ± 127.82 a</td>
<td>1361.09 ± 58.87 a</td>
<td>1084.24 ± 285.47 a</td>
<td>1187.44 ± 65.61 a</td>
<td>1243.41 ± 110.88 a</td>
<td>1154.40 ± 211.97 a,b,c</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>729.48 ± 349.61 a</td>
<td>780.90 ± 113.21 a</td>
<td>617.81 ± 419.42 a</td>
<td>472.01 ± 104.18 a</td>
<td>394.15 ± 167.38 a</td>
<td>454.90 ± 42.42 a,b,c</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>86.09 ± 0.91 b</td>
<td>65.17 ± 14.77 b</td>
<td>45.20 ± 21.33 a,b,c</td>
<td>45.20 ± 25.75 a,b,c</td>
<td>41.23 ± 6.34 a,b,c</td>
<td>20.96 ± 1.91 a,b,c</td>
</tr>
<tr>
<td>Linalool</td>
<td>17.99 ± 1.06 a</td>
<td>18.97 ± 3.79 a</td>
<td>18.98 ± 1.97 a</td>
<td>22.52 ± 0.03 a</td>
<td>17.62 ± 0.47 a</td>
<td>16.15 ± 0.05 a,b,c</td>
</tr>
<tr>
<td>1-octanol</td>
<td>13.14 ± 1.65 a,b,c</td>
<td>12.61 ± 1.16 a,b,c</td>
<td>18.55 ± 0.83 a</td>
<td>14.91 ± 1.29 a,b,c</td>
<td>14.71 ± 0.50 a,b,c</td>
<td>15.62 ± 0.13 a,b,c</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>24.05 ± 2.21 a,b,c</td>
<td>23.14 ± 3.95 a,b,c</td>
<td>26.37 ± 0.00 a,b,c</td>
<td>31.33 ± 1.66 b</td>
<td>30.20 ± 1.19 a</td>
<td>26.79 ± 0.69 a,b,c</td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>194.34 ± 20.03 a</td>
<td>174.50 ± 12.62 a</td>
<td>227.86 ± 29.74 a</td>
<td>244.89 ± 13.27 a</td>
<td>209.86 ± 19.23 a</td>
<td>193.43 ± 1.50 a,b,c</td>
</tr>
</tbody>
</table>

*S. cerevisiae* strains S26 and S29, with grape must or marc of the Touriga Nacional grape variety, after 96 h and applying or not MO. Values with the same superscript letter, for the same aromatic compound, are not significantly different (P<0.05).

**Note:**
- a, b, c indicate significant differences.
- MO refers to malolactic fermentation.
- MS (mass spectrometry).
- S26 (strain 26).
- S29 (strain 29).
- Others include a variety of compounds not specifically listed above.

**Table 4**

Average volatile compounds concentration (µg/L) determined by GC-MS. Results refer to the six deacidified wines through refermentation by *S. cerevisiae* strains S26 and S29, with grape must or marc of the Touriga Nacional grape variety, after 96 h and applying or not MO. Values with the same superscript letter, for the same aromatic compound, are not significantly different (P<0.05).
Figure 1