Effects of acetic acid, ethanol and SO_2 on the removal of volatile acidity from acidic wines by two S*accharomyces cerevisiae* commercial strains

Vilela-Moura A. [(1)], Schuller D. [(2)], 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

Running title: Biological removal of volatile acidity from wines

*For correspondence:

Manuela Côrte-Real

e-mail: mcortereal@bio.uminho.pt

Tel.: (+351) 253 604 314

Fax: (+351) 253 678 980.

ABSTRACT

Herein we report the influence of different combinations of initial concentration of acetic acid and ethanol on the removal of acetic acid from acidic wines by two commercial *Saccharomyces cerevisiae* strains S26 and S29. Both strains reduced the volatile acidity of an acidic wine (1.0 g l^{-1} acetic acid and 11% (v/v) ethanol) by 78% and 48%, respectively. Acetic acid removal by both strains was associated with a decrease in ethanol concentration of about 0.7 – 1.2% (v/v). Strain S26 revealed better removal efficiency due to its higher tolerance to stress factors imposed by acidic wines. We also demonstrate that the strong anti-oxidant and antiseptic effect of sulphur dioxide (SO_2) concentrations up to 170 mg l^{-1} inhibit the ability of both strains to reduce the volatile acidity of an acidic wine under our experimental conditions. Therefore, deacidification should be carried out either in wines stabilized by filtration or in wines with SO_2 concentrations below 75 mg l^{-1}. Deacidification of wines with the better performing strain S26 was associated with changes in the concentration of volatile compounds. The most pronounced increase was observed for isoamyl acetate (banana) and ethyl hexanoate (apple, pineapple), with an 18- and 25-fold increment, respectively, to values above the detection threshold. The acetaldehyde concentration of the deacidified wine was 2.3 times higher, and may have a detrimental effect on the wine aroma. In addition, deacidification led to increased fatty acids concentration, but still within the range of values described for spontaneous fermentations, and with apparently no negative impact on the organoleptical properties. We propose the use of *S. cerevisiae* strain S26 for the efficient reduction of the volatile acidity from acidic wines with acetic acid and ethanol concentrations not higher than 1.0 g l^{-1} and 11% (v/v), respectively.

INTRODUCTION

Acetic acid is the main component of the volatile acidity of wines and is therefore critical for wine quality. Its concentration in wines is approximately 0.5 g l^{-1} and should remain below 20 milliequivalents.l^{-1}, i.e. 1.2 g l^{-1} (expressed as acetic acid), according to European legislation (OIV 2009).

Quite a few authors have studied the production of volatile acidity by *Saccharomyces cerevisiae* under winemaking conditions with initial sugar concentrations around 200 g l^{-1}. Volatile acidity is formed at the beginning of cell growth (Alexandre et al. 1994; Coote and Kirsop 1974) and its production is affected by the yeast strain (Radler 1993; Giudici et al. 1995; Henschke 1997; Patel and Shibamoto 2002; Erasmus et al. 2004), the medium composition, vitamins, initial sugar concentration and fermentation conditions such as temperature variations (Monk and Cowley 1984). Wine yeasts produce acetic acid as a by-product of the hyperosmotic stress response caused by high sugar concentrations (>35 Brix) in grape must (Erasmus et al. 2004). In wines made from botrytized grapes, the increase of the initial sugar concentration (from 189 to 391 g l^{-1}) augments the volatile acidity concentration from 0.56 to 1.46 g l^{-1} (Lafon-Lafourcade and Ribéreau-Gayon 1977). It was shown that both the high sugar content and compounds like gluconic acid and glycerol produced due to *Botrytis* infection can affect the biological aging of the wine. In aging, if wine's gluconic acid content is more than 600 mg l^{-1}, heterolactic fermentations appear with certain intensity, producing high concentrations of lactic acid and volatile acidity (Ribéreau-Gayon et al. 1979; Perez et al. 1991). Other winemaking factors that favor the production of acetic acid by *S. cerevisiae* are: anaerobiosis, pH values below 3.1 or above 4.0 (Ribéreau-Gayon et al. 2000; Radler 1993). In addition, high acetate content in a wine, after a strong clarification of the must, is due to a depletion of yeast intracellular metabolites such as amino acids, unsaturated fatty acids, polyphenolic compounds and metals (Moruno et al. 1993). Overexpressing the glycerol 3-phosphate dehydrogenase gene, *GPD2*, caused *S. cerevisiae* to produce more than twice as much acetic acid as

the wild-type strain (S288C background) in anaerobic cell culture. However, deletion of the aldehyde dehydrogenase gene, *ALD6*, in wild-type and *GPD2* overexpressing strains decreased acetic acid production by three- and four-fold, respectively (Eglinton et al. 2002).

Effects derived from nutrient imbalance and competition between coexisting yeasts and bacterial populations during concurrent malolactic fermentations (Boulton et al. 1998) and citric acid metabolism (Davis et al. 1986) can also increase acetic acid content in wines. Malolactic fermentation performed by *Oenococcus oeni* and *Lactobacillus plantarum* modify the amino acid and volatile composition of the wine and also increase the initial volatile acidity (Lonvaud-Funel 1999). Acetic acid bacteria that can be found in fresh must (*Gluconobacter oxydans*) or species that predominate during fermentation (*Acetobacter pasteurianus* and *A. liquefaciens*) can also increase the acetic acid content of must or wines and might cause spoilage (Du Toit and Lambrechts 2002).

Few processing options are available to winemakers to remove sensorially objectionable levels of volatile acidity (above 1.0 g l^{-1}). Bioreduction methods using yeasts have been known for a long time. They basically consist in a refermentation associated with acetic acid consumption by yeasts (Ribéreau–Gayon et al. 2000; Vilela-Moura et al. 2008). However, they have not been sufficiently well characterised for commercial application.

Even though sugars are the preferential carbon and energy source of *S. cerevisiae*, non-fermentable substrates, such as acetic acid, can also be used for the generation of energy and cellular biomass (Schüller 2003). Although uptake of acetic acid may occur by passive diffusion, evidence for the existence of at least one acetate carrier in *S. cerevisiae* has been obtained (Casal et al. 1996; Paiva et al. 1999). The product of the gene *Jen1* is required for the uptake of lactate and other monocarboxylates in the yeast *S. cerevisiae* (Casal et al. 1999). A molecular approach addressing acetic acid induced stress response indicates the ubiquitin-mediated internalization of the aquaglyceroporin Fps1p, downregulating the flux of undissociated acetic acid into the cell (Mollapour and Piper 2007). Metabolic conversion of acetate into glucose-6-phosphate can be

divided into three separate pathways: production of acetyl-CoA, production of oxaloacetate by the glyoxylate cycle and gluconeogenesis (Schüller 2003; Dos Santos et al. 2003).

Grape must can be considered a culture medium that is far from optimum for most microorganisms. Upon inoculation, yeast cells must adapt to a fermentative environment that gradually changes during fermentation and that imposes multiple stress conditions such as high osmolarity (sugar concentration up to 300 g l^{-1}), low pH (2.9-3.8) (Pizarro et al. 2007), sulfur dioxide (SO_2) presence between 40 and 100 mg l^{-1} (Viegas et al. 1989), ethanol toxicity (Viegas et al. 1989), temperature variations (Pizarro et al. 2007) and increasing nitrogen limitation (Albers et al. 1996; Blateyron and Sablayrolles 2001; Mendes-Ferreira et al. 2004). A refermentation process, that aims to reduce excessive volatile acidity, imposes additional stress through elevated acetic acid concentrations. This may lead to a reduced cellular growth (Thomas and Davenport 1985; Pampulha and Loureiro 1989), induced cellular death (Pinto et al. 1989) and stuck fermentations (Rasmussen et al. 1995; Edwards et al. 1999; Eglinton and Henschke 1999).

Most of the SO_2 in wines is added as antioxidant at the beginning of fermentation to achieve microbiological control of must by limiting and/or preventing the propagation of undesirable yeasts and bacteria. However, a small amount of SO_2 is produced as a fermentation byproduct. SO_2 enters the yeast cell through diffusion and reacts, in the dissociated form, with cytoplasmatic enzymes, coenzymes and vitamins, leading ultimately to growth cessation and death (Romano and Suzzi 1992). As an antioxidant, SO_2 protects the fruit-like organoleptical qualities and supports wine color stability by inhibiting the activity of polyphenoloxidases (Boulton et al. 1998; Ribéreau-Gayon et al. 2000). SO_2 also prevents the conversion of acetaldehyde into ethanol, through inhibition of aldehyde dehydrogenase and binding with acetaldehyde (Frivik and Ebeler 2003). The rules of the International Organisation of Vine and Wine (OIV) consider 150 mg l^{-1} and 200 mg l^{-1} as maximum limits for final SO_2 concentrations of red and white wines, respectively. The maximum limit of 400 mg l^{-1} SO_2, applies to certain sweet white wines (OIV 2009).

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 distinctive capacity to consume acetic acid from a mixed culture medium containing two-thirds of a minimal medium and one third of an acidic white wine. When the media were supplemented with glucose (13% or 3.3 % w/v) and ethanol (4% or 10%, v/v) and strains were incubated under aerobic or limited aerobic conditions for 48 to 72 hours, the commercial strains S26 and S29 appeared to be the most promising candidates for efficient acetic acid removal. Strain S26 consumed 87% of acetic acid in a medium containing low glucose (3.3 %, w/v) and high ethanol (10%, v/v) concentration after 72 hours of incubation under aerobic conditions. Strain S29 consumed 83% of acetic acid under limited-aerobic conditions and in a medium containing high glucose (13 %, w/v) and low ethanol (4%, v/v) concentration after 48 hours of incubation. We also showed that the commercial *S. cerevisiae* strain S26 efficiently removes 61.5 % of the acetic acid when grown in an acidic white wine under limited-aerobic conditions (Vilela-Moura et al., 2008).

To further evaluate the applicability of *S. cerevisiae* strains in the deacidification of acidic wines, we herein assess acetic acid reduction by strains S26 and S29 under the very stressful conditions imposed by different combination of ethanol, acetic acid and SO_2 concentrations. We showed that strain S26 deacidifies wines containing up to 1.0 g l^{-1} acetic acid, 11% (v/v) ethanol and less than 100 mg l^{-1} SO_2 more efficiently than strain S29. Removal of excessive acetic acid by strain S26 exerts no major detrimental effect on wine volatile compounds.

MATERIALS AND METHODS

Microorganisms

In this study the *S. cerevisiae* commercial strains S26 and S29 (our internal references) were used. Both strains were kindly provided by Lalvin and Enoferm, respectively. The strains were kept at -80 ºC in micro tubes containing YPD broth (glucose 2%, w/v; peptone 1%, w/v; yeast extract 0.5%, w/v) supplemented with glycerol (30%, v/v).

Culture media and growth conditions

Frozen aliquots of yeast 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 during 48 hours at 25ºC prior to each experiment. Pre-cultures were grown overnight (25 ºC, 120 rpm) in 10 ml of a commercial acidic white wine to be tested and the cells were transferred to 250 ml Erlenmeyer flasks containing 230 ml of acidic wine, prepared as described in the following section. The initial cellular density was adjusted to 10^6 cells ml^{-1} ($OD_{640\ nm}$ 0.2), and incubation was carried out at 25ºC, 100 rpm. Throughout experiments, yeast cell concentration ($OD_{640\ nm}$) and viability (CFU/ml) was determined. All experiments were performed in triplicate.

Removal of acetic acid from acidic wines

Strains S26 and S29 were used to assess the influence of different ethanol and acetic acid concentrations on the removal of acetic acid from a commercial white wine (filter-sterilized, Millipore, 0.22 μm pore size) with the composition described in Table 1. Volatile acidity was adjusted to 1.0 g l^{-1}, 1.5 g l^{-1} and 1.75 g l^{-1} using glacial acetic acid (Merck, Darmstadt, Germany); ethanol was adjusted to 11% or 12% (v/v) using absolute ethanol (Merck, Darmstadt, Germany); the

pH was set to 3.5, using NaOH (0.1 M). The same wine was used to assess the influence of SO_2 addition (25, 50 and 100 mg l^{-1}), adding potassium metabisulphite (6%, w/v) after acetic acid, ethanol and pH adjustment to 1.0 g l^{-1}, 11% (v/v) and 3.5, respectively.

Analytical determinations

Acetic acid and ethanol concentrations were determined at the time points indicated using enzymatic kits (Enzytec, Scil Diagnostics, Viernheim, Germany). Analysis of the density, pH, alcohol concentration, volatile acidity, SO_2 and titratable acidity were performed as outlined in Table 1.

Solid-phase micro-extraction (SPME) extraction and GC-MS determination of aromatic compounds were carried out as previously described (Mendes-Ferreira et al. 2009). Briefly, SPME was achieved through adsorption of volatiles onto a fiber (100 µm polydimethylsiloxane –PDMS-, 85 µm Carboxen–polydimethylsiloxane -CAR/PDMS- and 50/30 µm Divinylbenzene/Carboxen/PDMS -DVB/CAR/PDMS). Extractions in headspace mode were carried out at 20 ± 1°C with magnetic stirring (1300 rpm). 2-octanol was used as an internal standard solution. Chromatographic analysis was performed, in the splitless mode, using an Agilent 6890 N gas chromatograph equipped with a 5973N mass spectrometer. The column employed was an Innovax capillary column, 30 m X 0.25 mm, with 0.5 µm film thickness (Agilent, Santa Clara, CA, USA) and helium (helium N60, Air Liquid, Portugal) was used as the carrier gas at 34 cm.s^{-1} average linear velocity. 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 ºC per minute up to 240 ºC, where it was held for 15 minutes. 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^{-1}. Spectra identification of sample compounds was supported by the Wiley database (Wiley/NBS Registry of Mass Spectral Data, 1989). Whenever

possible, identification was confirmed by comparing mass spectra and retention indices with those of authentic standards.

Statistical analysis

Acetic acid consumption and all the analytical parameters determined in the different assays were submitted to variance analysis (ANOVA) using the STATISTICA 7.0 software (StatSoft Inc., 2004). Tukey honestly significant difference (HSD) test was applied to the chemical data to determine the presence of significant differences between the analyzed samples; the model was statistically significant with a *P* value less than 0.05.

RESULTS

Combined effect of acetic acid and ethanol on the reduction of volatile acidity

Herein, we further assess the capacity of the commercial *S. cerevisiae* strains S26 and S29 to consume acetic acid under the very stressful growth conditions imposed by the combination of high ethanol (11 and 12%, v/v) and acetic acid (1.0 g l^{-1}, 1.5 g l^{-1} and 1.75 g l^{-1}) concentrations under limited aerobic conditions. As shown in Table 2, strains S26 and S29 reduced 78 % and 48%, respectively, of the acetic acid during 168 hours of incubation in an acidic wine with 11% (v/v) ethanol and 1.0 g l^{-1} acetic acid. Under these conditions, acetic acid reduction by strain S26 was significantly higher than strain S29. As expected, the titrable acidity decreased from 8.90 g l^{-1} to 3.77 g l^{-1} (S26) and 4.60 g l^{-1} (S29). With increasing initial acetic acid concentrations, the percentage of consumed acetic acid decreased by (i) 24.9% and 8.9% for S26 and S29 strains respectively, (wine with initial concentration of 1.5 g l^{-1} of acetic acid) and (ii) 21.7% and 14.7% for strains S26 and S29, respectively (wine with initial concentration of 1.75 g l^{-1} acetic acid). Some (not significant) ethanol consumption (0.7 to 1.3 %) was observed in all experiments. No significant

changes were observed for both strains regarding pH, total and free SO_2 concentration at the end of the incubation period of 168 hours.

For an initial ethanol concentration of 11% (v/v) only the acidic wine with an initial volatile acidity of 1.0 g l^{-1} was permissive for growth of strain S26 that concluded 3 cell divisions during 168 hours of incubation (Fig. 1). The most pronounced removal of acetic acid by both strains was not associated with cell growth. Strain S26 passed through a 24 h lag phase associated with the most evident acetic acid consumption (about 55%). In a second stage, cell density increased from 0.2 to 1.4 OD_{640nm}, but acetic acid removal was less efficient (about 23%). In parallel, the ethanol concentration decreased by 0.6 % (v/v). Contrarily, strain S29 showed no growth in wines with 11% (v/v) of ethanol at the acetic acid concentrations tested. This strain was however capable to consume about 40% of the acid during the first 48 hours of incubation, when the initial acetic acid concentration was 1.0 g l^{-1}, as previously described for strain S26. This happened probably because of the high inoculum's concentration (OD_{640nm} of 0.2, corresponding to10^6 CFU ml^{-1}). The lack of acetic acid consumption at later stages by both strains and higher initial acetic acid concentrations was most probably caused by metabolism inhibition, which is reflected by the loss of cellular viability after 96 hours. Both strains were not able to deacidify acidic wines with 12% (v/v) of ethanol and any of the three acetic acid concentrations tested (not shown).

Effect of sulphur dioxide on the removal of acetic acid from an acidic wine by strains S26 and S29

Considering that the SO_2 concentration of white wines should not exceed 200 mg l^{-1} (according the recommendations of the OIV), the effect of different SO_2 concentrations on acetic acid removal from an acidic white wine by strains S26 and S29 was also assessed. The volatile acidity and ethanol concentration of the commercial wine used (Table 1) was adjusted to 1.0 g l^{-1} and 11%

(v/v), respectively and the pH was set to 3.5. The wine was supplemented with SO_2 (25, 50 and 100 mg l^{-1}).

Table 3 shows that the total SO_2 concentration in the deacidified wine after 72 h was proportional to the three different amounts of SO_2 added to the wine. The initial concentration of acetic acid was not significantly reduced ($P \geq 0.05$) after deacidification with strains S26 and S29 indicating the strains´ inability to remove acetic acid from acidic wines that were supplemented with 25 mg l^{-1} of SO_2. For both strains and the wine with 1.0 g l^{-1} of acetic acid and 11% (v/v) of ethanol, the addition of 25 and 50 mg $l^{-1}$of SO_2 completely inhibited cell growth and induced loss of cell viability after 24 hours of inoculation. For higher SO_2 concentrations (100 mg l^{-1}) both strains started to die since the beginning of incubation (data not shown). The complete growth inhibition and cell death can be attributed to the strong anti-oxidant and antiseptic properties combined with the high ethanol and acetic acid concentrations.

Changes in wine aromatic compounds during deacidification with strain S26

As shown in the first section, strain S26 showed a higher resistance to the combined effects of ethanol and acetic acid and was also superior to strain S29 regarding acetic acid removal efficiency (Table 2). We therefore evaluated the impact of strain S26 on the aromatic profile after deacidification of an acidic wine with initial concentrations of 11 % (v/v) ethanol and 1.0 g l^{-1} of acetic acid. Strain S26 increased significantly the concentration of the following compounds of the ester fraction (Table 4): ethyl acetate (solvent like), isoamyl acetate (banana), ethyl propionate (ethereal, fruity, rum-like), ethyl isobutyrate (strawberry, ethereal, buttery notes), ethyl butyrate (pineaple notes), ethyl hexanoate (apple, pineapple, anise seed notes) that contribute to the wine`s bouquet in a positive way (excepting ethyl acetate). Isoamyl acetate and ethyl hexanoate were the only esters that increased above the detection thresholds of 30 µg l^{-1} and 5-14 µg l^{-1}, respectively.

Ethyl acetate and diethyl succinate were the esters present in highest concentrations in the deacidified wine. Ethyl acetate has a solvent like odor, considered to be a defect, but was found in concentrations lower than the detection threshold. The concentration of diethyl succinate (fruity - melon aroma) occurred in concentrations higher than the detection threshold in the uninoculated wine and did not change during deacidification.

Among the aldehydes and fusel alcohols, acetaldehyde concentration increased 2.3 fold to 225 mg l^{-1} after deacidification with strain S26. The agitation of the culture duplicated the initial dissolved O_2 from 4 mg l^{-1} to 8 mg l^{-1}, which explains the increased acetaldehyde concentration. This aldehyde has a grass or green-apple like aroma when above 100 mg l^{-1} (Carlton et al. 2007). Fusel alcohols (2-phenylethanol and isoamyl alcohol) cause off-flavors at high concentrations, whereas low concentrations of these compounds and their esters make an essential contribution to the aroma/flavor of wine. Isoamyl alcohol has a bitter, marzipan, burnt, whisky-like and harsh aroma and 2-phenylethanol, a compound with floral, rose-like notes. These two compounds were present in concentrations higher than their detection threshold, but there were no significant concentration differences between the acidic and the deacidified wines. The concentrations of the terpene alcohols linalool, α-terpineol (floral like odors) did not change significantly through deacidification. Citronellol concentration increased significantly, but remained below the detection limit.

The composition of the fatty acid fraction was also evaluated. Small amounts of these volatile compounds contribute positively to the wine quality, while excessive concentrations exert detrimental effects. Significant differences in their concentration resulted from the deacidification process. Butyric and isovaleric acids, not detectable in the acidic wine, increased to 0.6 and 0.3 mg l^{-1}, respectively after deacidification by strain S26; these concentrations were 3.7 and 10-fold higher than their detection threshold in wine, respectively. Hexanoic acid increased slightly but remained below the detection threshold. Octanoic acid has a grass acid like odor and occurred in lower

concentrations after deacidification, probably due to the conversion to the corresponding ester ethyl octanoate.

DISCUSSION

This publication adds new information on the effect of several wine parameters on removal of acetic acid from a white wine by two previously characterised commercial *S. cerevisiae* strains. We evaluated the combined effects of ethanol, acetic acid and SO_2 on the acetic acid removal efficiency of strains S26 and S29, using an acidic white wine. We found that strain S26 was able to grow in an acidic wine with 11% (v/v) of ethanol and 1.0 g l^{-1} of acetic acid after 24 hours of inoculation, and to consume 78% of the total amount of acetic acid after 168 hours. Under these conditions, strain S29 consumed just 48.3 % of the acetic acid, was unable to grow and lost viability after 96 hours. This indicates a lower tolerance of strain S29 to the combined effects of high concentration of acetic acid and ethanol. Both strains were unable to grow when ethanol concentration was adjusted to 12 % (v/v) and acetic acid concentrations were maintained (1.0 g l^{-1}, 1.5 g l^{-1}, 1.75 g l^{-1}). This shows that refermentation imposes very severe stress conditions and only few strains might be capable to cope with. Additional inhibitory effects can be exerted by sulphur dioxide (SO_2).

Sulphur dioxide has become practically obligatory in winemaking. This substance combines three important beneficial properties: antimicrobial and antioxidant activity, as well as the ability to synthetize non-volatile bisulfite adducts, which prevents their undesirable sensory properties. SO_2 combines also with oxygen and binds to sugars, aldehydes such as acetaldehyde and ketones, decreasing its properties as a wine stabilizing agent (Frivik and Ebeler 2003). Recently, it has become apparent that SO_2 can induce allergic reactions in humans (Ribéreau-Gayon et al. 2000) which led to the establishment of legal limits for its concentration in wine. When the concentration of total SO_2 was 95 mg l^{-1} (70 mg l^{-1} of the initial acidic wine + 25 mg l^{-1} of added SO_2), and still considerably below the SO_2 limit recommended by the OIV for white wines (200 mg l^{-1}) acetic acid

removal by both strains was completely inhibited. In fact, there was no significant reduction of volatile acidity and ethanol. Almost all the added SO_2 was combined. Therefore, the SO_2 levels of the acidic wines to be treated by the yeast should not exceed 75 mg l^{-1}. Deacidification should be preferentially carried out in wines stabilized with lower SO_2 concentrations or by filtration. However, it should be considered that these results were obtained in a micro-scale setting and still need to be evaluated in a winery large-scale approach.

Strain S26 was most efficient for biological deacidification of acidic wines and also showed a higher resistance to the combined effects of acetic acid and ethanol. Changes in volatile compounds associated with deacidification were therefore evaluated only for this strain. Both acetate and ethyl esters were present in significantly higher concentrations in the deacidified wine excepting ethyl-2 methylbutyrate, ethyl isovalerate and ethyl decanoate. The aromatic potential of these ester compounds, associated with fruity and floral notes, positively enhances the wine`s bouquet. The most pronounced increase was observed for isoamyl acetate (banana) and ethyl hexanoate (apple, pineapple), with an 18- and 25-fold increment, respectively, to values above the detection threshold. Acetate and ethyl esters are synthesized by carboxylesterases or transferases acting on acyl-CoA (Mckay 1993) by condensation of an alcohol and a coenzyme-A-activated acid (acyl-CoA). In *S. cerevisiae*, acetate esters result from the combination of acetyl-CoA with an alcohol, by the action of the alcohol acetyl transferases Atf1p and Atf2p (Lambrechts and Pretorius 2000). Ethyl esters are generated from acyl-CoA and ethanol by the action of Eht1p and Eeb1p (Mason and Dufour 2000; Saerens et al. 2006). The capacity of yeast to synthesise these compounds varies between strains (Lambrechts and Pretorius 2000; Wondra and Boveric 2001). The incubation temperature during the deacidification assay (25ºC) might have contributed to the formation of acetate and ethyl esters. Molina and collaborators (2007) showed that lower temperatures (15ºC) increased the concentration of ester compounds associated to fresh and fruity aromas. Higher temperatures (28ºC) increased the

concentration of compounds associated to flowery, banana and pineapple attributes, the predominant aromas in the S26-deacidified wine.

Acetaldehyde concentration increased to 225 mg l^{-1} after deacidification with strain S26. However, its initial concentration (94.8 mg l^{-1}) was already close to the upper limit of the concentration range found in white wines (Liu and Pilone 2000). This compound causes more concern for its aroma (grass, apple or sherry-like character when occurring in concentrations higher than 100 mg l^{-1}). This does not apply to all wine styles because high levels of acetaldehyde (up to 500 mg l^{-1}) are considered a unique feature of sherry wines (Liu and Pilone 2000). Besides, acetaldehyde binds sulphur dioxide and has therefore a negative impact on wine stability. Contrarily, lower acetaldehyde concentrations increase flavor complexity, due to the fruity and pleasant aroma, in particular in red wines (Frivik and Ebeler 2003). Aldehyde synthesis is affected by several factors such as the yeast strain, temperature, pH, nutrient availability, O_2 and SO_2 concentration. SO_2 is particularly important since it affects aldehyde dehydrogenase and thus the conversion of acetaldehyde into ethanol (Fivrik and Ebeler 2003). Besides, acetaldehyde is an intermediate product of yeast metabolism and a precursor of acetate, acetoin and ethanol (Romano et al. 1997). Its production through ethanol oxidation is strain dependent (Romano et al. 1994) and is favoured by O_2. In our previous work (Vilela-Moura et al. 2008) we showed that efficient acetic acid reduction requires some oxygen as provided by the limited-aerobic experimental setup used. Therefore, the expectation that this oxygen requirement had an impact on the acetaldehyde level, was confirmed. Nevertheless, we consider that the significance of increased acetaldehyde concentrations after deacidification still needs to be evaluated for different types of wines.

Fatty acids contribute positively to the wine quality when present in small concentrations, while excessive concentrations have detrimental effects. Their detection thresholds in water are respectively, 173 µg l^{-1} for butyric acid, 33.4 µg l^{-1} for isovaleric acid, 420-3000 µg l^{-1} for hexanoic acid, 500 – 8800 µg l^{-1} for octanoic acid and 1000 – 15000 µg l^{-1} for decanoic acid (Ferreira et al.

2000; Guth 1997). However, in spontaneously fermented wine these compounds may occur in concentrations higher than their detection threshold, namely, 650 μg l^{-1} for butyric acid; 51 μg l^{-1} for isovaleric acid; 2807 μg l^{-1} for hexanoic acid; 5711 μg l^{-1} for octanoic acid and 2033 μg l^{-1} for decanoic acid (Nurgel et al. 2002). Since the fatty acid concentrations we found in the acidic wine deacidified with strain S26 were close to those found in spontaneously fermented wine and had no detrimental effect on wine aroma (Nurgel et al. 2002), we infer that the observed increase in their concentrations had also no detrimental effect in deacidified wine aroma.

In general terms, the formation of new volatile compounds during the deacidification process altered the aromatic profile, increasing mainly the fraction of volatile ester compounds up to 25-fold. In contrast, the formation of ethyl acetate and acetaldehyde may cause some apprehension. However, only the human perception can reveal the true nature of the consequences of the deacidification process in terms of wine volatile complexity, and if pleasant aromatic compounds were formed, we may assume that acetaldehyde is not a major problem.

In summary, we propose the use of *S. cerevisiae* commercial strain S26 for the efficient reduction of the volatile acidity from acidic wines with acetic acid and ethanol concentrations not higher than 1.0 g l^{-1} and 11% (v/v), respectively.

ACKNOWLEDGEMENTS

This work was funded by Institute for Biotechnology and Bioengineering, Centre of Genetics and Biotechnology (IBB/CGB-UTAD) and by the projects PTDC/AGRALI/71460/2006, POCI/AGR/56102/2004 and PTDC/AGR-ALI/103392/2008 from the Portuguese Research Agency (Fundação para a Ciência e Tecnologia). The research leading to these results has also received funding from the European Community´s Seventh Framework Programme (FP7/2007-2013) under

grant agreement nº 232454. The authors thank Paula Ribeiro for SPME extractions and Virgílio Falco for support on GC-MS analysis of wines.

REFERENCES

Albers E, Larsson C, Liden G, Niklasson C, Gustafsson L (1996) Influence of the nitrogen source on *Saccharomyces cerevisiae* anaerobic growth and product formation. Appl Env Microbiol 62:3187-3195

Alexandre H, Nguyen Van Long T, Feuillat M, Charpentier C (1994) Contribution à l'étude des bourbes: influence sur la fermentescibilité des moûts. Rev Fr Œno 146:11–20

Boulton RB, Singleton VL, Bisson LF, Kunkee RE (1998) Yeasts and biochemistry of ethanol fermentation, principles and practices of winemaking, 1st edn. Springer, New York, pp 102–192

Blateyron L, Sablayrolles JM (2001) Stuck and slow fermentations in enology: statistical study of causes and effectiveness of combined additions of oxygen and diammonium phosphate. J Biosci Bioeng 91(2):184-189

Carlton WK, Gump B, Fugelsang K, Hasson AS (2007) Monitoring acetaldehyde concentrations during micro-oxygenation of red wine by headspace solid-phase microextraction with on-fiber derivatization. J Agric Food Chem 55:5620-5625

Casal M, Cardoso H, Leão C (1996) Mechanisms regulating the transport of acetic acid in *Saccharomyces cerevisiae*. Microbiol 142(6):1385-1390

Casal M, Paiva S, Andrade RP, Gancedo C, Leão C (1999) The lactate proton symport of *Saccharomyces cerevisiae* is encoded by *JEN1*. J Bacteriol 181:2620-2623

Coote N, Kirsop HH (1974) The content of some organic acids in beer and other fermented media. J Inst Brew 80:474–483

Davis CR, Wibowo DJ, Lee TH, Fleet GH (1986) Growth and metabolism of lactic acid bacteria during and after malolactic fermentation of wines at different pH. Appl Environ Microbiol 51(3):539-545

Delfini C, Cocito C, Bonino M (1999) A review: Biochemical and molecular mechanisms in *Saccharomyces cerevisiae* that are involved in the formation of some volatile compounds in wines. J Int Sci Vigne Vin 33(4):195-211

Dos Santos MM, Gombert AK, Christensen B, Olsson L, Nielsen J (2003) Identification of in vivo enzyme activities in the cometabolism of glucose and acetate by *Saccharomyces cerevisiae* by using 13C-labeled substrates. Eukaryot Cell 2(3):599-608

Du Toit WJ, Lambrechts MG (2002) The enumeration and identification of acetic acid bacteria from South African red wine fermentations. Int J Food Microbiol 74(1-2):57-64

Edwards CG, Reynolds AF, Rodriguez AV, Semon MJ, Mills JM (1999) Implication of acetic acid in the induction of slow/stuck grape juice fermentations and inhibition of yeast by *Lactobacillus sp*. Am J Enol Vitic 50(2):204-210

Eglinton JM, Henschke PA (1999) Restarting incomplete fermentations: the effect of high concentrations of acetic acid. Aust J Grape Wine Res 52:71-78

Eglinton JM, Heinrich AJ, Pollnitz AP, Langridge P, Henschke PA, Lopes MB (2002) Decreasing acetic acid accumulation by a glycerol overproducing strain of *Saccharomyces cerevisiae* by deleting the *ALD6* aldehyde dehydrogenase gene. Yeast 19(4):295–301

Erasmus DJ, Cliff M, van Vuuren HJJ (2004) Impact of yeast strain on the production of acetic acid, glycerol, and the sensory attributes of icewine. Am J Enol Vitic 55(4):371-378

1 Escudero A, Gogorza B, Melús MA, Ortín N, Cacho J, Ferreira V (2004) Characterization of the
2 aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. J
3 Agric Food Chem 52:3516-3524

4 Etievant PX (1991) Wine. In: Maarse H (ed) Volatile compounds in foods and beverages. 2nd edn.
5 Marcel Dekker, New York, pp 483–546

6 Ferreira V, Lopez R, Cacho JF (2000) Quantitative determination of the odorants of young red
7 wines from different grape varieties. J Sci Food Agric 80(11):1659-1667

8 Frivik S, Ebeler S (2003) Influence of sulfur dioxide on the formation of aldehydes in white wine.
9 Am J Enol Vitic 54(1):31-38

10 Giudici P, Zambonelli C, Passarelli P, Castellari L (1995) Improvement of wine composition with
11 cryotolerant *Saccharomyces* strains. Am J Enol Vitic 46:1:143-147

12 Guth H (1997) Identification of character impact odorants of different white wine varieties. J Agric
13 Food Chem 45:3027–3032

14 Henschke P (1997) Wine yeast. In: Zimmerman FK, Entian KD (ed) Yeast sugar metabolism,
15 biochemistry, genetics, biotechnology and applications. Technomic Publishing, Lancaster, pp 527–
16 560

17 Lafon-Lafourcade S, Ribéreau-Gayon P (1977) Origines de l'acidité volatile des grands vins
18 liquoreux. C R Acad Agric 9:551–557

19 Lambrechts MG, Pretorius IS (2000) Yeast and its importance to wine aroma - a review. S Afr J
20 Enol Vitic, 21 (Special Issue):97–129

Lonvaud-Funel A (1999) Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek 76:317–331

Liu SQ, Pilone GJ (2000) An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. Int J Food Sci Technol 35:49-61

Mollapour M, Piper P (2007) Hog1 mitogen-activated protein kinase phosphorylation targets the yeast Fps1 aquaglyceroporin for endocytosis, thereby rendering cells resistant to acetic acid. Mol Cell Biol 27:6446-6456

Mason AB, Dufour J (2000) Alcohol acetyltransferases and the significance of ester synthesis in yeast. Yeast 16:1287–1298

Mateo JJ, Jiménez M (2000) Monoterpenes in grape juice and wines. J Chromatography A 881:557-567

Mckay AM (1993) Microbial carboxylic ester hydrolases (EC 3.1.1) in food biotechnology-A review. Lett Appl Microbiol 16:1-6

Mendes-Ferreira A, Mendes-Faia A, Leão C (2004) Growth and fermentation patterns of *Saccharomyces cerevisiae* under different ammonium concentrations and its implications in winemaking industry. J Appl Microbiol 97(3):540-545

Mendes-Ferreira A, Barbosa C, Falco V, Leão C, Mendes-Faia A (2009) The production of hydrogen sulphide and other aroma compounds by wine strains of *Saccharomyces cerevisiae* in synthetic media with different nitrogen concentrations. J Ind Microbiol Biotechnol 36:571–583

Molina AM, Swiegers JH, Varela C, Pretorius IS, Agosin E (2007) Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Appl Microbiol Biotechnol 77:675–687

Monk PR, Cowley PJ (1984) Effect of nicotinic acid and sugar concentration of grape juice and temperature on accumulation of acetic acid yeast fermentation. J Ferment Technol 62:515–521

Moruno EG, Delfini C, Pessione E, Giunta C (1993) Factors affecting acetic acid production by yeasts in strongly clarified grape musts. Microbios 74(301):249-56

Nurgel C, Erten H, Canbas A¸ Cabaroglu T, Selli S (2002) Influence of *Saccharomyces cerevisiae* strains on fermentation and flavor compounds of white wines made from cv. Emir grown in Central Anatolia. J Ind Microbiol Biotechnol 29(1):28–33

Office International de la Vigne et du Vin (2009). International code of oenological practices. Paris: OIV, 259 p.

Office International de la Vigne et du Vin (1990). Recueil des méthodes internationales d´analyse des vins. Paris: OIV, 368 p.

Ong PKC, Acree TE (1999) Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. J Agric Food Chem 47:665-670

Paiva S, Althoff S, Casal M, Leão C (1999) Transport of acetate in mutants of *Saccharomyces cerevisiae* defective in monocarboxylate permeases. FEMS Microbiol Lett 170(2):301-306

Pampulha ME, Loureiro V (1989) Interaction of the effects of acetic acid and ethanol on inhibition of fermentation in *Saccharomyces cerevisiae*. Biotechnol Lett 2(4):269-274

Patel S, Shibamoto S (2002) Effect of different strains of *Saccharomyces cerevisiae* on production of volatiles in Napa gamay wine and petite syrah wine. J Agric Food Chem 50:5649–5653

Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC (2004) Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chem 84:585–590

Perez L, Valcarcel MJ, Gonzalez P, Domecq B (1991) Influence of *Botrytis* infection of the grapes on the biological aging process of Fino Sherry. Am J Enol Vitic 42(1):58-62

Pinto I, Cardoso H, Leão C, van Uden N (1989) High enthalpy and low enthalpy death in *Saccharomyces cerevisiae* induced by acetic acid. Biotechnol Bioeng 33(10):1350-1352

Pizarro F, Vargas FA, Agosin E (2007) A systems biology perspective of wine fermentations. Yeast 24(11):977-991

Rasmussen JE, Schultz E, Snyder RE, Jones RS, Smith CR (1995) Acetic acid as a causative agent in producing stuck fermentations. Am J Enol Vitic 46:278-280

Radler F (1993) Yeast metabolism of organic acids. In: Gram H (ed) Wine microbiology and biotechnology, Harwood Academic Publishers, Chur, pp 165–182

Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2000) The chemistry of wine and stabilization and treatments. Handbook of enology, vol. 2, 1st edn. Wiley, Chichester.

Ribéreau-Gayon P, Lafon-Lafourcade S, Dubourdieu D, Lucmaret V, Larue F (1979) Métabolisme de *Saccharomyces cerevisiae* dans le moût de raisins parasités par *Botrytis cinerea*. C R Acad Sci 289:441–444

Rizzon LA, Miele A (2004) Avaliação da cv. Tannat para elaboração de vinho tinto. Ciênc Tecnol Aliment 24(2):223-229

Romano P, Suzzi G (1992) Wine microbiology and biotechnology. Harwood Academic Publishers, Chur

Romano P, Suzzi G, Domizio P, Fatichenti F (1997) Secondary products formation as a tool for discriminating non-*Saccharomyces* wine strains. Strain diversity in non-*Saccharomyces* wine yeasts. Antonie van Leeuwenhoek 71:239–242

Romano P, Suzzi G, Turbanti L, Polsinelli M (1994) Acetaldehyde production in *Saccharomyces cerevisiae* wine yeasts. FEMS Microbiol Lett 118(3):213-218

Saerens SM, Verstrepen KJ, Van Laere SDM, Voet AR, Van Dijck P, Delvaux FR, Thevelein JM (2006) The *Saccharomyces cerevisiae EHT1* and *EEB1* genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. J Biological Chem 281(7):4446–4456

Schüller HJ (2003) Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. Curr Genet 43(3):139–160

Thomas S, Davenport RR (1985) *Zygosaccharomyces bailii*, a profile of characteristics and spoilage activities. Food Microbiol 2:157–169

Viegas CA, Rosa MF, Sá-Correia I, Novais JM (1989) Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. Appl Environ Microbiol 55(1):21-28

Vilela-Moura A, Schuller D, Mendes-Faia A, Côrte-Real M (2008) Reduction of volatile acidity of wines by selected yeast strains. Appl Microbiol Biotechnol 80(5):881-890

1 Wondra M, Boveric M (2001) Analyses of aroma components of Chardonnay wine fermented by
2 different yeast strains. Food Technol Biotechnol 39(2):141–148

3

1 Fig. 1

2 Growth, O.D. 640 nm (A), log CFU.ml-1 3 (B), acetic acid (C) and ethanol (D) consumption by *S.*

3 *cerevisiae* strains S26 (dark symbols) and S29 (open symbols) in acidic wines with 11% (v/v)

4 ethanol and 1.0 g l-1 (◆ ,◇), 1.5 g l-1 (■,□), or 1.75 g l-1 5 (▲,△) of acetic acid

5

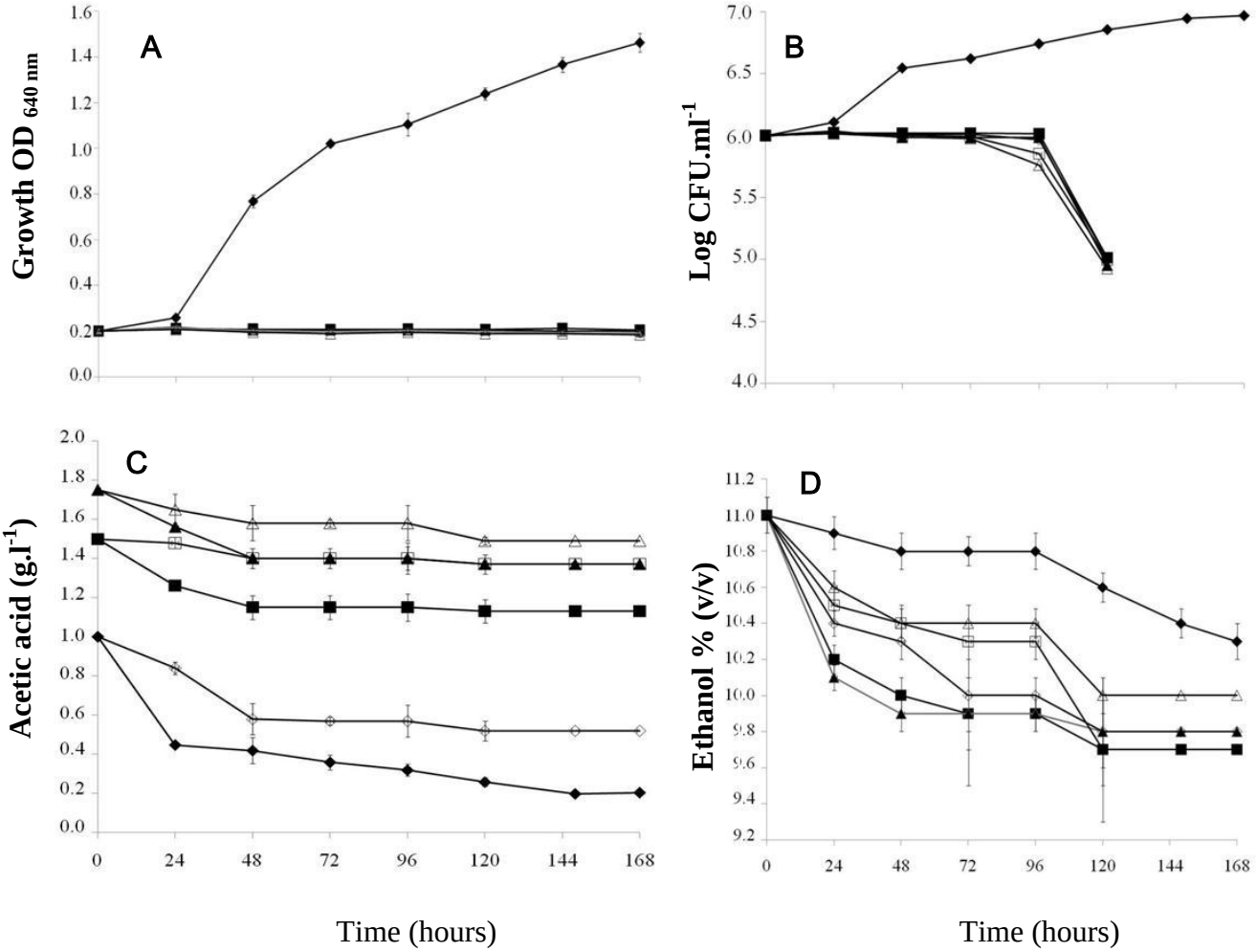
A
B
C
D
Growth OD 640 nm
Log CFU.ml-1
Acetic acid (g.l-1)
Ethanol % (v/v)
Time (hours)
Time (hours)

Fig. 1

1 Table 1

2 Physical and chemical characteristics of the wine used for deacidification assays

Chemical characteristics	White Wine	Analytical Methods (CEE N.º 2676/90)*
Density at 20ºC	0.9907	Densitometry
Free SO_2 (mg l^{-1})	3.2	Ripper Method
Total SO_2 (mg l^{-1})	70.3	Ripper Method
Volatile acidity (g l^{-1} acetic acid)	0.40	Destillation using a Cazenave-Ferré followed by titration with phenolphthalein
Residual sugar g l^{-1}	1.15	Lane-Eynon Method
Titratable acidity (g l^{-1} tartaric acid)	8.90	Titration with bromothymol blue
pH	3.10	Potentiometer
Alcoholic degree %, ethanol (v/v)	10.7	Distillation

3 * CEE N.º 2676/90 – Official Journal of the European Communities, 33, 3.10.1990. (ISSN 0257 – 7771)

Table 2

Effect of acetic acid on cell viability and oenological parameters of an acidic wine with an initial ethanol concentration of 11% (v/v) after 168 h deacidification by *S. cerevisiae* strains S26 and S29

Strains	$[Acetic\ acid]_i$ ($g\ l^{-1}$)	[Ethanol] % (v/v)	pH	Acetic acid (% consumption)	Titratable acidity ($g\ l^{-1}$)	[Total SO_2] ($mg\ l^{-1}$)	[Free SO_2] ($mg\ l^{-1}$)	$CFU.ml^{-1}$
S26	1.0	10.3±0.1 ^{b}	3.68±0.03 ^{b}	78.0±2.65 ^{e}	3.77±0.15 ^{b}	74.77±1.43 ^{b}	0.0±0.0 ^{a}	10^{7} ^{b}
S26	1.5	9.7±0.4 ^{a}	3.58±0.01 ^{a}	24.9±4.29 ^{c}	5.37±0.06 ^{a}	59.90±1.43 ^{a}	0.0±0.0 ^{a}	0 ^{a}
S26	1.75	9.8±0.2 ^{a,b}	3.57±0.01 ^{a}	21.7±0.99 $^{b, c}$	5.87±0.38 ^{a}	66.86±0.41 ^{a,b}	0.0±0.0 ^{a}	0 ^{a}
S29	1.0	9.8±0.2 ^{a,b}	3.61±0.02 ^{a}	48.3±4.73 ^{d}	4.60±0.10 ^{c}	64.75±0.98 ^{a,b}	0.0±0.0 ^{a}	0 ^{a}
S29	1.5	9.7±0.2 ^{a}	3.60±0.01 ^{a}	8.9± 3.08^{a}	5.50±0.40 ^{a}	66.93±9.40 ^{a,b}	0.0±0.0 ^{a}	0 ^{a}
S29	1.75	10.0±0.1 ^{a,b}	3.58±0.01 ^{a}	14.7±0.87 $^{a, b}$	5.80±0.20 ^{a}	65.18±3.82 ^{a,b}	0.0±0.0 ^{a}	0 ^{a}

i – Initial acetic acid concentration. The initial values of pH, titratable acidity, total and free SO_2 concentrations are referred in Table 1.The data are mean values of triplicate experiments with indication of standard deviation. Results obtained for strains and culture conditions with the same superscript letter are not significantly different ($P<0.05$)

Table 3

Effect of SO_2 addition on the oenological parameters of an acidic wine after 72 h deacidification with *S. cerevisiae* strains S26 and S29

Strains	$[SO_2]_i$ (mg l^{-1})	[Ethanol] % (v/v)	pH	[Acetic acid] (g l^{-1})	Titratable acidity (g l^{-1})	[Total SO_2] (mg l^{-1})	[Free SO_2] (mg l^{-1})	CFU.ml^{-1}
S26	25	10.6±0.2 ^{a}	3.49±0.01 ^{a}	0.99±0.03 ^{a}	5.21±0.04 ^{a}	93.68±8.71 ^{a}	2.17±0.65 ^{a}	0 ^{a}
S26	50	10.6±0.1 ^{a}	3.49±0.00 ^{a}	0.95±0.04 ^{a}	5.25±0.05 ^{a}	122.26±2.75 ^{b}	1.32±0.89 ^{a}	0 ^{a}
S26	100	10.6±0.1 ^{a}	3.47±0.01 ^{b}	0.99±0.03 ^{a}	5.14±0.04 ^{a,b}	173.01±2.18 ^{c}	0.96±0.32 ^{a}	0 ^{a}
S29	25	10.7±0.1 ^{a}	3.49±0.01 ^{a}	1.00±0.02 ^{a}	5.06±0.10 ^{b}	103.28±2.83 ^{a}	1.86±0.51 ^{a}	0 ^{a}
S29	50	10.5±0.1 ^{a}	3.49±0.01 ^{a}	0.94±0.03 ^{a}	5.13±0.03 ^{a,b}	123.14±2.62 ^{b}	2.84±0.59 ^{a}	0 ^{a}
S29	100	10.6±0.1 ^{a}	3.47±0.01 ^{b}	1.00±0.02 ^{a}	5.23±0.02 ^{a}	171.45±1.03 ^{c}	2.34±1.82 ^{a}	0 ^{a}

i – Initial SO_2 concentration. The initial values of pH, titratable acidity, total and free SO_2 concentrations are referred in Table 1. Results are mean values of triplicate experiments with their standard deviation. The initial concentrations of ethanol and acetic acid were 11% (v/v) and 1.0 g l^{-1}, respectively. Results obtained for strains and culture conditions with the same superscript letter are not significantly different (P<0.05)

Table 4

Concentration of wine aromatic compounds determined by GC-MS. Results refer to acidic white wine prior and after deacidification with *S. cerevisiae* S26 strain after 168 hours of deacidification. The odor description and detection threshold in wine refer to the references in the last column

		Concentration of wine aromatic compounds determined by GC-MS (present study)		Literature data		
Compounds		**Acidic wine ($\mu g\ l^{-1}$)**	**Deacidified wine ($\mu g\ l^{-1}$)**	**Odor description**	**Detection threshold in wine ($\mu g\ l^{-1}$)**	**References**
Ethyl acetate	**Esters**	407.5 ± 130.8 **a**	677.3 ± 126.2 **b**	Solvent like	7500 - 180000	Escudero et al. (2004); Guth (1997); Rizzon and Miele (2004)
Isoamyl acetate		1.9 ± 0.7 **a**	33.6 ± 9.4 **b**	Banana	30	Guth (1997)
2-Phenylethyl acetate		11.2 ± 1.7 **a**	16.1 ± 0.4 **a**	Roses, honey	250	Guth (1997)
Ethyl propionate		0.0 ± 0.0 **a**	13.4 ± 2.4 **b**	Ethereal, fruity, rum-like	1800	Étievant (1991)
Ethyl isobutyrate		0.0 ± 0.0 **a**	4.0 ± 1.2 **b**	Strawberry, ethereal, buttery, ripe	15	Ong and Acree (1999); Ferreira et al. (2000)
Ethyl butyrate		0.0 ± 0.0 **a**	15.2 ± 2.6 **b**	Pineapple	20	Escudero et al. (2004); Guth (1997)
Ethyl 2-methylbutyrate		0.0 ± 0.0 **a**	0.4 ± 0.6 **a**	Sweet, floral, fruity, apple	1-18	Guth (1997); Ferreira et al. (2000)
Ethyl isovalerate		0.0 ± 0.0 **a**	0.3 ± 0.5 **a**	Fruity	3	Ferreira et al. (2000)

Ethyl hexanoate		2.7 ± 0.4 [a]	68.6 ± 20.6 [b]	Anise seed, apple, pineapple	5-14	Guth (1997); Ferreira et al. (2000)
Ethyl octanoate		28.3 ± 1.8 [a]	52.0 ± 24.0 [a]	Sweet, cognac-apricot	2-5	Guth (1997); Ferreira et al. (2000)
Ethyl decanoate		5.0 ± 1.4 [a]	0.9 ± 0.2 [a]	Floral	200	Ferreira et al. (2000)
Diethyl succinate		7233.3 ± 10.7 [a]	7117.9 ± 26.0 [a]	Fruity, melon	1200	Peinado et al. (2004)
Acetaldehyde	**Others**	94815 ±261.6 [a]	225667±64088.6 [a]	Grass, green apple, sherry	100000	Carlton et al. (2007)
Benzaldehyde		61.2 ± 1.6 [b]	11.3 ± 0.1 [a]	Almond	3500	Delfini et al (1999)
Linalool		11.8 ± 0.3 [a]	12.2 ± 0.1 [a]	Rose	25	Ferreira et al. (2000)
α-Terpineol		30.7 ± 1.9 [a]	28.0 ± 1.3 [a]	Lily of the valley	300	Mateo and Jimenez (2000)
Citronellol		2.9 ± 0.1 [a]	4.6 ± 0.0 [b]	Citronella	100	Guth (1997)
2-phenylethanol		28642.5 ± 505.6 [a]	30472.5 ± 922.8 [a]	Roses	10000	Guth (1997)
Isoamyl alcohol		143970 ± 38183.8 [a]	140660 ± 1322.3 [a]	Marzipan, burnt, whisky-like	30000	Guth (1997)
Butyric acid	**Fatty acids**	0.0 ± 0.0 [a]	642.8 ± 17.3 [b]	Rancid, cheese	173	Ferreira et al. (2000)
Isovaleric acid		0.0 ± 0.0 [a]	315.4 ± 58.0 [b]	Rancid, sweaty	33.4	Ferreira et al. (2000)
Hexanoic acid		1638.0 ± 70.7 [a]	1967.5 ± 80.5 [b]	Sweaty, cheese notes	420 - 3000	Ferreira et al. (2000); Guth (1997)
Octanoic acid		2175.7 ± 14.1 [b]	1259.8 ± 109.7 [a]	Grass acid like	500-8800	Ferreira et al. (2000); Étievant (1991)

Decanoic acid	118.3 ± 2.4 [b]	67.5 ± 11.5 [a]	Soapy	1000-15000	Ferreira et al. (2000); Guth (1997)

Mean values of triplicate experiments are shown, with indication of standard deviation. Values for the same compound with the same superscript letter are not significantly different ($P<0.05$)