Effects of acetic acid, ethanol and SO$_2$ on the removal of volatile acidity from acidic wines by two *Saccharomyces cerevisiae* commercial strains

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Running title: Biological removal of volatile acidity from wines

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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 (SO₂) 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 SO₂ 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 
extensive 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 °C per minute up to 240 °C, 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 SO₂ 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 OD₆₄₀nm, 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 (OD₆₄₀nm of 0.2, corresponding to10⁶ 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 SO₂ concentration of white wines should not exceed 200 mg l⁻¹ (according the
recommendations of the OIV), the effect of different SO₂ 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 (pineapple 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, $\alpha$-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.
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.

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REFERENCES


Fig. 1

Growth, O.D. 640 nm (A), log CFU.ml-1 3 (B), acetic acid (C) and ethanol (D) consumption by S. cerevisiae strains S26 (dark symbols) and S29 (open symbols) in acidic wines with 11% (v/v) ethanol and 1.0 g l-1 (◆,◇), 1.5 g l-1 (■,□), or 1.75 g l-1 5 (▲,▲) of acetic acid.
Fig. 1
### Table 1

Physical and chemical characteristics of the wine used for deacidification assays

<table>
<thead>
<tr>
<th>Chemical characteristics</th>
<th>White Wine</th>
<th>Analytical Methods (CEE N.º 2676/90)*</th>
</tr>
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<tbody>
<tr>
<td>Density at 20ºC</td>
<td>0.9907</td>
<td>Densitometry</td>
</tr>
<tr>
<td>Free SO₂ (mg l⁻¹)</td>
<td>3.2</td>
<td>Ripper Method</td>
</tr>
<tr>
<td>Total SO₂ (mg l⁻¹)</td>
<td>70.3</td>
<td>Ripper Method</td>
</tr>
<tr>
<td>Volatile acidity (g l⁻¹ acetic acid)</td>
<td>0.40</td>
<td>Destillation using a Cazenave-Ferré followed by titration with phenolphthalein</td>
</tr>
<tr>
<td>Residual sugar g l⁻¹</td>
<td>1.15</td>
<td>Lane-Eynon Method</td>
</tr>
<tr>
<td>Titratable acidity (g l⁻¹ tartaric acid)</td>
<td>8.90</td>
<td>Titration with bromothymol blue</td>
</tr>
<tr>
<td>pH</td>
<td>3.10</td>
<td>Potentiometer</td>
</tr>
<tr>
<td>Alcoholic degree %, ethanol (v/v)</td>
<td>10.7</td>
<td>Distillation</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strains</th>
<th>[Acetic acid], g l(^{-1})</th>
<th>[Ethanol], % (v/v)</th>
<th>pH</th>
<th>Acetic acid (% consumption)</th>
<th>Titratable acidity, g l(^{-1})</th>
<th>[Total SO(_2)], mg l(^{-1})</th>
<th>[Free SO(_2)], mg l(^{-1})</th>
<th>CFU.ml(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S26</td>
<td>1.0</td>
<td>10.3±0.1 (^{b})</td>
<td>3.68±0.03 (^{b})</td>
<td>78.0±2.65 (^{e})</td>
<td>3.77±0.15 (^{b})</td>
<td>74.77±1.43 (^{b})</td>
<td>0.0±0.0 (^{a})</td>
<td>10(^{7}) (^{b})</td>
</tr>
<tr>
<td>S26</td>
<td>1.5</td>
<td>9.7±0.4 (^{a})</td>
<td>3.58±0.01 (^{a})</td>
<td>24.9±4.29 (^{c})</td>
<td>5.37±0.06 (^{a})</td>
<td>59.90±1.43 (^{a})</td>
<td>0.0±0.0 (^{a})</td>
<td>0 (^{a})</td>
</tr>
<tr>
<td>S26</td>
<td>1.75</td>
<td>9.8±0.2 (^{a,b})</td>
<td>3.57±0.01 (^{a})</td>
<td>21.7±0.99 (^{b,c})</td>
<td>5.87±0.38 (^{a})</td>
<td>66.86±0.41 (^{a,b})</td>
<td>0.0±0.0 (^{a})</td>
<td>0 (^{a})</td>
</tr>
<tr>
<td>S29</td>
<td>1.0</td>
<td>9.8±0.2 (^{a,b})</td>
<td>3.61±0.02 (^{a})</td>
<td>48.3±4.73 (^{d})</td>
<td>4.60±0.10 (^{c})</td>
<td>64.75±0.98 (^{a,b})</td>
<td>0.0±0.0 (^{a})</td>
<td>0 (^{a})</td>
</tr>
<tr>
<td>S29</td>
<td>1.5</td>
<td>9.7±0.2 (^{a})</td>
<td>3.60±0.01 (^{a})</td>
<td>8.9±3.08 (^{a})</td>
<td>5.50±0.40 (^{a})</td>
<td>66.93±9.40 (^{a,b})</td>
<td>0.0±0.0 (^{a})</td>
<td>0 (^{a})</td>
</tr>
<tr>
<td>S29</td>
<td>1.75</td>
<td>10.0±0.1 (^{a,b})</td>
<td>3.58±0.01 (^{a})</td>
<td>14.7±0.87 (^{a,b})</td>
<td>5.80±0.20 (^{a})</td>
<td>65.18±3.82 (^{a,b})</td>
<td>0.0±0.0 (^{a})</td>
<td>0 (^{a})</td>
</tr>
</tbody>
</table>

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

The effect of SO$_2$ addition on the oenological parameters of an acidic wine after 72 h deacidification with *S. cerevisiae* strains S26 and S29.

<table>
<thead>
<tr>
<th>Strains</th>
<th>[SO$_2$]$_i$</th>
<th>[Ethanol]</th>
<th>pH</th>
<th>[Acetic acid]</th>
<th>Titratable acidity</th>
<th>[Total SO$_2$]</th>
<th>[Free SO$_2$]</th>
<th>CFU.ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg l$^{-1}$)</td>
<td>% (v/v)</td>
<td>(g l$^{-1}$)</td>
<td>(g l$^{-1}$)</td>
<td>(mg l$^{-1}$)</td>
<td>(mg l$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S26</td>
<td>25</td>
<td>10.6±0.2</td>
<td>3.49±0.01</td>
<td>0.99±0.03</td>
<td>5.21±0.04</td>
<td>93.68±8.71</td>
<td>2.17±0.65</td>
<td>0</td>
</tr>
<tr>
<td>S26</td>
<td>50</td>
<td>10.6±0.1</td>
<td>3.49±0.00</td>
<td>0.95±0.04</td>
<td>5.25±0.05</td>
<td>122.26±2.75</td>
<td>1.32±0.89</td>
<td>0</td>
</tr>
<tr>
<td>S26</td>
<td>100</td>
<td>10.6±0.1</td>
<td>3.47±0.01</td>
<td>0.99±0.03</td>
<td>5.14±0.04</td>
<td>173.01±2.18</td>
<td>0.96±0.32</td>
<td>0</td>
</tr>
<tr>
<td>S29</td>
<td>25</td>
<td>10.7±0.1</td>
<td>3.49±0.01</td>
<td>1.00±0.02</td>
<td>5.06±0.10</td>
<td>103.28±2.83</td>
<td>1.86±0.51</td>
<td>0</td>
</tr>
<tr>
<td>S29</td>
<td>50</td>
<td>10.5±0.1</td>
<td>3.49±0.01</td>
<td>0.94±0.03</td>
<td>5.13±0.03</td>
<td>123.14±2.62</td>
<td>2.84±0.59</td>
<td>0</td>
</tr>
<tr>
<td>S29</td>
<td>100</td>
<td>10.6±0.1</td>
<td>3.47±0.01</td>
<td>1.00±0.02</td>
<td>5.23±0.02</td>
<td>171.45±1.03</td>
<td>2.34±1.82</td>
<td>0</td>
</tr>
</tbody>
</table>

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).
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.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Acidity of wine</th>
<th>Deacidified wine</th>
<th>Odor description</th>
<th>Detection threshold in wine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>407.5 ± 130.8</td>
<td>677.3 ± 126.2</td>
<td>Solvent like</td>
<td>7500 - 180000</td>
<td>Escudero et al. (2004); Guth (1997); Rizzon and Miele (2004)</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>1.9 ± 0.7</td>
<td>33.6 ± 9.4</td>
<td>Banana</td>
<td>30</td>
<td>Guth (1997)</td>
</tr>
<tr>
<td>2-Phenylethyl acetate</td>
<td>11.2 ± 1.7</td>
<td>16.1 ± 0.4</td>
<td>Roses, honey</td>
<td>250</td>
<td>Guth (1997)</td>
</tr>
<tr>
<td>Ethyl propionate</td>
<td>0.0 ± 0.0</td>
<td>13.4 ± 2.4</td>
<td>Ethereal, fruity, rum-like</td>
<td>1800</td>
<td>Étievant (1991)</td>
</tr>
<tr>
<td>Ethyl isobutyrate</td>
<td>0.0 ± 0.0</td>
<td>4.0 ± 1.2</td>
<td>Strawberry, ethereal, buttery, ripe</td>
<td>15</td>
<td>Ong and Acree (1999); Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Ethyl butyrate</td>
<td>0.0 ± 0.0</td>
<td>15.2 ± 2.6</td>
<td>Pineapple</td>
<td>20</td>
<td>Escudero et al. (2004); Guth (1997)</td>
</tr>
<tr>
<td>Ethyl 2-methylbutyrate</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.6</td>
<td>Sweet, floral, fruity, apple</td>
<td>1-18</td>
<td>Guth (1997); Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Ethyl isovalerate</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.5</td>
<td>Fruity</td>
<td>3</td>
<td>Ferreira et al. (2000)</td>
</tr>
</tbody>
</table>

*Notes: a, b indicate significant differences.*
<table>
<thead>
<tr>
<th>Compound</th>
<th>Value (± Standard Error)</th>
<th>Description</th>
<th>Concentration Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl hexanoate</td>
<td>2.7 ± 0.4&lt;br&gt;68.6 ± 20.6</td>
<td>Anise seed, apple, pineapple</td>
<td>5-14</td>
<td>Guth (1997); Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>28.3 ± 1.8&lt;br&gt;52.0 ± 24.0</td>
<td>Sweet, cognac-apricot</td>
<td>2-5</td>
<td>Guth (1997); Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>5.0 ± 1.4&lt;br&gt;0.9 ± 0.2</td>
<td>Floral</td>
<td>200</td>
<td>Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Diethyl succinate</td>
<td>7233.3 ± 10.7&lt;br&gt;7117.9 ± 26.0</td>
<td>Fruity, melon</td>
<td>1200</td>
<td>Peinado et al. (2004)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>9481.5 ± 261.6&lt;br&gt;225667 ± 64088.6</td>
<td>Grass, green apple, sherry</td>
<td>100000</td>
<td>Carlton et al. (2007)</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>61.2 ± 1.6&lt;br&gt;11.3 ± 0.1</td>
<td>Almond</td>
<td>3500</td>
<td>Delfini et al (1999)</td>
</tr>
<tr>
<td>Linalool</td>
<td>11.8 ± 0.3&lt;br&gt;12.2 ± 0.1</td>
<td>Rose</td>
<td>25</td>
<td>Ferreira et al. (2000)</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>30.7 ± 1.9&lt;br&gt;28.0 ± 1.3</td>
<td>Lily of the valley</td>
<td>300</td>
<td>Mateo and Jimenez (2000)</td>
</tr>
<tr>
<td>Citronellol</td>
<td>2.9 ± 0.1&lt;br&gt;4.6 ± 0.0</td>
<td>Citronella</td>
<td>100</td>
<td>Guth (1997)</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>2864.25 ± 505.6&lt;br&gt;30472.5 ± 922.8</td>
<td>Roses</td>
<td>10000</td>
<td>Guth (1997)</td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>143970 ± 38183.8&lt;br&gt;140660 ± 1322.3</td>
<td>Marzipan, burnt, whisky-like</td>
<td>30000</td>
<td>Guth (1997)</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.0 ± 0.0&lt;br&gt;642.8 ± 17.3</td>
<td>Rancid, cheese</td>
<td>173</td>
<td>Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>0.0 ± 0.0&lt;br&gt;315.4 ± 58.0</td>
<td>Rancid, sweaty</td>
<td>33.4</td>
<td>Ferreira et al. (2000)</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>1638.0 ± 70.7&lt;br&gt;1967.5 ± 80.5</td>
<td>Sweaty, cheese notes</td>
<td>420 - 3000</td>
<td>Ferreira et al. (2000); Guth (1997)</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>2175.7 ± 14.1&lt;br&gt;1259.8 ± 109.7</td>
<td>Grass acid like</td>
<td>500-8800</td>
<td>Ferreira et al. (2000); Étievant (1991)</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>118.3 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.5 ± 11.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Soapy</td>
<td>1000-15000</td>
</tr>
</tbody>
</table>

1 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)