

1 **Effects of acetic acid, ethanol and SO<sub>2</sub> on the removal of volatile acidity from acidic wines by**  
2 **two *Saccharomyces cerevisiae* commercial strains**

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1 **ABSTRACT**

2 Herein we report the influence of different combinations of initial concentration of acetic acid and  
3 ethanol on the removal of acetic acid from acidic wines by two commercial *Saccharomyces*  
4 *cerevisiae* strains S26 and S29. Both strains reduced the volatile acidity of an acidic wine (1.0 g l<sup>-1</sup>  
5 acetic acid and 11% (v/v) ethanol) by 78% and 48%, respectively. Acetic acid removal by both  
6 strains was associated with a decrease in ethanol concentration of about 0.7 – 1.2% (v/v). Strain S26  
7 revealed better removal efficiency due to its higher tolerance to stress factors imposed by acidic  
8 wines. We also demonstrate that the strong anti-oxidant and antiseptic effect of sulphur dioxide  
9 (SO<sub>2</sub>) concentrations up to 170 mg l<sup>-1</sup> inhibit the ability of both strains to reduce the volatile acidity  
10 of an acidic wine under our experimental conditions. Therefore, deacidification should be carried  
11 out either in wines stabilized by filtration or in wines with SO<sub>2</sub> concentrations below 75 mg l<sup>-1</sup>.  
12 Deacidification of wines with the better performing strain S26 was associated with changes in the  
13 concentration of volatile compounds. The most pronounced increase was observed for isoamyl  
14 acetate (banana) and ethyl hexanoate (apple, pineapple), with an 18- and 25-fold increment,  
15 respectively, to values above the detection threshold. The acetaldehyde concentration of the  
16 deacidified wine was 2.3 times higher, and may have a detrimental effect on the wine aroma. In  
17 addition, deacidification led to increased fatty acids concentration, but still within the range of  
18 values described for spontaneous fermentations, and with apparently no negative impact on the  
19 organoleptical properties. We propose the use of *S. cerevisiae* strain S26 for the efficient reduction  
20 of the volatile acidity from acidic wines with acetic acid and ethanol concentrations not higher than  
21 1.0 g l<sup>-1</sup> and 11% (v/v), respectively.

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## 1 INTRODUCTION

2 Acetic acid is the main component of the volatile acidity of wines and is therefore critical for wine  
3 quality. Its concentration in wines is approximately  $0.5 \text{ g l}^{-1}$  and should remain below 20  
4 milliequivalents. $\text{l}^{-1}$ , i.e.  $1.2 \text{ g l}^{-1}$  (expressed as acetic acid), according to European legislation (OIV  
5 2009).

6 Quite a few authors have studied the production of volatile acidity by *Saccharomyces cerevisiae*  
7 under winemaking conditions with initial sugar concentrations around  $200 \text{ g l}^{-1}$ . Volatile acidity is  
8 formed at the beginning of cell growth (Alexandre et al. 1994; Coote and Kirsop 1974) and its  
9 production is affected by the yeast strain (Radler 1993; Giudici et al. 1995; Henschke 1997; Patel  
10 and Shibamoto 2002; Erasmus et al. 2004), the medium composition, vitamins, initial sugar  
11 concentration and fermentation conditions such as temperature variations (Monk and Cowley 1984).

12 Wine yeasts produce acetic acid as a by-product of the hyperosmotic stress response caused by high  
13 sugar concentrations ( $>35$  Brix) in grape must (Erasmus et al. 2004). In wines made from botrytized  
14 grapes, the increase of the initial sugar concentration (from 189 to  $391 \text{ g l}^{-1}$ ) augments the volatile  
15 acidity concentration from  $0.56$  to  $1.46 \text{ g l}^{-1}$  (Lafon-Lafourcade and Ribéreau-Gayon 1977). It was  
16 shown that both the high sugar content and compounds like gluconic acid and glycerol produced  
17 due to *Botrytis* infection can affect the biological aging of the wine. In aging, if wine's gluconic acid  
18 content is more than  $600 \text{ mg l}^{-1}$ , heterolactic fermentations appear with certain intensity, producing  
19 high concentrations of lactic acid and volatile acidity (Ribéreau-Gayon et al. 1979; Perez et al.  
20 1991). Other winemaking factors that favor the production of acetic acid by *S. cerevisiae* are:  
21 anaerobiosis, pH values below 3.1 or above 4.0 (Ribéreau-Gayon et al. 2000; Radler 1993). In  
22 addition, high acetate content in a wine, after a strong clarification of the must, is due to a depletion  
23 of yeast intracellular metabolites such as amino acids, unsaturated fatty acids, polyphenolic  
24 compounds and metals (Moruno et al. 1993). Overexpressing the glycerol 3-phosphate  
25 dehydrogenase gene, *GPD2*, caused *S. cerevisiae* to produce more than twice as much acetic acid as

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

4 Effects derived from nutrient imbalance and competition between coexisting yeasts and bacterial  
5 populations during concurrent malolactic fermentations (Boulton et al. 1998) and citric acid  
6 metabolism (Davis et al. 1986) can also increase acetic acid content in wines. Malolactic  
7 fermentation performed by *Oenococcus oeni* and *Lactobacillus plantarum* modify the amino acid  
8 and volatile composition of the wine and also increase the initial volatile acidity (Lonvaud-Funel  
9 1999). Acetic acid bacteria that can be found in fresh must (*Gluconobacter oxydans*) or species that  
10 predominate during fermentation (*Acetobacter pasteurianus* and *A. liquefaciens*) can also increase  
11 the acetic acid content of must or wines and might cause spoilage (Du Toit and Lambrechts 2002).

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

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

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

3 Grape must can be considered a culture medium that is far from optimum for most microorganisms.  
4 Upon inoculation, yeast cells must adapt to a fermentative environment that gradually changes  
5 during fermentation and that imposes multiple stress conditions such as high osmolarity (sugar  
6 concentration up to 300 g l<sup>-1</sup>), low pH (2.9-3.8) (Pizarro et al. 2007), sulfur dioxide (SO<sub>2</sub>) presence  
7 between 40 and 100 mg l<sup>-1</sup> (Viegas et al. 1989), ethanol toxicity (Viegas et al. 1989), temperature  
8 variations (Pizarro et al. 2007) and increasing nitrogen limitation (Albers et al. 1996; Blateyron and  
9 Sablayrolles 2001; Mendes-Ferreira et al. 2004). A refermentation process, that aims to reduce  
10 excessive volatile acidity, imposes additional stress through elevated acetic acid concentrations.  
11 This may lead to a reduced cellular growth (Thomas and Davenport 1985; Pampulha and Loureiro  
12 1989), induced cellular death (Pinto et al. 1989) and stuck fermentations (Rasmussen et al. 1995;  
13 Edwards et al. 1999; Eglinton and Henschke 1999).

14 Most of the SO<sub>2</sub> in wines is added as antioxidant at the beginning of fermentation to achieve  
15 microbiological control of must by limiting and/or preventing the propagation of undesirable yeasts  
16 and bacteria. However, a small amount of SO<sub>2</sub> is produced as a fermentation byproduct. SO<sub>2</sub> enters  
17 the yeast cell through diffusion and reacts, in the dissociated form, with cytoplasmatic enzymes,  
18 coenzymes and vitamins, leading ultimately to growth cessation and death (Romano and Suzzi  
19 1992). As an antioxidant, SO<sub>2</sub> protects the fruit-like organoleptical qualities and supports wine color  
20 stability by inhibiting the activity of polyphenoloxidases (Boulton et al. 1998; Ribéreau-Gayon et  
21 al. 2000). SO<sub>2</sub> also prevents the conversion of acetaldehyde into ethanol, through inhibition of  
22 aldehyde dehydrogenase and binding with acetaldehyde (Frivik and Ebeler 2003). The rules of the  
23 International Organisation of Vine and Wine (OIV) consider 150 mg l<sup>-1</sup> and 200 mg l<sup>-1</sup> as maximum  
24 limits for final SO<sub>2</sub> concentrations of red and white wines, respectively. The maximum limit of 400  
25 mg l<sup>-1</sup> SO<sub>2</sub>, applies to certain sweet white wines (OIV 2009).

1 In our previous studies, the *S. cerevisiae* autochthonous strains 43C and 45C and the commercial  
2 strains S26, S29 and S30, as well as the non-*Saccharomyces* strains (*L. thermotolerans* 44C and *Z.*  
3 *bailii* ISA 1307) have demonstrated distinctive capacity to consume acetic acid from a mixed  
4 culture medium containing two-thirds of a minimal medium and one third of an acidic white wine.  
5 When the media were supplemented with glucose (13% or 3.3 % w/v) and ethanol (4% or 10%, v/v)  
6 and strains were incubated under aerobic or limited aerobic conditions for 48 to 72 hours, the  
7 commercial strains S26 and S29 appeared to be the most promising candidates for efficient acetic  
8 acid removal. Strain S26 consumed 87% of acetic acid in a medium containing low glucose (3.3 %,  
9 w/v) and high ethanol (10%, v/v) concentration after 72 hours of incubation under aerobic  
10 conditions. Strain S29 consumed 83% of acetic acid under limited-aerobic conditions and in a  
11 medium containing high glucose (13 %, w/v) and low ethanol (4%, v/v) concentration after 48  
12 hours of incubation. We also showed that the commercial *S. cerevisiae* strain S26 efficiently  
13 removes 61.5 % of the acetic acid when grown in an acidic white wine under limited-aerobic  
14 conditions (Vilela-Moura et al., 2008).

15 To further evaluate the applicability of *S. cerevisiae* strains in the deacidification of acidic wines,  
16 we herein assess acetic acid reduction by strains S26 and S29 under the very stressful conditions  
17 imposed by different combination of ethanol, acetic acid and SO<sub>2</sub> concentrations. We showed that  
18 strain S26 deacidifies wines containing up to 1.0 g l<sup>-1</sup> acetic acid, 11% (v/v) ethanol and less than  
19 100 mg l<sup>-1</sup> SO<sub>2</sub> more efficiently than strain S29. Removal of excessive acetic acid by strain S26  
20 exerts no major detrimental effect on wine volatile compounds.

21

## 1 MATERIALS AND METHODS

2

### 3 Microorganisms

4 In this study the *S. cerevisiae* commercial strains S26 and S29 (our internal references) were used.  
5 Both strains were kindly provided by Lalvin and Enoferm, respectively. The strains were kept at -80  
6 °C in micro tubes containing YPD broth (glucose 2%, w/v; peptone 1%, w/v; yeast extract 0.5%,  
7 w/v) supplemented with glycerol (30%, v/v).

8

### 9 Culture media and growth conditions

10 Frozen aliquots of yeast strains were streaked onto YPD plates (glucose 2%, w/v; peptone 1%, w/v;  
11 yeast extract 0.5%, w/v and agar 2%, w/v) and incubated during 48 hours at 25°C prior to each  
12 experiment. Pre-cultures were grown overnight (25 °C, 120 rpm) in 10 ml of a commercial acidic  
13 white wine to be tested and the cells were transferred to 250 ml Erlenmeyer flasks containing 230  
14 ml of acidic wine, prepared as described in the following section. The initial cellular density was  
15 adjusted to  $10^6$  cells ml<sup>-1</sup> (OD<sub>640 nm</sub> 0.2), and incubation was carried out at 25°C, 100 rpm.  
16 Throughout experiments, yeast cell concentration (OD<sub>640 nm</sub>) and viability (CFU/ml) was  
17 determined. All experiments were performed in triplicate.

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### 20 Removal of acetic acid from acidic wines

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

1 pH was set to 3.5, using NaOH (0.1 M). The same wine was used to assess the influence of SO<sub>2</sub>  
2 addition (25, 50 and 100 mg l<sup>-1</sup>), adding potassium metabisulphite (6%, w/v) after acetic acid,  
3 ethanol and pH adjustment to 1.0 g l<sup>-1</sup>, 11% (v/v) and 3.5, respectively.

#### 4 **Analytical determinations**

6 Acetic acid and ethanol concentrations were determined at the time points indicated using  
7 enzymatic kits (Enzytec, Scil Diagnostics, Viernheim, Germany). Analysis of the density, pH,  
8 alcohol concentration, volatile acidity, SO<sub>2</sub> and titratable acidity were performed as outlined in  
9 Table 1.

10 Solid-phase micro-extraction (SPME) extraction and GC-MS determination of aromatic compounds  
11 were carried out as previously described (Mendes-Ferreira et al. 2009). Briefly, SPME was  
12 achieved through adsorption of volatiles onto a fiber (100 µm polydimethylsiloxane –PDMS-, 85  
13 µm Carboxen–polydimethylsiloxane –CAR/PDMS- and 50/30 µm  
14 Divinylbenzene/Carboxen/PDMS -DVB/CAR/PDMS). Extractions in headspace mode were carried  
15 out at 20 ± 1°C with magnetic stirring (1300 rpm). 2-octanol was used as an internal standard  
16 solution. Chromatographic analysis was performed, in the splitless mode, using an Agilent 6890 N  
17 gas chromatograph equipped with a 5973N mass spectrometer. The column employed was an  
18 Innovax capillary column, 30 m X 0.25 mm, with 0.5 µm film thickness (Agilent, Santa Clara, CA,  
19 USA) and helium (helium N60, Air Liquid, Portugal) was used as the carrier gas at 34 cm.s<sup>-1</sup>  
20 average linear velocity. The desorption temperature was 270 °C during 10 min. The column was  
21 maintained at 40°C for 5 minutes after desorption, ramped at 4 °C per minute up to 200 °C, and  
22 then ramped at 10 °C per minute up to 240 °C, where it was held for 15 minutes. All mass spectra  
23 were acquired in electron impact (EI) mode at 70 eV, using full scan with a scan range of 26–250  
24 atomic mass units, at a rate of 6.12 scans.s<sup>-1</sup>. Spectra identification of sample compounds was  
25 supported by the Wiley database (Wiley/NBS Registry of Mass Spectral Data, 1989). Whenever



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

3

#### 4 **Statistical analysis**

5 Acetic acid consumption and all the analytical parameters determined in the different assays were  
6 submitted to variance analysis (ANOVA) using the STATISTICA 7.0 software (StatSoft Inc.,  
7 2004). Tukey honestly significant difference (HSD) test was applied to the chemical data to  
8 determine the presence of significant differences between the analyzed samples; the model was  
9 statistically significant with a *P* value less than 0.05.

10

### 11 **RESULTS**

12

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

14 Herein, we further assess the capacity of the commercial *S. cerevisiae* strains S26 and S29 to  
15 consume acetic acid under the very stressful growth conditions imposed by the combination of high  
16 ethanol (11 and 12%, v/v) and acetic acid (1.0 g l<sup>-1</sup>, 1.5 g l<sup>-1</sup> and 1.75 g l<sup>-1</sup>) concentrations under  
17 limited aerobic conditions. As shown in Table 2, strains S26 and S29 reduced 78 % and 48%,  
18 respectively, of the acetic acid during 168 hours of incubation in an acidic wine with 11% (v/v)  
19 ethanol and 1.0 g l<sup>-1</sup> acetic acid. Under these conditions, acetic acid reduction by strain S26 was  
20 significantly higher than strain S29. As expected, the titrable acidity decreased from 8.90 g l<sup>-1</sup> to  
21 3.77 g l<sup>-1</sup> (S26) and 4.60 g l<sup>-1</sup> (S29). With increasing initial acetic acid concentrations, the  
22 percentage of consumed acetic acid decreased by (i) 24.9% and 8.9% for S26 and S29 strains  
23 respectively, (wine with initial concentration of 1.5 g l<sup>-1</sup> of acetic acid) and (ii) 21.7% and 14.7%  
24 for strains S26 and S29, respectively (wine with initial concentration of 1.75 g l<sup>-1</sup> acetic acid). Some  
25 (not significant) ethanol consumption (0.7 to 1.3 %) was observed in all experiments. No significant

1 changes were observed for both strains regarding pH, total and free SO<sub>2</sub> concentration at the end of  
2 the incubation period of 168 hours.

3 For an initial ethanol concentration of 11% (v/v) only the acidic wine with an initial volatile acidity  
4 of 1.0 g l<sup>-1</sup> was permissive for growth of strain S26 that concluded 3 cell divisions during 168 hours  
5 of incubation (Fig. 1). The most pronounced removal of acetic acid by both strains was not  
6 associated with cell growth. Strain S26 passed through a 24 h lag phase associated with the most  
7 evident acetic acid consumption (about 55%). In a second stage, cell density increased from 0.2 to  
8 1.4 OD<sub>640nm</sub>, but acetic acid removal was less efficient (about 23%). In parallel, the ethanol  
9 concentration decreased by 0.6 % (v/v). Contrarily, strain S29 showed no growth in wines with  
10 11% (v/v) of ethanol at the acetic acid concentrations tested. This strain was however capable to  
11 consume about 40% of the acid during the first 48 hours of incubation, when the initial acetic acid  
12 concentration was 1.0 g l<sup>-1</sup>, as previously described for strain S26. This happened probably because  
13 of the high inoculum's concentration (OD<sub>640nm</sub> of 0.2, corresponding to 10<sup>6</sup> CFU ml<sup>-1</sup>). The lack of  
14 acetic acid consumption at later stages by both strains and higher initial acetic acid concentrations  
15 was most probably caused by metabolism inhibition, which is reflected by the loss of cellular  
16 viability after 96 hours. Both strains were not able to deacidify acidic wines with 12% (v/v) of  
17 ethanol and any of the three acetic acid concentrations tested (not shown).

18

### 19 **Effect of sulphur dioxide on the removal of acetic acid from an acidic wine by strains S26 and** 20 **S29**

21 Considering that the SO<sub>2</sub> concentration of white wines should not exceed 200 mg l<sup>-1</sup> (according the  
22 recommendations of the OIV), the effect of different SO<sub>2</sub> concentrations on acetic acid removal  
23 from an acidic white wine by strains S26 and S29 was also assessed. The volatile acidity and  
24 ethanol concentration of the commercial wine used (Table 1) was adjusted to 1.0 g l<sup>-1</sup> and 11%

1 (v/v), respectively and the pH was set to 3.5. The wine was supplemented with SO<sub>2</sub> (25, 50 and 100  
2 mg l<sup>-1</sup>).

3 Table 3 shows that the total SO<sub>2</sub> concentration in the deacidified wine after 72 h was proportional to  
4 the three different amounts of SO<sub>2</sub> added to the wine. The initial concentration of acetic acid was  
5 not significantly reduced ( $P \geq 0.05$ ) after deacidification with strains S26 and S29 indicating the  
6 strains' inability to remove acetic acid from acidic wines that were supplemented with 25 mg l<sup>-1</sup> of  
7 SO<sub>2</sub>. For both strains and the wine with 1.0 g l<sup>-1</sup> of acetic acid and 11% (v/v) of ethanol, the addition  
8 of 25 and 50 mg l<sup>-1</sup> of SO<sub>2</sub> completely inhibited cell growth and induced loss of cell viability after  
9 24 hours of inoculation. For higher SO<sub>2</sub> concentrations (100 mg l<sup>-1</sup>) both strains started to die since  
10 the beginning of incubation (data not shown). The complete growth inhibition and cell death can be  
11 attributed to the strong anti-oxidant and antiseptic properties combined with the high ethanol and  
12 acetic acid concentrations.

13

#### 14 **Changes in wine aromatic compounds during deacidification with strain S26**

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

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

6 Among the aldehydes and fusel alcohols, acetaldehyde concentration increased 2.3 fold to 225 mg l<sup>-1</sup>  
7 after deacidification with strain S26. The agitation of the culture duplicated the initial dissolved O<sub>2</sub>  
8 from 4 mg l<sup>-1</sup> to 8 mg l<sup>-1</sup>, which explains the increased acetaldehyde concentration. This aldehyde  
9 has a grass or green-apple like aroma when above 100 mg l<sup>-1</sup> (Carlton et al. 2007). Fusel alcohols  
10 (2-phenylethanol and isoamyl alcohol) cause off-flavors at high concentrations, whereas low  
11 concentrations of these compounds and their esters make an essential contribution to the  
12 aroma/flavor of wine. Isoamyl alcohol has a bitter, marzipan, burnt, whisky-like and harsh aroma  
13 and 2-phenylethanol, a compound with floral, rose-like notes. These two compounds were present  
14 in concentrations higher than their detection threshold, but there were no significant concentration  
15 differences between the acidic and the deacidified wines. The concentrations of the terpene alcohols  
16 linalool,  $\alpha$ -terpineol (floral like odors) did not change significantly through deacidification.  
17 Citronellol concentration increased significantly, but remained below the detection limit.

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

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

3

#### 4 **DISCUSSION**

5 This publication adds new information on the effect of several wine parameters on removal of  
6 acetic acid from a white wine by two previously characterised commercial *S. cerevisiae* strains. We  
7 evaluated the combined effects of ethanol, acetic acid and SO<sub>2</sub> on the acetic acid removal efficiency  
8 of strains S26 and S29, using an acidic white wine. We found that strain S26 was able to grow in an  
9 acidic wine with 11% (v/v) of ethanol and 1.0 g l<sup>-1</sup> of acetic acid after 24 hours of inoculation, and  
10 to consume 78% of the total amount of acetic acid after 168 hours. Under these conditions, strain  
11 S29 consumed just 48.3 % of the acetic acid, was unable to grow and lost viability after 96 hours.  
12 This indicates a lower tolerance of strain S29 to the combined effects of high concentration of acetic  
13 acid and ethanol. Both strains were unable to grow when ethanol concentration was adjusted to 12  
14 % (v/v) and acetic acid concentrations were maintained (1.0 g l<sup>-1</sup>, 1.5 g l<sup>-1</sup>, 1.75 g l<sup>-1</sup>). This shows  
15 that refermentation imposes very severe stress conditions and only few strains might be capable to  
16 cope with. Additional inhibitory effects can be exerted by sulphur dioxide (SO<sub>2</sub>).

17 Sulphur dioxide has become practically obligatory in winemaking. This substance combines three  
18 important beneficial properties: antimicrobial and antioxidant activity, as well as the ability to  
19 synthesize non-volatile bisulfite adducts, which prevents their undesirable sensory properties. SO<sub>2</sub>  
20 combines also with oxygen and binds to sugars, aldehydes such as acetaldehyde and ketones,  
21 decreasing its properties as a wine stabilizing agent (Frivik and Ebeler 2003). Recently, it has  
22 become apparent that SO<sub>2</sub> can induce allergic reactions in humans (Ribéreau-Gayon et al. 2000)  
23 which led to the establishment of legal limits for its concentration in wine. When the concentration  
24 of total SO<sub>2</sub> was 95 mg l<sup>-1</sup> (70 mg l<sup>-1</sup> of the initial acidic wine + 25 mg l<sup>-1</sup> of added SO<sub>2</sub>), and still  
25 considerably below the SO<sub>2</sub> limit recommended by the OIV for white wines (200 mg l<sup>-1</sup>) acetic acid

1 removal by both strains was completely inhibited. In fact, there was no significant reduction of  
2 volatile acidity and ethanol. Almost all the added SO<sub>2</sub> was combined. Therefore, the SO<sub>2</sub> levels of  
3 the acidic wines to be treated by the yeast should not exceed 75 mg l<sup>-1</sup>. Deacidification should be  
4 preferentially carried out in wines stabilized with lower SO<sub>2</sub> concentrations or by filtration.  
5 However, it should be considered that these results were obtained in a micro-scale setting and still  
6 need to be evaluated in a winery large-scale approach.

7 Strain S26 was most efficient for biological deacidification of acidic wines and also showed a  
8 higher resistance to the combined effects of acetic acid and ethanol. Changes in volatile compounds  
9 associated with deacidification were therefore evaluated only for this strain. Both acetate and ethyl  
10 esters were present in significantly higher concentrations in the deacidified wine excepting ethyl-2  
11 methylbutyrate, ethyl isovalerate and ethyl decanoate. The aromatic potential of these ester  
12 compounds, associated with fruity and floral notes, positively enhances the wine's bouquet. The  
13 most pronounced increase was observed for isoamyl acetate (banana) and ethyl hexanoate (apple,  
14 pineapple), with an 18- and 25-fold increment, respectively, to values above the detection threshold.

15 Acetate and ethyl esters are synthesized by carboxylesterases or transferases acting on acyl-CoA  
16 (Mckay 1993) by condensation of an alcohol and a coenzyme-A-activated acid (acyl-CoA). In *S.*  
17 *cerevisiae*, acetate esters result from the combination of acetyl-CoA with an alcohol, by the action  
18 of the alcohol acetyl transferases Atf1p and Atf2p (Lambrechts and Pretorius 2000). Ethyl esters are  
19 generated from acyl-CoA and ethanol by the action of Eht1p and Eeb1p (Mason and Dufour 2000;  
20 Saerens et al. 2006). The capacity of yeast to synthesise these compounds varies between strains  
21 (Lambrechts and Pretorius 2000; Wondra and Boveric 2001). The incubation temperature during  
22 the deacidification assay (25°C) might have contributed to the formation of acetate and ethyl esters.  
23 Molina and collaborators (2007) showed that lower temperatures (15°C) increased the concentration  
24 of ester compounds associated to fresh and fruity aromas. Higher temperatures (28°C) increased the

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

3 Acetaldehyde concentration increased to 225 mg l<sup>-1</sup> after deacidification with strain S26. However,  
4 its initial concentration (94.8 mg l<sup>-1</sup>) was already close to the upper limit of the concentration range  
5 found in white wines (Liu and Pilone 2000). This compound causes more concern for its aroma  
6 (grass, apple or sherry-like character when occurring in concentrations higher than 100 mg l<sup>-1</sup>). This  
7 does not apply to all wine styles because high levels of acetaldehyde (up to 500 mg l<sup>-1</sup>) are  
8 considered a unique feature of sherry wines (Liu and Pilone 2000). Besides, acetaldehyde binds  
9 sulphur dioxide and has therefore a negative impact on wine stability. Contrarily, lower  
10 acetaldehyde concentrations increase flavor complexity, due to the fruity and pleasant aroma, in  
11 particular in red wines (Frivik and Ebeler 2003). Aldehyde synthesis is affected by several factors  
12 such as the yeast strain, temperature, pH, nutrient availability, O<sub>2</sub> and SO<sub>2</sub> concentration. SO<sub>2</sub> is  
13 particularly important since it affects aldehyde dehydrogenase and thus the conversion of  
14 acetaldehyde into ethanol (Fivrik and Ebeler 2003). Besides, acetaldehyde is an intermediate  
15 product of yeast metabolism and a precursor of acetate, acetoin and ethanol (Romano et al. 1997).  
16 Its production through ethanol oxidation is strain dependent (Romano et al. 1994) and is favoured  
17 by O<sub>2</sub>. In our previous work (Vilela-Moura et al. 2008) we showed that efficient acetic acid  
18 reduction requires some oxygen as provided by the limited-aerobic experimental setup used.  
19 Therefore, the expectation that this oxygen requirement had an impact on the acetaldehyde level,  
20 was confirmed. Nevertheless, we consider that the significance of increased acetaldehyde  
21 concentrations after deacidification still needs to be evaluated for different types of wines.

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

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

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

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

17

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19

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- 3

## 1 REFERENCES

- 2 Albers E, Larsson C, Liden G, Niklasson C, Gustafsson L (1996) Influence of the nitrogen source  
3 on *Saccharomyces cerevisiae* anaerobic growth and product formation. Appl Env Microbiol  
4 62:3187-3195
- 5 Alexandre H, Nguyen Van Long T, Feuillat M, Charpentier C (1994) Contribution à l'étude des  
6 bourbes: influence sur la fermentescibilité des moûts. Rev Fr Œno 146:11–20
- 7 Boulton RB, Singleton VL, Bisson LF, Kunkee RE (1998) Yeasts and biochemistry of ethanol  
8 fermentation, principles and practices of winemaking, 1st edn. Springer, New York, pp 102–192
- 9 Blateyron L, Sablayrolles JM (2001) Stuck and slow fermentations in enology: statistical study of  
10 causes and effectiveness of combined additions of oxygen and diammonium phosphate. J Biosci  
11 Bioeng 91(2):184-189
- 12 Carlton WK, Gump B, Fugelsang K, Hasson AS (2007) Monitoring acetaldehyde concentrations  
13 during micro-oxygenation of red wine by headspace solid-phase microextraction with on-fiber  
14 derivatization. J Agric Food Chem 55:5620-5625
- 15 Casal M, Cardoso H, Leão C (1996) Mechanisms regulating the transport of acetic acid in  
16 *Saccharomyces cerevisiae*. Microbiol 142(6):1385-1390
- 17 Casal M, Paiva S, Andrade RP, Gancedo C, Leão C (1999) The lactate proton symport of  
18 *Saccharomyces cerevisiae* is encoded by *JEN1*. J Bacteriol 181:2620-2623
- 19 Coote N, Kirsop HH (1974) The content of some organic acids in beer and other fermented media. J  
20 Inst Brew 80:474–483

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

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

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

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

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

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

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

20 Erasmus DJ, Cliff M, van Vuuren HJJ (2004) Impact of yeast strain on the production of acetic  
21 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

- 1 Lonvaud-Funel A (1999) Lactic acid bacteria in the quality improvement and depreciation of wine.  
2 Antonie van Leeuwenhoek 76:317–331
- 3 Liu SQ, Pilone GJ (2000) An overview of formation and roles of acetaldehyde in winemaking with  
4 emphasis on microbiological implications. Int J Food Sci Technol 35:49-61
- 5 Mollapour M, Piper P (2007) Hog1 mitogen-activated protein kinase phosphorylation targets the  
6 yeast Fps1 aquaglyceroporin for endocytosis, thereby rendering cells resistant to acetic acid. Mol  
7 Cell Biol 27:6446-6456
- 8 Mason AB, Dufour J (2000) Alcohol acetyltransferases and the significance of ester synthesis in  
9 yeast. Yeast 16:1287–1298
- 10 Mateo JJ, Jiménez M (2000) Monoterpenes in grape juice and wines. J Chromatography A 881:557-  
11 567
- 12 McKay AM (1993) Microbial carboxylic ester hydrolases (EC 3.1.1) in food biotechnology-A  
13 review. Lett Appl Microbiol 16:1-6
- 14 Mendes-Ferreira A, Mendes-Faia A, Leão C (2004) Growth and fermentation patterns of  
15 *Saccharomyces cerevisiae* under different ammonium concentrations and its implications in  
16 winemaking industry. J Appl Microbiol 97(3):540-545
- 17 Mendes-Ferreira A, Barbosa C, Falco V, Leão C, Mendes-Faia A (2009) The production of  
18 hydrogen sulphide and other aroma compounds by wine strains of *Saccharomyces cerevisiae* in  
19 synthetic media with different nitrogen concentrations. J Ind Microbiol Biotechnol 36:571–583

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

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

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

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

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

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

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

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

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

- 1 Patel S, Shibamoto S (2002) Effect of different strains of *Saccharomyces cerevisiae* on production  
2 of volatiles in Napa gamay wine and petite syrah wine. *J Agric Food Chem* 50:5649–5653
- 3 Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC (2004) Comparative study of aromatic  
4 compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem*  
5 84:585–590
- 6 Perez L, Valcarcel MJ, Gonzalez P, Domecq B (1991) Influence of *Botrytis* infection of the grapes  
7 on the biological aging process of Fino Sherry. *Am J Enol Vitic* 42(1):58-62
- 8 Pinto I, Cardoso H, Leão C, van Uden N (1989) High enthalpy and low enthalpy death in  
9 *Saccharomyces cerevisiae* induced by acetic acid. *Biotechnol Bioeng* 33(10):1350-1352
- 10 Pizarro F, Vargas FA, Agosin E (2007) A systems biology perspective of wine fermentations. *Yeast*  
11 24(11):977-991
- 12 Rasmussen JE, Schultz E, Snyder RE, Jones RS, Smith CR (1995) Acetic acid as a causative agent  
13 in producing stuck fermentations. *Am J Enol Vitic* 46:278-280
- 14 Radler F (1993) Yeast metabolism of organic acids. In: Gram H (ed) *Wine microbiology and*  
15 *biotechnology*, Harwood Academic Publishers, Chur, pp 165–182
- 16 Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2000) *The chemistry of wine and*  
17 *stabilization and treatments. Handbook of enology, vol. 2, 1st edn. Wiley, Chichester.*
- 18 Ribéreau-Gayon P, Lafon-Lafourcade S, Dubourdieu D, Lucmaret V, Larue F (1979) Métabolisme  
19 de *Saccharomyces cerevisiae* dans le moût de raisins parasités par *Botrytis cinerea*. *C R Acad Sci*  
20 289:441–444

- 1 Rizzon LA, Miele A (2004) Avaliação da cv. Tannat para elaboração de vinho tinto. Ciênc Tecnol  
2 Aliment 24(2):223-229
- 3 Romano P, Suzzi G (1992) Wine microbiology and biotechnology. Harwood Academic Publishers,  
4 Chur
- 5 Romano P, Suzzi G, Domizio P, Faticenti F (1997) Secondary products formation as a tool for  
6 discriminating non-*Saccharomyces* wine strains. Strain diversity in non-*Saccharomyces* wine  
7 yeasts. Antonie van Leeuwenhoek 71:239–242
- 8 Romano P, Suzzi G, Turbanti L, Polsinelli M (1994) Acetaldehyde production in *Saccharomyces*  
9 *cerevisiae* wine yeasts. FEMS Microbiol Lett 118(3):213-218
- 10 Saerens SM, Verstrepen KJ, Van Laere SDM, Voet AR, Van Dijck P, Delvaux FR, Thevelein JM  
11 (2006) The *Saccharomyces cerevisiae* *EHT1* and *EEB1* genes encode novel enzymes with medium-  
12 chain fatty acid ethyl ester synthesis and hydrolysis capacity. J Biological Chem 281(7):4446–4456
- 13 Schüller HJ (2003) Transcriptional control of nonfermentative metabolism in the yeast  
14 *Saccharomyces cerevisiae*. Curr Genet 43(3):139–160
- 15 Thomas S, Davenport RR (1985) *Zygosaccharomyces bailii*, a profile of characteristics and spoilage  
16 activities. Food Microbiol 2:157–169
- 17 Viegas CA, Rosa MF, Sá-Correia I, Novais JM (1989) Inhibition of yeast growth by octanoic and  
18 decanoic acids produced during ethanolic fermentation. Appl Environ Microbiol 55(1):21-28
- 19 Vilela-Moura A, Schuller D, Mendes-Faia A, Côrte-Real M (2008) Reduction of volatile acidity of  
20 wines by selected yeast strains. Appl Microbiol Biotechnol 80(5):881-890

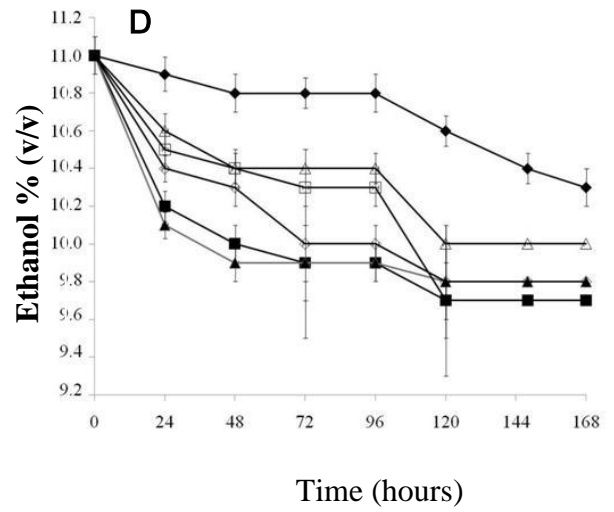
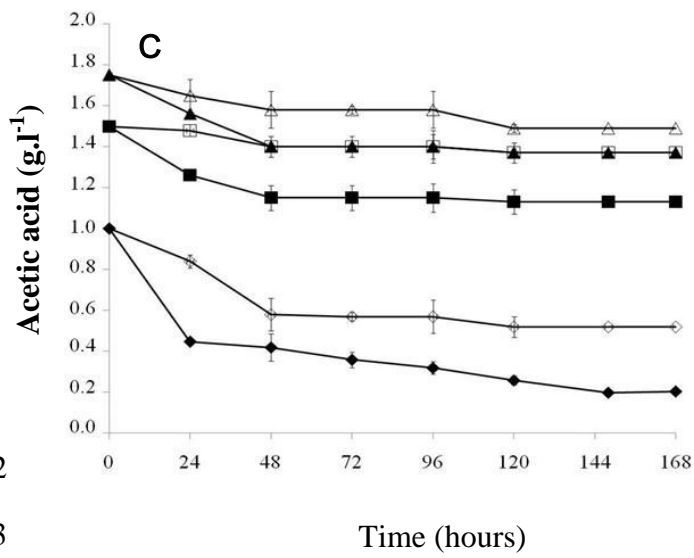
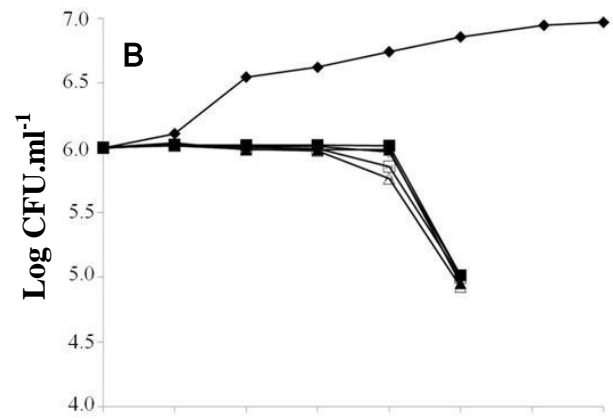
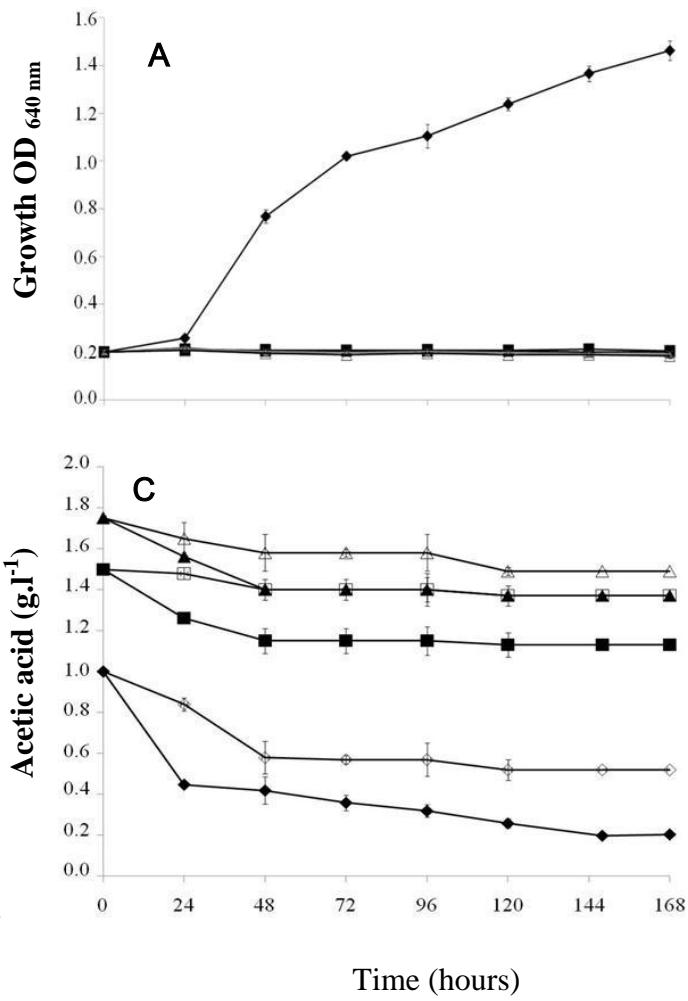


1 Wondra M, Boveric M (2001) Analyses of aroma components of Chardonnay wine fermented by  
2 different yeast strains. *Food Technol Biotechnol* 39(2):141–148

3

1 Fig. 1  
2 Growth, O.D. 640 nm (A), log CFU.ml<sup>-1</sup> 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<sup>-1</sup> (◆,◇), 1.5 g l<sup>-1</sup> (■,□), or 1.75 g l<sup>-1</sup> 5 (▲,△) of acetic acid  
5

1



2

3

4 **Fig. 1**

5

1 Table 1

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

Chemical characteristics	White Wine	Analytical Methods (CEE N.° 2676/90)*
Density at 20°C	0.9907	Densitometry
Free SO <sub>2</sub> (mg l <sup>-1</sup> )	3.2	Ripper Method
Total SO <sub>2</sub> (mg l <sup>-1</sup> )	70.3	Ripper Method
Volatile acidity (g l <sup>-1</sup> acetic acid)	0.40	Distillation using a Cazenave-Ferré followed by titration with phenolphthalein
Residual sugar g l <sup>-1</sup>	1.15	Lane-Eynon Method
Titrateable acidity (g l <sup>-1</sup> tartaric acid)	8.90	Titration with bromothymol blue
pH	3.10	Potentiometer
Alcoholic degree %, ethanol (v/v)	10.7	Distillation

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

## 1 Table 2

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  
 3 deacidification by *S. cerevisiae* strains S26 and S29

Strains	[Acetic acid] <sub>i</sub> (g l <sup>-1</sup> )	[Ethanol] % (v/v)	pH	Acetic acid (% consumption)	Titrateable acidity (g l <sup>-1</sup> )	[Total SO <sub>2</sub> ] (mg l <sup>-1</sup> )	[Free SO <sub>2</sub> ] (mg l <sup>-1</sup> )	CFU.ml <sup>-1</sup>
S26	1.0	10.3±0.1 <sup>b</sup>	3.68±0.03 <sup>b</sup>	78.0±2.65 <sup>e</sup>	3.77±0.15 <sup>b</sup>	74.77±1.43 <sup>b</sup>	0.0±0.0 <sup>a</sup>	10 <sup>7b</sup>
S26	1.5	9.7±0.4 <sup>a</sup>	3.58±0.01 <sup>a</sup>	24.9±4.29 <sup>c</sup>	5.37±0.06 <sup>a</sup>	59.90±1.43 <sup>a</sup>	0.0±0.0 <sup>a</sup>	0 <sup>a</sup>
S26	1.75	9.8±0.2 <sup>a,b</sup>	3.57±0.01 <sup>a</sup>	21.7±0.99 <sup>b,c</sup>	5.87±0.38 <sup>a</sup>	66.86±0.41 <sup>a,b</sup>	0.0±0.0 <sup>a</sup>	0 <sup>a</sup>
S29	1.0	9.8±0.2 <sup>a,b</sup>	3.61±0.02 <sup>a</sup>	48.3±4.73 <sup>d</sup>	4.60±0.10 <sup>c</sup>	64.75±0.98 <sup>a,b</sup>	0.0±0.0 <sup>a</sup>	0 <sup>a</sup>
S29	1.5	9.7±0.2 <sup>a</sup>	3.60±0.01 <sup>a</sup>	8.9± 3.08 <sup>a</sup>	5.50±0.40 <sup>a</sup>	66.93±9.40 <sup>a,b</sup>	0.0±0.0 <sup>a</sup>	0 <sup>a</sup>
S29	1.75	10.0±0.1 <sup>a,b</sup>	3.58±0.01 <sup>a</sup>	14.7±0.87 <sup>a,b</sup>	5.80±0.20 <sup>a</sup>	65.18±3.82 <sup>a,b</sup>	0.0±0.0 <sup>a</sup>	0 <sup>a</sup>

4 i – Initial acetic acid concentration. The initial values of pH, titrateable acidity, total and free SO<sub>2</sub> concentrations are referred in Table 1. The data are mean  
 5 values of triplicate experiments with indication of standard deviation. Results obtained for strains and culture conditions with the same superscript letter are  
 6 not significantly different ( $P < 0.05$ )

7

1 Table 3

2 Effect of SO<sub>2</sub> addition on the oenological parameters of an acidic wine after 72 h deacidification with *S. cerevisiae* strains S26 and S29

Strains	[SO <sub>2</sub> ] <sub>i</sub> (mg l <sup>-1</sup> )	[Ethanol] % (v/v)	pH	[Acetic acid] (g l <sup>-1</sup> )	Titrateable acidity (g l <sup>-1</sup> )	[Total SO <sub>2</sub> ] (mg l <sup>-1</sup> )	[Free SO <sub>2</sub> ] (mg l <sup>-1</sup> )	CFU.ml <sup>-1</sup>
S26	25	10.6±0.2 <sup>a</sup>	3.49±0.01 <sup>a</sup>	0.99±0.03 <sup>a</sup>	5.21±0.04 <sup>a</sup>	93.68±8.71 <sup>a</sup>	2.17±0.65 <sup>a</sup>	0 <sup>a</sup>
S26	50	10.6±0.1 <sup>a</sup>	3.49±0.00 <sup>a</sup>	0.95±0.04 <sup>a</sup>	5.25±0.05 <sup>a</sup>	122.26±2.75 <sup>b</sup>	1.32±0.89 <sup>a</sup>	0 <sup>a</sup>
S26	100	10.6±0.1 <sup>a</sup>	3.47±0.01 <sup>b</sup>	0.99±0.03 <sup>a</sup>	5.14±0.04 <sup>a,b</sup>	173.01±2.18 <sup>c</sup>	0.96±0.32 <sup>a</sup>	0 <sup>a</sup>
S29	25	10.7±0.1 <sup>a</sup>	3.49±0.01 <sup>a</sup>	1.00±0.02 <sup>a</sup>	5.06±0.10 <sup>b</sup>	103.28±2.83 <sup>a</sup>	1.86±0.51 <sup>a</sup>	0 <sup>a</sup>
S29	50	10.5±0.1 <sup>a</sup>	3.49±0.01 <sup>a</sup>	0.94±0.03 <sup>a</sup>	5.13±0.03 <sup>a,b</sup>	123.14±2.62 <sup>b</sup>	2.84±0.59 <sup>a</sup>	0 <sup>a</sup>
S29	100	10.6±0.1 <sup>a</sup>	3.47±0.01 <sup>b</sup>	1.00±0.02 <sup>a</sup>	5.23±0.02 <sup>a</sup>	171.45±1.03 <sup>c</sup>	2.34±1.82 <sup>a</sup>	0 <sup>a</sup>

3 i – Initial SO<sub>2</sub> concentration. The initial values of pH, titrateable acidity, total and free SO<sub>2</sub> concentrations are referred in Table 1. Results are mean values of  
 4 triplicate experiments with their standard deviation. The initial concentrations of ethanol and acetic acid were 11% (v/v) and 1.0 g l<sup>-1</sup>, respectively. Results  
 5 obtained for strains and culture conditions with the same superscript letter are not significantly different (*P*<0.05)

6  
7

1 Table 4

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

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

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



**Decanoic acid**

118.3 ± 2.4<sup>b</sup>

67.5 ± 11.5<sup>a</sup>

Soapy

1000-15000

Ferreira et al. (2000); Guth (1997)

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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  
2 significantly different (P<0.05)

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