

1 **The impact of acetate metabolism on yeast fermentative performance and wine**  
2 **quality: reduction of volatile acidity of grape musts and wines**

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1 **Abstract**

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3 Acetic acid is the main component of the volatile acidity of grape musts and wines. It can be  
4 formed as a by-product of alcoholic fermentation or as a product of the metabolism of acetic  
5 and lactic acid bacteria, which can metabolise residual sugars to increase volatile acidity.

6 Acetic acid has a negative impact on yeast fermentative performance and affects the quality  
7 of certain types of wine when present above a given concentration. In this mini-review we  
8 present an overview of fermentation conditions and grape-must composition favouring  
9 acetic acid formation, as well the metabolic pathways leading to its formation and  
10 degradation by yeast. The negative effect of acetic acid on the fermentative performance of  
11 *Saccharomyces cerevisiae* will also be covered, including its role as a physiological inducer  
12 of apoptosis. Finally, currently available wine deacidification processes and new proposed  
13 solutions based on zymological deacidification by select *S. cerevisiae* strains will be  
14 discussed.

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## 2 **Production of acetic acid in grape must and wine**

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4 Volatile acidity is derived from acids of the acetic series present in wine both in the free  
5 state and combined as salts (OIV 2009). The volatile acidity of wines must always be low  
6 (Boulton et al. 1996). In excessive quantities, volatile acids are seen as a spoilage  
7 characteristic conferring the wine an acrid taste and the unpleasant vinegar aroma. The main  
8 component of the volatile acidity of wines is acetic acid, which typically occurs in wines in  
9 concentrations ranging from 0.2 to 0.6  $\text{g l}^{-1}$ , but they may be higher under certain conditions  
10 (Bely et al. 2003). The OIV (2010) refers that the maximum acceptable limit for volatile  
11 acidity in most wines is 1.2  $\text{g l}^{-1}$  of acetic acid. The aroma threshold for acetic acid depends  
12 on the wine variety and style. Ribéreau-Gayon et al. (2006a) refers that an acetic acid  
13 concentration of at least 0.90  $\text{g l}^{-1}$  is required to produce a noticeable bitter, sour aftertaste in  
14 wine, though it does not cause a strong odor.

15 High levels of volatile acidity can however be acceptable in some types of wine such as  
16 icewines (Erasmus et al. 2004) and botritized wines, with a maximum acetic acid  
17 concentration of 2.1  $\text{g l}^{-1}$  (OIV 2010). Acetic acid can be formed at any time from the  
18 beginning of wine production (in grapes) until the final product (bottled wine), as a bacterial  
19 or yeast metabolite (Table 1). It can be produced before alcoholic fermentation by bacterial  
20 spoilage in *Botrytis cinerea*-infected grapes. This fungal infection leads to a ruptured grape  
21 berry skin, allowing access of bacteria to the berry's interior. *Acetobacter* species can  
22 dominate on the surface of rotten grapes, using the ethanol produced by wild yeasts as their  
23 preferred carbon source, though *Gluconobacter* species are also usually present on grapes  
24 (Du Toit and Lambrechts 2002).

1 Acetic acid is also formed as a by-product of alcoholic fermentation by *Saccharomyces*  
2 *cerevisiae*. Studies on the production of volatile acidity by *S. cerevisiae* under winemaking  
3 conditions showed that this acid is mainly formed at the beginning of alcoholic fermentation  
4 (Alexandre et al. 1994; Coote and Kirsop 1974) and its production is affected by different  
5 factors, namely the yeast strain (Erasmus et al. 2004; Orlic' et al. 2010; Patel and Shibamoto  
6 2002; Shimazu and Watanabe 1981; Torrens et al. 2008), grape-must composition (Delfini  
7 and Costa 1993), and fermentation conditions such as nitrogen content (Barbosa et al. 2009;  
8 Vilanova et al. 2007), vitamins, initial sugar concentration (Radler 1993) and other physical  
9 factors such as temperature (Monk and Cowley 1984; Llauradó et al. 2005; Beltran et al.  
10 2008). Wine yeasts also produce acetic acid to equilibrate the redox balance in response to  
11 the hyperosmotic stress caused by high sugar concentrations, which can be especially severe  
12 in the high °Brix (>35 °Brix) grape-must (Erasmus et al. 2004) and in wines made from  
13 botritized grapes (Amerine et al. 1972). It has been shown that compounds like gluconic acid  
14 and glycerol produced as a consequence of *Botrytis* infection can affect the biological aging  
15 of this type of wines. Indeed, gluconic acid can be metabolised by heterofermentative lactic  
16 acid bacteria, which produce high concentrations of lactic acid and volatile acidity  
17 (Ribéreau-Gayon et al. 1979; Perez et al. 1991). Anaerobiosis, pH values below 3.1 or above  
18 4.0, and excessive grape-must clarification are among other factors that favour the  
19 production of acetic acid by *S. cerevisiae* (Ribéreau-Gayon et al. 2006b). Variations in acetic  
20 acid production in natural *S. cerevisiae* strains can also have a genetic basis. A study using  
21 genome hybridization on DNA microarrays revealed that when asparagine is used as a major  
22 nitrogen source, acetic acid production is inversely associated with asparaginase type I  
23 activity and linked the production of this acid to nitrogen assimilation and the CO<sub>2</sub>  
24 production rate (Marullo et al. 2007). Acetate is also secreted in high levels by certain  
25 yeasts, such as *Dekkera* and its anamorph *Brettanomyces*, that have attracted attention as

1 spoilage agents of wine (Sponholz 1993; Gerós et al. 2000a; Pretorius 2000). Other apiculate  
2 wine yeasts, mainly species of *Hanseniaspora*, anamorph of *Kloeckera* (Romano et al. 1992;  
3 Ciani and Maccarelli 1998) as well as wine species of the genus *Candida* (Fleet and Heard  
4 1993), involved in the early phase of both spontaneous and inoculated fermentations, can  
5 lead to a high content of acetic acid in wine. *Saccharomyces ludwigii* is another spoilage  
6 frequently isolated from wine at the end of the fermentation process and during wine  
7 storage. Some strains from this species, known for its high alcohol tolerance and high  
8 resistance to antimicrobial compounds, produce undesirable amounts (more than 0.75 g l<sup>-1</sup>)  
9 of acetic acid (Romano et al. 1999). Malolactic fermentation, the decarboxylation of malic  
10 acid into lactic acid by lactic acid bacteria, is associated with changes in the amino acid and  
11 volatile composition of the wine and also increases the initial volatile acidity (Lonvaud-  
12 Funel 1999; Pozo-Bayon et al. 2005). Acetate is produced by starter cultures of *Oenococcus*  
13 *oeni* under pantothenic acid deprivation due to CoA deficiency (Richter et al. 2001). Other  
14 factors contributing to the excessive formation of acetic acid during grape-must fermentation  
15 are, among others, products derived from nutrient imbalance and competition between  
16 coexisting yeasts and bacterial populations during concurrent malolactic fermentations  
17 (Boulton et al. 1996; Moruno et al. 1993). Lactic acid bacteria (Cogan 1987) and/or acetic  
18 acid bacteria (*Acetobacter pasteurianus* and *A. liquefaciens*) that survive during  
19 fermentation can also increase the acetic acid content of wines and may cause wine spoilage  
20 (Du Toit and Lambrechts 2002). Even after bottling, red wines may under peculiar  
21 circumstances carry a small population of acetic acid bacteria that can proliferate in bottles  
22 stored in an upright position, spoiling the wine (Bartowsky and Henschke 2008).

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24 **Acetic acid metabolism in yeast**

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1 As referred above, acetic acid in grape-must or wines can be the product of bacterial or yeast  
2 metabolism. Herein both the anabolic and catabolic pathways of acetic acid in yeast will be  
3 covered with regards to their implications in the production and quality of wine. The ability  
4 of yeasts to catabolise acetic acid can be especially exploited to develop methods for the  
5 zymological deacidification of grape-musts or wines. This issue will be discussed below.

6 The actual yeast biochemical pathways contributing to acetic acid formation in wine have  
7 not yet been clearly elucidated (Boulton et al. 1996, Ribéreau-Gayon et al. 2006b). Several  
8 enzymatic reactions have been suggested to contribute to acetic acid formation by yeast  
9 during beer fermentations (Jost and Piendl 1975): (i) reversible formation from acetyl Co-A  
10 and acetyl adenylate through acetyl Co-A synthetase (ACS), (ii) cleavage of citrate by citrate  
11 lyase (iii) production from pyruvate by pyruvate dehydrogenase (PDH), yielding acetyl Co-  
12 A that can be hydrolysed into acetate through acetyl Co-A hydrolase (iv) reversible  
13 formation from acetyl-phosphate by acetyl kinase and (v) oxidation of acetaldehyde by  
14 aldehyde dehydrogenase (ALD). It is known that  $\text{NADP}^+$ -dependent ALD is active during  
15 alcoholic fermentation while PDH activity is limited under anaerobic conditions (Ribéreau-  
16 Gayon et al. 2006b). When pyruvate dehydrogenase is repressed, the PDH bypass still  
17 allows the formation of acetyl Co-A from pyruvate, used for example to synthesize fatty  
18 acids. This pathway (Fig. 1) involves the sequential transformation of pyruvate to  
19 acetaldehyde (through pyruvate decarboxylase), acetate (through ALD) and acetyl-CoA  
20 (through ACS). Therefore, under anaerobiosis, yeast with the lowest ALD activity and the  
21 highest ACS activity produce the least amount of acetic acid (Verduyn et al. 1990). This  
22 observation supports the proposal that acetic acid is mainly produced through the PDH  
23 bypass though, as mentioned above, other suggested enzymatic reactions may be involved.  
24 Yeast cultures exposed to oxygen and actively synthesizing fatty acids for growth may  
25 produce acetic acid upon entry into anaerobic conditions as a mechanism for the

1 regeneration of free Co-A (Fig. 1) needed for other biosynthetic activities (Boulton et al.  
2 1996). This mechanism may explain the accumulation of acetic acid by yeasts with a  
3 shortage of pantothenic acid, a precursor of Co-A (Ribéreau-Gayon et al. 2006b). Acetate  
4 formation may also play a physiological role in the regeneration of reducing equivalents  
5 (NADH and NADPH) that are essential for the maintenance of the redox balance (Saint-Prix  
6 et al. 2004; Remize et al. 2000).

7 Acetate, like other non-fermentable substrates such as ethanol, glycerol and lactate, can be  
8 used as a sole carbon source for the generation of energy and cellular biomass under aerobic  
9 conditions (Barnett et al. 1990; Schuller 2003). In *S. cerevisiae*, acetate transport and  
10 metabolism are subject to glucose repression similarly to the utilization of many other  
11 alternative carbon sources, since glucose is the preferential carbon and energy source of this  
12 species. Hence, when grown in medium containing glucose and acetic acid, this yeast  
13 displays a diauxic growth with consumption of acetic acid only after glucose exhaustion  
14 (Casal et al. 1996; Rodrigues 1998). This behaviour is also described for other yeast species  
15 like *Candida utilis* (Leão and Van Uden 1986), *Torulasporea delbrueckii* (Casal and Leão  
16 1995) and *Dekkera anomala* (Gerós et al. 2000b). However, as referred below, some  
17 commercial *S. cerevisiae* wine strains are able to consume acetic acid in the presence of  
18 glucose (Vilela-Moura et al. 2008). This behaviour resembles that of species  
19 *Zygosaccharomyces bailii* ISA 1307, which displays a biphasic growth in medium  
20 containing a mixture of glucose and acetic acid; the first phase is associated with  
21 simultaneous consumption of glucose and acetic acid, and the second with the utilization of  
22 the remaining acid (Sousa et al. 1998). It was proposed that regulation of both membrane  
23 transport and ACS are important for the ability of *Z. bailii* to metabolise acetic acid in the  
24 presence of glucose. Perfusion experiments also showed that *Z. bailii* is more resistant than  
25 *S. cerevisiae* to short-term intracellular pH changes caused by acetic acid (Arneborg et al.

1 2000). These physiological traits are responsible for the high resistance of the species in  
2 environments containing mixtures of sugars and acetic acid such as those often present  
3 during wine fermentation.

4 Catabolism of acetic acid in yeast, including its cellular uptake, is obviously important to  
5 promote its degradation and therefore reduce acetic acid concentration in grape-musts and  
6 wines. Acetic acid entry into the cells depends on the extracellular pH and growth  
7 conditions. In glucose-repressed yeast cells at low pH, where acetic acid is mostly  
8 undissociated ( $pK_a$  is 4.75), it enters mainly by facilitated diffusion (Casal et al. 1996).  
9 Ethanol enhances the passive influx of labeled acetic acid, which follows first-order kinetics  
10 with a rate constant that increases exponentially with ethanol concentration (Casal et al.  
11 1998). More recently, it was shown that deletion of *FPS1*, coding for an aquaglyceroporin  
12 channel, abolishes acetic acid accumulation at low pH (Mollapour and Piper 2007). When  
13 grown at low pH, *S. cerevisiae* acquires enhanced resistance to acetic acid through loss of  
14 Fps1p mediated by transient activation of the Hog1p mitogen-activated protein kinase.  
15 Hog1p directly phosphorylates Fps1p, targeting this channel for endocytosis and degradation  
16 in the vacuole. Evidence for the existence of at least two acetate carriers in de-repressed *S.*  
17 *cerevisiae* cells has been obtained (Casal et al. 1996; Paiva et al. 1999). It is known that  
18 Jen1p is required for the uptake of lactate in *S. cerevisiae* and can also transport other  
19 monocarboxylates, including acetate (Casal et al. 1999). The protein Ady2p was later found  
20 to be essential for acetate transport activity in acetic acid-grown cells (Paiva et al. 2004).  
21 When available as the sole carbon and energy source, acetate is metabolised to acetyl  
22 coenzyme A (acetyl Co-A) by one of the two ACS proteins: Acs1p (peroxisomal) or Acs2p  
23 (cytosolic). Acetyl Co-A is then oxidized in the tricarboxylic acid cycle after entering  
24 mitochondria. It is also used to produce succinate and hence replenish the cell with  
25 biosynthetic precursors by entering the glyoxylate cycle, which involves the key enzymes

1 isocitrate lyase and malate synthase outside the mitochondria. In addition, acetyl Co-A is  
2 used for synthesis of macromolecules, which requires active gluconeogenesis (dos Santos et  
3 al. 2003; Kruckeber and Dickinson 2004).

4 The aforementioned studies have characterized the transport and catabolism of acetic acid in  
5 yeast using mainly synthetic media. Therefore, further work is required to assess how these  
6 two steps in acetic acid catabolism are affected by the stress conditions present in grape-  
7 musts and wines.

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### 9 **Cytotoxic effect of acetic acid on the fermentative yeast *S. cerevisiae* and its role as** 10 **physiological inducer of apoptosis**

11

12 Acetic acid can affect the metabolic activity of fermentative yeast and give rise to sluggish  
13 or stuck fermentations (Alexandre and Charpentier 1998). Therefore, in light of the  
14 biotechnological relevance of *S. cerevisiae*, the cytotoxic effects induced by this and other  
15 weak carboxylic acids on fermentative yeast have been the subject of extensive research. It  
16 was shown that when acetic acid enters cells by simple diffusion it dissociates if the  
17 extracellular pH is lower than the intracellular pH. This leads to intracellular acidification,  
18 anion accumulation (Casal et al. 1996) and inhibition of cellular metabolic activity, namely  
19 fermentation (Pampulha and Loureiro 1989). Studies on enzymatic activities showed that  
20 enolase is the glycolytic enzyme most affected by acetic acid, resulting in an alteration of  
21 glycolysis (Pampulha and Loureiro 1990). Moreover, acetic acid compromises the cellular  
22 viability of *S. cerevisiae* under certain conditions, and ultimately results in two types of cell  
23 death, high and low enthalpy cell death (Pinto et al. 1989). Assessment of cellular structural  
24 and functional changes induced by acetic acid in *S. cerevisiae* by flow cytometry pointed to  
25 an intracellular localization of the acetic acid cellular target(s) (Ludovico 1999; Prudêncio et

1 al. 1998). Identification of morphological, structural and functional cellular death markers  
2 allowed the characterization of the cell death process. High doses of acetic acid (120-200  
3 mM) lead to a necrotic phenotype in exponential phase cells of *S. cerevisiae* whereas low  
4 doses (20-80 mM) trigger a programmed cell death (PCD) exhibiting characteristics of  
5 mammalian apoptosis (Ludovico et al. 2001).

6 Alterations associated with cell death induced by low levels of acid include: (i)  
7 cycloheximide-inhibitable chromatin condensation along the nuclear envelope verified by  
8 transmission electron microscopy and DAPI staining; (ii) exposure of phosphatidylserine on  
9 the surface of the cytoplasmic membrane, revealed by the FITC–annexin V reaction; and  
10 (iii) the occurrence of DNA strand breaks, demonstrated by the TUNEL assay. Pulsed field  
11 gel electrophoresis of chromosomal DNA from stationary phase cells dying by apoptosis  
12 after exposure to acetic acid (175 mM) revealed DNA breakdown into fragments of several  
13 hundred kilobases, consistent with the higher order chromatin degradation preceding DNA  
14 laddering in apoptotic mammalian cells (Ribeiro et al. 2006). Subsequent studies  
15 demonstrated the involvement of mitochondria in the *S. cerevisiae* PCD process triggered by  
16 acetic acid, indicating that, like in mammalian cells, PCD in yeast can be mediated by  
17 mitochondria. Biochemical and molecular evidence provided by such studies included the  
18 accumulation of mitochondrial reactive oxygen species (ROS), transient hyperpolarization  
19 followed by depolarization, decrease in cytochrome oxidase activity affecting mitochondrial  
20 respiration, and release of lethal factors like cytochrome *c* (Ludovico et al. 2002). ROS, in  
21 particular hydrogen peroxide, are mediators rather than by-products in *S. cerevisiae* cells  
22 committed to apoptosis triggered by acetic acid (Giannattasio et al. 2005). Mitochondrial  
23 outer membrane permeabilization (MOMP) is a crucial step in the apoptotic pathway. This  
24 triggers the release of proteins from the mitochondrial intermembrane space into the cytosol,  
25 where they ensure propagation of the apoptotic cascade and execution of cell death. Opening

1 of a mitochondrial pore called the permeability transition pore (PTP), which leads to the  
2 swelling of mitochondria and rupture of the mitochondrial outer membrane, has been put  
3 forward as one of the mechanisms underlying mammalian MOMP. Although the molecular  
4 composition of the pore is not completely defined, it has been proposed that its major  
5 components are the adenine nucleotide transporter (ANT), the voltage dependent anion  
6 channel (VDAC) and cyclophilin D (for a review, see Crompton 1999; Martinou et al. 2000;  
7 Bras et al. 2005). Yeast genetic approaches revealed that while deletion of POR1 (yeast  
8 VDAC) enhances apoptosis triggered by acetic acid, absence of ADP/ATP carrier (AAC)  
9 proteins (yeast orthologues of ANT) protects cells exposed to acetic acid (Pereira et al.  
10 2007). Absence of AAC proteins does not completely prevent acetic acid-induced apoptosis,  
11 suggesting that alternative redundant pathways are involved. One such pathway may be the  
12 translocation of Aif1p, the yeast apoptosis inducing factor, from the mitochondria to the  
13 nucleus in response to acetic acid (Wissing et al. 2004). Other mitochondrial proteins have  
14 been implicated in the execution of the yeast apoptotic program induced by acetic acid,  
15 including those involved in fission/fusion, namely Fis1p, Dnm1p, Mdv1p (Fannjiang et al.  
16 2004) and Nucl1p, the yeast ortholog of the mammalian endonuclease G (Buttner et al.  
17 2007). Ysp2p is another mitochondrial protein with a direct function in mitochondria-  
18 mediated PCD, since its absence hinders mitochondrial thread-to-grain transition and  
19 confers resistance to acetic acid-induced PCD (Sokolov et al. 2006). Caspases (cysteine  
20 aspartic proteases), key components of the mammalian apoptotic machinery, have a crucial  
21 role in cell dismantling. The metacaspase Yca1p, the only yeast ortholog of mammalian  
22 caspases identified so far, is activated in cells undergoing acetic acid-induced apoptosis in a  
23 manner strongly dependent on the cell growth phase (Pereira et al. 2007). Since cells lacking  
24 Yca1p undergo apoptosis in response to acetic acid, though more slowly than wild type  
25 cells, a caspase-independent pathway was also proposed (Guaragnella et al. 2006). Besides

1 Yca1p, the Kex1p protease, involved in programmed cell death caused by defective N-  
2 glycosylation, also contributes to the active cell death program induced by acetic acid stress  
3 (Hauptmann et al. 2006). Transient proteasome activation is also necessary for protein  
4 degradation during acetic acid-induced apoptosis (Valenti et al. 2008). The occurrence of  
5 mitochondrial degradation following apoptosis induction is a common feature of  
6 mammalian cells (reviewed in Tolkovsky et al. 2002). This event usually occurs through an  
7 autophagic process that shows selectivity for mitochondria, termed mitophagy (Lemasters  
8 2005). Recent evidence supports the view that the PTP could be the trigger for  
9 mitochondrial degradation (Rodriguez-Enriquez et al. 2006; Kim et al. 2007). In yeast cells  
10 undergoing apoptosis, mitochondrial degradation has also been reported (Fannjiang et al.  
11 2004). Selective removal of mitochondria was reported following heterologous expression  
12 of Bax (Kissova et al. 2007), mitochondrial dysfunction (Priault et al. 2005), osmotic  
13 swelling (Nowikovskiy et al. 2007) and in yeast stationary phase cells (Tal et al. 2007).  
14 However, removal of mitochondria is not always dependent on the autophagic machinery  
15 (Matsui et al. 2006). It was recently found that autophagy is not active during acetic acid-  
16 induced apoptosis (Pereira et al. 2010). Alternatively, the vacuolar protease Pep4p is  
17 translocated to the cytosol and, together with the AAC proteins, plays an important role in  
18 mitochondrial degradation. Moreover, it was proposed that the AAC proteins relay a signal  
19 of mitochondrial dysfunction, targeting their destruction. Another work also documented the  
20 involvement of the vacuole in the apoptotic process. Deletion of class C vacuolar protein  
21 sorting genes results in drastically enhanced sensitivity to treatment with acetic acid and lead  
22 to a necrotic death (Schauer et al. 2009). These results unveil a complex regulation and  
23 interplay between mitochondria and the vacuole in yeast PCD.

24 As described above, acetic acid-induced death has been characterized to a great extent, and  
25 we are beginning to understand the intricate interplay between the large number of players

1 involved in this response. However, there are no studies available regarding the  
2 characterization of cell death in response to ethanol and acetic acid. Ethanol-induced cell  
3 death of *S. cerevisiae* also exhibits features of apoptosis and is mediated by the  
4 mitochondrial fission protein Fis1 (Kitagaki et al. 2007), and it is known that octanoic and  
5 decanoic acid enhance ethanol induced cell death (Sá-Correia 1986). It will be important to  
6 confirm whether cell death occurs through a regulated process in the presence of ethanol and  
7 acetic acid, and to assess its implications on yeast fermentative performance. These studies  
8 will contribute to overcoming limitations in large-scale fermentation processes, such as  
9 those utilized in the production of alcoholic beverages and ethanol-based biofuels.

10

## 11 **Current methods and new solutions for the reduction of volatile acidity in wines and** 12 **grape musts**

13

14 Several methodologies aiming to decrease excessive volatile acidity of acidic wines have  
15 been proposed, which include: i) microbial stabilization of the acidic wine followed by  
16 mixture with other wines; ii) use of membrane processes such as reverse osmosis and  
17 nanofiltration (Fugelsang and Edwards 2007); iii) biological removal of acetic acid through  
18 refermentation (Ribéreau- Gayon et al. 1975; Ribéreau- Gayon et al. 2000). The first  
19 approach relies on microbial stabilization of the acidic wine, which is then blended with  
20 other wines with low acetic acid content, but the resulting wine usually has reduced  
21 commercial value (Zoecklein et al. 1995). Alternatively, the acidic wine can be sold for  
22 distillation purposes to obtain ethanol, also with economical losses. Reverse osmosis (RO)  
23 and nanofiltration can also be used for the deacidification of acidic wines. These techniques  
24 yield an acetic acid rich-permeate which is then treated by ion exchange to remove the acid  
25 (Boulton et al. 1996). RO is similar to membrane filtration and removes many types of large

1 molecules and ions from solutions by applying pressure to the solution when it is on one  
2 side of a selective membrane. It removes particles larger than 0.1 nm, whereas nano- ultra-  
3 and microfiltration remove particle sizes larger than 1, 3, and 50 nm, respectively. The  
4 separation efficiency is dependent on solute concentration, pressure and water flux rate.  
5 Several companies market RO systems for volatile acidity reduction. Vinovation, a  
6 Californian company, proposes coupling reverse osmosis and anion exchange resins,  
7 whereby the permeate of reverse osmosis (containing mainly water, ethanol and acetic acid)  
8 is coupled with an ion exchange resin for volatile acidity removal. The treated permeate is  
9 then combined with the retentate. The company VA Filtration proposes a combination of RO  
10 and selective adsorption of acetic acid. A third approach consists of the combination of two  
11 stages of RO, where the targeted acid of the first permeate is transferred in a salty form and  
12 then retained by the second stage RO membrane (Massot et al. 2008).

13 Several approaches have been developed for biodeacidification in order to achieve wines  
14 with a fine balance between sugar and acid contents, but they are limited to the metabolism  
15 of malic acid (Bony et al. 1997; Husnik et al. 2006; Husnik et al. 2007; Main et al. 2007;  
16 Silva et al. 2003; Sousa et al. 1995; Volschenk et al. 1997). Nonetheless, a genetically  
17 modified strain that substantially decreases acetate yield has been obtained (Remize et al.  
18 2000). However, due to the controversial discussion regarding the use of genetically  
19 modified food in Europe, it is likely that such strains will not be used for winemaking in the  
20 near future (Schuller and Casal 2005; Schuller 2010). Alternatively, abnormally high  
21 concentrations of acetic acid can be removed from wines by refermentation (Riberéau-  
22 Gayon et al. 1975). In this process, one third of an acidic wine is mixed with two thirds of  
23 freshly crushed grapes or of the residual marc from the fermentation of a finished wine  
24 (remaining pulp, after draining the newly made wine), such that the volatile acidity of this  
25 mixture does not exceed  $0.73 \text{ g l}^{-1}$  of acetic acid. This rather empirical approach reduces

1 volatile acidity to values in the range of 0.37 g l<sup>-1</sup> of acetic acid and implies low costs.  
2 However, it harbors the risk of unexpected final results and detrimental effects on  
3 fermentation since the involved yeast flora is largely unknown (Zoecklein et al. 1995). In an  
4 approach to search for yeasts that are the most suitable for a deacidification process, 135  
5 yeast isolates and 9 commercial *S. cerevisiae* strains were characterized regarding their  
6 ability to use glucose and acetic acid simultaneously. The most promising strains  
7 (commercial strains S26 and S29) were then evaluated in synthetic media containing acidic  
8 wines that were supplemented with high glucose/low ethanol or low glucose/high ethanol  
9 concentrations. This simulates the refermentation of a wine with grape-must from the  
10 beginning of fermentation or with the residual marc from a finished wine, respectively. Both  
11 strains remove over 80% of the acetic acid, though strain S29 is more efficient under the  
12 first condition, with limited aerobiosis, whereas strain S26 is more efficient under the second  
13 condition, in an aerobic environment (Vilela-Moura et al. 2008). However, even the low  
14 amounts of oxygen required under the limited-aerobic conditions tested might compromise  
15 the application of the strains in refermentation processes. Therefore, acetic acid removal  
16 from acidic white or red wines by the S26 and S29 strains was also evaluated at a pilot scale  
17 under enological conditions. When grape-must is used for the supplementation of acidic  
18 white wines, strains S26 and S29 still reduce approximately half the acetic acid and exhaust  
19 the sugar. Similar results were obtained for mixtures of acidic red wines with grape-must or  
20 residual marc, which were not improved by micro-oxygenation (MO). This study also  
21 showed that lower concentrations of acetic acid do not always correlate with higher sensory  
22 classification. Indeed, although the red wines obtained by refermentation with the grape-  
23 must have a somewhat lower acetic acid concentration, those obtained by marc addition  
24 when strain S26 is used without MO achieve the best sensory scores. A separate study found

1 that the volatile aroma compound composition is not affected by MO, but rather by the  
2 refermentation process itself (Vilela-Moura et al. 2010a).

3 The reduction in acetic acid by the strains mentioned above was also assessed under the very  
4 stressful conditions imposed by the combination of ethanol, acetic acid, and SO<sub>2</sub> to evaluate  
5 their applicability in the deacidification of different types of acidic wines (Vilela-Moura et  
6 al. 2010b). Both S26 and S29 strains efficiently reduce the volatile acidity (78% and 48%)  
7 from acidic wines with acetic acid and ethanol concentrations not higher than 1.0 gL<sup>-1</sup> and  
8 11% (v/v), respectively. However, the strong anti-oxidant and antiseptic effect of sulphur  
9 dioxide (SO<sub>2</sub>) concentrations in the range of 95 - 170 mgL<sup>-1</sup> inhibits the reduction of volatile  
10 acidity. Deacidification by strain S26 is associated with significantly increased  
11 concentrations of wine aromatic compounds, such as isoamyl acetate (banana) and ethyl  
12 hexanoate (apple, pineapple) but acetaldehyde concentration also increases slightly (Vilela-  
13 Moura et al. 2010b). Efficient removal of acetic acid from an acidic wine (1.1 gL<sup>-1</sup> acetic  
14 acid, 12.5% ethanol, pH 3.12) is also observed when *S. cerevisiae* S26 cells are entrapped in  
15 double-layer alginate-chitosan beads, a method which would allow for facilitated separation  
16 of the yeast from the finished wine (Vilela-Moura, unpublished data).

17 The aforementioned studies support the use of refermentation processes using select  
18 commercial yeast strains as enological practices to correct grape-musts or wines with  
19 excessive volatile acidity. Moreover, they provide the basis of efficient and inexpensive  
20 alternative deacidification methods that contribute to improving the quality of wines with  
21 excessive levels of volatile acidity.

22

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24

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7

8 Figure 1. Schematic representation of the main reactions and enzymes involved in acetic acid  
9 metabolism in yeast. When pyruvate dehydrogenase (PDH) is repressed, acetyl-  
10 CoA is synthesized through the PDH bypass (grey arrows) which involves the  
11 sequential action of pyruvate decarboxylase (PDC), aldehyde dehydrogenase  
12 (ALD) and acetyl CoA synthetase (ACS). Acetyl-CoA is used for fatty acid  
13 synthesis, or oxidized in the tricarboxylic acid cycle (TCA) after entering  
14 mitochondria. Acetyl-CoA can be converted to acetate and generate Co-A by  
15 acetyl-CoA hydrolase (ACH).

16

## 17 **References**

- 18 Alexandre H, Charpentier C (1998) Biochemical aspects of stuck and sluggish fermentation  
19 in grape must. *J Ind Microbiol Biotechnol* 20:20–27
- 20 Alexandre H, Nguyen Van Long T, Feuillat M, Charpentier C (1994) Contribution à l'étude  
21 des bourbes: influence sur la fermentescibilité des moûts. *Rev Fr Eno* 146:11–20
- 22 Amerine MA, Berg HW, Cruess WV (1972) *The Technology of Wine Making*. The Avi  
23 Publishing Company (eds.) 3rd edn. Westport, CT
- 24 Arneborg N, Jespersen L, Jakobsen M (2000) Individual cells of *Saccharomyces cerevisiae*  
25 and *Zygosaccharomyces bailii* exhibit different short-term intracellular pH responses  
26 to acetic acid. *Arch Microbiol* 174:125-128
- 27 Barbosa C, Falco V, Mendes-Faia A, Mendes-Ferreira A (2009) Nitrogen addition  
28 influences formation of aroma compounds, volatile acidity and ethanol in nitrogen  
29 deficient media fermented by *Saccharomyces cerevisiae* wine strains. *J Biosci*  
30 *Bioeng* 108:99-104
- 31 Barnett JA, Payne RW, Yarrow D (1990) *Yeast. Characteristics and Identification*.  
32 Cambridge University Press (eds) 2nd edn. Cambridge

- 1 Bartowsky EJ, Henschke PA (2008) Acetic acid bacteria spoilage of bottled red wine - a  
2 review. *Int J Food Microbiol* 125:60-70
- 3 Beltran G, Novo M, Guillamon JM, Mas A, Rozes N (2008) Effect of fermentation  
4 temperature and culture media on the yeast lipid composition and wine volatile  
5 compounds. *Int J Food Microbiol* 121:169-177
- 6 Bely M, Rinaldi A, Dubourdiou D (2003) Influence of assimilable nitrogen on volatile  
7 acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. *J*  
8 *Biosci Bioeng* 96:507-512
- 9 Bony M, Bidart F, Camarasa C, Ansanay V, Dulau L, Barre P, Dequin S (1997) Metabolic  
10 analysis of *S. cerevisiae* strains engineered for malolactic fermentation. *FEBS Letters*  
11 410:452-456
- 12 Boulton RB, Singleton VL, Bisson LF, Kunkee RE (1996) Principles and Practices of  
13 Winemaking. Chapman & Hall (eds) 1st edn. New York, USA
- 14 Bras M, Queenan B, and Susin SA (2005) Programmed cell death via mitochondria:  
15 different modes of dying. *Biochemistry* 70:231–239
- 16 Buttner S, Eisenberg T, Carmona-Gutierrez D, Ruli D, Knauer H, Ruckenstuhl C, Sigrist C,  
17 Wissing S, Kollroser M, Frohlich KU, Sigrist S, Madeo F (2007) Endonuclease G  
18 regulates budding yeast life and death. *Mol Cell* 25:233-246.
- 19 Casal M, Leao C (1995) Utilization of short-chain monocarboxylic acids by the yeast  
20 *Torulaspora delbrueckii*: Specificity of the transport systems and their regulation.  
21 *Biochim Biophys Acta* 1267:122-130
- 22 Casal M, Cardoso H, Leao C (1996) Mechanisms regulating the transport of acetic acid in  
23 *Saccharomyces cerevisiae*. *Microbiology* 142:1385-1390
- 24 Casal M, Cardoso H, Leao C (1998) Effects of ethanol and other alkanols on transport of  
25 acetic acid in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 64:665-668
- 26 Casal M, Paiva S, Andrade RP, Gancedo C, Leao C (1999) The lactate-proton symport of  
27 *Saccharomyces cerevisiae* is encoded by JEN1. *J Bacteriol* 181:2620-2623
- 28 Ciani M, Maccarelli F (1998) Oenological properties of non-*Saccharomyces* yeasts associated  
29 with wine-making. *World J Microbiol Biotechnol* 14:199–203.
- 30 Cogan TM (1987) Co-metabolism of citrate and glucose by *Leuconostoc* spp.: Effects on  
31 growth, substrate and products. *J Appl Bacteriol* 63:551–558
- 32 Coote N, Kirsop HH (1974) The content of some organic acids in beer and other fermented  
33 media. *J Inst Brew* 80:474–483
- 34 Crompton, M (1999) The mitochondrial permeability transition pore and its role in cell  
35 death. *Biochem J* 341: 233–249

- 1 Delfini C and Costa A (1993) Effects of the grape must lees and insoluble materials on the  
2 alcoholic fermentation rate and on the production of acetic acid, pyruvic acid and  
3 acetaldehyde. *Am J Enol Viticult* 44:86-92
- 4 dos Santos MM, Gombert AK, Christensen B, Olsson L, Nielsen J (2003) Identification of  
5 in vivo enzyme activities in the cometabolism of glucose and acetate by  
6 *Saccharomyces cerevisiae* by using <sup>13</sup>C-labeled substrates. *Eukaryot Cell* 2:599-608
- 7 Du Toit WJ, Lambrechts MG (2002) The enumeration and identification of acetic acid  
8 bacteria from south african red wine fermentations. *Int J Food Microbiol* 74:57-64
- 9 Erasmus DJ, Cliff M, van Vuuren HJJ (2004) Impact of yeast strain on the production of  
10 acetic acid, glycerol, and the sensory attributes of Icewine. *Am J Enol Viticult*  
11 55:371-378
- 12 Fannjiang Y, Cheng WC, Lee SJ, Qi B, Pevsner J, McCaffery JM, Hill RB, Basanez G,  
13 Hardwick JM (2004) Mitochondrial fission proteins regulate programmed cell death  
14 in yeast. *Genes Dev* 18:2785-2797
- 15 Fleet G H, Heard G M (1993) Yeasts-Growth during Fermentation. In: Fleet G H (ed.),  
16 Wine Microbiology and Biotechnology Harwood Academic Publishers, Chur,  
17 Switserzland, pp. 27-54
- 18 Fugelsang KC, Edwards CG (2007) Wine Microbiology. Practical Applications and  
19 Procedures. Springer Science Business Media (eds) 2nd edn. New York, USA
- 20 Gerós H, Azevedo MM, Cássio F (2000a) Biochemical studies on the production of acetic  
21 acid by the yeast *Dekkera anomala*. *Food Technol and Biotechnol* 38:59-62
- 22 Gerós H, Cassio F, Leão C (2000b) Utilization and transport of acetic acid in *Dekkera*  
23 *anomala* and their implications on the survival of the yeast in acidic environments. *J*  
24 *Food Prot* 63:96-101
- 25 Giannattasio S, Guaragnella N, Côrte-Real M, Passarella S, Marra E (2005) Acid stress  
26 adaptation protects *Saccharomyces cerevisiae* from acetic acid-induced programmed  
27 cell death. *Gene* 354:93-98
- 28 Guaragnella N, Pereira C, Sousa MJ, Antonacci L, Passarella S, Côrte-Real M, Marra E,  
29 Giannattasio S (2006) Yca1 participates in the acetic acid induced yeast programmed  
30 cell death also in a manner unrelated to its caspase-like activity. *FEBS Lett*  
31 580:6880-6884
- 32 Hauptmann P, Riel C, Kunz-Schughart LA, Frohlich KU, Madeo F, Lehle L (2006) Defects  
33 in n-glycosylation induce apoptosis in yeast. *Mol Microbiol* 59:765-778
- 34 Husnik JJ, Volschenk H, Bauer J, Colavizza D, Luo Z, van Vuuren HJ (2006) Metabolic  
35 engineering of malolactic wine yeast. *Metabolic Engineering* 8:315-323
- 36 Husnik JJ, Delaquis PJ, Cliff MA, van Vuuren HJJ (2007) Functional analyses of the  
37 malolactic wine yeast ml01. *Am J Enol Viticult* 58:42-52

- 1 Jost and Piendl (1975) Technological influences on the formation of acetate during  
2 fermentation. *Am Soc Brew Chem* 34:31–37
- 3 Joyeux A, Lafon-Lafourcade S, Ribéreau-Gayon P, (1984a) Evolution of acetic acid bacteria  
4 during fermentation and storage of wine. *Appl. Environ. Microbiol.* 48:153–156.  
5
- 6 Joyeux A, Lafon-Lafourcade S, Ribéreau-Gayon P (1984b) Metabolism of acetic acid bacteria in  
7 grape must: consequences on alcoholic and malolactic fermentation. *Sci. Aliments* 4, 247–255.
- 8 Kim I, Rodriguez-Enriquez S, Lemasters JJ (2007) Selective degradation of mitochondria by  
9 mitophagy. *Arch Biochem Biophys* 462:245-253
- 10 Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, Camougrand N (2007) Selective and  
11 non-selective autophagic degradation of mitochondria in yeast. *Autophagy* 3:329-336
- 12 Kitagaki H, Araki Y, Funato K, Shimoi H (2007) Ethanol-induced death in yeast exhibits  
13 features of apoptosis mediated by mitochondrial fission pathway. *FEBS Lett*  
14 581:2935-2942
- 15 Kruckeber AL, Dickinson JR (2004) Carbon metabolism. In: Dickinson JR and Schweizer  
16 M (eds) *The metabolism and molecular physiology of Saccharomyces cerevisiae*.  
17 CRC Press, New York, pp 42-76
- 18 Leão C, Van Uden N (1986) Transport of lactate and other short-chain monocarboxylates in  
19 the yeast *Candida utilis*. *Appl Microbiol Biotechnol* 23:389-393
- 20 Lemasters JJ (2005) Selective mitochondrial autophagy, or mitophagy, as a targeted defense  
21 against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res* 8:3-  
22 5
- 23 Llauradó JM, Rozès N, Constantí M, Mas A (2005) Study of some *Saccharomyces*  
24 *cerevisiae* strains for winemaking after preadaptation at low temperatures. *J Agric*  
25 *Food Chem* 53:1003-11
- 26 Lonvaud-Funel A (1999) Lactic acid bacteria in the quality improvement and depreciation of  
27 wine. *Antonie Van Leeuwenhoek* 76:317–331
- 28 Ludovico P (1999) Efeitos do ácido acético no potencial de membrana mitocondrial e sua  
29 relação com a perda de integridade e viabilidade celular em *Zygosaccharomyces*  
30 *bailii* e *Saccharomyces cerevisiae*. Estudos por citometria de fluxo e  
31 espectrofluorimetria. Tese de Mestrado, Universidade do Minho
- 32 Ludovico P, Sousa MJ, Silva MT, Leao C, Côrte-Real M (2001) *Saccharomyces cerevisiae*  
33 commits to a programmed cell death process in response to acetic acid. *Microbiology*  
34 147:2409-2415
- 35 Ludovico P, Rodrigues F, Almeida A, Silva MT, Barrientos A, Côrte-Real M (2002)  
36 Cytochrome *c* release and mitochondria involvement in programmed cell death  
37 induced by acetic acid in *Saccharomyces cerevisiae*. *Mol Biol Cell* 13:2598-2606

- 1 Main GL, Threlfall RT, Morris JR (2007) Reduction of malic acid in wine using natural and  
2 genetically enhanced microorganisms. *Am J of Enol Viticult* 58:341-345
- 3 Martinou, JC, Desagher S, and Antonsson B (2000) Cytochrome *c* release from  
4 mitochondria: all or nothing. *Nat Cell Biol* 2:41-43
- 5 Marullo P, Aigle M, Bely M, Masneuf-Pomarede I, Durrens P, Dubourdieu D, Yvert G  
6 (2007) Single qtl mapping and nucleotide-level resolution of a physiologic trait in  
7 wine *Saccharomyces cerevisiae* strains. *FEMS Yeast Res* 7:941-952
- 8 Massot A, Mietton-Peuchot M, Peuchot C, Milisic V (2008) Nanofiltration and reverse  
9 osmosis in winemaking. *Desalination* 231:283-289
- 10 Matsui M, Yamamoto A, Kuma A, Ohsumi Y, and Mizushima N (2006) Organelle  
11 degradation during the lens and erythroid differentiation is independent of  
12 autophagy. *Biochem Biophys Res Commun* 339:485-489
- 13 Mollapour M, Piper PW (2007) Hog1 mitogen-activated protein kinase phosphorylation  
14 targets the yeast Fps1 aquaglyceroporin for endocytosis, thereby rendering cells  
15 resistant to acetic acid. *Mol Cell Biol* 27:6446-6456
- 16 Monk PR, Cowley PJ (1984) Effect of nicotinic acid and sugar concentration of grape juice  
17 and temperature on accumulation of acetic acid yeast fermentation. *J Ferment*  
18 *Technol* 62:515-521
- 19 Moruno EG, Delfini C, Pessione E, Giunta C (1993) Factors affecting acetic acid production  
20 by yeasts in strongly clarified grape musts. *Microbios* 74:249-256
- 21 Nowikovsky K, Reipert S, Devenish RJ, Schweyen RJ (2007) Mdm38 protein depletion  
22 causes loss of mitochondrial K<sup>+</sup>/H<sup>+</sup> exchange activity, osmotic swelling and  
23 mitophagy. *Cell Death Differ* 14:1647-1656
- 24 Office Internationale de la Vigne et du Vin (2009) Compendium of international methods of  
25 wine and must analysis. Vol1 OIV, Paris, p 419 p  
26
- 27 Office Internationale de la Vigne et du Vin (2010) International code of oenological  
28 practices. OIV, Paris, p 274 p  
29
- 30 Orlić S, Arroyo-López FN, Huić-Babić K, Lucilla I, Querol A, Barrio E (2010) A  
31 comparative study of the wine fermentation performance of *Saccharomyces*  
32 *paradoxus* under different nitrogen concentrations and glucose/fructose ratios. *J Appl*  
33 *Microbiol* 108:73-80
- 34 Paiva S, Althoff S, Casal M, Leao C (1999) Transport of acetate in mutants of  
35 *Saccharomyces cerevisiae* defective in monocarboxylate permeases. *FEMS*  
36 *Microbiol Lett* 170:301-306
- 37 Paiva S, Devaux F, Barbosa S, Jacq C, Casal M (2004) Ady2p is essential for the acetate  
38 permease activity in the yeast *Saccharomyces cerevisiae*. *Yeast* 21:201-210

- 1 Pampulha MA and Loureiro-Dias MC (1989) Combined effect of acetic acid, pH and  
2 ethanol on intracellular pH of fermenting yeast. *Appl Microbiol Biotechnol* 31:547–  
3 550
- 4 Pampulha MA and Loureiro-Dias MC (1990) Activity of glycolytic enzymes of  
5 *Saccharomyces cerevisiae* in the presence of acetic acid. *Appl Microbiol Biotechnol*  
6 34:375–380
- 7 Patel S, Shibamoto S (2002) Effect of different strains of *Saccharomyces cerevisiae* on  
8 production of volatiles in Napa Gamay wine and Petite Syrah wine. *J Agric Food*  
9 *Chem* 50:5649–5653
- 10 Pereira C, Camougrand N, Manon S, Sousa MJ, Côte-Real M (2007) ADP/ATP carrier is  
11 required for mitochondrial outer membrane permeabilization and cytochrome *c*  
12 release in yeast apoptosis. *Mol Microbiol* 66:571-582
- 13 Pereira C, Chaves S, Alves S, Salin B, Camougrand N, Manon S, Sousa MJ, Côte-Real M  
14 (2010) Mitochondrial degradation in acetic acid-induced yeast apoptosis: The role of  
15 Pep4 and the ADP/ATP carrier. *Mol Microbiol* 76:1398-410
- 16 Perez L, Valcarcel MJ, Gonzalez P, Domecq B (1991) Influence of *Botrytis* infection of the  
17 grapes on the biological aging process of Fino Sherry. *Am J Enol Viticult* 42:58-62
- 18 Pinto, I, Cardoso, H, Leão, C (1989) High enthalpy and low enthalpy death in  
19 *Saccharomyces cerevisiae* induced by acetic acid. *Biotechnol Bioeng* 33:1350-1352
- 20 Pozo-Bayon MA, E GA, Polo MC, Tenorio C, Martin-Alvarez PJ, Calvo de la Banda MT,  
21 Ruiz-Larrea F, Moreno-Arribas MV (2005) Wine volatile and amino acid  
22 composition after malolactic fermentation: Effect of *Oenococcus oeni* and  
23 *Lactobacillus plantarum* starter cultures. *J Agric Food Chem* 53:8729-8735
- 24 Pretorius IS (2000) Tailoring wine yeast for the new millennium: Novel approaches to the  
25 ancient art of winemaking. *Yeast* 16:675-729
- 26 Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, Martinou JC (2005) Impairing the  
27 bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast.  
28 *Cell Death Differ* 12:1613–1621
- 29 Prudêncio C, Sansonetty F, Côte-Real M (1998) Flow cytometric assessment of cell  
30 structural and functional changes induced by acetic acid in the yeasts  
31 *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae*. *Cytometry* 31:307-313
- 32 Radler F (1993) Yeasts-metabolism of organic acids.. In: Fleet,G.H. (ed.), *Wine*  
33 *Microbiology and Biotechnology* Harwood Academic Publishers, Chur, Switzerzland,  
34 pp. 165–223
- 35 Remize F, Andrieu E, Dequin S (2000) Engineering of the pyruvate dehydrogenase bypass  
36 in *Saccharomyces cerevisiae*: Role of the cytosolic Mg<sup>2+</sup> and mitochondrial K<sup>+</sup>  
37 acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic  
38 fermentation. *Appl and Environ Microbiol* 66:3151-3159

- 1 Ribeiro GF, Côrte-Real M, Johansson B (2006) Characterization of DNA damage in yeast  
2 apoptosis induced by hydrogen peroxide, acetic acid, and hyperosmotic shock. *Mol*  
3 *Biol Cell* 17:4584-4591
- 4 Ribéreau-Gayon P, Lafon-Lafourcade S, Dubourdiou D, Lucmaret V, Larue F (1979)  
5 Métabolisme de *Saccharomyces cerevisiae* dans le moût de raisins parasités par  
6 *Botrytis cinerea*. *C R Acad Sci Fr* 289:441-444
- 7 Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdiou D (2006a). Alcohols and Other Volatile  
8 Compounds, In: John Wiley & Sons (Ed.), *The Chemistry of Wine Stabilization and Treatments*,  
9 2nd edn.: *Handbook of Enology*, vol. 2, pp. 51-64. Chichester, England
- 10 Ribéreau-Gayon P, Dubourdiou D, Donèche B, Lonvaud A, (2006b). John Wiley & Sons,  
11 Ltd (eds) *The Microbiology of Wine and Vinifications*, 2nd edn.: *Handbook of*  
12 *Enology*, vol. 1, Chichester, England
- 13 Rodrigues F (1998) Estudos sobre a regulação do metabolismo intracelular de ácido acético  
14 na levedura *Zygosaccharomyces bailli* ISA 1307. Tese de Mestrado, Universidade do  
15 Minho, Braga
- 16 Rodriguez-Enriquez S, Kim I, Currin RT, and Lemasters JJ (2006) Tracker dyes to probe  
17 mitochondrial autophagy (mitophagy) in rat hepatocytes. *Autophagy* 2:39-46
- 18 Richter H, Vlad D, Uden G (2001) Significance of pantothenate for glucose fermentation by  
19 *Oenococcus oeni* and for suppression of the erythritol and acetate production. *Arch*  
20 *Microbiol* 75:26-31
- 21 Romano P., Suzzi G., Comi G, Zironi R (1992) Higher alcohol and acetic acid production by  
22 apiculate wine yeasts. *J Appl Bacteriol* 73:126-130  
23
- 24 Romano P, Marchese R, Laurita C, Salzano G and Turbanti L (1999) Biotechnological suitability  
25 of *Saccharomyces ludwigii* for fermented beverages. *World J. Microbiol. Biotechnol.*  
26 15:451-545
- 27 Sá-Correia I (1986) Synergistic effects of ethanol, octanoic, and decanoic acids on the  
28 kinetics and the activation parameters of thermal death in *Saccharomyces bayanus*.  
29 *Biotechnol Bioeng* 28:761-763
- 30 Saint-Prix F, Bonquist L, Dequin S (2004) Functional analysis of the ADL gene family of  
31 *Saccharomyces cerevisiae* during anaerobic growth on glucose: The NADP<sup>+</sup>-  
32 dependent Ald6p and Ald5p isoforms play a major role in acetate formation.  
33 *Microbiology* 150:2209-2220
- 34 Schauer A, Knauer H, Ruckenstuhl C, Fussi H, Durchschlag M, Potocnik U, Frohlich KU  
35 (2009) Vacuolar functions determine the mode of cell death. *Biochim Biophys Acta*  
36 1793:540-545
- 37 Schuller D, Casal M (2005) The use of genetically modified *Saccharomyces cerevisiae*  
38 strains in the wine industry. *Appl Microbiol Biotechnol* 68:292-304

- 1 Schuller D (2010) Better yeast for better wine - genetic improvement of *Saccharomyces*  
2 *cerevisiae* winemaking strains. In: Rai M, Kövics G (eds) Progress in mycology.  
3 Scientific publishers (India), Jodhpur, pp 1-51
- 4 Schuller H J (2003) Transcriptional control of nonfermentative metabolism in the yeast  
5 *Saccharomyces cerevisiae*. Curr Genet 43:139-160
- 6 Silva S, Ramon-Portugal F, Andrade P, Abreu S, Texeira MD, Strehaiano P (2003) Malic  
7 acid consumption by dry immobilized cells of *Schizosaccharomyces pombe*.  
8 American Journal of Enology and Viticulture 54:50-55
- 9 Shimazu Y, Watanabe M. (1981) Effects of yeast strains and environmental conditions on  
10 formation of organic acid in must during fermentation. J Ferment Technol, 59:27–32
- 11 Sokolov S, Knorre D, Smirnova E, Markova O, Pozniakovsky A, Skulachev V, Severin F  
12 (2006) Ysp2 mediates death of yeast induced by amiodarone or intracellular  
13 acidification. Biochim Biophys Acta 1757:1366-70.
- 14 Sousa MJ, Mota M, Leão C (1995) Effects of ethanol and acetic acid on the transport of  
15 malic acid and glucose in the yeast *Schizosaccharomyces pombe*: implications in  
16 wine deacidification. FEMS Microbiol Lett. 126:197-202.
- 17 Sousa MJ, Rodrigues F, Côrte-Real M, Leao C (1998) Mechanisms underlying the transport  
18 and intracellular metabolism of acetic acid in the presence of glucose in the yeast  
19 *Zygosaccharomyces bailii*. Microbiology 144:665-670
- 20 Sponholz W R (1993) Wine spoilage by microorganisms. In: Fleet,G.H. (ed.), Wine  
21 Microbiology and Biotechnology Harwood Academic Publishers, Chur, Switserzland,  
22 pp. 395– 420
- 23 Tal R, Winter G, Ecker N, Klionsky DJ, Abeliovich H (2007) Aup1p, a yeast mitochondrial  
24 protein phosphatase homolog, is required for efficient stationary phase mitophagy  
25 and cell survival. J Biol Chem 282:5617-5624
- 26 Tolkovsky AM, Xue L, Fletcher GC, Borutaite V (2002) Mitochondrial disappearance from  
27 cells: A clue to the role of autophagy in programmed cell death and disease?  
28 Biochimie 84:233-240
- 29 Torrens J, Urpi P, Riu-Aumatell M, Vichi S, Lopez-Tamames E, Buxaderas S (2008)  
30 Different commercial yeast strains affecting the volatile and sensory profile of cava  
31 base wine. Int J Food Microbiol 124:48-57
- 32 Valenti D, Vacca RA, Guaragnella N, Passarella S, Marra E, Giannattasio S (2008) A  
33 transient proteasome activation is needed for acetic acid-induced programmed cell  
34 death to occur in *Saccharomyces cerevisiae*. FEMS Yeast Res 8:400–404
- 35 Verduyn C, Postma E, Scheffers WA, Van Dijken JP (1990) Physiology of *Saccharomyces*  
36 *cerevisiae* in anaerobic glucose-limited chemostat cultures. J Gen Micro 136:359-  
37 403

- 1 Vilanova M, Ugliano M, Varela C, Siebert T, Pretorius IS, Henschke PA (2007) Assimilable  
2 nitrogen utilisation and production of volatile and non-volatile compounds in  
3 chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. Appl  
4 Microbiol Biotechnol 77:145-157
- 5 Vilela-Moura A, Schuller D, Mendes-Faia A, Côte-Real M (2008) Reduction of volatile  
6 acidity of wines by selected yeast strains. Appl Microbiol Biotechnol 80:881-890
- 7 Vilela-Moura A, Schuller D, Mendes-Faia A, Corte-Real M (2010a) Effect of refermentation  
8 conditions and micro-oxygenation on the reduction of volatile acidity by commercial  
9 *S. cerevisiae* strains and their impact on the aromatic profile of wines. Int J Food  
10 Microbiol 141:165–172
- 11 Vilela-Moura A, Schuller D, Mendes-Faia A, Côte-Real M (2010b) Effects of acetic acid,  
12 ethanol, and SO<sub>2</sub> on the removal of volatile acidity from acidic wines by two  
13 *Saccharomyces cerevisiae* commercial strains. Appl Microbiol Biotechnol 87:1317-  
14 1326
- 15 Volschenk H, Viljoen M, Grobler J, Bauer F, Lonvaud-Funel A, Denayrolles M, Subden  
16 RE, VanVuuren HJJ (1997) Malolactic fermentation in grape musts by a genetically  
17 engineered strain of *Saccharomyces cerevisiae*. American Journal of Enology and  
18 Viticulture 48:193-197
- 19 Wissing S, Ludovico P, Herker E, Buttner S, Engelhardt SM, Decker T, Link A, Proksch A,  
20 Rodrigues F, Côte-Real M, Frohlich KU, Manns J, Cande C, Sigrist SJ, Kroemer G,  
21 Madeo F (2004) An AIF orthologue regulates apoptosis in yeast. J Cell Biol  
22 166:969-974
- 23 Zoecklein BW, Fugelsang KC, Gump BH, Nury FS (1995) Wine Analysis and Production.  
24 Chapman & Hall (eds.) 1st edn, New York, NY, USA
- 25