

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 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 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 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 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 CO₂
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⁻¹)
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 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.

8

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 g l^{-1} of acetic acid. This rather empirical approach reduces

1 volatile acidity to values in the range of 0.37 gL⁻¹ 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 SO₂ 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 g l⁻¹ and
8 11% (v/v), respectively. However, the strong anti-oxidant and antiseptic effect of sulphur
9 dioxide (SO₂) concentrations in the range of 95 - 170 mg l⁻¹ 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 g l⁻¹ 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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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

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