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

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Abstract

Acetic acid is the main component of the volatile acidity of grape musts and wines. It can be formed as a by-product of alcoholic fermentation or as a product of the metabolism of acetic and lactic acid bacteria, which can metabolise residual sugars to increase volatile acidity. Acetic acid has a negative impact on yeast fermentative performance and affects the quality of certain types of wine when present above a given concentration. In this mini-review we present an overview of fermentation conditions and grape-must composition favouring acetic acid formation, as well the metabolic pathways leading to its formation and degradation by yeast. The negative effect of acetic acid on the fermentative performance of *Saccharomyces cerevisiae* will also be covered, including its role as a physiological inducer of apoptosis. Finally, currently available wine deacidification processes and new proposed solutions based on zymological deacidification by select *S. cerevisiae* strains will be discussed.

Production of acetic acid in grape must and wine

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

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

Acetic acid is also formed as a by-product of alcoholic fermentation by *Saccharomyces cerevisiae*. Studies on the production of volatile acidity by *S. cerevisiae* under winemaking conditions showed that this acid is mainly formed at the beginning of alcoholic fermentation (Alexandre et al. 1994; Coote and Kirsop 1974) and its production is affected by different factors, namely the yeast strain (Erasmus et al. 2004; Orlić et al. 2010; Patel and Shibamoto 2002; Shimazu and Watanabe 1981; Torrens et al. 2008), grape-must composition (Delfini and Costa 1993), and fermentation conditions such as nitrogen content (Barbosa et al. 2009; Vilanova et al. 2007), vitamins, initial sugar concentration (Radler 1993) and other physical factors such as temperature (Monk and Cowley 1984; Llauradó et al. 2005; Beltran et al. 2008). Wine yeasts also produce acetic acid to equilibrate the redox balance in response to the hyperosmotic stress caused by high sugar concentrations, which can be especially severe in the high ºBrix (>35 ºBrix) grape-must (Erasmus et al. 2004) and in wines made from botritized grapes (Amerine et al. 1972). It has been shown that compounds like gluconic acid and glycerol produced as a consequence of *Botrytis* infection can affect the biological aging of this type of wines. Indeed, gluconic acid can be metabolised by heterofermentative lactic acid bacteria, which produce high concentrations of lactic acid and volatile acidity (Ribéreau-Gayon et al. 1979; Perez et al. 1991). Anaerobiosis, pH values below 3.1 or above 4.0, and excessive grape-must clarification are among other factors that favour the production of acetic acid by *S. cerevisiae* (Ribéreau-Gayon et al. 2006b). Variations in acetic acid production in natural *S. cerevisiae* strains can also have a genetic basis. A study using genome hybridization on DNA microarrays revealed that when asparagine is used as a major nitrogen source, acetic acid production is inversely associated with asparaginase type I activity and linked the production of this acid to nitrogen assimilation and the CO_2 production rate (Marullo et al. 2007). Acetate is also secreted in high levels by certain yeasts, such as *Dekkera* and its anamorph *Brettanomyces*, that have attracted attention as

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

Acetic acid metabolism in yeast

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

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

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

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

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

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

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

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

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

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

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

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

of a mitochondrial pore called the permeability transition pore (PTP), which leads to the swelling of mitochondria and rupture of the mitochondrial outer membrane, has been put forward as one of the mechanisms underlying mammalian MOMP. Although the molecular composition of the pore is not completely defined, it has been proposed that its major components are the adenine nucleotide transporter (ANT), the voltage dependent anion channel (VDAC) and cyclophilin D (for a review, see Crompton 1999; Martinou et al. 2000; Bras et al. 2005). Yeast genetic approaches revealed that while deletion of POR1 (yeast VDAC) enhances apoptosis triggered by acetic acid, absence of ADP/ATP carrier (AAC) proteins (yeast orthologues of ANT) protects cells exposed to acetic acid (Pereira et al. 2007). Absence of AAC proteins does not completely prevent acetic acid-induced apoptosis, suggesting that alternative redundant pathways are involved. One such pathway may be the translocation of Aif1p, the yeast apoptosis inducing factor, from the mitochondria to the nucleus in response to acetic acid (Wissing et al. 2004). Other mitochondrial proteins have been implicated in the execution of the yeast apoptotic program induced by acetic acid, including those involved in fission/fusion, namely Fis1p, Dnm1p, Mdv1p (Fannjiang et al. 2004) and Nuc1p, the yeast ortholog of the mammalian endonuclease G (Buttner et al. 2007). Ysp2p is another mitochondrial protein with a direct function in mitochondria-mediated PCD, since its absence hinders mitochondrial thread-to-grain transition and confers resistance to acetic acid-induced PCD (Sokolov et al. 2006). Caspases (cysteine aspartic proteases), key components of the mammalian apoptotic machinery, have a crucial role in cell dismantling. The metacaspase Yca1p, the only yeast ortholog of mammalian caspases identified so far, is activated in cells undergoing acetic acid-induced apoptosis in a manner strongly dependent on the cell growth phase (Pereira et al. 2007). Since cells lacking Yca1p undergo apoptosis in response to acetic acid, though more slowly than wild type cells, a caspase-independent pathway was also proposed (Guaragnella et al. 2006). Besides

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

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

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

Current methods and new solutions for the reduction of volatile acidity in wines and grape musts

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

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

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

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

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

The reduction in acetic acid by the strains mentioned above was also assessed under the very stressful conditions imposed by the combination of ethanol, acetic acid, and SO_2 to evaluate their applicability in the deacidification of different types of acidic wines (Vilela-Moura et al. 2010b). Both S26 and S29 strains efficiently reduce the volatile acidity (78% and 48%) from acidic wines with acetic acid and ethanol concentrations not higher than 1.0 gl^{-1} and 11% (v/v), respectively. However, the strong anti-oxidant and antiseptic effect of sulphur dioxide (SO_2) concentrations in the range of 95 - 170 mgl^{-1} inhibits the reduction of volatile acidity. Deacidification by strain S26 is associated with significantly increased concentrations of wine aromatic compounds, such as isoamyl acetate (banana) and ethyl hexanoate (apple, pineapple) but acetaldehyde concentration also increases slightly (Vilela-Moura et al. 2010b). Efficient removal of acetic acid from an acidic wine (1.1 gl^{-1} acetic acid, 12.5% ethanol, pH 3.12) is also observed when *S. cerevisiae* S26 cells are entrapped in double-layer alginate-chitosan beads, a method which would allow for facilitated separation of the yeast from the finished wine (Vilela-Moura, unpublished data).

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

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

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