Influence of Yeasts on Wine Acidity: New Insights into *Saccharomyces cerevisiae*

Vion Charlotte[1,2#], Nadine Yeramian[3#], Ana Hranilovic[4], Isabelle Masneuf-Pomarède[2], Philippe Marullo [1,2*]

[1]Biolaffort, Bordeaux, FRANCE

[2] UMR 1366 Œnologie, Université de Bordeaux, INRAE, Bordeaux INP, BSA, ISVV

[3]Microbiology Division, Department of Biotechnology and Food Science, Faculty of Science-University of Burgos, Spain

[4] Laffort, Bordeaux, FRANCE

[#]These authors contributed equally to this work

*Corresponding author: philippe.marullo@u-bordeaux.fr

Abstract

Climate change is strongly affecting the winemaking sector, notably by decreasing wine acidity due to lower malic acid levels in the grapes. Wine-related microorganisms can greatly affect the organic acid contained in wines as they are able to metabolise or synthesise different acids. Major advances in biochemistry, ecophysiology and molecular biology have led to numerous yeast strains being selected for that have specific oenological properties, including acidity modulation. The yeast *Saccharomyces cerevisiae* is the most extensively studied species, harbouring both malic acid-consuming and producing strains which are of interest in various vinification itineraries. Yeast-derived acidification of wines can indeed be achieved via malic acid production by *S. cerevisiae*, as well as via lactic acid production by *Lachancea thermotolerans*. Co-fermentations of these two species become promising tools to manage wine acidity while ensuring fermentation

completion and wine quality. Deacidification of wines via malic acid consumption is relevant in cooler winemaking regions, and/or for shortening malolactic fermentation and thereby increasing wine stability. This review delivers an in-depth overview of the effect of various oenologically relevant yeasts on wine acidity, with a focus on the latest findings on novel (de)acidifying *S. cerevisiae* strains.

Malic acid, yeast species, acidity, microbiology

Introduction

Climate change is a direct consequence of global warming, representing the greatest environmental challenge to be faced by humanity. Steady increases in carbon dioxide and other human-made emissions accentuate the greenhouse effect, with a direct rise in temperatures which drastically impact agricultural production. Climate is crucial to the concept of *terroir*; therefore, its modification largely affects the development and the quality of grapes (van Leeuwen & Darriet, 2016). Variations in climatic conditions lead to advanced phenology (E. Duchêne & Schneider, 2005; van Leeuwen & Darriet, 2016), with subsequent maturation phases coinciding with warmer summer periods. This trend shortens the grape ripening season, which may not be compatible with the production of high-quality wines, especially in continental regions (van Leeuwen & Darriet, 2016). Temperature increase affects multiple compositional parameters of grapes, including higher sugar concentrations (Coombe, 1987; Nistor et al., 2018), minor synthesis of anthocyanins (Arrizabalaga et al., 2018; Coombe, 1987) and decreases in titratable acidity due mainly to lower malic and tartaric acid concentrations. In turn, the resulting wines have higher alcohol content and altered aroma composition and sensorial properties (Bureau et al., 2000; E. Duchêne & Schneider, 2005; van Leeuwen & Darriet, 2016). Due to warming combined with sever dryness, traditional wine regions are becoming less suitable for viticulture; in parallel, other regions in northern Europe, where vineyard cultivation was unimaginable until recently, are benefiting from new climatic conditions, more suitable to growing certain grape varieties (Fraga et al., 2013).

It is well known that titratable acidity decrease is mainly due to malic acid degradation, as high temperatures accelerate malate respiration during ripening. Tartaric acid degradation is less rapid (Kliewer, 1971) and relatively stable in response to temperature variations (É. Duchêne, 2016), thus varieties with high tartaric acid concentrations are better adapted to climate change (Poni et al., 2018).

As reviewed by several authors (Chidi et al., 2018; Frost et al., 2017; Volschenk et al., 2006), acidity is of primary importance for wine balance and its overall sensory profile, including taste, aroma, and mouthfeel. Wines that are too acidic are perceived by consumers as being sour and too sharp. Conversely, wines with very low acidity are described as being flabby and flat, and as having less defined aromas and flavours, and reduced persistence on the palate (Malfeito-

Ferreira, 2021). More generally, acidity contributes to ‘freshness’, a feature sought by consumers in modern wines. Acidity directly modifies wine flavour components Bureau et al., 2000 and colour (Conde et al., 2007), since pH directly impacts anthocyanins absorbance. Thus, controlling wine acidity is a key factor for various components of wine quality. Moreover, insufficient acidity in grapes and wines negatively impacts their microbial stability due to reduction of the molecular sulfur dioxide (SO_2) fraction that is lowered at higher pH (Divol et al., 2012). Thus, increased additions of sulfur dioxide (SO_2) are required to reach the same level of antioxidant and antimicrobial effectiveness. As a consequence, the production of acetic acid by lactic acid bacteria in juices with a high pH can be observed. This practice, however, may not be compatible with increasing consumer demands for wines with lower SO_2 content. In this context, grape growers and winemakers seek multidisciplinary solutions for adapting their viticultural and oenological practices to preserve the overall quality of the grapes and resulting wines (Dequin et al., 2017).

Several chemical and biological solutions for modulating acidity can be applied before, after or during Alcoholic Fermentation (AF). Physiochemical methods for acidity adjustment have been thoroughly reviewed elsewhere (Volschenk et al., 2006). The most common method for chemical deacidification consists of adding calcium or potassium carbonate ($CaCO_3$ or K_2CO_3, respectively) to wine in order to induce a reaction with tartaric acid and precipitation as either potassium bitartrate or calcium bitartrate. Wine acidity can also be corrected via blending strategies with the grape juice/wine of different acidity levels (Comuzzo & Battistutta, 2019). Acidification is mostly achieved by the addition of tartaric acid, the strongest organic acid found in wine and which has the highest impact on pH. Other organic acids, such as lactic acid, malic acid, and citric acid, can be used as acidulants. Recently, fumaric acid was also authorised as a wine additive, but only to inhibit malolactic fermentation (OIV, 2021).

Modern winemaking seeks to limit the amount of additives in wines, and biological approaches for managing wine acidity are thus preferred. Wine acidity is modulated by various wine-related microorganisms, in particular by yeasts during AF and lactic acid bacteria during malolactic fermentation (MLF). During MLF, the L-malic acid is converted into L-lactic acid and CO_2 via the activity of the malolactic enzyme (MLE EC 4.1.1.101) found in some lactic acid bacteria belonging to the genera *Oenococcus, Lactiplantibacillus, Fructilactobacillus, Lentilactobacillus* , and *Pediococcus* (Sumby et al., 2014). Under certain conditions (high sugar concentration, lack of nitrogen and high pH), alcoholic fermentation may become sluggish or stop

suddenly while the sugars are still in the process of fermenting to ethanol; lactic bacteria take over and metabolise the sugar into acetic acid and D- or D and L-lactic acids (lactic spoilage) (Ribereau-Gayon, Dubourdieu, et al., 2006). Besides the action of lactic bacteria (LAB), yeasts can also modulate wine acidity. In this review, we explore acidifying and de-acidifying yeast properties. First, a brief overview of the key organic acids that play a role in wine acidity is given. Second, the metabolic origin and the pathways involved in the biosynthesis and catabolism of organic acid are described, as well as the phenotypic variability that could be generated using both genetically modified (GM) and non-GM approaches. Finally, the third and fourth sections are dedicated to biological deacidification and acidification of wines, respectively.

The origin of acidity in grape juices

1. A brief definition of wine acidity

Acidity in wine can be defined by two main parameters: the pH and the Titratable Acidity (TA) (Ribereau-Gayon, Glories, et al., 2006). Broadly, pH is defined by the expression: pH = log 1 /[H+] = - log [H+]; the pH of a wine is the measure of free protons concentration in the solution, calculated as pH = - log[H+], while TA refers to the concentration of titratable H3O+ ions in wine. In hydroalcoholic solutions like wine, weak organic acids are partially dissociated, and their dissociation degree is represented by their pKa. The lower the pKa, the stronger the dissociation and in turn the concentration of H_3O^+ ions in the solution. Typical values for white wine are a pH of 3.0-3.4 and a TA of 6-9 g/L as tartaric acid, and for red wine a pH of 3.3-3.7 and a TA of 5-8 g/L as tartaric acid (Waterhouse et al., 2016). The TA is a good proxy for the perceived sourness in wine, while pH is weakly correlated with sourness perception (Plane et al., 1980). In practice, the goals of achieving low wine pH and of avoiding excessively high TA (and thus sourness) often compete with each other. In addition to pH and TA, another parameter of oenological importance is the buffer capacity. The buffer capacity can be defined as the ability of a solution to maintain a stable pH upon addition of a strong acid or base. This property is directly correlated with the concentration of weak acids and their conjugate bases. Consequently, wines with higher TA have a higher buffering capacity.

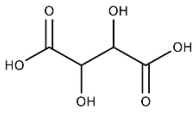
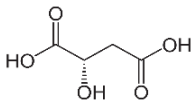
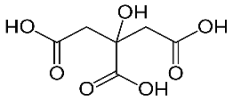
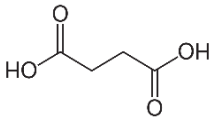
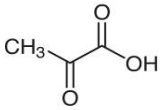
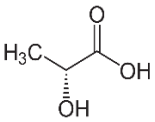
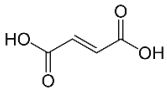
2. Organic acids and wine acidity

The main organic acids that contribute to wine TA are presented in Table 1. Two of them, tartaric acid and malic acid, contribute to up to 90% of the titratable acidity of grape juices and

wines. Tartaric acid is present in some fruits (Jantwal et al., 2022) and notably in grapes, in which it represents quantitatively the main organic acid of grape juice and wine (Ribereau-Gayon, Glories, et al., 2006). The isomer of tartaric acid found in the grape is the L (+) form. Its concentration varies between 2 and 10 g/L (Chidi et al., 2018). Malic acid takes its name from the apple (*malus* in Latin), in which it is present in high concentrations. Mature grapes contain between 2 and 6.5 g/L of L-malic acid (Chidi et al., 2018). This C4-dicarboxylic organic acid takes three acid-base forms: malic acid (H2M), hydrogen malate (HM) or malate (M). In grape juice and wine, the protonated forms are predominant (pK_{a1} = 3.40) while malate is mostly found in cytosolic conditions (pK_{a2} = 5.11). Malic acid has two stereoisomeric forms (L and D), but only the L-isomer exists naturally.

The concentrations of organic acids in grapes are influenced by many factors, including grape variety, ripening stage, climatic conditions, soil potassium levels, plant nutrition, and canopy management, as reviewed elsewhere (Gerós et al., 2012; Volschenk et al., 2006). While both tartaric and malic acids can be found in grapes early in the growing season, their behaviour during ripening and winemaking differs. Tartaric acid is synthesised during initial berry cell division and remains stable more or less throughout the ripening process of healthy berries. It is not metabolised during winemaking but can be lost through physiochemical mechanisms like precipitation. While malic acid is present at very high concentrations prior to *véraison*, it is actively metabolised during berry ripening and is significantly impacted by microbial activity, as described in the following sections of this review. Other organic acids that modulate wine acidity, such as succinic, lactic, citric and acetic acids, can be synthetised or metabolised by yeasts and bacteria during winemaking. Finally, gluconic acid, naturally present in trace amounts in healthy grapes, is found in a larger concentration in wines produced from rotten grapes; in fact, *Botrytis cinerea* and acetic bacteria are able to produce gluconic acid by glucose oxidation (Ribereau-Gayon, Dubourdieu, et al., 2006)

Table 1. Main organic acids present in healthy grape must and wine, sourced from Waterhouse *et al*. 2016.

Acid	Structure	pK_a in water*	Typical concentrations in grape juice (g/L)	Typical concentrations in wine (g/L)	Source**
Tartaric		2.98; 4.34	2-10	2-10	G
Malic		3.40; 5.11	1-7	0.5-7	G, Y
Citric		3.13; 4.76; 6.4	0.1-0.7	0.1-0.8	G, Y
Succinic		4.21; 5.64	0	0.5-1.5	Y
Pyruvic		2.4	0	0.01-0.5	Y
Lactic		3.86	0	0-3	LAB, Y
Acetic		4.76	0	0.1-0.5	Y, LAB, AAB
Fumaric		3.03; 4.44	0-0.1	0-0.1	G,Y

**Polyprotic acids have one pK_a for each -COOH group. The pK_a values in water are slightly different to those in wines, as they are affected by ethanol concentration, ionic strength, and temperature. As a rule of thumb, the first pK_a of an organic acid is 0.10-0.15 units higher in wine than water and 0.10-0.15 units lower for second pK_a.*

*** G: grape, Y: yeast, LAB: lactic acid bacteria, AAB : acetic acid bacteria*

Metabolic pathways of organic acids in yeast.

Organic acids constitute branch points of many catabolic routes. Pyruvic acid is the end point of glycolysis, while citrate, malate, fumarate, and succinate are the main metabolites of tricarboxylic acid (TCA) and glyoxylate cycles. Organic acids are building blocks involved in the biosynthesis of amino acids and *fusel* alcohols. Moreover, they play a central role in oxidoreductive

reactions necessary for catabolic and anabolic pathway homeostasis. Interestingly, most organic acids participate in metabolic reactions in cytosol, peroxisome, and the mitochondrial matrix, which are catalysed by specific isoforms. This compartmentalisation, as well as the existence of membrane shuttle systems, add complexity to our understanding of the metabolic flux of these compounds. As previously reviewed, the transfers between yeast compartments play an essential role in the homeostasis of oxidoreductive cofactors ($NAD(P)^+$/NAD(P)H) within the mitochondrial matrix (Bakker et al., 2001). The complex interconnection of organic acids is outlined in Figure 1, representing the central metabolism map of the model species *Saccharomyces cerevisiae* that has been widely investigated. Interestingly, *Schizosaccharomyces pombe* and *Lachancea thermotolerans* species, which may participate in AF, have particular metabolic features in their malate and lactate metabolism (blue and green inserts, respectively). The following paragraphs highlight the general biochemical and enzymatic aspects of organic acid metabolism, which are crucial for understanding the biological variations during the winemaking process. Each enzymatic reaction is described by its EC identifier as well as by the name of the *S cerevisiae* protein(s).

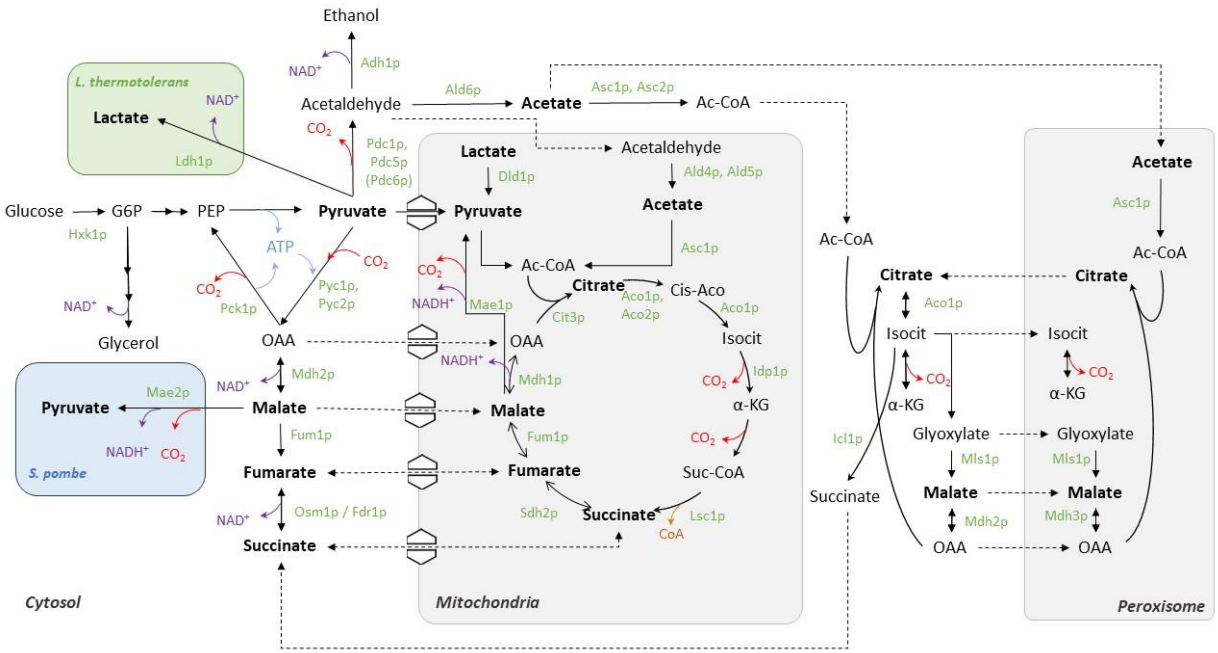


Figure 1. Interconnection of organic acids in the central carbon metabolism of *S. cerevisiae*. Organic acids in bold are those routinely quantified in wine.

1. Pyruvic acid

Pyruvic acid is the end-product of glycolysis and is produced by the irreversible

dephosphorylation of phosphoenolpyruvate (PEP) by the pyruvate kinase (Cdc19p/Pyk2p EC 2.7.1.40). In the presence of oxygen, this acid is carried in the mitochondrial matrix and incorporated into the TCA cycle by the pyruvate dehydrogenase complex (Pdh-cpx EC 1.2.4.1), where it is fully oxidised through the respiration chain that provides the cell with energy (ATP). Pyruvate can also be decarboxylated in acetaldehyde by the cytosolic pyruvate decarboxylase. Acetaldehyde is then converted into acetate which results in the production of cytosolic acetyl-CoA, which plays a role in the biosynthesis of fatty acids during alcoholic fermentation. This shunt is known as the pyruvate dehydrogenase by-pass (Flikweert et al., 1996; Remize et al., 2000). In hypoxic conditions, ATP is produced exclusively via glycolysis and must be constantly reduced for regenerating the oxidized form (NAD^+), which is essential for the continuation of glycolysis. The cytosolic reduction of pyruvate can provide NAD^+ by different metabolic routes. In higher eucaryotes, pyruvate is reduced to lactic acid, which also occurs for some yeast species (see below). Alternatively, pyruvate follows the pathway of AF, which is a common feature of fermenting yeast species. Briefly, during alcoholic fermentation, pyruvate is decarboxylated and then reduced to ethanol by the subsequent actions of pyruvate decarboxylase (Pdc1p/Pdc5p. EC 4.1.1.72/43) and cytosolic alcohol dehydrogenase (Adh1p: EC 1.1.1.1). Alternatively, pyruvate can be reduced to malate (via oxaloacetate) in the cytoplasm or oxidised to citrate, isocitrate and α-ketoglutarate through the oxidative branch of the TCA cycle. Pyruvate is therefore the origin of all the organic acids in wine, as discussed below. This also explains its very low concentrations at the end of fermentation.

2. Malic acid

2.1. L(-) Malic acid production pathways

In fungi, malic acid is produced from pyruvate via four main routes, as described below.

(1) In the presence of oxygen and in functional mitochondria, malic acid is produced in the mitochondrial matrix from fumarate, which is in turn formed by the succinate dehydrogenase complex (SDH-cpx, EC 1.3.5.1). These steps belong to the oxidative branch of the TCA cycle. The conversion of fumarate to malate is catalysed via the activity of fumarase (Fum1p, EC 4.2.1.2), which has a much higher affinity for fumarate than for malate (Pines et al., 1996). Interestingly, this enzyme can be located in both the cytosol and the mitochondrial matrix, which depend on the shunt activity of glyoxylate (Regev-Rudzki et al., 2009). Therefore, it is difficult to discriminate

between the cytosolic and the mitochondrial production of succinate and fumarate from malate.

(2) During alcoholic fermentation, the TCA cycle is split in two branches (oxidative and reductive) at SDH-cpx level due to lack of oxygen as the final electron acceptor. However, C4 organic acids (malate, fumarate, and succinate) can still be produced in the mitochondria from oxaloacetate by the reductive branch of TCA (Camarasa et al., 2003). This pathway requires the reduction of oxaloacetate to malate by the mitochondrial isoform of the malate dehydrogenase (Mdh1p, EC 1.1.1.37). In *S. cerevisiae*, this enzyme has a low Km for both malate and oxaloacetate and is active in both directions (Minard & McElister-Henn, 1994; Pines et al., 1996, 1997).

(3) The third route is the cytosolic production of C4 organic acids that follows a parallel path to the reductive branch of TCA. Since oxaloacetate is exclusively produced by the cytosolic pyruvate carboxylase activity (Pyc1p/Pyc2p EC 6.4.1.1), C4 acids are derived from cytosolic oxaloacetate when glucose is the sole carbon source. This anabolic reaction is essential for gluconeogenesis and plays a decisive role in the biosynthesis of aspartate from a fermentable carbon source (Stucka et al., 1991). The presence of cytosolic malate dehydrogenase (Mdh2p EC 1.1.1.38) allows the direct reduction of oxaloacetate in malate without any mitochondrial transport. In *S. cerevisiae*, the cytosolic isoform has a strong affinity for oxaloacetate (Km = 0.07 mM) and controls malic acid production (Pines et al., 1997). This cytoplasmic reaction can provide an alternative pool of NAD^+ at the beginning of alcoholic fermentation, supplementing NAD^+ generation via glycerol biosynthesis during the glycerol-pyruvic fermentation. The activity of cytosolic malate dehydrogenase is negatively regulated by glucose at the transcriptional and post transcriptional levels (Minard & McElister-Henn, 1994) and the role of this route is minor in high gravity matrices. However, cytosolic Mdh2p isoform is routinely quantified during alcoholic fermentation (Blein-Nicolas et al., 2015) via proteomics and its role in malic acid homeostasis still needs to be clarified.

(4) The fourth production route of malic acid involves the condensation and acetyl-CoA and glyoxylate, catalysed by malate synthase (Mls1p EC 2.3.3.9). Although the glyoxylate cycle is involved in the utilisation of lipidic sources in peroxisome, this protein, which is subject to glucose catabolic repression, is also situated in the cytoplasm in the presence of ethanol (Kunze et al., 2002) and has been quantified by proteomics during AF (Blein-Nicolas et al., 2015).

The mechanisms triggering the expulsion of malic acid outside the cell have been poorly documented, but Salmon (Salmon, 1987a) has reported that the export of malic acid depends on an

active transporter and provided preliminary evidence of a malic efflux dependent on glucose (Casal et al., 2008).

2.2. Malic acid degradation pathways

During vinification, malic acid is partially degraded by fermenting yeasts. First, malic acid can be converted into other C4 organic acids via the glyoxylate and TCA cycles as described above. In addition, malate may be assimilated as a carbon source by the malic enzyme. Yeasts decarboxylate malic acid into pyruvic acid by the NADH-dependent malic enzyme (Mae1p, EC 1.1.1.38) (Boles et al., 1998). This enzyme requires divalent cations (Mn^{2+} or Mg^{2+}) as cofactors and may have different compartmentation depending on the yeast species. In *S. pombe*, the decarboxylation of malic acid occurs in the cytosol and the Km of malic enzyme has a strong affinity for malic acid (Km = 3.2 mM). In *S. cerevisiae*, the enzyme is located in the mitochondria and exhibits a much higher Km (50 mM) (Saayman & Viljoen-Bloom, 2006).

3. Lactic acid

Lactic acid is a monoprotic acid (pK_a 3.86) that is mostly produced by the malolactic enzyme of bacteria as the L-isomer. Its concentration range in wine mostly depends on malolactic fermentation, which is beyond the scope of this review. *S. cerevisiae* strains do not produce significant amounts of D-lactic acid since this organic acid is mostly consumed to produce pyruvate in respiratory conditions (Lodi & Ferrero, 1993). In contrast, other fermenting yeasts, such as *L. thermotolerans,* can produce high amounts of L-lactic acid through the direct reduction of pyruvate by the cytosolic lactate dehydrogenase (Ldh1p/Ldh3p, EC 1.1.1.27). The molecular mechanisms underlying lactic acid biosynthesis at the expense of ethanol or any other metabolite in *L. thermotolerans* are still poorly understood, as well as the genetic basis of a high inner-strain variation in this trait (Banilas et al., 2016; Hranilovic et al., 2018). Based on the whole genome sequence, *L. thermotolerans* possesses three Ldhp and two Adhp paralogues. Their expression was recently analysed in a study that provides initial information on molecular mechanisms of differential lactic acid production in *L. thermotolerans* (Sgouros et al., 2020). This revealed the up-regulation of *LDH2* in high-lactate producing strains, with no further differences in the expression of other genes (i.e., *LDH1*, *LDH3*, *ADH1* and *ADH2*) at the early stationary phase.

Moreover, it is unclear whether the formation of lactic acid from pyruvate due to the inherent *LDH* activity serves to replenish oxidised NAD^+ that has been depleted as a result of

glycolysis, which is in yeasts primarily achieved through alcoholic fermentation. However, while ethanol can leave the cell via passive diffusion, lactic acid has to be actively transported at the expense of ATP, as it has a high intercellular pH and is present in a dissociated form. To maintain the proton motive force and the intercellular pH, protons must be exported via the plasma membrane H^+-ATPase at the expense of one ATP per proton. Although the exact mechanisms are still unknown, the export of lactate (*i.e.*, dissociated anion) can also be ATP-dependent (Sauer et al., 2010). According to these authors, once exported, lactic acid has a low extracellular pH and is present in its protonated form and can thus permeate the cell membrane via passive diffusion, perpetuating the energy-requiring cycle. The recycling of NADH via the lactic acid pathway therefore appears to be more costly for the cell compared to the ethanol pathway. The physiological and/or evolutionary benefits of the simultaneous accumulation of ethanol and lactic acid are unclear, but this strategy might be useful for out-competing microorganisms that co-exist within the same niche, comparable to the 'make-accumulate-consume' strategy in *S. cerevisiae* (Hagman et al., 2013). Altogether, this warrants further research on central carbon metabolism in *L. thermotolerans*, particularly on the regulatory framework of the redox balance, through studies purposely designed to quantify the microbial growth and evolution of metabolites in conjunction with transcriptomics.

4. Acetic acid

Acetic acid is the main volatile acid in wine and is a byproduct of microbial metabolism. It is considered an undesirable compound and constitutes an organoleptic default in wine at high concentration. Except in the case of wine spoilage by lactic and acetic acid bacteria, acetic acid is mostly produced by fermenting yeasts at the beginning of alcoholic fermentation in amounts ranging from 200 to 600 mg/L (Vilela-Moura et al., 2011).

The metabolic pathway of acetate under the anaerobic conditions resulting from the acetic acid in the grape juice occurs mostly via the pyruvate dehydrogenase bypass, which reroutes part of acetaldehyde in acetate by the main cytosolic isoform of aldehyde dehydrogenase (Ald6p, EC 1.2.1.3) (Postma et al., 2022; Remize et al., 2000). The acetic acid formed is then transformed into Acetyl-CoA by the acetyl-CoA synthetase (Acs1p, EC 6.2.1.1). The resulting acetyl-CoA might be used in fatty acids biosynthesis or enter the mitochondria for further oxidation via the tricarboxylic cycle. The mitochondrial isoenzyme Aldp5 is also implicated in acetate formation in oenological

conditions (Saint-Prix et al., 2004).

Acetic acid production can be partially linked to glycerol production in specific conditions (Eglinton et al., 2002). Remarkably, a high sugar concentration (> 300 g/L) triggers an overproduction of glycerol by yeasts in response to osmotic stress (Blomberg, 2000). This glycerol synthesis leads to an overflow of oxidated NAD^+. This response is coupled with an overproduction of acetic acid due to the overexpression of *ALD2* and *ALD3* genes, regenerating NADH (Navarro-Aviño et al., 1999).

5. Citric, fumaric, α-ketoglutaric, and succinic acids

TCA acids are typical by-products of AF and can be found in wines in variable concentrations. During AF, succinate can be formed via both branches of the TCA cycle: 1) the oxidative branch of the TCA pathway, or 2) by the TCA reductive pathway via fumarate reductase. In the second case, the TCA cycle proceeds from oxaloacetate via malate to succinate but does not progress any further as the SDH complex is not functional during AF (Wales et al., 1980). Additional succinate is formed by oxidative decarboxylation of α-ketoglutarate when glutamate is present in the medium. As well as being produced by the TCA pathway, succinic acid can also be synthetised from isocitrate via the glyoxylate shunt. This reaction is catalysed by isocitrate lyase (Icl1p, EC 4.1.3.1) (Fernandez et al., 1992). However, enzyme is induced by growth on ethanol and repressed by growth on glucose (Raab & Lang, 2011) and thus might play a minor role during the alcoholic fermentation (Klerk, 2010).

Fumarate is an intermediary of the TCA cycle and can be formed by the reductive pathway and catalysed by the fumarate synthase (Fum1p, EC 4.2.1.2) that has both mitochondrial and cytosolic localization (Wu & Tzagoloff, 1987). Citrate is part of the TCA cycle and can be formed by the condensation of oxaloacetate and acetyl-CoA. This reaction is catalysed by citrate synthase (Cit1p, EC 2.3.3.1) which is subjected to glucose repression (Rosenkrantz et al., 1994). Cit1p has peroxisomal isoenzyme, Cit2p, which is involved in the glyoxylate cycle. It also catalyses the condensation of oxaloacetate and acetyl-CoA to form citrate. In the TCA cycle, citrate is converted into cis-aconitate, then isocitrate is converted into α-ketoglutarate by aconitase (Aco1p, EC 4.2.1.3) (Gangloff et al., 1990) followed by isocitrate dehydrogenase (Idp1p, EC 1.1.1.42). This conversion of citrate to α-ketoglutarate is also possible in the cytosol, as the Aco1p localisation is dual. In addition, Idp1p has a paralog, Idp2, which is the cytosolic isoenzyme (Postma et al., 2022).

Genetic levers for controlling the organic acid content of wines.

1. Genetically modified yeast strains

In recent decades, several attempts have been made to modulate acidity by using genetically modified (GM) yeasts, mostly focusing on the modulation of lactic and malic acids. Some of them have been applied at industrial scale.

1.1. Lactic acid overproduction

Advances have been made in the genetical engineering of *S. cerevisiae* strains to increase lactate yields for oenological use. These strains were obtained by implementing the heterologous expression of the *L-LDH* gene of *Lactobacillus casei*, which was controlled by the Adh1p promoter (Dequin & Barre, 1994). This resulted in the simultaneous conversion of glucose to both ethanol and lactate in a laboratory growth medium, with up to 20% (w/v) of the glucose transformed into L-lactate. In a follow-up study, eight commercial wine starters were engineered for lactic acid production and characterised under oenological conditions (Dequin et al., 1999). Depending on the strain, lactic acid levels in a synthetic grape juice ranged from 1.6 to 4.1 g/L, whereas the corresponding parental strains formed less than 0.2 g/L. The matrix-derived impact on final lactate yields was further trialled using the strain that produced the largest amounts of this metabolite. Wines obtained from seven grape musts contained between 2.6 and 8.6 g/L of lactic acid, highlighting the impact of grape juice composition on the pathway. The final acidity was affected by the lactic acid concentration, as well as the buffering capacities of each grape juice; for example, a lactic acid concentration of 5.7 g/L decreased the pH of one wine by 0.11 and another by 0.36 units. Despite the slower CO_2 production rate, the development of the engineered strain remained unaffected, as did the volatile acidity production. The acidified wines also showed up to 0.25% v/v lower ethanol content compared to the control strain as a result of partial carbon diversion from ethanol to lactate (Dequin et al., 1999). Because lactic acid serves as a final electron sink, its formation results in the reduction of equimolar amounts of alcohol without affecting the intracellular redox balance. This is of additional value, since the wines which are deficient in acidity often contain overly high ethanol levels. However, given that the concentrations of lactic acid required to decrease ethanol content by 1% v/v exceed 15 g/L, any major decreases via this strategy are likely to impart excessive acidity to wines (Tilloy et al., 2015).

1.2. Malic acid degradation

In *S. cerevisiae*, malic acid degradation is incomplete due to several factors. The transport of this acid into the cell is inefficient (Salmon, Vezinhet & Barre., 1987) and the activity of its malic enzyme is moderate due to its mitochondrial localisation and its high Km value (see above). To overcome these limitations, Volschenk *et al.* (1997) proposed the heterologous expression of the genes *mae1* and *mae2* of *S. pombe* using a genetic engineering approach. These genes encode for a transmembrane malic acid transporter (Grobler et al., 1995) and a cytosolic malic isoform (Viljoen et al., 1994), respectively. This GM *S. cerevisiae* strain degraded up to 8 g/L of malic acid, greatly exceeding the *S. cerevisiae* malate depletion rate (0 to 3 g/L) (Volschenk et al., 2001) and avoiding off flavours produced by *S. pombe*.

1.3. Malic acid transformation in lactic acid

To address the unpredictability of malolactic fermentation (MLF), several studies have attempted to consume malic acid via *S. cerevisiae* during alcoholic fermentation. Different teams have proposed introducing the malolactic enzyme in *S. cerevisiae* by cloning the malolactic gene *MLES* of *Lactococcus lactis* (Ansanay et al., 1993; Denayrolles et al., 1995). However, the transformation of malate into lactate was incomplete due to the lack the pump for malic acid uptake in *S. cerevisiae* (Ansanay et al., 1996). To overcome this, different strains of *S. cerevisiae* co-expressing the malic transporter encoded by the gene *mae1* of *S. pombe* and the *Lactococcus lactis* malolactic gene *MLES* were proposed (Bony et al., 1997; Volschenk et al., 1997). The combined action of these enzymes led to successful and complete malolactic fermentation by yeast without the use of lactic bacteria.

In an attempt to include the MLF step in the alcoholic fermentation process at industrial scale, the ML01 strain was genetically modified to conduct malolactic fermentation (Husnik et al., 2007). This genetically modified wine yeast was a "*Prise de Mousse"* strain. It contains the malate transporter gene (*MAE1*) from *S. pombe* and the malolactic gene (*MLEA*) from *Oenococcus oeni*. It is capable of decarboxylating up to 9.2 g/L of malate to equimolar amounts of lactate during alcoholic fermentation. Sensory analyses have confirmed that it is suitable for winemaking.

1.4. Malic acid overproduction

The inability to use genetically modified yeast in industrial fermentations has limited the implementation of genetic engineering strategies for managing wine acidity. Interestingly GM strains have

been created to overproduce malic acid in a non-oenological context. Zelle *et al.* have shown that efficient malate production can be achieved by improving the following cytosolic pathway: conversion of glucose to pyruvate through glycolysis, followed by carboxylation of pyruvate to oxaloacetate (by Pyc2p) and reduction of oxaloacetate to malate (by the cytosolic isoenzyme Mdh2p) (Zelle et al., 2008). They evaluated the impact of three genetic modifications: i) overexpression of the native pyruvate dehydrogenase encoded by *PYC2*, ii) high expression of an allele of *MDH3* from which the encoded malate dehydrogenase was retargeted to the cytosol - *MDH3* encodes the peroxisomal isoenzyme of the malate dehydrogenase, but Mdh3p will be used preferentially over the cytosolic Mdh2p, because the latter is subject to catabolite inactivation, which is undesirable for the cultivation on glucose, and iii) expression of the *Sz. pombe* malate transporter in the *S. cerevisiae* strain. The cumulative effect of these three genetic modifications was stronger than a single modification and the resulting engineered strain produced up to 59 g/L of malic acid.

2. Natural genetic variations found in *S. cerevisiae* populations.

Recent studies have focused on elucidating the natural variation in the production of organic acids by fermenting *S. cerevisiae* strains in an oenological context using quantitative genetics approaches. Several QTLs were linked to the variation of succinate production located on the chromosome IV, VI, XI, XV, XIII, and XIV (Ambroset et al., 2011; Eder et al., 2018; Salinas et al., 2012). For succinic acid production, the impact of two genes *FLX1* (Chr IX QTL) and *MDH2* (Chr XV QTL) were experimentally validated. *FLX1* encodes a transporter of flavin adenine dinucleotide (FAD) across the mitochondrial membrane that can modulate the activity of the succinate dehydrogenase. *MDH2* encodes the cytosolic malate dehydrogenase involved in malate/oxalacetate interconversion that play a role in the glyoxylate cycle. More recently, the genetic determinism of malic acid has also been investigated in a multi-environmental QTL-mapping program (Peltier et al., 2021). The percentage of malic acid consumed by a wide population of yeast strains was calculated (MAC%) and eleven QTLs linked to malic acid consumption were identified (Peltier et al., 2021; Vion et al., 2021). Six genes affecting the variation of MAC% among progeny were validated by functional genetics experiments. The genes *MAE1*, *PYC2*, and *SDH2* are directly related to malic acid, pyruvic acid and oxaloacetate metabolism and their position on the metabolic map are shown in Figure 2. *MAE1* encodes the mitochondrial malic enzyme, *PYC2* encodes an isoform of pyruvate kinase, and *SDH2* the catalytic subunit of the succinate dehydrogenase complex. Interestingly, the gene *MAE1* carries a single nonsynonymous allelic variation MAE^{I605V} that has been previously described to modify the

production of branched ethyl esters, with are directly connected to malic acid catabolism (Eder *et al.* 2018). In addition, two other genes, *PMA1* and *PNC1,* have a role in proton and NAD^+/NADH, H^+ homeostasis. Finally, the gene *YBL036c* encodes for a putative alanine racemase with a connection to the mitochondrial pyruvate pool. Interestingly, most of the allelic forms of QTLs involved in malic acid consumption were derived from the same parental strain. Phylogenomic analyses demonstrated that those alleles were derived from the *flor* yeast genome (Peltier et al., 2021), which constitutes a specific genetic group of wine yeasts (Coi et al., 2017). *Flor* yeasts are adapted to surviving in harsh environments that are depleted of sugar and rich in ethanol. Recently, we demonstrated that, compared to other *S. cerevisiae* strains, the *flor* yeast population can consume a large fraction of malic acid present in grape juice (Vion, Le Mao, et al., 2023).

The expression and contribution of the different QTLs mapped for malic acid consumption have been investigated in breeding programmes aiming to control the malic acid level at the end of the AF. First, a marker-assisted selection of malic-consuming strains was achieved demonstrating that individuals carrying a high proportion of *enhancer* alleles statistically consumed more malic acid than those carrying a proportion of *preserver* alleles. Although each allele had a low impact on the final MAC% value, their cumulative effect strongly impacted the MAC% (Vion et al., 2021). Second, malic producer strains were obtained by crossing together strains consuming low amounts of malic acid. After two cycles of segregation and selection, individuals producing up to 3.5 g/L of malic acid at the end of alcoholic fermentation were obtained. These extreme strains were significantly enriched in *preserver* alleles (Vion, Muro et al., 2023).

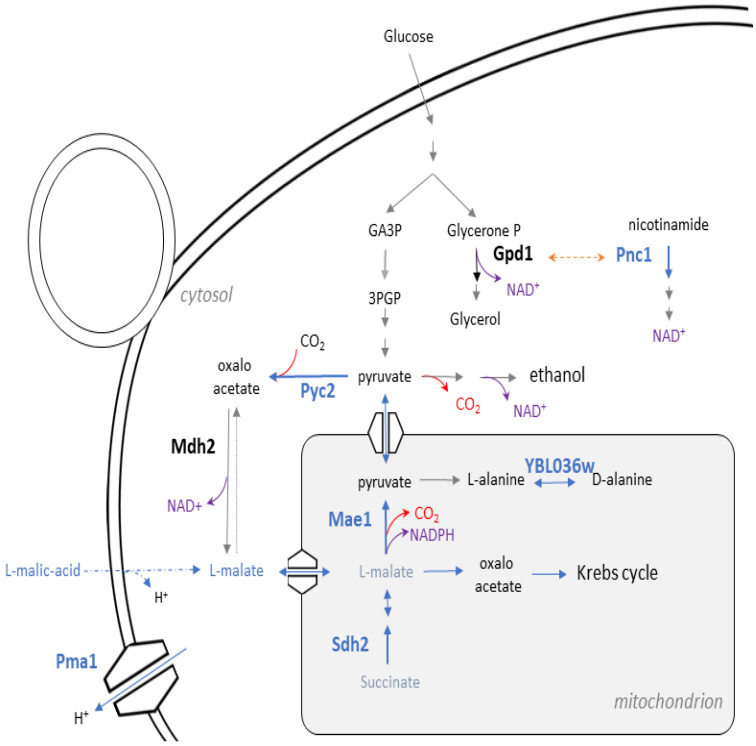


Figure 2. Metabolomic map of *S. cerevisiae*. Genes impacting Malic acid consumption (MAC%) are shown in blue. Figure inspired from Peltier et al. (2021).

Microbiological applications for reducing wine acidity during alcoholic fermentation.

Deacidification of wine may be necessary for maintaining a good sensorial balance in terms of a sweet and sour. In red wines, it is used for two main reasons: i) to facilitate the beginning of MLF, since LAB are inhibited by a low pH (Ribéreau-Gayon, P., Dubourdien, D., Donéche, 2006), and ii) to impact the sensory perception of wines, as high acidity may cause excessive sourness and negatively impact other wine sensory parameters (e.g., astringency) (Sowalsky & Noble, 1998). Since tartaric acid is not metabolised by yeasts (Gao & Fleet, 1995), the reduction of acidity during alcoholic fermentation is due to the consumption of malic acid by the fermenting yeast. This degradation significantly modifies wine TA and pH. The amount of malic acid consumed by yeast depends on many genetic factors that have been discussed in the previous section regarding *S. cerevisiae*. In addition, major differences exist between yeast species that are mostly due to three biochemical features: i) the presence of a specific transporter in the cell, ii) the affinity of the malic enzyme for malic acid, and iii) the cellular location of the malic enzyme. In this section, technological details regarding three yeasts species that have been used for reducing wine acidity will be discussed, as well as their respective uses in winemaking.

1. Contribution of *Saccharomyces cerevisiae*

Several studies have investigated the ability of *S. cerevisiae* strains to consume malic acid during alcoholic fermentation. Some strains have been reported to consume up to 45 % of malic acid, while the role of other strains is to conserve acidity and consume little or no malic acid (Delcourt et al., 1995; Peltier et al., 2018; Redzepovic et al., 2003). The natural variability of *Saccharomyces* strains regarding the consumption of malic acid in different grape juices has been recently reevaluated for genetically distinct populations (Vion, Le Mao, et al., 2023). The *Flor* yeast population consumed significantly more malic acid than wine and fruit populations. This higher consumption might be regarded as a sign of the adaptation of these yeasts to growing in harsh media with depleted sugars and high ethanol concentrations. This property might be due to complex genetic regulation and adaptation, as indicated by the recent findings discussed in the previous section. Indeed, *flor* yeasts have been reported to shift to oxidative metabolism when sugar is depleted (David-Vaizant & Alexandre, 2018). They have also shown higher intracellular

metabolic load than wine yeast (Vion, Brambati, et al., 2023). Hence, *flor* yeasts might be able to consume more malic acid at the end of fermentation than wine yeasts. By using genetic selection strategies, strains able to consume around 70 % of malic acid have been successfully obtained (Vion et al., 2021), enabling efficient wine acidity management. Such strains have proven to facilitate malolactic fermentation by reducing wine malic acid concentration and increasing its pH (Vion et al., 2021). To our knowledge, no study has shown consumption higher than 80% of initial malic acid or less than 0.5 g/L of malic acid remaining after fermentation by a strain of *S. cerevisiae*, regardless of the initial medium.

Despite this huge variability, *S. cerevisiae* is considered a relatively poor metaboliser of extracellular malate compared to other species. This is due to the weak malate dehydrogenase (Mdh2p) affinity for malate (Pines et al., 1996), the mitochondrial location of the malic enzyme (Mae1p) and its low affinity for malate (Km = 50 mM) (Boles et al., 1998). In addition, malic acid has been reported to enter the cell in its undissociated form (H2M) by simple diffusion due to the lack of active transport of malate through the membrane (Salmon, 1987). Malic acid has two pKa (pK_{a1} = 3.40 and pK_{a2} = 5.11), while the pH of grape juice ranges between 3.2 and 4.0. Extracellular malic acid can be found mostly in its undissociated (H2M) and mono-dissociated (HM) forms. Once it enters the cell, it acquires its deprotonated form (M). A proton pump ensures the exit of H^+ and helps maintain an intracellular pH of around 5-6. When entering the cell by diffusion, malic acid is in its undissociated form, which represents about 50% of the total malic acid available in grape juice at a pH of 3.5. As low pH values enhance the H2M/HM ratio, more di-protonated form is consumed, triggering the deacidification of the medium. This explains why more malic acid is consumed in grape juice at higher acidity levels. For all these reasons, *S. cerevisiae* consumes less malic acid than other yeasts, such as *Z. bailii* or *S. pombe*.

Malic acid consumption by *S. cerevisiae* depends on environmental factors, such as grape juice pH, and the concentration of assimilable nitrogen (Delcourt et al., 1995; Vilanova et al., 2007). Several studies have indicated that a high initial malic acid concentration will lead to its greater consumption (Delcourt et al., 1995; Vion, Muro et al., 2023) with malic acid production repressed in what would normally be malic acid-producing yeasts (Farris et al., 1989; Yéramian et al., 2007). However, Redzepovic *et al.* did not report any differences in malic acid consumption between two media with 3 g/L and 8 g/L of initial malic acid (Redzepovic et al., 2003). Low biotin content also favours malic acid degradation (Salmon, Vezinhet, & Barre, 1987; Schwartz & Radler,

1988), as does an elevated glucose concentration (Delcourt et al., 1995). Finally, low pH promotes the consumption of malate (Delcourt et al., 1995; Ramon-Portugal et al., 1999), since malic acid enters the cell in its undissociated form by simple diffusion. Finally, the addition of thiamine also facilitates malic acid consumption by *S. cerevisiae* (Carre et al., 1983).

2. Contribution of *Schizosaccharomyces pombe*

The genus *Schizosaccharomyces* encompasses four related species (*S. japonicus, S. octosporus, S. cryophilus,* and *S. pombe*) (Hironori, 2014), the latter being particularly efficient for malic acid consumption. *S. pombe* is mostly isolated from tropical regions and from high sugar habitats (Jeffares, 2018) but is rarely detected in winemaking conditions, because it is out-competed by *S. cerevisiae* (Yokotsuka et al., 1993). It is characterised by its ability to completely metabolise the malic acid from grapes. This specific feature is due to the action of a constitutive active malic acid transporter encoded by the *mae2* gene (Grobler et al., 1995). The incorporated malic acid is decarboxylated to pyruvic acid by the malic enzyme (in presence of NAD^+ and one of the divalent cations Mn^{2+} or Mg^{2+}) (Osothsilp & Subden, 1986). The high affinity of the malic enzyme for its substrate (Km 3.2 mM) and its cytosolic location contribute to the stronger efficiency of malo-ethanolic fermentation with respect to *S. cerevisiae*. The resulting pyruvate follows the alcoholic fermentation pathway, producing ethanol and CO_2. In this pathway, known as malo-ethanolic fermentation, one molecule of malic acid is fermented to produce one molecule of ethanol and two molecules of CO_2 in anaerobic conditions (Volschenk et al., 2003). In *S. Pombe,* both malic acid transporter and malic enzyme activities are induced by the presence of malic acid in the medium (Osothsilp & Subden, 1986).

Several authors have proposed adding *S. pombe* in grape juices for either partial or complete consumption of malic acid as an alternative to MLF (S. Benito et al., 2012; Ciani et al., 2009; Redzepovic et al., 2003). The proposed itineraries involve pure culture fermentations of *S. pombe,* and their co-cultures with *S. cerevisiae* or, as described more recently, with *L. thermotolerans* (Á. Benito et al., 2015). To date, only one strain of *S. pombe* is commercially available in an immobilised form (Suárez-Lepe et al., 2012) for uses in a controlled biological deacidification process; in this process, the immobilized *S. pombe* cells use malic acid (Ciani et al., 2009), whereas *S. cerevisiae* achieves fermentation using almost all the available sugar. Despite the advantages of deacidifying wines with *S. pombe*, its industrial use in winemaking is limited due to the production of off-flavours including acetic acid (S. Benito et al., 2012) and a loss in typicity and fruitiness

(Carre et al., 1983; Redzepovic et al., 2003).

3. Contribution of *Zygosaccharomyces bailii*

Zygosaccharomyces bailii is a fructophilic yeast which can degrade high concentrations of malic acid during alcoholic fermentation (Baranowski & Radler, 1984). This species is considered a spoilage organism in the food industry because of its strong resistance to weak organic acids, chemical preservatives (sulfites, sorbic acid), ethanol, and high sugar concentrations (Martorell et al., 2007; Radler et al., 1993; Sousa et al., 1996). Different studies have reported the use of this species in wineries for mixed fermentation with *S. cerevisiae* (Escribano et al., 2018; Escribano-Viana et al., 2019; Garavaglia et al., 2015). *Z. bailii* preferably degrades fructose, followed by glucose; malic acid is only degraded during the glucose degradation step. Most of malate is oxidatively decarboxylated to pyruvate by the malic enzyme, while a small fraction is reduced by fumarase and fumarase reductase (Kuczynski & Radler, 1982). The malic enzyme of *Z. bailii*, has a notable affinity for malate (K_m=10 mM) and is constitutively expressed (Baranowski & Radler, 1984). The same authors reported that this species has a L-malate transporter which is induced by glucose and inactivated by fructose. These properties allow *Z. bailii* to metabolise large amount of malic acid or acetic acid (Rodrigues et al., n.d.) in the presence of glucose. Although *Z. bailii* cannot be used alone as a starter for winemaking, the use of multi-starters that comprise a strain of *S. cerevisiae* and a non-*Saccharomyces* yeast for fermentation are being increasingly studied for different purposes, such as biological deacidification, bio-protection, and confering aroma complexity to wines. In this light of this, active dried yeasts of *Z. bailli* have become available (Ciani et al., 2009).

Microbiological applications for enhancing wine acidity during alcoholic fermentation.

The main purposes of acidifying wines using organic acids are to increase TA and decrease pH, which can be necessary to maintain the freshness of a wine. The indirect aims are to enhance and stabilise the colour and the tannin structure of the wine, and to prevent microbial spoilage. The appropriate acidity levels help preserve wine over time, and leads to a reduction in sulfur dioxide content and microbiological stabilisation.

Acid-producing yeasts are generally less common than non-acid-producing yeasts

(Kuczynski & Radler, 1982) because of their slightly lower rate of multiplication and growth. For this reason, acid-producing strains are rarely dominant in natural yeast populations of grape must. Nevertheless, if a sufficiently large population of acid-producing strains is inoculated in the must, they can become dominant and increase the acidity of the resulting wine.

1. Malic acid production during wine fermentation

The ability of *S. cerevisiae* to produce malate in an oenological context has been poorly documented. Earlier studies have reported that concentrations of 1 g/L can be reached under optimal pH and temperature (Farris et al., 1989; Yéramian et al., 2007) in wine making conditions. Recently, malic acid-producing *S. cerevisiae* strains were selected for preserving wine acidity during alcoholic fermentation. These strains were able to produce up to 3.5 g/L of malic acid and to decrease the wine pH up 0.5 units compared to fermentations conducted with malic consuming strains (Vion, Muro, et al., 2023). Cryotolerant yeasts, such as *Saccharomyces uvarum*, tend to produce more malic acid than *S. cerevisiae* (Coloretti et al., 2002; Fatichenti et al., 1984; Schwartz & Radler, 1988) due to their psychrophilic properties. This feature is mostly shared by hybrids between *S. cerevisiae* and *S. uvarum* (Origone et al., 2018), which have been proposed as a solution for coping with both drops in acidity and high sugar levels in grape juices. A recent comparison of *S cerevisiae* and *S. uvarum* strains confirmed the high malic acid production of the latter species (Vion, Le Mao, et al., 2023).

In addition to strain variability, fermentation conditions can largely influence malic acid production. Oenological conditions are in fact not optimal for malate synthesis. High pH (around 5), low initial malic acid content, and low yeast-assimilable nitrogen (YAN) concentrations were instead found to promote the production of malate by *S. cerevisiae* (Salmon et al., 1987; Schwartz & Radler, 1988; Yéramian et al., 2007). Despite suboptimal conditions, some yeast strains can anabolise malic acid during AF (Fatichenti et al., 1984; Flikweert et al., 1996; Schwartz & Radler, 1988). In general, malic acid production is greater when the initial level of malic acid in grapes is low (Davaux, 2001; Ramon-Portugal et al., 1999; Vion, Muro et al., 2022; Yéramian et al., 2007). Recently, we demonstrated that the high production of malic acid partially negatively affects the fermentation performance of acidifying strains (Vion, Muro, et al., 2022). This finding suggests a phenotypic trade-off between fermentation completion and malic acid production.

2. Lactic acid production during alcoholic fermentation

Lactic acid is a microbially-derived wine acid, and a permitted oenological acidulant under most regulations (Waterhouse et al., 2016). It is often described as a 'soft' and 'mild' acid, in contrast to the descriptors 'green' and 'harsh' which are more often used to describe malic and tartaric acids. However, the pertinence of such attributes remains elusive. It is particularly unclear whether the 'softer' acidity perception of lactic acid simply reflects the partial deacidification of wine via malolactic fermentation. Despite such ambiguities, acidification by lactic acid has certain advantages: it is not lost by precipitation (as is the case with tartaric acid) due to the solubility of both potassium and calcium salts, nor prone to microbial degradation.

2.1. The lactic producing species *Lachancea thermotolerans*

The yeast *L. thermotolerans* is an occasional constituent of the grape/wine microbiome, and it is also found in a range of other natural anthropic habitats worldwide (Hranilovic et al., 2017). Like other yeast species, *L. thermotolerans* populations can be differentiated by both geographic origin and the ecological niche of isolation, and this differentiation is reflected in the phenotypic level in terms of the oenological performance of the strain (Hranilovic et al., 2018). The metabolic hallmark of *L. thermotolerans* is L-lactic acid production concomitant to alcoholic fermentation. The maximum reported concentrations are 16.6 g/L (Banilas et al., 2016), which by far exceed those recorded for any non-GM yeast, but this trait is highly strain dependent (Banilas et al., 2016; Hranilovic et al., 2018); for example, the final levels of lactic acid formed in fermentations of the same grape juice using 94 different *L. thermotolerans* strains ranged between 1.8 to 12 g/L (Hranilovic et al., 2018). In mixed cultures of *L. thermotolerans* and *S. cerevisiae*, used in 'dry' wine production, levels of lactic acid production depend on the *L. thermotolerans* strain as well as on the yeast inoculation regimes. Due to the antagonistic activity of *S. cerevisiae* towards *L. thermotolerans*, mediated by mechanisms of cell-cell contact and secretion of antimicrobial peptides (Kemsawasd et al., 2015), co-inoculations generally lead to less lactic acid production compared to the sequential inoculations (Gobbi et al., 2012; Kapsopoulou et al., 2007; Sgouros et al., 2020). In the latter innoculations, a longer delay in *S. cerevisiae* inoculation results in a higher metabolic contribution of *L. thermotolerans*. According to Kapsopoulou *et al.* (2007) 0.18 g/L of lactic acid is produced in co-inoculated fermentation. A tenfold increase (1.8 g/L) was recorded when inoculation with *S. cerevisiae* was delayed for one day, whereas a two- and three-day delay in occulation resulted in the production of 4.28 g/L and 5.13 g/L of lactic acid respectively. In terms

of acidity modulation, *L. thermotolerans* strains are also capable of partially degradating up to 20% of malic acid, and their acetic acid production is low and rather invariant (Hranilovic et al., 2018). When using *L. thermotolerans,* the final wine pH can decrease by about 0.5 units, which represents a dramatic acidification capacity. Depending on the strain and the fermentation conditions, these wines have been found to also contain either comparable amounts of or up to 1.6% v/v less ethanol compared to their respective *S. cerevisiae* monocultures (Gobbi et al., 2012; Kapsopoulou et al., 2007; Sgouros et al., 2020). The lower ethanol content is in line with the partial diversion of carbon flux from ethanol to lactic acid, but more detailed studies on the carbon flux of different *L. thermotolerans* strains are required.

2.2. The contribution of other yeast species in the production of lactic acid.

Under oenological conditions, *S. cerevisiae* strains produce very little (if any) D- or L-lactic acid via reduction of pyruvate by NAD-dependent D- and L-LDHs in mitochondria (Dequin & Barre, 1994). Information on the ability of yeasts (other than *L. thermotolerans*) to produce lactic acid is limited and few systematic screenings for this trait have been carried out (Sauer et al., 2010). An agar plate-based assay 'LASSO' has been developed (Witte et al., 1989) for the detection of lactic acid production and was used to screen a collection of 100 yeast strains. Only two strains were able to produce lactic acid, and they were both identified as *L. thermotolerans*. This assay was revisited only recently and modified to a liquid format (225 µL) for multi-well plates (Osburn et al., 2018). In a study focusing on the selection of yeasts for sour-style beer production without the use of LAB, strains of four other species were able to produce lactic acid: *Lachancea fermentati*, *Hanseniaspora vinae*, *Schizosaccharomyces japonicus* and *Wickerhamomyces anomalus* (Osburn et al., 2018). All these species were, to a certain degree, evaluated for their winemaking potential (Domizio et al., 2018; Medina et al., 2013; Padilla et al., 2018; Porter et al., 2019), but, to the best of our knowledge, without delivering any striking results regarding lactic acid or wine acidity modulation. One exception is *Sz. japonicus*, which has been reported to decrease total acidity in wine in both pure cultures and co-cultures with *S. cerevisiae* due to its ability to degrade malic acid (Domizio et al., 2018). To date, the most extensive characterisation of lactic acid production by yeasts other than *L. thermotolerans* is available for *L. fermentati*. Final lactic acid concentrations in beers produced by *L. fermentati* depended on the strain and fermentation conditions, with maximal values of 1.6 g/L (Bellut et al., 2019, 2020; Osburn et al., 2018). Lower inoculation rates in combination with increased fermentation temperatures boosted lactic acid production, as did

higher initial glucose concentrations (Bellut et al., 2020). A comparison of whole genome sequences of strains with differential lactic acid production has revealed a mutation in a low lactic acid production strain, resulting in a premature stop codon in a homologue *S. cerevisiae JEN1* (Bellut et al., 2020). This gene encodes for a monocarboxylate transporter involved in the export of lactic acid, thus providing a tentative explanation for the different lactic acid production capacities found in studies of *L. fermentati* strains. This further highlights the scant knowledge on lactic acid biosynthesis by yeasts.

3. Yeast production of succinic acid.

Succinic acid is the weakest wine acid ($pKa_1 = 4.18$ and $pKa_2 = 5.23$). Although it is absent in grapes, it is the main carboxylic acid to be produced by yeasts during alcoholic fermentation, mainly during their exponential growth (Thoukis et al., 1965). The yeast strain strongly influences succinic acid production. For example, the cryotolerant strain *S. uvarum* produces larger amount of succinic acid than non-cryotolerant strains (Vion, Le Mao, et al., 2023). *S. uvarum* produces between 1-2 g/L of succinic acid, whereas *S. cerevisiae* produces 0.5 to 1.5 g/L during alcoholic fermentation. Interestingly, a positive correlation has been found between high malic acid production and succinic acid production (Vion, Muro, et al., 2022), which indicates that the production of both acids might be partially coupled. Furthermore, Bach *et al.* have also reported a positive correlation between α-aminobutyric acid (GABA) content in grape juice and succinate production (Bach et al., 2009). Its production is stimulated at low TA and a pH of 4-4.4 (Thoukis et al., 1965); however, this pH range does not correspond to the usual wine pH variation. In addition, the formation of succinic acid increases with nitrogen concentrations of up to 500 mg/L. Succinic acid production also increases with temperature within the range of 10-30°C, but it diminishes after 40°C (Shimazu & Waranabe, 1981). A linear correlation exists between glucose concentration (up to 8%) and the formation of succinic acid independently of nitrogen source. Finally, *S. cerevisiae* produces considerably more succinic acid when SO_2 is absent in the medium (Shimazu & Waranabe, 1981).

Conclusion

The acidity of wine is a key component of its overall quality. With climate change posing a significant challenge to the winemaking industry, the emergence of yeast strains for wine acidity

693 management has become an essential tool for winemakers. Current trends mainly focus on
694 acidification to improve the analytical and sensory profiles of wines in the context of climate
695 change. Deacidification can nonetheless be of interest to reduce acidity in cooler regions as well as
696 to shorten malolactic fermentation by lowering malic acid content post-AF. Besides the use of
697 specific Saccharomyces cerevisiae strains, novel oenological practices also combine the use of
698 different non-Saccharomyces yeasts to either increase or decrease wine acidity. A diverse range of
699 yeast starters could therefore be used in specific vinification strategies tailored to the climate,
700 terroir, and desired wine style.
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References

1.

Ambroset, C., Petit, M., Brion, C., Sanchez, I., Delobel, P., Guerin, C., Chiapello, H., Nicolas, P., Bigey, F., Dequin, S., Blondin, B., Fay, J. C., Guérin, C., Chiapello, H., Nicolas, P., Bigey, F., Dequin, S., & Blondin, B. (2011). Deciphering the Molecular Basis of Wine Yeast Fermentation Traits Using a Combined Genetic and Genomic Approach. *Genes|Genomes|Genetics*, *1*(4), 263–281. https://doi.org/10.1534/g3.111.000422

Ansanay, V., Dequin, S., Blondin, B., & Barre, P. (1993). Cloning, sequence and expression of the gene encoding the malolactic enzyme from Lactococcus lactis. *FEBS Letters*, *332*(1–2), 74–80. https://doi.org/10.1016/0014-5793(93)80488-g

Ansanay, V., Dequin, S., Camarasa, C., Schaeffer, V., Grivet, J. P., Blondin, B., Salmon, J. M., & Barre, P. (1996). Malolactic fermentation by engineered Saccharomyces cerevisiae as compared with engineered Schizosaccharomyces pombe. *Yeast*, *12*(3), 215–225. https://doi.org/10.1002/(SICI)1097-0061(19960315)12:3<215::AID-YEA903>3.0.CO;2-M

Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J. J., Hilbert, G., & Pascual, I. (2018). Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. *Plant Science*, *267*, 74–83. https://doi.org/10.1016/j.plantsci.2017.11.009

Bach, B., Sauvage, F.-X., Dequin, S., & Camarasa, C. (2009). Role of γ-Aminobutyric Acid as a Source of Nitrogen and Succinate in Wine. *American Journal of Enology and Viticulture*, *60*(4), 508. https://doi.org/10.5344/ajev.2009.60.4.508

Bakker, B. M., Overkamp, K. M., Van Maris, A. J. A., Kötter, P., Luttik, M. A. H., Van Dijken, J. P., & Pronk, J. T. (2001). Stoichiometry and compartmentation of NADH metabolism in Saccharomyces cerevisiae. *FEMS Microbiology Reviews*, *25*(1), 15–37. https://doi.org/10.1016/S0168-6445(00)00039-5

Banilas, G., Sgouros, G., & Nisiotou, A. (2016). Development of microsatellite markers for Lachancea thermotolerans typing and population structure of wine-associated isolates. *Microbiological Research*, *193*, 1–10. https://doi.org/10.1016/j.micres.2016.08.010

Baranowski, K., & Radler, F. (1984). The glucose-dependent transport of L-malate in Zygosaccharomyces bailii. *Antonie van Leeuwenhoek*, *50*, 329–340.

Bellut, K., Krogerus, K., & Arendt, E. K. (2020). Lachancea fermentati Strains Isolated From Kombucha: Fundamental Insights, and Practical Application in Low Alcohol Beer Brewing. *Frontiers in Microbiology*, *11*(April), 1–21. https://doi.org/10.3389/fmicb.2020.00764

Bellut, K., Michel, M., Hutzler, M., Zarnkow, M., Jacob, F., De Schutter, D. P., Daenen, L., Lynch, K. M.,

Zannini, E., & Arendt, E. K. (2019). Investigation into the Potential of Lachancea fermentati Strain KBI 12.1 for Low Alcohol Beer Brewing. *Journal of the American Society of Brewing Chemists*, *77*(3), 157–169. https://doi.org/10.1080/03610470.2019.1629227

Benito, Á., Calderón, F., Palomero, F., & Benito, S. (2015). Combine use of selected schizosaccharomyces pombe and lachancea thermotolerans yeast strains as an alternative to the traditional malolactic fermentation in red wine production. *Molecules*, *20*(6), 9510–9523. https://doi.org/10.3390/molecules20069510

Benito, S., Palomero, F., Morata, A., Calderón, F., & Suárez-Lepe, J. A. (2012). New applications for Schizosaccharomyces pombe in the alcoholic fermentation of red wines. *International Journal of Food Science and Technology*, *47*(10), 2101–2108. https://doi.org/10.1111/j.1365-2621.2012.03076.x

Blein-Nicolas, M., Albertin, W., Da Silva, T., Valot, B., Balliau, T., Masneuf-Pomarède, I., Bely, M., Marullo, P., Sicard, D., Dillmann, C., De Vienne, D., & Zivy, M. (2015). A systems approach to elucidate heterosis of protein abundances in yeast. *Molecular and Cellular Proteomics*, *14*(8), 2056–2071. https://doi.org/10.1074/mcp.M115.048058

Blomberg, A. (2000). Metabolic surprises in Saccharomyces cerevisiae during adaptation to saline conditions: Questions, some answers and a model. *FEMS Microbiology Letters*, *182*(1), 1–8. https://doi.org/10.1016/S0378-1097(99)00531-5

Boles, E., De Jong-Gubbels, P., & Pronk, J. T. (1998). Identification and characterization of MAE1, the Saccharomyces cerevisiae structural gene encoding mitochondrial malic enzyme. *Journal of Bacteriology*, *180*(11), 2875–2882. https://doi.org/10.1128/jb.180.11.2875-2882.1998

Bony, M., Bidart, F., Camarasa, C., Ansanay, V., Dulau, L., Barre, P., & Dequin, S. (1997). Metabolic analysis of S. cerevisiae strains engineered for malolactic fermentation. *FEBS Letters*, *410*(2–3), 452–456. https://doi.org/10.1016/S0014-5793(97)00637-6

Bureau, S. M., Razungles, A. J., & Baumes, R. L. (2000). The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *Journal of the Science of Food and Agriculture*.

Camarasa, C., Grivet, J. P., & Dequin, S. (2003). Investigation by 13C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways of succinate formation in Saccharomyces cerevisiae during anaerobic fermentation. *Microbiology*, *149*(9), 2669–2678. https://doi.org/10.1099/mic.0.26007-0

Carre, E., Lafon-lafourcade, S., & Bertrand, A. (1983). Desacidification Biologique Des Vins Blancs Secs Par Fermentation De L'Acide Malique Par Les Levures. *Connaissance Vigne Vin*, *17*, 43–53.

Casal, M., Paiva, S., Queirós, O., & Soares-Silva, I. (2008). Transport of carboxylic acids in yeasts. *FEMS Microbiology Reviews*, *32*(6), 974–994. https://doi.org/10.1111/j.1574-6976.2008.00128.x

Chidi, B. S., Bauer, F. F., & Rossouw, D. (2018). *Organic Acid Metabolism and the Impact of Fermentation*

Practices on Wine Acidity : A Review. *39*(2).

Ciani, M., Comitini, F., Mannazzu, I., & Domizio, P. (2009). Controlled mixed culture fermentation: A new perspective on the use of non-Saccharomyces yeasts in winemaking. *FEMS Yeast Research*, *10*(2), 123–133. https://doi.org/10.1111/j.1567-1364.2009.00579.x

Coi, A. L., Bigey, F., Mallet, S., Marsit, S., Zara, G., Gladieux, P., Galeote, V., Budroni, M., Dequin, S., & Legras, J. L. (2017). Genomic signatures of adaptation to wine biological ageing conditions in biofilm-forming flor yeasts. *Molecular Ecology*, *26*(7), 2150–2166. https://doi.org/10.1111/mec.14053

Coloretti, F., Zambonelli, C., Castellari, L., Tini, V., & Rainieri, S. (2002). The effect of DL-malic acid on the metabolism of L-malic acid during wine alcoholic fermentation. *Food Technology and Biotechnology*, *40*(4), 317–320.

Comuzzo, P., & Battistutta, F. (2019). Acidification and pH Control in Red Wines. In *Red Wine Technology*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814399-5.00002-5

Conde, C., Silva, P., Fontes, N., C.P.Dias, A., Tavares, R. M. ;, Sousa, M. J. ;, Agasse, A., Delrot, S., & Geros, H. (2007). Biochemical Changes throughout Grape Berry Development and Fruit and Wine Quality. *Food*, 157–180. https://doi.org/10.1016/B978-0-12-811155-0.00007-7

Coombe, B. G. (1987). Influence of Temperature on Composition and Quality of Grapes. *Acta Horticulturae*.

Davaux, F. (2001). L'acidité du mout au vin: d'autres alternatives à l'acidification par l'acide tartrique. *Comment Maitriser l'acidité Du Vin*, 23–27.

David-Vaizant, V., & Alexandre, H. (2018). Flor yeast diversity and dynamics in biologically aged wines. *Frontiers in Microbiology*, *9*(SEP), 1–16. https://doi.org/10.3389/fmicb.2018.02235

Delcourt, F., Taillandier, P., Vidal, F., & Strehaiano, P. (1995). Influence of pH, malic acid and glucose concentrations on malic acid consumption by Saccharomyces cerevisiae. *Applied Microbiology and Biotechnology*, *43*(2), 321–324. https://doi.org/10.1007/BF00172832

Denayrolles, M., Aigle, M., & Lonvaud-Funel, A. (1995). Functional expression in Saccharomyces cerevisiae of the Lactococcus lactis mleS gene encoding the malolactic enzyme. *FEMS Microbiology Letters*, *125*(1), 37–43. https://doi.org/10.1111/j.1574-6968.1995.tb07332.x

Dequin, S., Baptista, E., & Barre, P. (1999). Acidification of Grape Musts by Saccharomyces cerevisiae Wine Yeast Strains Genetically Engineered to Produce Lactic Acid. *Am. J. Enol. Vitic*, *50*.

Dequin, S., & Barre, P. (1994). Mixed Lactic Acid-Alcoholic Fermentation by Saccharomyes cerevisiae Expressing the Lactobacillus casei L(+)-LDH. *Nature Biotechnology*.

Dequin, S., Escudier, J. L., Bely, M., Noble, J., Albertin, W., Masneuf-Pomarède, I., Marullo, P., Salmon, M. J., Sablayrolles, J. M., & Ollat, N. (2017). How to adapt winemaking practices to modified grape composition under climate change conditions. In *Journal International des Sciences de la Vigne et du*

Vin (Vol. 51, Issue 2). https://doi.org/10.20870/oeno-one.2016.0.0.1584

Divol, B., Du Toit, M., & Duckitt, E. (2012). Surviving in the presence of sulphur dioxide: Strategies developed by wine yeasts. *Applied Microbiology and Biotechnology*, *95*(3), 601–613. https://doi.org/10.1007/s00253-012-4186-x

Domizio, P., Lencioni, L., Calamai, L., Portaro, L., & Bisson, L. F. (2018). Evaluation of the yeast schizosaccharomyces japonicus for use in wine production. *American Journal of Enology and Viticulture*, *69*(3), 266–277. https://doi.org/10.5344/ajev.2018.18004

Duchêne, É. (2016). HOW CAN GRAPEVINE GENETICS CONTRIBUTE TO THE ADAPTATION TO CLIMATE CHANGE? *Oeno One*, *50*(3), 113–124. https://doi.org/https://doi.org/10.20870/oeno-one.2016.50.3.98

Duchêne, E., & Schneider, C. (2005). Grapevine and Climatic changes: a glance at the situation in Alsace. *Agron. Sustain. Dev*, 93–99. https://doi.org/10.1051/agro:2004057

Eder, M., Sanchez, I., Brice, C., Camarasa, C., Legras, J. L., & Dequin, S. (2018). QTL mapping of volatile compound production in Saccharomyces cerevisiae during alcoholic fermentation. *BMC Genomics*, *19*(1), 1–19. https://doi.org/10.1186/s12864-018-4562-8

Eglinton, J. M., Heinrich, A. J., Pollnitz, A. P., Langridge, P., Henschke, P. A., & De Barros Lopes E, M. (2002). Decreasing acetic acid accumulation by a glycerol overproducing strain of Saccharomyces cerevisiae by deletiong the ALD6 aldehyde dehydrogenase gene. *Yeast*, *19*(4), 295–301. https://doi.org/10.1002/yea.834

Escribano, R., González-Arenzana, L., Portu, J., Garijo, P., López-Alfaro, I., López, R., Santamaría, P., & Gutiérrez, A. R. (2018). Wine aromatic compound production and fermentative behaviour within different non-Saccharomyces species and clones. *Journal of Applied Microbiology*, *124*(6), 1521–1531. https://doi.org/10.1111/jam.13735

Escribano-Viana, R., Portu, J., Garijo, P., López, R., Santamaría, P., López-Alfaro, I., Gutiérrez, A. R., & González-Arenzana, L. (2019). Effect of the sequential inoculation of non-saccharomyces/Saccharomyceson the anthocyans and stilbenes composition of tempranillo wines. *Frontiers in Microbiology*, *10*(APR), 1–10. https://doi.org/10.3389/fmicb.2019.00773

Farris, G., Fatichenti, F., & Deiana, P. (1989). Incidence De La Temperature Et Du pH Sur La Production D'Acide Malique Par Saccharomyces cerevisiae. In *Journal International des Sciences de la Vigne et du Vin* (Vol. 23).

Fatichenti, F., Farris, G. A., Deiana, P., & Ceccarelli, S. (1984a). Malic acid production and consumption by selected of Saccharomyces cerevisiae under anaerobic and aerobic conditions. *Applied Microbiology and Biotechnology*, *19*(6), 427–429. https://doi.org/10.1007/BF00454382

Fatichenti, F., Farris, G. A., Deiana, P., & Ceccarelli, S. (1984b). Malic acid production and consumption

by selected of Saccharomyces cerevisiae under anaerobic and aerobic conditions. *Applied Microbiology and Biotechnology*, *19*(6), 427–429. https://doi.org/10.1007/BF00454382

Fernandez, E., Moreno, F., & Rodicio, R. (1992). The ICL1 gene from Saccharomyces cerevisiae. *European Journal of Biochemistry*, *204*(3), 947–1189.

Flikweert, M. T., Van der Zanden, L., Janssen, W. M. TH. M., Steensma, H. Y., Van Dijken, J. P., & Pronk, J. T. (1996). Pyruvate Decarboxylase : An Indispensable Enzyme for Growth of Saccharomyces cerevisiae on Glucose. *Yeast*, *12*, 247–257. https://doi.org/10.1016/s0021-9258(19)76974-9

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., & Santos, J. A. (2013). Future scenarios for viticultural zoning in Europe: ensemble projections and uncertainties. *International Journal of Biometeorology*, *57*(6), 909–925. https://doi.org/10.1007/s00484-012-0617-8

Frost, S. C., Harbertson, J. F., & Heymann, H. (2017). A full factorial study on the effect of tannins, acidity, and ethanol on the temporal perception of taste and mouthfeel in red wine. *Food Quality and Preference*, *62*(July 2016), 1–7. https://doi.org/10.1016/j.foodqual.2017.05.010

Gangloff, S., Marguet, D., & Lauquin, G. (1990). Molecular cloning of the yeast mitochondrial aconitase gene (ACO1) and evidence of a synergistic regulation of expression by glucose plus glutamate. *Molecular and Cellular Biology*, *10*(7), 3551–3561.

Gao, C., & Fleet, G. H. (1995). Degradation of malic and tartaric acids by high density cell suspensions of wine yeasts. In *Food Microbiology* (Vol. 12).

Garavaglia, J., Schneider, R. de C. de S., Camargo Mendes, S. D., Welke, J. E., Zini, C. A., Caramão, E. B., & Valente, P. (2015). Evaluation of Zygosaccharomyces bailii BCV 08 as a co-starter in wine fermentation for the improvement of ethyl esters production. *Microbiological Research*, *173*, 59–65. https://doi.org/10.1016/j.micres.2015.02.002

Gerós, H., Chaves, M. M., & Delrot, S. (2012). The biochemistry of the grape berry. *The Biochemistry of the Grape Berry*. https://doi.org/10.2174/97816080536051120101

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., & Ciani, M. (2012). Lachancea thermotolerans and Saccharomyces cerevisiae in simultaneous and sequential co-fermentation: A strategy to enhance acidity and improve the overall quality of wine. *Food Microbiology*. https://doi.org/10.1016/j.fm.2012.10.004

Grobler, J., Bauer, F., Subden, R. E., & Van Vuuren, H. J. J. (1995). The mae1 gene of Schizosaccharomyces pombe encodes a permease for malate and other C4 dicarboxylic acids. *Yeast*, *11*(15), 1485–1491. https://doi.org/10.1002/yea.320111503

Hagman, A., Säll, T., Compagno, C., & Piskur, J. (2013). Yeast "Make-Accumulate-Consume" Life Strategy Evolved as a Multi-Step Process That Predates the Whole Genome Duplication. *PLoS ONE*, *8*(7), e68734. https://doi.org/10.1371/journal.pone.0068734

Hironori, N. (2014). Schizosaccharomyces japonicus: the fission yeast is a fusion of yeast and hyphae. *Yeast*, *31*, 83–90. https://doi.org/10.1002/yea

Hranilovic, A., Bely, M., Masneuf-Pomarede, I., Jiranek, V., & Albertin, W. (2017). The evolution of Lachancea thermotolerans is driven by geographical determination, anthropisation and flux between different ecosystems. *PLoS ONE*, *12*(9), 1–17. https://doi.org/10.1371/journal.pone.0184652

Hranilovic, A., Gambetta, J. M., Schmidtke, L., Boss, P. K., Grbin, P. R., Masneuf-Pomarede, I., Bely, M., Albertin, W., & Jiranek, V. (2018). Oenological traits of Lachancea thermotolerans show signs of domestication and allopatric differentiation. *Scientific Reports*, *8*(1), 14812. https://doi.org/10.1038/s41598-018-33105-7

Husnik, J. I., Delaquis, P. J., Cliff, M. A., & van Vuuren, H. J. J. (2007). Functional Analyses of the malolactic wine yeast ML01. *American Journal of Enology and Viticulture*, *58*(Maicas 2001), 1.

Jantwal, A., Durgapal, S., Upadhyay, J., Joshi, T., & Kumar, A. (2022). Tartaric acid. *Antioxidants Effects in Health: The Bright and the Dark Side*, 485–492. https://doi.org/10.1016/B978-0-12-819096-8.00019-7

Jeffares, D. C. (2018). The natural diversity and ecology of fission yeast. *Yeast*, *35*(3), 253–260. https://doi.org/10.1002/yea.3293

Kapsopoulou, K., Mourtzini, A., Anthoulas, M., & Nerantzis, E. (2007). Biological acidification during grape must fermentation using mixed cultures of Kluyveromyces thermotolerans and Saccharomyces cerevisiae. *World Journal of Microbiology and Biotechnology*, *23*(5), 735–739. https://doi.org/10.1007/s11274-006-9283-5

Kemsawasd, V., Branco, P., Almeida, M. G., Caldeira, J., Albergaria, H., & Arneborg, N. (2015). Cell-to-cell contact and antimicrobial peptides play a combined role in the death of Lachanchea thermotolerans during mixed-culture alcoholic fermentation with Saccharomyces cerevisiae. *FEMS Microbiology Letters*, *362*(14), 1–8. https://doi.org/10.1093/femsle/fnv103

Klerk, J.-L. De. (2010). *Succinic acid production by wine yeasts* (Issue March).

Kliewer, W. M. (1971). Effect of Day Temperature and Light Intensity on Concentration of Malic and Tartaric Acids in Vitis vinifera L. Grapes. *Journal of the American Society for Horticultural Science*, *96*, 372–377.

Kuczynski, J. T., & Radler, F. (1982). The Anaerobic Metabolism of Malate of Saccharomyces bailii and the Partial Purification and Characterization of Malic Enzyme. *Archives of Microbiology*, *131*, 266–270.

Kunze, M., Kragler, F., Binder, M., Hartig, A., & Gurvitz, A. (2002). Targeting of malate synthase 1 to the peroxisomes of Saccharomyces cerevisiae cells depends on growth on oleic acid medium. *European Journal of Biochemistry*, *269*, 915–922.

Lodi, T., & Ferrero, I. (1993). Isolation of the DLD gene of Saccharomyces cerevisiae encoding the mitochondrial enzyme D-lactate ferricytochrome c oxidoreductase. *Molecular & General Genetics : MGG*, *238*(3), 315–324.

Malfeito-Ferreira, M. (2021). Fine wine flavour perception and appreciation: Blending neuronal processes, tasting methods and expertise. *Trends in Food Science & Technology*, *115*, 332–346. https://doi.org/https://doi.org/10.1016/j.tifs.2021.06.053

Martorell, P., Stratford, M., Steels, H., Fernández-Espinar, M. T., & Querol, A. (2007). Physiological characterization of spoilage strains of Zygosaccharomyces bailii and Zygosaccharomyces rouxii isolated from high sugar environments. *International Journal of Food Microbiology*, *114*(2), 234–242. https://doi.org/10.1016/j.ijfoodmicro.2006.09.014

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M. E., Barquet, M., Gaggero, C., Dellacassa, E., & Carrau, F. (2013). Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with Hanseniaspora vineae. *Food Chemistry*, *141*(3), 2513–2521. https://doi.org/10.1016/j.foodchem.2013.04.056

Minard, K. I., & McElister-Henn, L. (1994). Glucose-Induced Phosphorylation of the MDH2 Isoenzyme of Malate Dehydrogenase in Saccharomyces cerevisiae. *Archives of Biochemistry and Biophysics*, 302–309.

Navarro-Aviño, J. P., Prasad, R., Miralles, V. J., Benito, R. M., & Serrano, R. (1999). A proposal for nomenclature of aldehyde dehydrogenases in Saccharomyces cerevisiae and characterization of the stress-inducible ALD2 and ALD3 genes. *Yeast*, *15*(10 A), 829–842. https://doi.org/10.1002/(SICI)1097-0061(199907)15:10A<829::AID-YEA423>3.0.CO;2-9

Nistor, E., Dobrei, A. G., Dobrei, A., & Camen, D. (2018). Growing season climate variability and its influence on sauvignon Blanc and Pinot Gris Berries and Wine Quality: Study case in Romania (2005-2015). *South African Journal of Enology and Viticulture*, *39*(2), 196–207. https://doi.org/10.21548/39-2-2730

OIV. (2021). *RESOLUTION OIV-OENO 581A-2021*.

Origone, A. C., Rodríguez, M. E., Oteiza, J. M., Querol, A., & Lopes, C. A. (2018). Saccharomyces cerevisiae × Saccharomyces uvarum hybrids generated under different conditions share similar winemaking features. *Yeast*, *35*(1), 157–171. https://doi.org/10.1002/yea.3295

Osburn, K., Amaral, J., Metcalf, S. R., Nickens, D. M., Rogers, C. M., Sausen, C., Caputo, R., Miller, J., Li, H., Tennessen, J. M., & Bochman, M. L. (2018). Primary souring: A novel bacteria-free method for sour beer production. *Food Microbiology*, *70*, 76–84. https://doi.org/10.1016/j.fm.2017.09.007

Osothsilp, C., & Subden, R. E. (1986). Isolation and characterization of Schizosaccharomyces pombe mutants with defective NAD-dependent malic enzyme. *Canadian Journal of Microbiology*.

940 Padilla, B., Gil, J. V., & Manzanares, P. (2018). Challenges of the non-conventional yeast
941 wickerhamomyces anomalus in winemaking. *Fermentation*, *4*(3).
942 https://doi.org/10.3390/fermentation4030068
943 Peltier, E., Bernard, M., Trujillo, M., Prodhomme, D., Barbe, J. C., Gibon, Y., & Marullo, P. (2018). Wine
944 yeast phenomics: A standardized fermentation method for assessing quantitative traits of
945 Saccharomyces cerevisiae strains in enological conditions. *PloS One*, 1–23.
946 https://doi.org/10.1101/191353
947 Peltier, E., Vion, C., Abou Saada, O., Friedrich, A., Schacherer, J., & Marullo, P. (2021). Flor Yeasts Rewire
948 the Central Carbon Metabolism During Wine Alcoholic Fermentation. *Frontiers in Fungal Biology*,
949 *2*(October). https://doi.org/10.3389/ffunb.2021.733513
950 Pines, O., Even-Ram, S., Elnathan, N., Battat, E., Aharonov, O., Gibson, D., & Goldberg, I. (1996). The
951 cytosolic pathway of L-malic acid synthesis in Saccharomyces cerevisiae: The role of fumarase.
952 *Applied Microbiology and Biotechnology*, *46*(4), 393–399. https://doi.org/10.1007/s002530050835
953 Pines, O., Shemesh, S., Battat, E., & Goldberg, I. (1997). Overexpression of cytosolic malate dehydrogenase
954 (MDH2) causes overproduction of specific organic acids in Saccharomyces cerevisiae. *Applied*
955 *Microbiology and Biotechnology*, *48*(2), 248–255. https://doi.org/10.1007/s002530051046
956 Plane, R., Mattick, L., & Weirs, L. (1980). An acidity index for the taste of wines. *American Journal of*
957 *Enology and Viticulture*, *31*, 258–265.
958 Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Matarrese, A. M. S.,
959 Gallotta, A., Bellincontro, A., Mencarelli, F., & Tombesi, S. (2018). Grapevine quality: A multiple
960 choice issue. *Scientia Horticulturae*, *234*, 445–462.
961 https://doi.org/https://doi.org/10.1016/j.scienta.2017.12.035
962 Porter, T. J., Divol, B., & Setati, M. E. (2019). Lachancea yeast species: Origin, biochemical characteristics
963 and oenological significance. *Food Research International*, *119*(October 2018), 378–389.
964 https://doi.org/10.1016/j.foodres.2019.02.003
965 Postma, E., Couwenberg, L., van Roosmalen, R., Geelhoed, J., de Groot, P., & Daran-Lapujade, P. (2022).
966 Top-Down, Knowledge-Based Genetic Reduction of Yeast central Carbon Metabolism. *MBio*, 1–6.
967 Raab, A. M., & Lang, C. (2011). Oxidative versus reductive succinic acid production in the yeast
968 Saccharomyces cerevisiae. *Bioengineered Bugs*, *2*(2). https://doi.org/10.4161/bbug.2.2.14549
969 Radler, F., Herzberger, S., Schonig, I., & Schwarz, P. (1993). Investigation of a killer strain of
970 Zygosaccharomyces bailii. *Journal of General Microbiology*, *139*(3), 495–500.
971 https://doi.org/10.1099/00221287-139-3-495
972 Ramon-Portugal, F., Seiller, I., Taillandier, P., Favarel, J. L., Nepveu, F., & Strehaiano, P. (1999). Kinetics
973 of Production and Consumption of Organic Acids during Alcoholic Fermentation by Saccharomyces

cerevisae. *Food Technology and Biotechnology*, *37*(4), 235–240.

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., & Viljoen-Bloom, M. (2003). Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation. *International Journal of Food Microbiology*, *83*(1), 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3

Regev-rudzki, N., Battat, E., Goldberg, I., & Pines, O. (2009). Dual localization of fumarase is dependent on the integrity of the glyoxylate shunt. *Molecular Microbiology*, *72*(March), 297–306. https://doi.org/10.1111/j.1365-2958.2009.06659.x

Remize, F., Andrieu, E., & Dequin, S. (2000). Engineering of the pyruvate dehydrogenase bypass in Saccharomyces cerevisiae: Role of the cytosolic Mg2+ and mitochondrial K+ acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation. *Applied and Environmental Microbiology*, *66*(8), 3151–3159. https://doi.org/10.1128/AEM.66.8.3151-3159.2000

Ribéreau-Gayon, P., Dubourdien, D., Donéche, B. & A. L. (2006). *Handbook of Enology Volume 1 The Microbiology of Wine and Vinifications 2 nd Edition Handbook of Enology Volume 1 The Microbiology of Wine and Vinifications 2 nd Edition* (Vol. 1).

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., & Lonvaud, A. (2006). Handbook of Enology: The Microbiology of Wine and Vinifications: Second Edition. In *Handbook of Enology: The Microbiology of Wine and Vinifications: Second Edition* (Vol. 1). https://doi.org/10.1002/0470010363

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of enology Volume 2 The chemistry of wine stabilization and treatments*.

Rodrigues, F., Joã O Sousa, M., Ludovico, P., Santos, H., Cô Rte-Real, M., Leã, C., & Bassilana, M. (n.d.). *The Fate of Acetic Acid during Glucose Co-Metabolism by the Spoilage Yeast Zygosaccharomyces bailii*. https://doi.org/10.1371/journal.pone.0052402

Rosenkrantz, M., Kell, C. S., Pennell, E. A., Webster, M., & Devenish, L. J. (1994). Distinct upstream activation regions for glucose-repressed and derepressed expression of the yeast citrate synthase gene CIT1. *Current Genetics*, *25*(3), 185–195. https://doi.org/10.1007/BF00357161

Saayman, M., & Viljoen-Bloom, M. (2006). The Biochemistry of Malic Acid Metabolism by Wine Yeasts – A Review. *South African Journal of Enology & Viticulture*, *27*(2), 113–122. https://doi.org/10.21548/27-2-1612

Saint-Prix, F., Bönquist, L., & Dequin, S. (2004). Functional analysis of the ALD gene family of Saccharomyces cerevisiae during anaerobic growth on glucose: The NADP+-dependent Ald6p and Ald5p isoforms play a major role in acetate formation. *Microbiology*, *150*(7), 2209–2220. https://doi.org/10.1099/mic.0.26999-0

Salinas, F., Cubillos, F. A., Soto, D., Garcia, V., Bergström, A., Warringer, J., Ganga, M. A., Louis, E. J.,

Liti, G., & Martinez, C. (2012). The genetic basis of natural variation in oenological traits in Saccharomyces cerevisiae. *PloS One*, *7*(11), e49640. https://doi.org/10.1371/journal.pone.0049640

Salmon, J. M. (1987). l-Malic-acid permeation in resting cells of anaerobically grown Saccharomyces cerevisiae. *BBA - Biomembranes*, *901*(1), 30–34. https://doi.org/10.1016/0005-2736(87)90253-7

Salmon, J. M., Vezinhet, F., & Barre, P. (1987). Anabolic role of l-malic acid in Saccharomyces cerevisiae in anaerobiosis during alcoholic fermentation. *FEMS Microbiology Letters*, *42*(2–3), 213–220. https://doi.org/10.1111/j.1574-6968.1987.tb02075.x

Sauer, M., Porro, D., Mattanovich, D., & Branduardi, P. (2010). 16 years research on lactic acid production with yeast–ready for the market? *Biotechnology and Genetic Engineering Reviews*, *27*(1), 229–256. https://doi.org/10.1080/02648725.2010.10648152

Schwartz, H., & Radler, F. (1988). Formation of L(-)malate by Saccharomyces cerevisiae during fermentation. *Applied Microbiology and Biotechnology*, *27*(5–6), 553–560. https://doi.org/10.1007/BF00451631

Sgouros, G., Mallouchos, A., Filippousi, M. E., Banilas, G., & Nisiotou, A. (2020). Molecular characterization and enological potential of a high lactic acid-producing lachancea thermotolerans vineyard strain. *Foods*, *9*(5). https://doi.org/10.3390/foods9050595

Shimazu, Y., & Waranabe, M. (1981). Effects of Yeast Strains and Environmental Conditions on Formation of Organic Acids in Must during Fermentation. *Fermentation Technology*, *59*(1), 27–32.

Sousa, M. J., Miranda, L., Côrte-Real, M., & Leão, C. (1996). Transport of acetic acid in Zygosaccharomyces bailii: Effects of ethanol and their implications on the resistance of the yeast to acidic environments. *Applied and Environmental Microbiology*, *62*(9), 3152–3157. https://doi.org/10.1128/aem.62.9.3152-3157.1996

Sowalsky, R. A., & Noble, A. C. (1998). Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. *Chemical Senses*, *23*(3), 343–349. https://doi.org/10.1093/chemse/23.3.343

Stucka, R., Dequin, S., Salmon, J. M., & Gancedo, C. (1991). DNA sequences in chromosomes 11 and VII code for pyruvate carboxylase isoenzymes in Saccharomyces cerevisiae: analysis of pyruvate carboxylase-deficient strains. *MGG Molecular & General Genetics*, *229*(2), 307–315. https://doi.org/10.1007/BF00272171

Suárez-Lepe, J. A., Palomero, F., Benito, S., Calderón, F., & Morata, A. (2012). Oenological versatility of Schizosaccharomyces spp. *European Food Research and Technology*, *235*(3), 375–383. https://doi.org/10.1007/s00217-012-1785-9

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2014). Implications of new research and technologies for malolactic fermentation in wine. In *Applied Microbiology and Biotechnology* (Vol. 98, Issue 19, pp.

8111–8132). Springer Verlag. https://doi.org/10.1007/s00253-014-5976-0

Thoukis, G.-, Ueda, M., & Wright, D. (1965). The Formation Of Succinic Acid During Alcoholic Fermentation. *American Journal of Enology and Viticulture*, *16*.

Tilloy, V., Cadière, A., Ehsani, M., & Dequin, S. (2015). Reducing alcohol levels in wines through rational and evolutionary engineering of Saccharomyces cerevisiae. *International Journal of Food Microbiology*, *213*, 49–58. https://doi.org/10.1016/j.ijfoodmicro.2015.06.027

van Leeuwen, C., & Darriet, P. (2016). The Impact of Climate Change on Viticulture and Wine Quality. *Journal of Wine Economics*, *11*(1), 150–167. https://doi.org/10.1017/jwe.2015.21

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I. S., & Henschke, P. A. (2007). Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wine yeasts. *Applied Microbiology and Biotechnology*, *77*(1), 145–157. https://doi.org/10.1007/s00253-007-1145-z

Vilela-Moura, A., Schuller, D., Mendes-Faia, A., Silva, R. D., Chaves, S. R., Sousa, M. J., & Côrte-Real, M. (2011). The impact of acetate metabolism on yeast fermentative performance and wine quality: Reduction of volatile acidity of grape musts and wines. *Applied Microbiology and Biotechnology*, *89*(2), 271–280. https://doi.org/10.1007/s00253-010-2898-3

Viljoen, M., Subden, R. E., Krizus, A., & Van Vuuren, H. J. (1994). Molecular analysis of the malic enzyme gene (mae2) of Schizosaccharomyces pombe. *Yeast (Chichester, England)*, *10*(5), 613–624. https://doi.org/10.1002/yea.320100506

Vion, C., Brambati, M., Costa, G. Da, Richard, T., & Marullo, P. (2023). Endo metabolomic profiling of flor and wine yeasts reveals a positive correlation between intracellular metabolite load and the specific glycolytic flux during wine fermentation. *Frontiers in Microbiology*, *October*, 1–15. https://doi.org/10.3389/fmicb.2023.1227520

Vion, C., Le Mao, I., Yeramian, N., Muro, M., Bernard, M., Da Costa, G., Richard, T., & Marullo, P. (2023). Targeted 1-H-NMR wine analyses revealed specific metabolomic signatures of yeast populations belonging to the Saccharomyces genus (submitted). *Foom Microbiol Submitted*.

Vion, C., Muro, M., Bernard, Margaux, Richard, B., Fautre, V., Yeramian, N., Masneuf-pomarède, I., Tempère, S., & Marullo, P. (2023). New malic acid producer strains of Saccharomyces cerevisiae for preserving wine acidity during alcoholic fermentation. *Food Microbiology*, *112-104209*. https://doi.org/10.1016/j.fm.2022.104209

Vion, C., Peltier, E., Bernard, M., Muro, M., & Marullo, P. (2021). Marker Assisted Selection of malic-consuming Saccharomyces cerevisiae strains for winemaking. Efficiency and limits of a QTL's driven breeding program. *Journal of Fungi*, *March*, 17–24. https://doi.org/10.20944/preprints202103.0132.v1

Volschenk, H., van Vuuren, H. J. J., & Viljoen-Bloom, M. (2003). Malo-ethanolic fermentation in Saccharomyces and Schizosaccharomyces. *Current Genetics*, *43*(6), 379–391. https://doi.org/10.1007/s00294-003-0411-6

Volschenk, H., van Vuuren, H. J. J., & Viljoen-Bloom, M. (2006). Malic Acid in Wine: Origin, Function and Metabolism during Vinification. *South African Journal of Enology & Viticulture*, *27*(2). https://doi.org/10.21548/27-2-1613

Volschenk, H., Viljoen, M., Grobler, J., Petzold, B., Bauer, F., Subden, R. E., Young, R. A., Lonvaud, A., Denayrolles, M., & Van Vuuren, H. J. J. (1997). Engineering pathways for malate degradation in saccharomyces cerevisiae. *Nature Biotechnology*, *15*(3), 253–257. https://doi.org/10.1038/nbt0397-253

Volschenk, H., Viljoen-Bloom, M., Subden, R. E., & Van Vuuren, H. J. J. (2001). Malo-ethanolic fermentation in grape must by recombinant strains of Saccharomyces cerevisiae. *Yeast*, *18*(10), 963–970. https://doi.org/10.1002/yea.743

Wales, D. S., Cartledge, T. G., & Lloyd, D. (1980). Effects of glucose repression and anaerobiosis on the activities and subcellular distribution of tricarboxylic acid cycle and associated enzymes in Saccharomyces carlsbergensis. *Journal of General Microbiology*, *116*(1), 93–98. https://doi.org/10.1099/00221287-116-1-93

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). Acids. In *Understanding Wine Chemistry* (pp. 19–33). Wiley. https://doi.org/10.1002/9781118730720.ch3

Witte, V. ;, Krohn, U. ;, & Emis C., C. (1989). *Characterization of yeasts with high L [+] -lactic acid production : Lactic acid specific soft-agar overlay (LASSO) and TAFE-patterns*. *29*, 707–716.

Wu, M., & Tzagoloff, A. (1987). Mitochondrial and cytoplasmic fumarases in Saccharomyces cerevisiae are encoded by a single nuclear gene FUM1. *Journal of Biological Chemistry*, *262*(25), 12275–12282. https://doi.org/10.1016/s0021-9258(18)45347-1

Yéramian, N., Chaya, C., & Suárez Lepe, J. A. (2007a). L-(-)-malic acid production by Saccharomyces spp. during the alcoholic fermentation of wine. *Journal of Agricultural and Food Chemistry*, *55*(3), 912–919. https://doi.org/10.1021/jf061990w

Yéramian, N., Chaya, C., & Suárez Lepe, J. A. (2007b). L-(-)-malic acid production by Saccharomyces spp. during the alcoholic fermentation of wine. *Journal of Agricultural and Food Chemistry*, *55*(3), 912–919. https://doi.org/10.1021/jf061990w

Yokotsuka, K., Otaki, A., Naitoh, A., & Tanaka, H. (1993). Controlled Simultaneous Deacidification and Alcohol Fermentation of a High-Acid Grape Must Using Immobilized Yeasts, Schizosaccharomyces pombe and Saccharomyces cerevisiae. *American Journal of Enology and Viticulture*, *44*(4).

Zelle, R. M., De Hulster, E., Van Winden, W. A., De Waard, P., Dijkema, C., Winkler, A. A., Geertman, J.

M. A., Van Dijken, J. P., Pronk, J. T., & Van Maris, A. J. A. (2008). Malic acid production by Saccharomyces cerevisiae: Engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. *Applied and Environmental Microbiology*, *74*(9), 2766–2777. https://doi.org/10.1128/AEM.02591-07