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Review

Biological management of acidity in wine industry: A review

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ABSTRACT

Climate change is generating several problems in wine technology. One of the main ones is lack of acidity and difficulties performing malolactic fermentation to stabilize wines before bottling. Among the different available acidity management technologies, such as direct acid addition, ion exchange resins, electro-membrane treatments, or vineyard management, the microbiological option is reliable and deeply studied. The main approach is the increase in malic acid content because of the metabolism of specific *Saccharomyces* strains and to increase lactic acid because of the metabolism of *Lachancea* genus. Other non-*Saccharomyces* yeasts, such as *Starmerella bacillaris* or *Candida stellata* can also acidify significantly because of the production of pyruvic or succinic acid. Wine industry needs the removal of malic acid in most red wines before bottling to achieve wine stability. *Oenococus oeni* performs the malolactic fermentation of red wines on most conditions because of the metabolization of malic acid into lactic acid. However, modern oenology challenges such as high ethanol concentrations, high pH or low levels of malic acid have made researchers to look for other options to reduce potential risks of deviation. Other wine-related microorganisms able to de-acidify malic acid have appeared as interesting alternatives for specific difficult scenarios. *Lactiplantibacillus plantarum* and *Schizosaccharomyces* genus make up nowadays the main studied alternatives.

1. Introduction

The management of wine acidity has become complex during the last years because of the climate change influence. Some wine regions located in warm viticulture areas traditionally showed low acidity, while other viticulture regions considered from template climates start to suffer from lack of acidity (Mendes Ferreira and Mendes-Faia, 2020). Several strategies related to increase acidity try nowadays to solve the problem. The most known ones are direct acid addition, ion exchange resins, electro membrane treatments, vineyard management or acidifier microorganisms (Berbegal et al., 2019; Comuzzo and Battistutta, 2019). High pH grape juices may generate technical problems with difficult solutions during alcoholic fermentation.

The main acids that determine total acidity in wine are tartaric, malic, lactic, and citric acids. From a sensory point of view tartaric and citric acid influence freshness sensations while malic acid is harsh although their influence depends on the concentration. Tartaric acid is chemically unstable as it can precipitate as tartrates when it combines with potassium cations, generating significant decreases in total acidity and pH increases. Malic and citric acids are unstable from a microbiological point of view and may originate undesirable re-fermentation, turbidity, and volatile acidity problems. Malic acid is unstable as lactic bacteria can metabolize it into lactic acid and CO_2. For that reason, most red wines perform malolactic fermentation before bottling. Overripe musts may show final concentrations in malic acid below 1 g/L (Benito, 2020) while grape juices from cool Atlantic regions can reach concentrations over 6 g/L. The genus *Schizosaccharomyces* may also metabolize malic acid into ethanol and CO_2 during alcoholic fermentation (Benito, 2019a). Citric acid is also unstable as lactic bacteria may metabolize it into acetic acid and diacetyl (Capozzi et al., 2021; Sumby

Abbreviations: TA, Total Acidity; MA, Malic Acid; SA, Succinic Acid; LT, *Lachancea thermotolerans*; SC, *Saccharomyces cerevisiae*; GMO, Genetically Modified Organisms; ↑, Increase; ↓, Decrease; n.d., No data available.

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et al., 2019). Lactic acid has the advantage of being stable while the other major acids, tartaric, malic, and citric, show chemical or microbial instabilities that may decrease wine quality. Additionally, it is the cheapest option from an economical point of view. Table 1 summarizes the importance of the main acids present in wine from a winemaking point of view.

There are several biological strategies to improve the acidity of wine. *Saccharomyces* genus can improve acidity, increasing the concentration of malic or lactic acid in small amounts below 1 g/L for natural strains. However, genetically modified *Saccharomyces* strains can significantly influence the acidity, although most countries legislate its use (Benito, 2019b; Maicas, 2021). *L. thermotolerans* has become the most reliable biological option to acidify wine, as it can increase the final concentration of lactic acid in several grams per liter, influencing significantly, the final acidity and pH of wine (Vicente et al., 2021b). Other non-conventional yeasts, such as *Starmerella bacillaris* or *Candida stellata*, may also slightly improve final lactic acid or succinic acid concentration. Table 2 summarizes the main studied biological acidification strategies for winemaking.

The main microbiological de-acidification strategies focus on malic acid metabolism. The climate change generated grape musts in some viticulture regions with low concentrations, close to 0.5 g/L, while some grape musts from Atlantic regions may show final concentrations up to 7 g/L (Benito, 2020). Most red wines perform malic acid stabilization before bottling to avoid undesirable re-fermentations during the commercialization phase. The most popular microbiological option to stabilize red wine from a microbiological point of view is the use of *Oenococcus oeni* that metabolizes malic acid into CO_2 and lactic acid. Modern oenology developed other options, such as the use of other lactic bacteria, such as *Lactiplantibacillus plantarum* (Urbina et al., 2021) or the yeast *Schizosaccharomyces pombe* (Benito, 2019a). These new options could perform better in specific scenarios, reducing some potential risks mainly related to *O. oeni* heterofermentative metabolism. Another option is the use of genetically modified *Saccharomyces* yeasts, although most wine-producer countries legislate its use (Benito et al., 2019; Maicas, 2021). Table 3 summarizes the main studied biological deacidification strategies for winemaking.

Last studies combine different biological acidification and de-acidification options to increase the acidity while achieving microbial stability. Some of those strategies combine *L. thermotolerans* (Benito, 2018a; Vicente et al., 2021b) with *S. pombe* (Benito, 2020) or *O. oeni* (Snyder et al., 2021) or *L. plantarum* (Urbina et al., 2021). Table 4 summarizes the main studied combinations of biological acidifiers and de-acidifiers.

Three sections make up the present work, regarding the biological acidification and deacidification processes available in winemaking. Each section details an explanation of the involved microorganisms. Regarding the biological acidification section, *S. cerevisiae* and *L thermotolerans* are the most important options. Whereas in the deacidification processes, the main implicates are *O. oeni*, *S. pombe* and *L. plantarum.* A last section introduces the studied combinations between acidifiers and de-acidifiers microorganisms.

2. Biological acidification

2.1. *Saccharomyces cerevisiae: malic and lactic acid increasing.*

2.1.1. *Malic acid formation by Saccharomyces cerevisiae*

During alcoholic fermentation, specific *S. cerevisiae* strains may produce moderate amounts of malic acid below 1 g/L (Table 2; Su et al., 2014; Yéramian et al., 2007). Yeasts produce L-(-)-malic acid via the fumarate pathway catalyzed by cytosolic or mitochondrial fumarase, or via oxaloacetic acid catalyzed by malate dehydrogenase (MDH) (Yéramian et al., 2007). A *S. cerevisiae* sake yeast produced 2.9 fold more (326 mg/L) malic acid than a commercial yeast control (111 mg/L), this increased production is related to a higher activity of malate dehydrogenase and a lower mitochondrial activity of the strain studied (Nakayama et al., 2012).

One study reports 10 strains of *S. cerevisiae* out of 282 possess the ability to produce malic acid during alcoholic fermentation, while most *S. cerevisiae* consume it. Malic acid production varied from 0.39 to 0.76 g/L (Yéramian et al., 2007). These strains that come from warm regions tend to preserve or produce malic acid, while the ones from cool regions have consumption tendency. Fermentation conditions also significantly influenced the malic acid production. Low temperature, high pH, low sugar content, low malic acid, and yeast assimilable nitrogen concentration are parameters related to malic acid rises. Increases in the concentrations of pyruvate and fumarate also rises the malic acid final content.

As malic acid production following *S. cerevisiae* strain selection processes is moderate, reaching increases below 1 g/L, some researchers improved this parameter through molecular engineering. Overexpression of an isoenzyme of malate dehydrogenase (Mdh2p) can cause high production yield of malic acid up to 12 g/L (Su et al., 2014). Malic acid production may be controlled by three different mechanisms using genetic modifications: overexpression of pyruvate carboxylase (encoded by PYC2 gene), overexpression of malate dehydrogenase (encoded by MDH3 gene), and expression of malate transporter (encoded by SpMAE1

Table 1
Summary of the importance of the main acids present in wine from a winemaking point of view.

	Origin	Wine concentration (g/L)	Acidification effect in total acidity	Advantages	Disadvantages Un-stability	Price
Tartaric acid	Grape Addition	2–6	1 g/L → 1 g/L↑ pH → 0.1 ↓	Freshness	K^+ precipitation	5.50–6.74 €/kg E-334
Malic acid	Grape Addition Yeasts	0.2–7	1 g/L → 1.12 g/L↑		Lactic Bacteria (CO_2 + Lactic acid) Harsh (sensory)	7.02–9.23 €/kg E-296
Lactic acid	Lactic Bacteria *Lachancea* Other yeasts Addition	0–3	1 g/L → 0.83 g/L ↑	Softer than malic acid	Stable	3.13–4.40 €/kg E-270
Citric acid	Grape Addition	0–1	1 g/L → 1.07 g/L ↑	Freshness	Lactic Bacteria (Acetic acid ↑ + Diacetyl ↑)	5.25–6.26 €/kg E-330
Acetic acid	Acetic bacteria Lactic bacteria Yeast	0.2–1	1 g/L → 1.25 g/L ↑		Stable Negative (> 0.8 g/L)	

↑: Increase, ↓: Decrease.
The prices of acids are the ones the two main Spanish marketers offer in 2022 (www.agrovin.com;www.dolmarproductos.com). The acidification effects of the different acids were calculated using a calculator of wine acidity (www.az3oeno.com/calculadoras/conversion-de-acidez/).

Table 2
Summary of the main studied biological acidification strategies for winemaking.

	Principle	Acidification Effect	Advantages	Disadvantages	Price
Saccharomyces malic acid formation (Yéramian et al., 2007; Nakayama et al., 2012; Su et al., 2014)	MA ↑ Microbial metabolism Strain selection GMO	Strain selection: MA → 0.3–0.7 g/L ↑ pH → 0.10 ↓ GMO: MA → 12 g/L ↑		Malic acid is not microbially stable Malic acid can be harsh in high concentrations GMO legislation	Commercial dry yeast price n.d. for GMO
Saccharomyces lactic acid formation (Dequin et al., 1999; Dequin and Barre, 1994)	LA ↑ Microbial metabolism GMO	Strain selection: Traces GMO: LA → 2.6–8.6 g/L ↑ pH → 0.23–0.35 ↓	Lactic acid stability	GMO legislation	n.d. for GMO
Saccharomyces genus succinic acid formation (Chidi et al., 2015; Pascual et al., 2017)	SA ↑ Microbial metabolism	SA → 0.5–1.8 g/L ↑ Total acidity→1.3 g/L ↑ pH → 0.1 ↓		Succinic acid sensory properties	Commercial dry yeast price: 500 g 53.00 €
Lachancea thermotolerans lactic acid production (Benito, 2018a; Vicente et al., 2021b)	LA ↑ Microbial metabolism	LA → 1–9 g/L ↑ pH → 0.1–0.5 ↓	Lactic acid stability color intensity ↑, aroma ↑, acetic acid ↓, ethanol ↓	Ethanol and SO_2 resistance of *L. thermotolerans*.	Commercial dry yeast: 500 g 40 to 90 €
Starmerella bacillaris (Vilela, 2019)	α-ketoglutaric and PA ↑ Microbial metabolism	TA → 0.5–1.1 g/L ↑	Alcohol↓ Acetic Acid↓ Wine aroma ↑ Color intensity ↑		
Candida stellata (Ciani and Ferraro, 1998; Ciani et al., 2000)	SA ↑ Microbial metabolism	SA → 1.83 g/L ↑ TA → 2 g/L ↑ pH → 0.1 ↓		Succinic acid sensory properties Sensitive to low pH	

MA: Malic acid, LA: Lactic acid, SA: Succinic acid, PA: Pyruvic acid, TA: Total Acidity, GMO: Genetically Modified Organisms, ↑: Increase, ↓: Decrease.
The range of the prices of microorganisms is the one the main Spanish marketers offer in 2022 (Agrovin, Spain; Lallemand, Canada; Hansen, Denmark).

gene) (Zelle et al., 2011). Other studies identified genes related to high malic acid production, such as MDH2 and FUM1 (Asano et al., 2001) or stress response genes, such as HSP12 (Oba et al., 2011). However, genetically modified organisms are occasionally considered potential food-safety hazards and several countries have strict legislations related to them (Maicas, 2021).

2.1.2. Lactic acid formation during alcoholic fermentation

Saccharomyces cerevisiae may produce trace amounts of lactic acid during alcoholic fermentation because of inefficiency of lactic dehydrogenases (LDH) in mitochondria (Dequin et al., 1999). However, unless genetically modified *S. cerevisiae* strains, lactic acid production is very low and does not affect significantly total acidity. The expression of a gene from *Lactobacillus casei* for LDH allows producing 10 g/L lactic acid fermenting on a synthetic medium (Dequin and Barre, 1994). The metabolically engineered strain had a new pathway for lactic acid production from pyruvate. LDH gene expressed in a wine yeast strain that fermented seven different grape musts produced lactic acid that varied from 2.6 to 8.6 g/L (Table 2). All the wines significantly decreased their pH from the initial must pH value from 0.23 to 0.35 units.

2.1.3. Other organic acids produced by Saccharomyces genus

Saccharomyces genus may release other organic acids different from malic, acetic, or lactic acids that may influence total acidity of wine (Volschenk et al., 2017). The main acids are succinic, α-ketoglutaric, pyruvic and fumaric acid (Chidi et al., 2015) that are intermediates or by-products of TCA cycle or glycolysis. TCA cycle generates succinic acid under anaerobic conditions. Pyruvic acid and α-ketoglutaric are mainly intermediates in glycolysis (Chidi et al., 2015). One study compared the organic acid production of some widely commercial wine strains (EC1118, DV10, VIN13, BM45 and 285) (Chidi et al., 2015), reporting a high strain variability. VIN13 strain was the highest producer of succinic acid in anaerobic (0.575–0.611 g/L) and aerobic (2.195–3.816 g/L) conditions. The rest of the strains showed lower succinic acid production in anaerobic (0.296–0.418 g/L) and aerobic (1.133–1.834 g/L) conditions, with DV10 strain having the lowest production (0.198 g/L in anaerobic conditions and 0.855–1.709 g/L in aerobic conditions). Pyruvic acid production was the most variable outcome; however, the scattering had no pattern. In addition, pyruvic acid reaches its maximum value from the second to the fourth day of alcoholic fermentation before decreasing fast. The average highest concentrations varied from 150 to 250 mg/L for *S. cerevisiae* and the effect on pH is not significant because of the low concentration.

A commercial product based in *S. cerevisiae*, Ionys™ (Lallemand, Canada) was developed after the evolution of a parental strain of *S. cerevisiae* under hyperosmotic conditions. These conditions forced the high osmolarity glycerol pathway allowing the production of less alcohol and more glycerol to protect cells as well as some punctual mutations in several cells. Ionys™ (Lallemand, Canada) can reduce pH by 0.1 units and increase total acidity by 1.3 g/L, because of increases in succinic acid and α-ketoglutaric acids during TCA cycle (Pascual et al., 2017).

2.2. Lachancea thermotolerans: a great lactic acid producer

Lachancea thermotolerans, formerly known as *Kluyveromyces thermotolerans*, is a non-conventional yeast that has a rather similar shape and slightly smaller size than *S. cerevisiae* that makes them difficult to distinguish under microscopic observations (Fig. 1) (Benito, 2018a). *L. thermotolerans* have become the most popular non-*Saccharomyces* in viticulture areas that suffer from lack of acidity because of its unique ability to generate lactic acid during alcoholic fermentation from sugar

Table 3
Summary of the main studied biological de-acidification strategies for winemaking.

	Principle	De-acidification effect	Advantages	Disadvantages	Price
Oenococcus oeni Malic acid metabolism (Sumby et al., 2014; Pardo and Ferrer, 2018; Sumby et al., 2019)	MA ↓ Microbial metabolism Malolactic Fermentation	MA 1 g/L ↓ → TA 0.3 g/L ↓ → pH 0.03 ↓	Classical secondary fermentation. Wine microbial stability. Esters↑, terpenes↑, haze↓, acetaldehyde↓, SO_2↓	Sensitivity to high ethanol and sulfur concentrations and low temperatures. Biogenic amines, ethyl carbamate, color intensity losses.	Commercial dry bacteria 60–90 €/kg
Schizosaccharomyces pombe Malic acid metabolism (Benito, 2019a)	MA ↓ Microbial metabolism Maloethanolic fermentation	MA 1 g/L ↓ → TA 1.12 g/L ↓ → pH 0.11 ↓	Fast wine microbial stability. Color intensity ↑, Biogenic amines ↓, Ethyl carbamate ↓, Higher alcohols ↓, Polysaccharides ↑	For not selected strains: Acetic acid ↑, sulfhidric acid ↑, acetaldehyde ↑	Commercial liquid yeast 90 €/kg
Lactiplantibacillus plantarum Malic acid metabolism (Pardo and Ferrer, 2018; Brizuela et al., 2019)	MA ↓ Microbial metabolism Malolactic Fermentation	MA 1 g/L ↓ → TA 0.3 g/L ↓ → pH 0.03 ↓	Fast wine microbial stability. Homofermentative character, Color intensity ↑, Esters↑, thiols↑, acetic acid↓, biogenic amines ↓	Sensitivity to high ethanol and sulfur concentrations. Limited de-acidification activity. Better performance at high pH Possible incompatibilities Ethyl carbamate Ethyl phenols ↑	Commercial liquid bacteria 100 €/kg
Saccharomyces GMO (Benito, 2019b; Maicas, 2021)	MA ↓ Microbial metabolism Malolactic Fermentation	MA 1 g/L ↓ → TA 0.3 g/L ↓ → pH 0.03 ↓	Fast wine microbial stability during alcoholic fermentation.	GMO legislation	n.d.

MA: Malic acid, TA: Total Acidity, GMO: Genetically Modified Organisms, ↑: Increase, ↓: Decrease, n.d: No data available.
The range of the prices of microorganisms is the one the main Spanish marketers offer in 2022 (Agrovin, Spain; Lallemand, Canada; Hansen, Denmark; Bioenologia, Italy).

Table 4
Summary of the main studied combinations of biological acidifiers and de-acidifiers.

	Principle	De-acidification effect	Acidification effect	Advantages	Disadvantages	Price
L. thermotolerans × *S. pombe* (Benito, 2020)	LA ↑ MA ↓ Combined microbial metabolism	Total removal of unstable malic acid	LA → 1.63–2.77 g/L ↑ pH → 0.2–0.3 ↓	Lactic acid stability Malic acid removal color intensity ↑, aroma ↑, acetic acid ↓, ethanol ↓	*L. thermotolerans:* ethanol and SO_2 resistance. *S. pombe* strain must be selected.	Commercial *L. thermotolerans*: 500 g 40 to 90 $ Commercial *S. pombe*: 1 L 90 €
L. thermotolerans × *O. oeni* × *S. cerevisiae* (Snyder et al., 2021; Urbina et al., 2021)	Combined microbial metabolism	Total removal of unstable malic acid	LA → 2.91–11.1 g/L ↑ pH → 0.23–0.61 ↓	Lactic acid stability Malic acid removal	Heterofermentative sugar metabolism of *O. oeni* may increase acetic acid during long alcoholic fermentations.	Commercial *L. thermotolerans*: 500 g 40 to 90 $ Commercial dry bacteria 60–90 €/kg
L. thermotolerans × *L. plantarum* × *S. cerevisiae* (Urbina et al., 2021)	Combined microbial metabolism	Total removal of unstable malic acid	LA → 2.88 g/L ↑ pH → 0.26 ↓	Lactic acid stability Malic acid removal color intensity ↑, aroma ↑, acetic acid ↓, ethanol ↓	Limited de-acidification activity of *L. plantarum* when malic acid concentration is high.	Commercial *L. thermotolerans*: 500 g 40 to 90 $ Commercial liquid bacteria 100 €/kg

MA: Malic acid, LA: Lactic acid, ↑: Increase, ↓: Decrease.

metabolism (Benito, 2018a; Hranilovic et al., 2018; Porter et al., 2019; Vilela, 2019). The specie *L. thermotolerans* is the best biological option to acidify wine (Table 2), as most scientific articles report significant acidifications and pH reductions because of lactic acid production while *S. cerevisiae* only produces trace amounts of lactic acid. As the interest in *L. thermotolerans* has increased during the last years, seven commercial

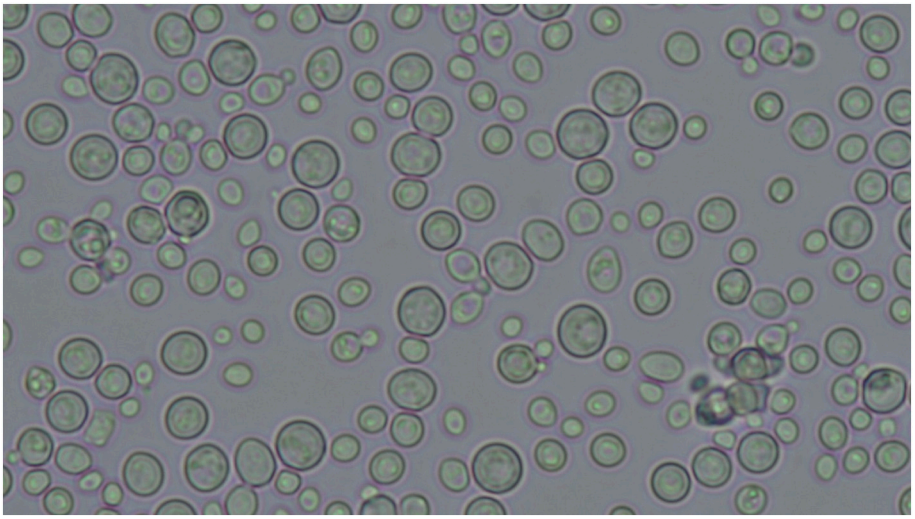

Fig. 1. Detail of microscopic observation of a combined fermentation between yeasts of the *Lachancea thermotolerans* species (small rounded cells) and yeasts of the *Saccharomyces cerevisiae* species (large rounded cells). *Lachancea thermotolerans* cells are slightly smaller.

strains are nowadays available in market, the first appeared in 2013 (Vejarano and Gil-Calderón, 2021).

The first acidification wine-related works employing *L. thermotolerans* took place in the Mediterranean countries Greece and Italy, where several wine regions are areas that suffer from climate change (Benito, 2018a; Vicente et al., 2021b). The studies also focused on early maturing varieties. Later, Spain applied *L. thermotolerans* in early Spanish grape varieties such as Tempranillo (Benito et al., 2015a) with risk of over-ripeness in warm geographies, or in grape varieties considered neutral because of their high productivity characterized by low acidity and sugar concentrations, such as the most planted grape variety Airen (Benito et al., 2016b).

2.2.1. *L. thermotolerans acidifying capacity*

The yeast species *L. thermotolerans* can improve the total acidity of wines through its unique ability to generate L-lactic acid during alcoholic fermentation from the metabolism of fermentative sugars without significantly consuming malic acid or increasing volatile acidity (Benito, 2018a; Vicente et al., 2021b). This metabolism differs from that of lactic acid bacteria mainly based on malic acid (Benito, 2020). Lactic acid has the advantage of being stable, while most major acids in wine have chemical or microbiological instabilities.

The acidifying capacity described in the scientific literature for the species *L. thermotolerans* varies from 1 to 9 g/L in lactic acid (Table 2) and from 1 to 6 g/L in total acidity (Benito, 2018a). The pH reductions vary from 0.1 and 0.5 units depending on the amount of L-lactic acid generated during alcoholic fermentation. These variations depend mainly on the selected strain, fermentation conditions, and inoculation modality (Sgouros et al., 2020; Vicente et al., 2021b). The sequential inoculation modality is the one that gets the best results by allowing *L. thermotolerans* to ferment in purity for a longer period without competence (Table 5). Initial co-inoculations with other yeast species, such as *S. cerevisiae* or *S. pombe*, report lower acidification effects because of the initial competence performed by those more fermentative microorganisms.

Scientific literature generally describes *L. thermotolerans* wines as being better from a sensory point of view than *S. cerevisiae* controls, mainly because of a better balance between acidity and other taste properties.

Lactic acid production involves lactate dehydrogenase (LDH) enzyme. LDH enzyme catalyzes pyruvate as an intermediate in glycolysis into lactic acid (Sauer et al., 2010). There are three genes related to lactate dehydrogenase have been identified that inter-convert pyruvate into lactic acid, accompanied by the interconversion of NADH and NAD+ (Sgouros et al., 2020). A recent study has researched the genes and pathways included in improved lactic acid production (Gatto et al., 2020). Lactate production from pyruvate occurs with *ldh* gene. Other option evaluated the transport of monocarboxylic acid across plasma membrane since intracellular accumulation activates feedback inhibition of LDH gene and decreases lactic acid yield. Genes involve in this transport are JEN1 and ADY2. Eleven strains showed lactic acid yield was between 2.8 and 12 g/L. Characterization of loci related to lactic acid production evaluated the genes (*ldh1*, *ldh2*, *ldh3*, *jen1*, *ady2*) believed to be related to improved lactic acid production in the strains and comparing their lactic acid production. The strains with high lactic acid production showed higher correlations with *ldhs* and *jen1* amino acid sequence. In line with previous studies (Soares-Silva et al., 2007), *jen1* and *ady2* gene effect on lactic acid accumulation was found to be not much effective. Moreover, the *ldh* gene expressions on high lactic acid producer strains were more prominent. Fig. 2 summarizes the fermentative sugar metabolism of *L. thermotolerans*.

2.2.2. *Main limitations of L. thermotolerans*

Despite the great competitive advantage that *L. thermotolerans* species possesses for being able to increase the acidity of wines, it also has some limitations (Table 2) that make it difficult to use when compared to classic fermentative yeasts (Vicente et al., 2021b).

L. thermotolerans has a moderate fermentative power and cannot ferment in ethanol concentrations higher than 9–10% (v/v). Although this fermentative power is higher than most other non-*Saccharomyces*, it is not enough to ferment a regular dry wine, which values usually vary from 12 to 15% (v/v). This limitation obliges combining it with another, more fermentative yeast genera, such as *Saccharomyces* or *Schizosaccharomyces* (Benito, 2020) in order to guarantee the total fermentation of the sugars from the must. Another important limitation is the resistance to sulfur dioxide, which rarely exceeds 20 mg/L (Benito, 2018a, 2018b) of free sulfur dioxide, although some selected strains can tolerate up to 40 mg/L (Vicente et al., 2021b). This limitation restricts the use of *L. thermotolerans* to grapes with good sanitary characteristics that do not require high corrective additions of SO_2. Another option is the use of alternatives to SO_2 such as chitosan, lysozyme or ascorbic acid that do not inhibit the development of *L. thermotolerans* while protecting wine or must against spoilage microorganisms and oxidation. The fermentation kinetics are very slow at temperatures lower than 20 °C and the production of lactic acid may be very small, below 0.3 g/L (Benito et al., 2015b).

Table 5
Summary of the effect of sequential fermentations between *L. thermotolerans* (LT) and *S. cerevisiae* (SC) over wine acidity for different studies.

	Must	SC	SC + LT	SC…LT	LT	Reference
Lactic acid (g/L)	n.d.	0.032	0.18	1.80 24 h 4.20 48 h 5.13 72 h	n.d.	(Kapsopoulou et al., 2007)
Total acidity (g/L)	7.4	7.50	8.10	9.44 24 h 11.84 48 h 12.60 72 h	n.d.	
pH	3.5	3.43	3.46	3.37 24 h 3.26 48 h 3.20 72 h	n.d.	
Lactic acid (g/L)	n.d.	n.d.	n.d	n.d.	n.d.	(Comitini et al., 2011)
Total acidity (g/L)	n.d.	7.12	9.00	n.d.	n.d.	
pH	3.2	3.16	2.97	n.d.	n.d.	
Lactic acid (g/L)	n.d.	0.16	0.81	0.76 24 h 1.55 48 h	3.42	(Gobbi et al., 2013) Laboratory-scale
Total acidity (g/L)	5.55	7.26	9.20	9.26 24 h 9.33 48 h	9.53	
pH	3.61	3.53	3.46	3.47 24 h 3.33 48 h	3.40	
Lactic acid (g/L)	n.d.	0.20	2.35	6.38	n.d.	(Gobbi et al., 2013)
Total acidity (g/L)	7.35	7.03	9.33	12.45	n.d.	Industrial-scale
pH	3.24	3.37	3.29	3.21	n.d.	
Lactic acid (g/L)	<0.1	0.0	n.d.	0.22	n.d.	(Benito et al., 2015b)
Total acidity(g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.26	3.39	n.d.	3.38	n.d.	
Lactic acid (g/L)	<0.1	0.01	n.d.	2.75	n.d.	(Benito et al., 2015a)
Total acidity(g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.92	3.95	n.d.	3.74	n.d.	
Lactic acid (g/L)	<0.1	0.02	0.24	3.18	n.d.	(Benito et al., 2016b)
Total acidity (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.68	3.74	3.71	3.52	n.d.	
Lactic acid (g/L)	<0.1	0.01	n.d.	2.96	n.d.	(Benito et al., 2016a)
Total acidity (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.88	3.90	n.d.	3.71	n.d.	
Lactic acid (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	(Balikci et al., 2016)
Total acidity (g/L)	4.22	5.00	5.40	5.98 24 h 6.28 48 h 6.22 72 h	6.29	
pH	3.37	3.28	3.28	3.36	3.37	
Lactic acid (g/L)	<0.1	0.01	n.d.	2.77	n.d.	(Benito et al., 2017)
Total acidity (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.64	3.68	n.d.	3.5	n.d.	
Lactic acid (g/L)	n.d.	0.21	n.d.	1.51	n.d.	(Dutraive et al., 2019)
Total acidity (g/L)	8.7	n.d.	n.d.	n.d.	n.d.	
pH	2.9	3.1	n.d.	3.1	n.d.	
Lactic acid (g/L)	<0.1	0.01	n.d.	1.63	n.d.	(Benito et al., 2019)
Total acidity (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	
pH	3.61	3.64	n.d.	3.47	n.d.	
Lactic acid (g/L)	n.d.	0.54	n.d.	6.60	n.d.	(Morata et al., 2019)
Total acidity (g/L)	n.d.	3.05	n.d.	6.55	n.d.	
pH	3.9	4.20	n.d.	3.63	n.d.	
Lactic acid (g/L)	n.d.	0.1	n.d.	0.2	0.2	(Blanco et al., 2020)
Total acidity (g/L)	5.7	5.6	n.d.	5.7	5.8	Treixadura grape variety
pH	3.51	3.45	n.d.	3.42	3.39	

(*continued on next page*)

Table 5 (*continued*)

	Must	SC	SC + LT	SC…LT	LT	Reference
Lactic acid (g/L)	n.d.	0.2	n.d	7.2	7.1	(Blanco et al., 2020)
Total acidity (g/L)	4.6	5.3	n.d.	10.3	10.1	Mencia grape variety
pH	3.57	3.74	n.d.	3.57	3.54	
Lactic acid (g/L)	n.d.	<0.6	2.3	10.4	n.d.	(Sgouros et al., 2020)
Total acidity (g/L)	5.46	6.3	9.2	15.5	n.d.	Pasteurized must
pH	3.42	3.53	3.38	3.24	n.d.	
Lactic acid (g/L)	n.d.	<0.6	<0.6	5.5	n.d.	(Sgouros et al., 2020)
Total acidity (g/L)	5.48	6.1–6.2	6.1	10.2	n.d.	Natural must
pH	3.31	3.29–3.39	3.27	3.15	n.d.	
Lactic acid (g/L)	n.d.	0.4	0.6–5	1–8.1	n.d.	(Hranilovic et al., 2021)
Total acidity (g/L)	n.d.	5	5.2–8.9	5.1–11.1	n.d.	
pH	3.9	3.86	3.49–3.85	3.36–3.58	n.d.	

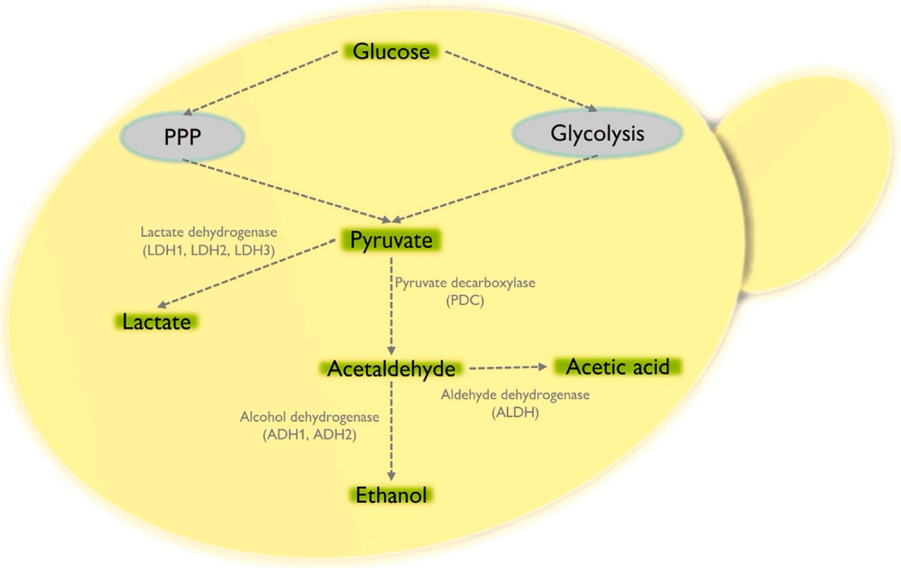

Fig. 2. Fermentative sugar metabolism of *L. thermotolerans* adapted from Kyoto Encyclopedia of Genes and Genomes pathway database.

Although there is no direct relationship between histamine precursors such as the amino acid histidine and the final content of the biogenic amine, some studies report a higher content of this precursor in the final wine fermented with *L. thermotolerans* or aged with its lees (Belda et al., 2016; Benito et al., 2016a). Other undesirable defects, such as the generation of high concentrations of isovaleric acid or acetoin, has been occasionally reported (Benito, 2018a). Recent studies describe incompatibilities depending on the strain used with other microorganisms used in oenology, such as lactic acid bacteria (Urbina et al., 2021). Therefore, the *L. thermotolerans* strains used in winemaking must follow selection processes based on these parameters before using them at industrial scale.

2.2.3. Other virtues of L. thermotolerans different from lactic acid production

Several scientific studies regarding *L. thermotolerans* described it as generating reduced concentrations of acetic acid compared to controls fermented with *S. cerevisiae*. Concentrations are usually lower than 0.2 g/L in acetic acid (Benito, 2018a; Vilela, 2018). Besides increasing the acidity of wines and improving the sensory perception of wines produced from musts that suffer from lack of acidity, the generation of higher concentrations of fruity fermentative esters also significantly improves the evaluation of consumers (Dutraive et al., 2019). Studies carried out on red wines always describe increases in color intensities of about 10% attributed to the greater coloring effect of the anthocyanins in the wine when pH decreases because of the lactic acid generation (Benito, 2020). Other positive characteristics (Table 2) sometimes described for fermentations carried out by *L. thermotolerans* are reduced alcoholic degree, higher concentrations in glycerol, polysaccharides, thiols, and terpenes or decreases in the final content of higher alcohols, ochratoxin A, acetaldehyde, and ethanol (Vicente et al., 2021b). However, these characteristics vary widely depending on the strain used, so prior selection processes are necessary if these goals are the primary aim of winemaking.

2.3. Other non-Saccharomyces: no-conventional alternatives in wine industry

Apart from *L. thermotolerans*, there are other non-conventional yeast with some effects on a particular acid production or total acidity (Table 2).

2.3.1. Starmerella bacillaris/Candida zemplinina

Other non-conventional yeasts can be beneficial for wine

acidification; one of them is *Candida zemplinina* (basionym of *Starmerella bacillaris*) that was firstly isolated from Tokaj wine grapes, often isolated from overripe or botrytized grapes (Ciani et al., 2016). Its low ethanol yield, high glycerol production and its fructophilic character attracted special attention in modern enology. *C. zemplinina* appears even in the end of alcoholic fermentation, which suggests that some strains have high alcohol tolerance and fermentative power (Rantsiou et al., 2012). *C. zemplinina* is also osmotolerant and acidogenic, which makes it rather adapted to sweet wine fermentation (Vilela, 2019).

C. zemplinina is a prominent producer of pyruvic acid in anaerobic conditions because of its glycerol-pyruvic pathway preference. Some strains of C. *zemplinina* produce about 100 mg/L of pyruvic acid, while the *S. cerevisiae* controls only produce about 20 mg/L, what suggests that under limited oxygen environments, *C. zemplinina* may form several organic acids via TCA cycle. In addition, production of 2-oxoketoglutaric acid may have a key role in acidogenic attributes of *C. zemplinina* (Goold et al., 2017; Magyar et al., 2014).

Combined fermentations between C. *zemplinina* and *S. cerevisiae* on four white grape musts showed higher final total acidity concentrations than the pure *S. saccharomyces* control (Englezos et al., 2018). The chardonnay wine showed a final total acidity of 6.3 g/L for pure *S. cerevisiae* fermentation, while the sequential fermentation had 7.1 g/L. The other trials showed similar results, sequential fermentation showed higher total acidities from 0.5 g/L to 1.1 g/L. Primary organic acid formation (tartaric, malic, lactic, citric, and succinic acid) cannot explain the increase in acidity. As mentioned before, other acids, such as α-ketoglutaric or pyruvic acid, must source the increase. Moreover, the consumption of malic acid was relatively lower in sequential fermentation with 0.5 g/L compared to 0.7 g/L consumption observed in pure *S. cerevisiae* fermentation (that corresponds to 28% and 36% reduction in malic acid content, respectively) (Englezos et al., 2018). In addition, the mixed fermentation resulted in higher esters and thiols concentrations, which improved wine aroma in Sauvignon blanc wine samples (Englezos et al., 2018). In another study (Castrillo et al., 2019), sequential fermentation of C. *zemplinina* and *Saccharomyces cerevisiae* produced the lowest alcohol content and highest total acidity compared to pure *Saccharomyces* fermentations and sequential fermentations made with *L. thermotolerans*, *Torulaspora delbrueckii*, *Metschnikowia fructicola*.

Because of the reduction in pH, *C. zemplinina* may also influence wine color, and pyruvic acid may combine with anthocyanins, forming Vitisin A that is a very stable color pigment (Romboli et al., 2015). *C. zemplinina* is also a low acetic acid producer, its co-inoculation with *S. cerevisiae* showed 0.3 g/L less acetic acid compared to a pure *S. cerevisiae* control (Rantsiou et al., 2012).

2.3.2. *Candida stellata*

Candida stellata is one non-conventional yeast that appears regularly in considerable amounts (between 5% to 12%, or even up to 50% of the total population) in most early spontaneous fermentations (Combina et al., 2005; Torija et al., 2001). High populations are common in overripe or botrytized grape berries. Although it is not a great fermenter, it can occasionally remain active until the end of alcoholic fermentation (García et al., 2018), depending on vitamins such as biotin or thiamine. It is sensitive to low pH, conflicting with the aim of acidification (García et al., 2018). *C. stellata* may produce succinic acid up to 1.83 g/L, while the *S. cerevisiae* control showed a maximum of 0.45 g/L. The sequential fermentation ended with a final concentration of 1.10 g/L of succinic acid (Ciani and Ferraro, 1998). Other study reports increases of 2 g/L of total acidity in sequential fermentations involving *C. stellata* compared to the *S. cerevisiae* control. Nevertheless, the final succinic acid concentration does not totally explain the final increase in total acidity. Other research also shows improved production of succinic acid (Ciani et al., 2000).

2.4. *Lactiplantibacillus plantarum*

Although *Lactiplantibacillus plantarum* is better known because of its ability to reduce acidity due to the malolactic fermentation processes (Brizuela et al., 2019). Some studies describe it as a biological acidification option. *L. plantarum* can produce lactic acid from malic acid degradation but also from sugar metabolism, but without increasing acetic acid (Pardo and Ferrer, 2018). One study report and acidification effect that reduced the pH in 0.5 units, *L. plantarum* generated 8.3 g/L of lactic acid during the fermentation processes. Malic acid metabolism produced 1.95 g/L of lactic acid, while sugar metabolism produced 6.35 g/L (Onetto and Bordeu, 2015). The acidification effect increases if *L. plantarum* is inoculated before *S. cerevisiae* in combined fermentations. The consumption of sugar suggests that this method can also reduce the final potential ethanol concentration because of the sugar reduction.

3. Biological de-acidification

3.1. *Oenococcus oeni: the traditional option for malolactic fermentation*

Classical winemaking practices contain two fermentation processes, firstly an alcoholic fermentation mainly conducted by selected strains of *S. cerevisiae* and secondly a malolactic fermentation performed by lactic acid bacteria once alcoholic fermentation is over. *O. oeni* is the main microorganism involved in the malolactic fermentation of wines. The elimination of malic acid is crucial since it decreases the risk of bottle re-fermentation and turbidity in red wines. Fig. 3 shows cells of *O. oeni* performing a malolactic fermentation after alcoholic fermentation once there are not any residual sugars.

3.1.1. *O. oeni de-acidifying capacity*

The de-acidification activity takes place through the malic acid decarboxylation that generates lactic acid (Fig. 4). The reduction of acidity depends on the initial malic acid concentration that varies in must from 0.3 to 7 g/L (Table 1). Losing 1 g/L of malic acid equals 1.12 g/L in tartaric acid, while the earning of 1 g/L of lactic acid equals 0.83 g/L in tartaric acid. The final balance is loss of about 0.3 g/L of total acidity in tartaric acid for each gram of malic acid metabolized during malolactic fermentation (Table 3).

3.1.2. *Main limitations of O. oeni malolactic fermentation*

O. oeni is sensitive to high concentrations of ethanol over 15% (v/v), low temperatures below 18 °C and free sulfur dioxide levels over 10 mg/L (Table 3) (Sumby et al., 2014). Other secondary known limiting parameters are lack of nutrients, medium fatty acids, or phage infections (Sumby et al., 2019). Although malolactic fermentation is a process needed before bottling red wines, on some occasions can negatively influence the color intensity, volatile acidity, biogenic amines, ethyl carbamate and sensory properties (Benito, 2019b).

Acetic acid production is a collateral effect of malolactic fermentation. Under control fermentations, it increases between 0.05 and 0.1 g/L because of citric and pentose degradation. However, in uncontrolled situations where *O. oeni* consumes residual hexoses, the increases are higher and usually significantly reduce the final quality of wine. If *O. oeni* consumes hexoses may produce high concentrations of diacetyl that can mask desired aromas. Several studies observed color losses during malolactic fermentation processes that vary from 8 to 30% (Benito, 2020). Those color losses take place because of pH increases that influence anthocyanins coloration, lactic bacteria glycosidase enzymes, absorption of anthocyanins and reduction of acetaldehyde, that decreases vitisin B formation (Benito, 2020).

3.1.3. *Other virtues of O. oeni*

Although the primary advantage of *O. oeni* is to get stable and softer wines because of the malic acid metabolism. Malolactic fermentation is a complex process that can improve other quality parameters (Table 3)

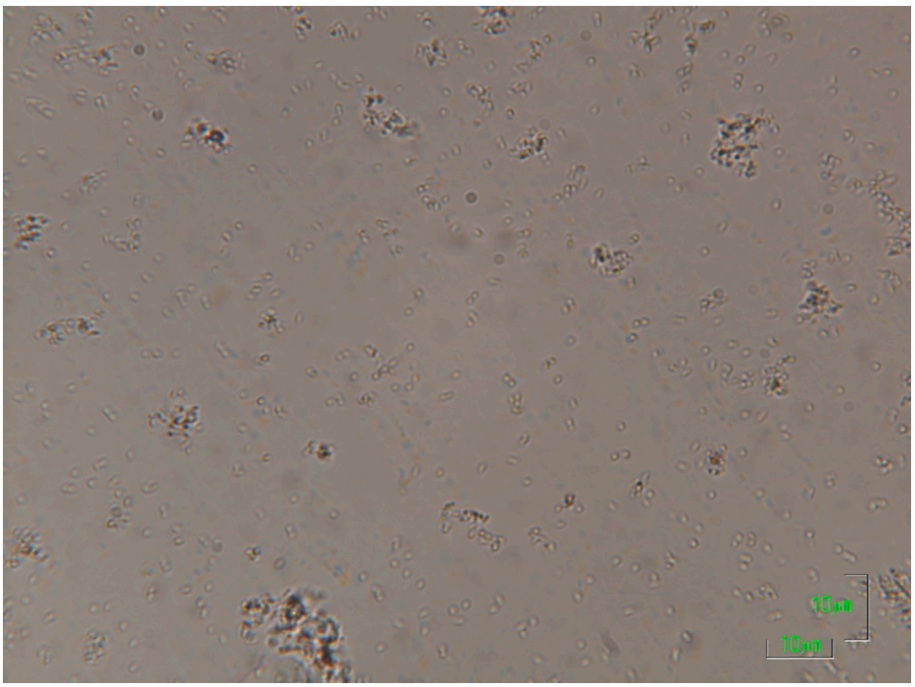

Fig. 3. Detail of microscopic observation of a malolactic fermentation carried out by *Oenococcus oeni*.

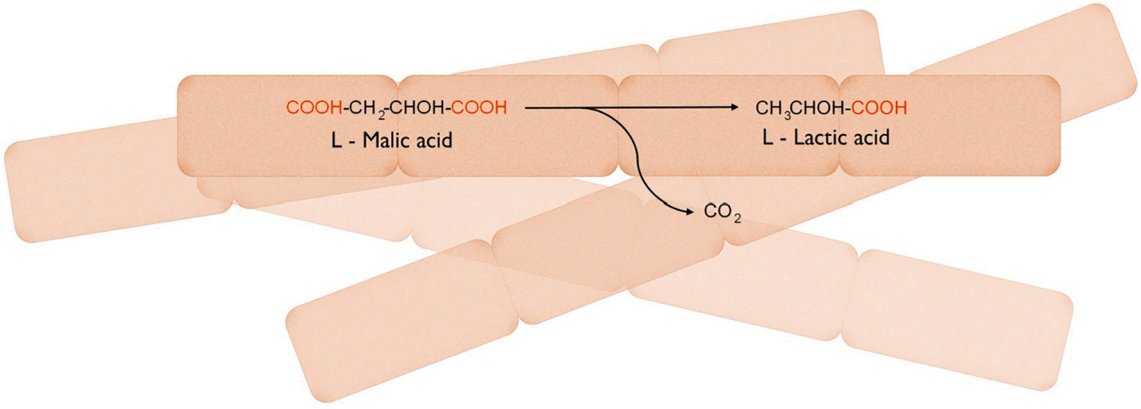

Fig. 4. Detail of malic acid decarboxylation process by *Oenococcus oeni*.

because of additional virtues of *O. oeni* (Capozzi et al., 2021; Virdis et al., 2021). Several works report improvements in aroma composition (Pripis-Nicolau et al., 2004; Knoll et al., 2010; Antalick et al., 2012; Costello et al., 2013; Bartowsky et al., 2015; Fia et al., 2018; Gammacurta et al., 2018; Lasik-Kurdyś et al., 2018;), protein stabilization (Virdis et al., 2021), clarification (Rodríguez et al., 2019; Virdis et al., 2021), acetaldehyde reduction (Osborne et al., 2000; De Orduña, 2010; Virdis et al., 2021). Most studies show that these positive characteristics of the species *O. oeni* are strain-dependent (Capozzi et al., 2021; Virdis et al., 2021). This strain dependency allows performing selection processes to increase those virtues.

3.1.4. Co-inoculation versus sequential inoculation

When co-inoculations with *S. cerevisiae* take place properly without alcoholic fermentation stopping or sluggish, the final wines show less acetic acid, more color intensity, and less diacetyl (Knoll et al., 2012; Urbina et al., 2021). Some studies report notable increases in volatile acidity that vary from 0.05 to 0.22 g/L for long alcoholic fermentations (Urbina et al., 2021). Authors explain the effect due to the heterofermentative character of *O. oeni* that allows it to metabolize hexoses into acetic acid. However, several other works did not observe this effect in fast and uninterrupted alcoholic fermentations (Knoll et al., 2012; Pardo and Ferrer, 2018).

3.2. Lactiplantibacillus plantarum: a recent alternative for malolactic fermentation

There are several lactic bacteria species different from *O. oeni* that can perform malolactic fermentation in winemaking (Capozzi et al., 2021; Virdis et al., 2021). *Lactiplantibacillus plantarum* (Fig. 7) appeared during the last years as an alternative to the classic *O. oeni* malolactic fermentation (Brizuela et al., 2019). Its primary advantage is its homofermentative character (Table 3), that makes it not able to metabolize hexoses and to increase the volatile acidity that may decrease wine quality. The development of recent freeze-drying commercial products that allow proper preservation processes has increased its popularity among the winemakers (Krieger-Weber et al., 2020). Several studies show it can perform better than *O. oeni* in specific scenarios related to warm viticultural areas where initial grape juices show high concentrations of sugar over 250 g/L and high pH close to 4 (Urbina

et al., 2021).

3.2.1. De-acidifying capacity of L. plantarum

The studies that compare *L. plantarum* with *O. oeni* report a very similar de-acidifying capacity (Gardoni et al., 2021). The malic acid-deacidification process lengths from 2 to 4 days for the co-inoculations with *S. cerevisiae* during the first stages of alcoholic fermentation. *L. plantarum* also possesses the malolactic enzyme encoding gene (Brizuela et al., 2019; Du Toit et al., 2011) although it cannot metabolize sugars.

3.2.2. Main limitations of L. plantarum use

Most *L. plantarum* strains do not tolerate ethanol concentrations higher than 8–10% (v/v) (Table 3) (Brizuela et al., 2019). Therefore, *L. plantarum* must metabolize malic acid during the first stages of alcoholic fermentation before the level of ethanol becomes too high. However, some modern studies show specific strains tolerate high concentrations of ethanol and can perform a sequential malolactic fermentation after alcoholic fermentations, as *O. oeni* does (Pardo and Ferrer, 2018).

Although the de-acidification is quite effective in wines with low concentrations of malic acid like those of warm viticulture areas (Urbina et al., 2021). When the concentrations of malic acid are higher than 5 g/L, although it always performs significant de-acidifications, it does not reach the 100% degradation of malic acid reaching values of about 80% (Gardoni et al., 2021). Some studies report incompatibility problems with *Saccharomyces* and non-*Saccharomyces* strains (Du Plessis et al., 2019; Gardoni et al., 2021; Russo et al., 2020).

Other secondary reported disadvantages are risk of ethyl carbamate (Brizuela et al., 2019; Capozzi et al., 2012) and ethyl phenols (Couto et al., 2006; Silva et al., 2011; Capozzi et al., 2021).

3.2.3. Other virtues of Lactiplantibacillus plantarum use

The prime virtue of *L. plantarum* compared to *O. oeni* is its homofermentative character of malic acid metabolism that reduces the risk of undesirable acetic acid increases in wine (Table 3). Several works report other secondary improvements such as aroma composition (Mtshali et al., 2010; Lerm et al., 2011; Esteban-Torres et al., 2013; Iorizzo et al., 2016; Takase et al., 2018; Brizuela et al., 2019; Capozzi et al., 2021), spoilage microorganisms managment (Du Toit et al., 2011; Dean et al., 2012; De Senna and Lathrop, 2017; López-Seijas et al., 2020) and biogenic amines control (Brizuela et al., 2019).

3.3. Schizosaccharomyces pombe: a malic acid-consuming yeast

The yeast species *Schizosaccharomyces pombe* possesses the unique ability to metabolize malic acid to carbon dioxide and ethanol through malic-alcoholic fermentation (Benito, 2019a) (Fig. 5). This metabolism allows the malic de-acidification of musts that occasionally present excessive acidity due to lack of ripening in the grape (Benito et al., 2015b). However, modern red winemaking use *S. pombe* to eliminate small amounts of malic acid in excessively mature red wines characterized by low concentrations of malic acid, high alcohol content and high pH reaching microbiological stability. In those situations, it is possible to avoid malolactic fermentations from a preventive point of view that would develop in conditions of high pH and alcoholic degree. *S. pombe* possesses a peculiar rectangular morphology and fission reproduction that makes very easy to identify it after inoculation in wine (Fig. 6).

3.3.1. De-acidifying capacity of S. pombe

Many studies describe *S. pombe* and other yeasts of the same genus as capable of consuming malic acid in its entirety in musts with a high content of malic acid greater than 5 g/L (Benito et al., 2016c; Benito, 2019a). The increase can be greater than 0.4 pH units for a Riesling must with an initial concentration of malic acid close to 5 g/L (Gardoni et al., 2021) or less than 0.1 pH units in musts with initial malic acid content below 0.5 g/L of the Garnacha grape variety (Benito, 2020). Consumption of malic acid takes place shortly after the inoculation of *S. pombe* in the must during the alcoholic fermentation.

3.3.2. Comparison of S. pombe with lactic acid bacteria

Although one of the primary objectives of the use of species belonging to the genus *Schizosaccharomyces* is to get a microbiological stabilization from the point of view of malic acid that avoids possible unwanted re-fermentations after bottling the wine. Very few studies include controls that compare *Schizosaccharomyces* fermentations with the classic malic stabilization process carried out by lactic bacteria (Benito, 2020; Gardoni et al., 2021). This situation makes it difficult to draw conclusions regarding whether it is convenient to use it as an alternative to classical conventional processes in certain circumstances.

There is only one study to date that compares all the main microbiological options for de-acidification in wine, including controls de-acidified by *S. pombe*, *O. oeni* and *L. plantarum* (Gardoni et al., 2021). *S. pombe* achieved in this study the complete elimination of malic acid during alcoholic fermentation, fermenting alone and combined with *L. thermotolerans*. While initial co-inoculations between *S. cerevisiae* and *O. oeni* or *L. plantarum* achieved de-acidification of about 80% for malic acid during alcoholic fermentation for an initial high malic acid concentration close to 5 g/L. The classic process of alcoholic fermentation by *S. cerevisiae* followed by malolactic fermentation by *O. oeni* also achieved a 100% malic de-acidification, although it required an

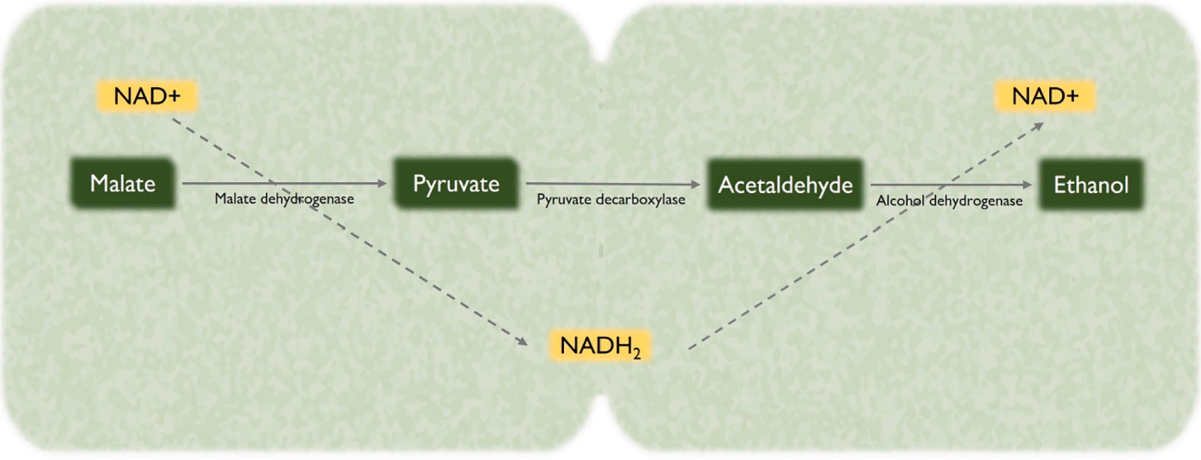

Fig. 5. Detail of malo-ethanolic fermentation process by *Schizosaccharomyces pombe*.

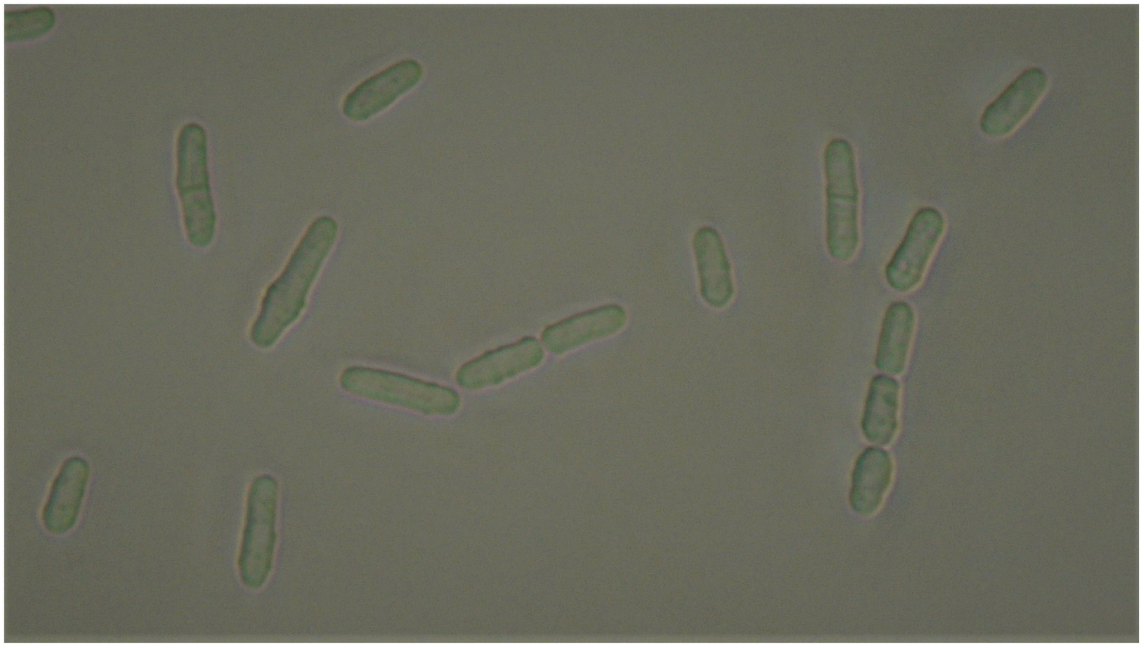

Fig. 6. Detail of microscopic observation of a fermentation carried out by *Schizosaccharomyces pombe*. Rectangular cells and reproduction by fission and sporulation take place.

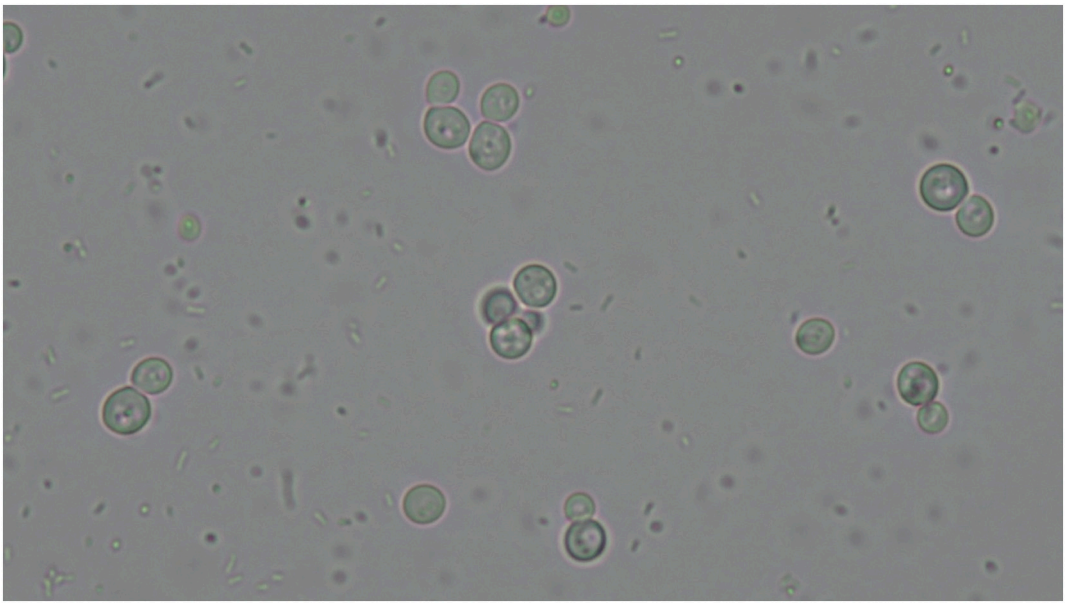

Fig. 7. Detail of microscopic observation of a malolactic fermentation carried out by *Lactiplantibacillus plantarum* (small long cells) and *Saccharomyces cerevisiae* (big cells).

additional 24 days at controlled temperature to carry out the malolactic fermentation.

All the studies that investigated the influence of *S. pombe* on wine color that include controls that perform classic malolactic fermentation, describe color intensity losses during malolactic fermentation of between 10 and 20% and increases in volatile acidity between 0.1 and 0.2 g/L. This effect did not take place in the alcoholic fermentations carried out by *S. pombe* (Benito et al., 2017; Benito, 2020).

3.3.3. Main limitations of S. pombe use

Although practically all strains of *S. pombe* have a great capacity for malic acid de-acidification, most strains of *S. pombe* show limitations that make it difficult to apply them to modern winemaking. The major limitation described for the oenological applications of the species *S. pombe* is the tendency of most strains of the species and the genus to produce high concentrations of acetic acid (Benito et al., 2016a; Benito, 2019a). Currently, researchers have solved this limitation with selective strain processes, fed-batch fermentation, or enrichment of the medium with the nutrient magnesium (Benito, 2019a, 2020). Another limitation is the tendency of most strains of the genus *Schizosaccharomyces* to produce high concentrations of hydrogen sulfide and acetaldehyde. The limited incidence of *S. pombe* in nature made it difficult to perform strain-selective processes for representative universes unless selective-differential media are used (Benito, 2018b). In juices with very high

concentrations of malic acid above 7 g/L, the final ethanol concentration can significantly increase because of the malo-alcoholic fermentation (Minnaar et al., 2021).

3.3.4. Other virtues of S. pombe

S. pombe has a high fermentation power similar to that of *S. cerevisiae*, which makes some strains capable of fermenting wines to concentrations in ethanol greater than 15% (v/v) (Benito, 2019a). *S. pombe* can produce wines free of some problems that affect food safety (Table 3) such as biogenic amines, ethyl carbamate and ochratoxin A (Benito, 2019a, 2019b). *S. pombe* can increase the color intensity of wines and its stability (Benito et al., 2015a, 2017). This is because of the elimination of malolactic fermentation and its associated loss of color. S. *pombe* generates highly stable anthocyanin compounds such as vitisins A and B. *S. pombe* is the yeast documented as the largest producer of polysaccharides (Benito et al., 2019). Most of the studies that have studied aromatic profiles of wines fermented by *S. pombe* coincide with their low productivity of higher alcohols (Scansani et al., 2020).

3.4. Saccharomyces cerevisiae: a double role in malic acid metabolism

Saccharomyces cerevisiae can significantly reduce the initial concentration of malic acid present in grape juice during alcoholic fermentation. There is a great variability that varies from production of malic acid (opposite effect) to degradation of malic acid down to 50% (Benito, 2019a). The degradation is higher for higher initial concentrations of malic acid. However, natural strains of *S. cerevisiae* cannot remove all the initial malic acid and to guarantee the microbial stability.

Genetically modified *S. cerevisiae* strains can totally metabolize or to degrade the malic acid. These GMOs may contain the genes ML01 or ECMO01. One gene allows performing the malo-alcoholic fermentation as *S. pombe* species does, while the other allows performing malolactic fermentation as *O. oeni* species does. Both options allow removing all malic acid from the media directly during the alcoholic fermentation without perform a malolactic fermentation, reducing the production time and potential risks. However, the use of GMO remains controversial, and several countries legislate their use (Benito, 2019b; Maicas, 2021), not allowing it or obliging to show it to the consumer on the label.

3.5. Other non-Saccharomyces able to degrade significantly malic acid

Several species of non-*Saccharomyces* such as *T. delbrueckii* (Benito, 2018b), *L. thermotolerans* (Blanco et al., 2020; Vicente et al., 2021b) or *M. pulcherrima* (Vicente et al., 2020) possess strain-dependent ability to degrade significantly malic acid in about 20%. Other species, such as *Issatchenkia orientalis* (Kim et al., 2008; Seo et al., 2007), *Pichia kudriavzevii* (Del Mónaco et al., 2014; Vicente et al., 2021a) or *Issatchenkia terricola* (Shi et al., 2019), may degrade significantly malic acid down to 50%, which is very interesting in order to deacidify very sharp acidic wines from Atlantic regions. However, only *Schizosaccharomyces* genus (Benito, 2019a) may totally consume all the initial malic acid down to 100% to achieve malic acid microbial stability.

3.6. Biological de-acidification comparisons

Nowadays, there is only one scientific study that allows comparing all the available microbiological options that allow to totally de-acidify malic acid in wine (Gardoni et al., 2021). The study fermented a Riesling grape juice with an initial content in malic acid of 4.7 g/L, pH of 3.09 and total acidity of 10.48 g/L. The article used different combinations between *O. oeni* and *L. plantarum* with *S. cerevisiae* before and after the alcoholic fermentation and pure fermentations of *S. pombe* and a combination with *L. thermotolerans*. *S. pombe* could metabolize all the malic acid during the alcoholic fermentation while combined fermentations between *S. cerevisiae* and *O. oeni* or *L. plantarum* metabolized up to 80% during alcoholic fermentation. However, other studies report reductions of 100% for *O. oeni* or *L. plantarum* during alcoholic fermentation, in combined fermentations with *S. cerevisiae* for musts that contained less initial malic acid.

The study also reports a slightly increase in ethanol for *S. pombe* pure fermentations and acetic acid increases for *O. oeni* and *S. cerevisiae* initial co-inoculations. Fermentations involving *S. pombe* showed the lowest final concentrations in higher alcohols, while fermentations involving *L. thermotolerans* showed the highest final concentrations in esters.

4. Combination of acidification and de-acidification biological strategies

New winemaking trends start to combine microorganisms able to acidify with microorganisms able to de-acidify that allow reaching the microbial stability from a malic acid point of view just after alcoholic fermentation under difficult alcoholic fermentation situations (Table 4). These studies focus on warm viticulture areas where there is a need of acidification while the concentrations of malic acid are exceptionally low due to the high maturity of grapes. In those scenarios the grape juices show low malic acid concentration below 1 g/L or even close to 0 g/L, high pH close to 4 and high sugar concentrations over 250 g/L (Benito, 2020). Winemakers look for strategies to avoid performing classical malolactic fermentations in wines over 15% (v/v) of ethanol content that usually suffer from long alcoholic fermentation endings, pH over 4 and risk of residual sugar. In those specific situations, conventional *O. oeni* malolactic fermentations can deteriorate the final wine quality due to high final contents of acetic acid, diacetyl, and biogenic amines. For that reason, winemakers start to use *L. plantarum* or *S. pombe* to stabilize wine during alcoholic fermentation removing the lesser amounts of malic acid. However, although those processes achieve malic acid stabilization, they even reduce the low acidity of the initial grape juice. For that reason, *L. thermotolerans* compensates the loss of acidity generating lactic acid in combined inoculations.

4.1. L. thermotolerans and S. pombe combination: acidification and stability after alcoholic fermentation without bacteria use

The combination of *L. thermotolerans* with another powerful fermentative yeast, such as *S. pombe*, allows avoiding performing malolactic fermentation in wines with high ethanol concentrations and high pH levels from warm viticulture areas or overripe grapes. In this combination, *L. thermotolerans* increases the acidity, generating lactic acid, while *S. pombe* consumes the unstable malic acid and ends the alcoholic fermentation (Benito, 2020). The result is a wine stabilized from a malic acid point of view and acidified during alcoholic fermentation. Therefore, the wine does not need to undergo malolactic fermentation after alcoholic fermentation.

There are nine scientific articles that studied the biotechnology showing similar conclusions (Benito, 2020; Gardoni et al., 2021). However, four studies do not include a control that performed classical malolactic fermentation being impossible to compare both processes. The studies report the new alternative to be slightly slower than the *S. cerevisiae* alcoholic fermentations control but to be always faster than the classic sequential fermentations between *S. cerevisiae* and *O. oeni* in about 17 to 25 days. The studies always report higher increases in final values than the classical methodology in lactic acid, glycerol and color that vary from 0.5 to 2.5 g/L, from 0.27 to 0.71 g/L and from 17 to 26% respectively. Although the differences significantly vary depending on the study, the studies always report lower final concentrations than the classical methodology in acetic acid, ethyl acetate, diacetyl, urea, and biogenic amines.

4.2. *L. thermotolerans, O. oeni and S. cerevisiae combination: acidification and stability after alcoholic fermentation*

This novel alternative option is based on using *L. thermotolerans* to increase acidity while *O. oeni* metabolizes malic acid into lactic acid during the beginning of alcoholic fermentation (Urbina et al., 2021; Snyder et al., 2021). The main objective of this combination is reducing the production hours in winemaking while increasing wine acidity. The main disadvantage of this combination is the heterofermentative character of *O. oeni* that may consume sugar during long alcoholic fermentations endings increasing the final concentration in acetic acid and Diacetyl. This combination requires a late inoculation of a *Saccharomyces* strain able to inhibit a great development of *O. oeni* and able to finish the alcoholic fermentation without entering in sluggish or stopping. There are only nowadays two scientific articles that deal with the topic (Urbina et al., 2021; Snyder et al., 2021). They show that if the alcoholic fermentation develops properly in an appropriate time no undesirable effects take place. The studies report a significant increase in final color intensity of about 20% when compared to the classical sequential fermentation between *S. cerevisiae* and *O. oeni*.

4.3. *L. thermotolerans, L. plantarum and S. cerevisiae combination: acidification and stability after alcoholic fermentation*

This novel alternative option is based on using *L. thermotolerans* to increase acidity while *L. plantarum* metabolizes malic acid into lactic acid during the beginning of alcoholic fermentation (Urbina et al., 2021). The main objective of this combination is avoiding the heterofermentative character of *O. oeni* that may consume sugar during long alcoholic fermentations of grapes juices with high sugar content. However, this combination needs a late inoculation of *Saccharomyces* to ensure a proper alcoholic fermentation ending in wines of elevated potential ethanol content.

There is only one study that reports results regarding this new biotechnology (Urbina et al., 2021). The article shows bigger final values than the conventional method based on *S. cerevisiae* and *O. oeni* sequential fermentations in lactic acid, color intensity, ethyl lactate, 2-phenyl ethyl acetate and glycerol in about 2 g/L, 20%, 50%, 35% and 0.5 g/L correspondingly. Furthermore, the novel choice produces wines with smaller final contents than the conventional methodology in acetic acid, diacetyl, ethyl acetate, pH, ethanol, and 1-propanol in 0.1 g/L, 70%, 20%, 0.2, 0.3% (v/v) and 30% correspondingly.

5. Conclusion

Modern winemaking bases the nowadays microbiological management of wine acidity on acidification to improve the sensorial perception in warm viticultural regions and de-acidification to improve the sensorial perception of wines from Atlantic areas or to achieve microbial stability in red wines before bottling.

The main microbiological option to acidify wines that suffer from lack of acidity is *L. thermotolerans* that can increase lactic acid in several grams per liter and to reduce the pH in several decimal units. Other options are some strains of *S. cerevisiae* able to produce small concentrations of malic, lactic, or succinic acid. *S. bacillaris* can produce α-ketoglutaric acid and pyruvic acid and *C. stellata* is able to produce significant concentrations of succinic acid.

The classical option to de-acidify wine is lactic bacteria of species *O. oeni* that can metabolize malic acid into lactic acid. During the last years, new options based on other lactic bacteria, such as *L. plantarum* or yeasts such as *S. pombe* that can metabolize malic acid into ethanol, are reliable in specific situations related to high potential ethanol concentrations and high pH and risk of residual sugars.

New trends combine acidifier microorganisms and de-acidifier microorganisms during alcoholic fermentation to correct acidity and to stabilize wines from a microbiological point of view just after alcoholic fermentation. These trends are of great interest in warm viticulture areas, nevertheless the number of scientific studies is nowadays limited, and it should increase in the future.

Genetically modified yeasts are very efficient acidifying or de-acidifying wines. However, most countries legislate the use of GMOs.

Future studies must compare the different biological acidification and de-acidification options to determinate which one is the most appropriated deepening on each situation.

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