

**REVIEW ARTICLE**

Wine acidification methods: a review

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ABSTRACT

Global warming is directly linked to a lower concentration in organic acids in grape berries, leading to higher pHs in wine. Because of this lack of acidity, many important factors are impacted, as wine acidity and pH play a crucial role in various equilibriums. Indeed, the lower acidity and the higher pH modify the parameters of wine, such as free and molecular sulfur dioxide availability, colour and sensory aspects. Therefore, it is an ongoing challenge for winemakers to deal with wine acidification and thus preserve wine physico-chemical properties and prevent early spoilage due to microbiological instability induced by high pH. Different acidification methods are allowed by the OIV, chemical acidification being one the most common, followed by physical acidification and microbiological acidification. This review examines these three methods of acidification. The first part details chemical acidification and gives a complete description of various organic acids used in winemaking, and their different properties and regulations; the second part focuses on physical acidification, such as cation exchange resins and electrodialysis; and the last part briefly reviews the novelty of microbiological acidification in wine.

KEYWORDS: wine, acidification, sulphur dioxide, climate change, organic acids, electrodialysis



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INTRODUCTION

Over the last few years, climate change and rising temperatures have had an undeniably significant effect on the grape and wine industry. Harvest dates have considerably advanced in the last decades. Data from Johannisberg (Rheingau, Germany) have shown that, on average, harvest now takes place two to three weeks earlier than the harvest from the late 18th and the early 20th centuries (Stock *et al.*, 2005); this is negatively affecting wine quality.

Because of rising temperatures, berries are maturing much more quickly, with higher sugar levels and lower organic acid concentrations. The trend of higher pH values leads to an increasing risk of microbial infection and generally unstable wines. pH directly impacts the efficiency of sulphur dioxide (SO₂), which is mainly found in wine in two forms: free SO₂ and bound SO₂. Free SO₂ exists in three forms: molecular SO₂, bisulphite (HSO₃⁻) and sulphite (SO₃²⁻); the ratio of these three forms depends on the wine pH. Bisulphite is the main form of free SO₂ (94 to 99 %) found in wine. Bisulphite binds with acetaldehyde, which is a by-product of the oxidation of ethanol and is desirable in sherry wines, but considered a defect in table wines. Sulphites, on the other hand, are present in very small quantities (0.01 to 0.12 %) and help prevent direct oxidation by removing free oxygen and reactive oxygen forms. Sulphites also deactivate the polyphenoloxidase enzyme, which is responsible for enzymatic browning. Molecular SO₂ comprises 0.5 to 6 % of total free SO₂ and has the effect of an anti-septic. The typical levels of molecular SO₂ needed to achieve microbial stability are between 0.6 and 0.8 mg/L (Ribéreau-Gayon *et al.*, 2012). Higher pH leads to the lower antibacterial activity of SO₂, meaning that higher amounts are needed to successfully protect wines from early spoilage and organoleptic alterations (Lafon-Lafourcade and Peynaud, 1970). Today, to attain the same microbial stability levels in wine (pH 4, 15 % alcohol per volume stored at 15 °C), free SO₂ levels would need to be between 95 and 126 mg/L. These values imply that total SO₂ in wine can exceed legal limits set by the International Organization of Vine and Wine (OIV Resolution OENO 09/1998).

These characteristics (i.e., high pH and SO₂), make it very challenging to control bacterial growth. High pH (> 3.5) and low molecular SO₂ encourage the formation of undesirable volatile compounds, such as mousey off-flavours, via lactic acid bacteria (Costello *et al.*, 1993; Grbin *et al.*, 1996). Mousey off flavours are associated with the smell of poorly-maintained rodent cages. However, some of the descriptors can vary; for example, popcorn and wet cloth. Bacteria, such as *Lactobacillus sp.* and *Pediococcus sp.*, are known to grow in these conditions and are responsible for the production of undesirable volatile compounds; they are thus considered to induce spoilage in wine.

An optimal pH level is not only necessary for wine stability and microbiological equilibrium, but is also directly linked to wine colour and sensory properties. Climate change has been proved to affect anthocyanins, which has an impact on wine

colour. Higher temperatures in vineyards (30 °C and higher) lead to lower levels of anthocyanins (Buttrose *et al.*, 1971; Spayd *et al.*, 2002; Tarara *et al.*, 2008). Wine pH is known to play an important role in the colour of wine, because it affects the equilibrium between different forms of anthocyanins (Brouillard and Delaporte, 1977). It can also condition certain polymerisation reactions or the condensation of red wine pigments (Gil *et al.*, 2012).

Furthermore, wine pH greatly impacts wine sensory perception. Total acidity and pH are often associated with the freshness of wine. Wine acidity, and more importantly wine pH, play an important role in the preservation of wine aroma and flavour. Wine pH can also play a major role in the sensation of sourness and astringency (Sowalsky and Noble, 1998).

Dealing with Wine acidity is thus a very important challenge, especially in the face of climate change. Different methods have been applied (Table 1), the main one being the acidification of must and wine, which consists of increasing total acidity and thus decreasing pH (OIV, 2017). All acidification practices are performed so that the initial acidity is not increased by more than 54 meq/L, equivalent to 4 g/L of tartaric acid (OIV, 2017). In Europe, the maximum acidity permitted for must is 1.5 g/L and 2.5 g/L for wine in tartaric acid equivalents (Regulation EU 1308, 2013).

TABLE 1. Recommended techniques for must and wine acidification¹.

Technique	Uses
Chemical acidification	Must & wine
Microbiological acidification	Must
Cation exchange resin	Must & wine
Electromembrane treatments	Must & wine

¹ (OIV, 2017)

This review thus focuses on the chemical, physical and microbiological acidification of must and wine.

CHEMICAL ACIDIFICATION

Chemical acidification is a process used to adjust acidity levels in wine and is the most popular method for pH reduction in winemaking. The most commonly used acid in this process is tartaric acid, which is naturally found in grapes and plays a significant role in the taste and structure of the wine. However, in addition to tartaric acid, we will be discussing other possible and important acidifying agents, such as malic, citric, lactic and fumaric acid, which are used by winemakers to ensure the highest quality of their product.

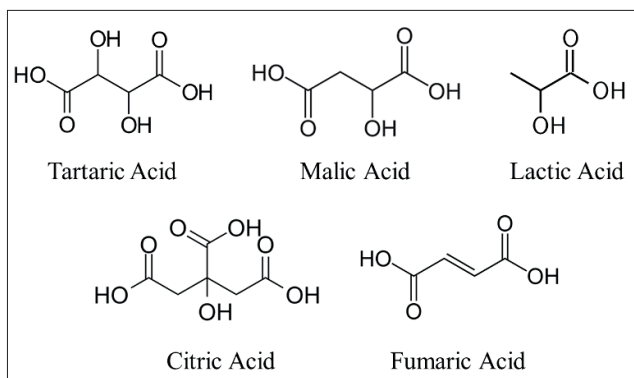


FIGURE 1. Organic acids.

1. Tartaric acid

Dihydroxybutanedioic acid (Figure 1), commonly known as tartaric acid (TA) is a diprotic acid that has two carboxylic groups whose pKas (at 25 °C) are 2.98 and 4.34 (Figure 2) (Lide *et al.*, 2005). It is commonly used as an acidulant for when small acidity corrections are needed. TA has a sour taste and gives food a sharp, tart flavour. It is often added as an antioxidant (E334) to products like carbonated beverages, fruit jellies and effervescent tablets. Out of all the organic acids known to have an effect on microorganisms, TA is the least antimicrobial and inhibits less microbial growth (Gurtler and Mai, 2014).

TA is the strongest acid and naturally present in grapes in amounts of between 5 and 10 g/L. The isomer found in grapes is the L-(+)-TA form and is synthesised from glucose (Saito and Kasai, 1978). TA was the first acid to be permitted by the OIV for acidification purposes. TA is mainly used in wine post-fermentation, but can also be used in must when the pH is higher than 3.5. When added to wine or must, tartaric acid dissociates, forming hydronium ions, which

increase total acidity and bitartrate. At a pH of 3.5, 23.4 % of TA is present in its undisassociated form (H_2T), 67.5 % in its bitartrate form (HT^-) and 9.1 % in its totally disassociated form (T^{2-}). The bitartrate (HT^-) often reacts with potassium to form potassium bitartrate, also known as potassium hydrogen tartrate (KHT). KHT has a very low solubility in water and can crystallise, a phenomenon often referred to as tartaric precipitation. TA precipitation is a well-known problem and can occur unpredictably. KHT crystals are harmless, but are considered unappealing by the consumer. To help prevent this phenomenon, winemakers remove the excess salt by cooling the wine at $-4\text{ }^\circ\text{C}$ over several days to induce KHT precipitation prior to bottling. A limitation of this method, known as cold stabilization (Maujean, 1994), is that it is not possible to have full control over the remaining KHT concentration. This problem has led to the development of other KHT removal techniques, like ion exchange resins (Mourgues, 1993) and electro dialysis (Escudier *et al.*, 1993) described hereafter.

The instability of calcium tartrate (CaT) in wine is another issue linked to TA. Similar to potassium bitartrate (KHT), calcium can react with the bitartrate ion (HT^-) to form calcium tartrate. The chances of precipitation occurring increase when calcium levels reach 60 mg/L in red wine and 80 mg/L in white wine (Ribéreau-Gayon *et al.*, 2012). While less common than potassium bitartrate instability, calcium tartrate instability is more problematic, as it is not as easily prevented by traditional stabilisation methods, such as cold treatment stabilisation (Maujean *et al.*, 1984). One approach to preventing the precipitation of CaT crystals is the use of electro dialysis in the same way it is used for KHT removal.

Thus, while tartaric acid plays a critical role in shaping the taste and quality of wine, its interaction with other compounds in wine, particularly calcium and potassium, presents challenges that require careful management in order

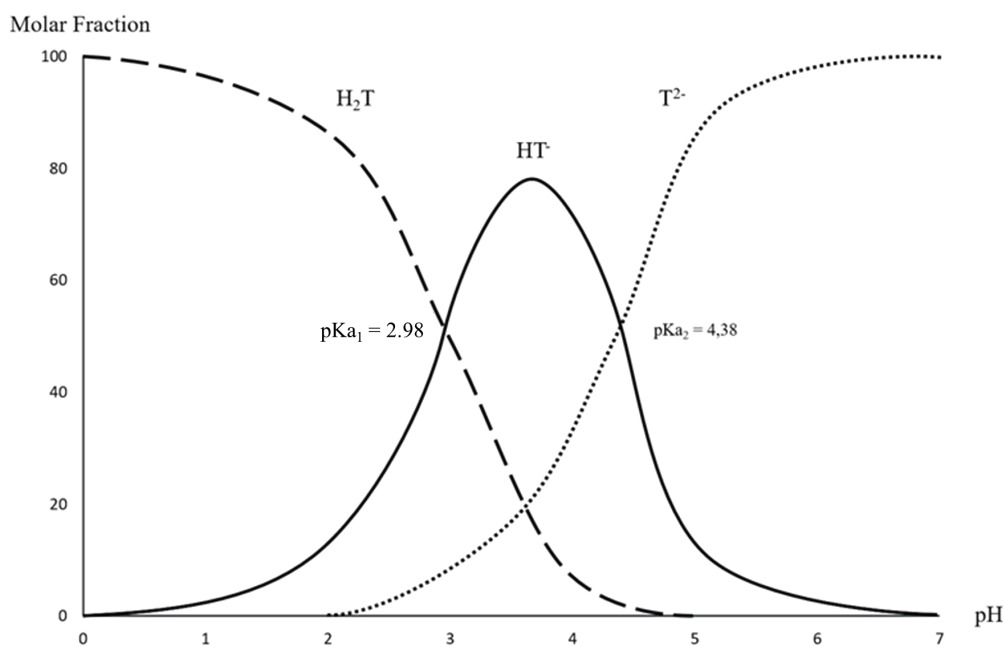


FIGURE 2. Dissociation curve of tartaric acid.

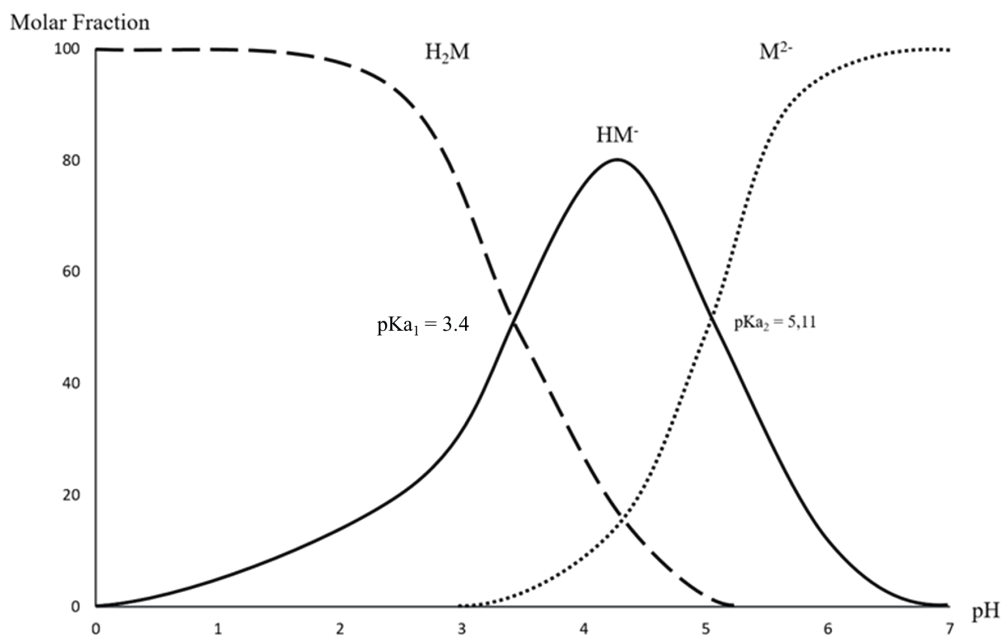


FIGURE 3. Dissociation curve of malic acid.

to maintain the stability and aesthetic appeal of the final product. Moreover, when added in high amounts, TA may induce a bitter taste and increase the wine's astringency, and it is responsible for the tart taste.

2. Malic acid

Malic acid (MA) (Figure 1), or 2-hydroxybutanedioic acid, is a diprotic acid, whose pKas at 25 °C are 3.40 and 5.11 (Figure 3) (Lide *et al.*, 2005). At a pH of 3.5, 47 % of the total concentration of MA is present in its undisassociated form (H₂M), 51.7 % in the disassociated form (HM⁻) and only 1.2 % in the totally disassociated form (M²⁻) (Usseglio-Tomasset, 1985).

MA is the most widespread fruit acid. It has a flavour reminiscent of apple and sourness and contributes to the sour taste in unripe apples. It is mainly used as an acidulant (E296) in apple-containing products, such as cider, because of its taste. MA has a direct impact on the organoleptic properties of wines; it increases the sourness and freshness of wines while reducing the pH (Amerine and Ough, 1970; Carvalho *et al.*, 2001). Before veraison, MA concentration can be found in quantities as high as 25 g/L. By harvest, the malic acid concentration in berries decreases sharply to between 1 and 6.5 g/L (Ribéreau-Gayon *et al.*, 2012; Ruffner *et al.*, 1982). This reduction in MA concentration is due to the respiration process during which malic acid is metabolised. In warmer climates, the loss of malic acid through respiration is more pronounced. Malic acid concentration levels are directly linked to maturity and temperature (Buttrose *et al.*, 1971; Kliewer, 1971).

During winemaking, L-malic acid undergoes malolactic fermentation (MLF) and is transformed to lactic acid by the action of lactic acid bacteria (LAB). MLF occurs often in red wines and seldomly in white wines. The removal of malic acid via MLF usually induces an average increase in pH of 0.1 to 0.3 (Margalit, 1997). When added prior to fermentation,

a racemic mixture of malic acid is used, of which most of the L-malic acid will be removed by MLF and, because of its resistance to microbial attack, D-malic acid will remain, maintaining the wine at a low pH.

Adding malic acid to finished wine could be useful for red wines from warmer regions, where hot temperatures lead to lower concentrations of malic acid. This acid, however, makes a favourable substrate for the growth of LAB. Due to this characteristic, the use of MA for acidification should not be contemplated for adjusting pH or total acidity; rather, this substance can be considered for enabling LAB to carry out their own metabolic pathways, boosting the aromatic and gustatory complexity of the final product. However, because MA addition can initiate a second MLF, it can lead to the wine becoming cloudy and slightly sparkling.

3. Lactic acid

Lactic acid (LA) (Figure 1), 2-hydroxypropanoic acid, is a monoprotic acid, whose pKa at 25 °C is 3.86 (Figure 4) (Lide *et al.*, 2005), meaning that it is a weaker acid than TA and MA. In the food industry, LA is used as a preservative, a curing agent and a flavouring agent (E270).

LAB synthesises LA from malic acid via MLF. Because LA is less acidic than MA, MLF decreases the total acidity and, as mentioned earlier, induces an average increase in pH of 0.1 to 0.3 (Margalit, 1997). MLF can be avoided in order to maintain the higher acidity of wines from warm areas, for example. However, allowing wines to undergo MLF before bottling increases stability. LA helps increase the acidity of wine by prolonging the sensory perception of sourness (Carvalho *et al.*, 2001).

There are three advantages to adding lactic acid to wine: it can be added just before bottling without risk of precipitation; it produces a rounder and smoother mouthfeel than malic acid; and it gives the wine a sweet taste.

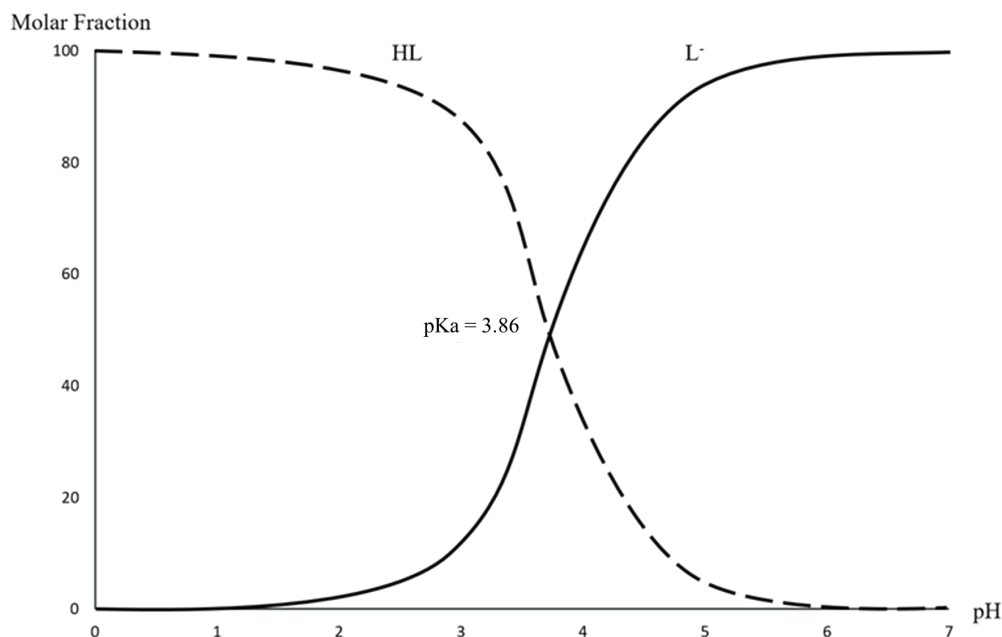


FIGURE 4. Dissociation curve of lactic acid.

Being the weakest acid permitted by the OIV for wine acidification purposes, LA must be added in higher quantities to achieve the same pH decrease as malic or tartaric acids.

4. Citric acid

The 2-hydroxypropane-1,2,3-tricarboxylic acid, also known as citric acid (CA) (Figure 1), is a triprotic acid, whose pKa at 25 °C are 3.13, 4.76 and 6.40 (Figure 5) (Lide *et al.*, 2005). CA is a naturally occurring acid in many fruits and vegetables, especially citrus-like fruits. It is a weak acid often used as a natural preservative in many foods (E330), or to add a sour taste to drinks or food. CA is a very important metabolite in the Krebs cycle (Ribéreau-Gayon *et al.*, 2012).

In wine, CA is commonly found in quantities within the range of 0.1 to 0.7 g/L. Wine bacteria use CA for metabolism. It is first degraded into acetic acid and pyruvic acid (Shimazu *et al.*, 1985), and the pyruvic acid is then metabolised forming lactic acid and a fraction of diacetyl, acetoin and 2,3-butanediol. An increase in CA concentration is correlated with an increase in diacetyl concentration. CA can help antioxidants by chelating metal ions and thus helping prevent browning (Jiang and Fu, 1998).

CA addition in must is only permitted by the OIV to prevent iron hazes; the remaining residue should not exceed 1 g/L (Zoecklein, 2012). When added to wine, CA enhances the taste of many white wines, while contributing to a citric character. The main disadvantage of this acid is its microbial instability, as it increases the growth of unwanted microorganisms in the wine. LAB can metabolise citric acid to form acetic acid and diacetyl (Capozzi *et al.*, 2021; Sumby *et al.*, 2019).

5. Fumaric acid

Fumaric acid (Figure 1), (2E)-but-2-enedioic acid, is a diprotic acid whose pKas at 25 °C are 3.02 and 4.38 (Figure 6) (Lide *et al.*, 2005). Fumaric acid (FA) originally derived

its name from the plant, *Fumaria officinalis*, from which this organic acid was isolated for the first time (Roa Engel *et al.*, 2008). FA is an intermediary metabolite in the citric acid cycle, being converted into L-malic acid through the action of the fumarase enzyme (Akiba *et al.*, 1984; Pines *et al.*, 1996). Due to its low molecular weight, 116.073 g/mol, FA has a greater buffering capacity than other food grade acids at a pH of around 3.0.

FA is the cheapest of the food-grade acids and is non-toxic; it has been used as an antibacterial agent and acidulant in the food and juice industry since 1946 (Das *et al.*, 2016; Straathof and van Gulik, 2012). It is classified as a food additive other than sweeteners and colouring with the E number E297 under the European Union Commission Regulations N° 1129/2011. It has a fruit-like flavour.

Because of its dicarboxylic group, FA has a high acidifying power. It is naturally present in small quantities in both the red and white berries of grapes (from 5.11 to 10.69 mg/L) (Eyduran *et al.*, 2015; García Romero *et al.*, 1993). In addition to pH reduction, FA can be used for its antibacterial properties, which has been demonstrated in different food and drink, such as non-heat processed vegetables, apple cider inoculated with *E.Coli* and vacuum-packaged ground beef (Comes and Beelman, 2002; Lu *et al.*, 2011; Podolak *et al.*, 1996). FA also has antifungal properties (Akao and Kuroda, 1991).

Due to its action of lowering pH, FA also limits bacterial development and growth (Gurtler and Mai, 2014). When added before MLF, it not only decreases pH but also inhibits LAB activities by affecting the biosynthesis of pyrimidines (Cofran and Meyer, 1970; Pilone *et al.*, 1974; Silver and Leighton, 1982); this was observed when FA was added in amounts of between 0.4 and 1.5 g/L after alcoholic fermentation (Bauer and Dicks, 2004). More recently, FA has been shown to completely inhibit the growth of *O. Oeni*

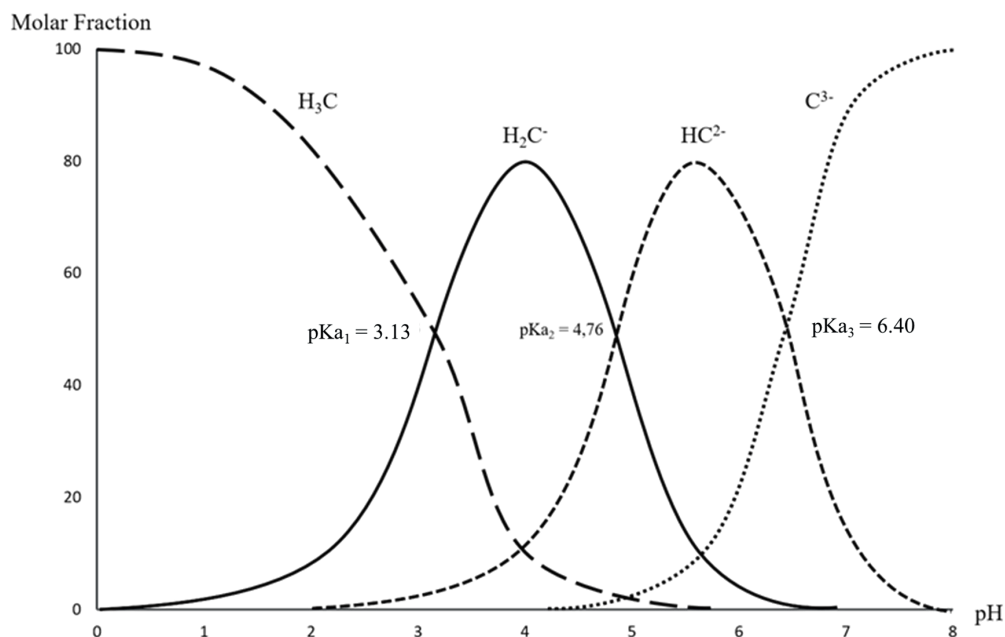


FIGURE 5. Dissociation curve of citric acid.

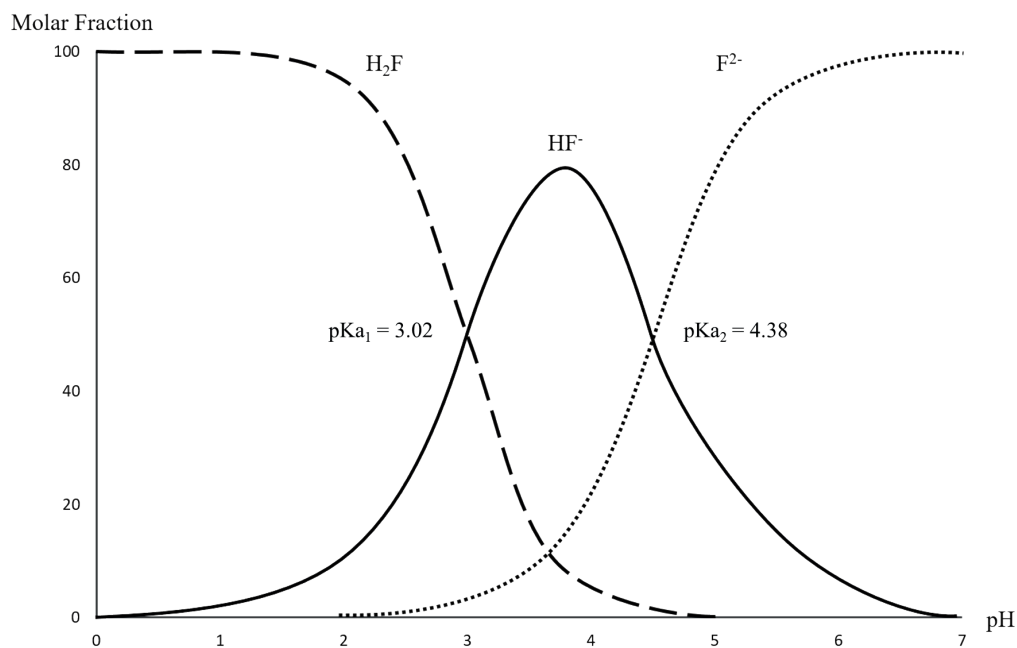


FIGURE 6. Dissociation curve of fumaric acid.

when in amounts of 0.3 to 0.6 g/L at a pH of 3.3 (Morata *et al.*, 2019). A study of the addition of FA to different varieties has shown that it has a long-lasting effect on *O. Oeni* and could thus be a more permanent solution than classical SO₂ addition (Morata *et al.*, 2023).

The FA threshold in white wine has been described by Ough (1963) as being 1 g/L, which is the lowest threshold compared to tartaric and citric acid. The sensory threshold of FA in red wine is around 1387 mg/L (Gancel *et al.*, 2022). FA is perceived as being more sour than citric acid and malic acid

(Buechsenstein and Ough, 1979). A recent study has shown that in concentrations of 0.6 g/L FA improves the perception of acidity and body in wine (Morata *et al.*, 2019).

However, FA can be challenging to work with since it is very difficult to dissolve in water, as it has a solubility of less than 10 g/L (Yang *et al.*, 2011). FA solubility in wine is around 15 g/L at 25 °C, making it one of the least soluble organic acids in wine in comparison with tartaric, malic and citric acid (respectively 1049.3 g/L, 1047.0 g/L, 1079.7 g/L) (Gancel *et al.*, 2022).

TABLE 2. Summary of organic acids and their potential for wine acidification use.

	M _w g/mol	pKa at 25°C	Solubility in SB - CS wine at 25°C g/L	Concentration required for lowering pH by 0.1 in SB - CS wine* g/L	Thresholds in SB wine g/L	Thresholds in CS wine g/L
Tartaric acid	150.087	2.98 – 4.34 ¹	1049.3 – 922.3 ²	0.34 – 0.31 ²	1.6 ²	1.1 ²
Malic acid	134.087	3.40 – 5.11 ¹	1047.0 – 932.3 ²	0.51 – 0.391 ²	1.3 ²	0.8 ²
Lactic acid	90.080	3.86 ¹	–	0.44 – 0.45 ²	1.6 ²	1.9 ²
Citric acid	192.124	3.13 – 4.76 – 6.40 ¹	1079.7 – 1056.7 ²	0.47 – 0.53 ²	2.3 ²	1.3 ²
Fumaric acid	116.073	3.02 – 4.38 ¹	14.9 – 9.3 ²	0.22 – 0.20 ²	1.9 ²	1.3 ²

SB = Sauvignon blanc, CS = Cabernet-Sauvignon

* Initial wine pH: SB = 3.34, CS = 3.61

¹ Lide *et al.*, 2005. ² Gancel *et al.*, 2022

FA in concentrations of up to 3 g/L is already permitted for wine acidification purposes in countries like the USA, Canada and Chile (Smith and Hong-Shum, 2008). The OIV has adopted a new resolution for the authorisation of FA addition in wine (300 to 600 mg/L) in order to prevent MLF (OIV-OENO 581A-2021). It is yet to be permitted for acidification purposes in OIV member countries.

PHYSICAL ACIDIFICATION

As seen in the previous section, the addition of organic acids to wine to reduce its pH is now a common practice. However, it can be challenging to control this pH reduction, as well as to overcome the adverse effects and difficulties of working with organic acids, such as tartaric acid precipitation, the risk of a second MLF if malic acid is used, or the difficulty in dissolving fumaric acid. Many physical acidification methods have been developed that can be used as alternatives to some traditional oenological practices.

1. Ion exchange resins

The use of cation exchange resins is one available option for concurrently lowering pH, decrease cation concentration and restrict the formation of tartrate salts (Esau and Amerine, 1966; Mira *et al.*, 2006).

Acidification using ion exchange resins is a process consisting of treating wine through a polymerised insoluble granular medium in order to exchange positive or negative ions. This medium is composed of a polymeric matrix of styrene and divinylbenzene, which is attached to different ionised functional groups (carboxylic acid or sulfonic acid for acidic resins and various types of amino groups for basic exchangers). Three different techniques involving ion exchange resins exist: i) the use of a cation exchange resin from which the protons (H⁺) replace the potassium ions in the wine, ii) the use of an anion exchange resin from which the tartrate ion is exchanged for hydroxyl (OH⁻), and iii)

the use of a mixture of both resins, with both potassium and tartrate ions being exchanged for H⁺ and OH⁻, such that the potassium bitartrate is swapped for water. As per the 442/2012 and 443/2012 resolution of the OIV and the 144/2013 EU regulation, only the first method using the cation exchange resin is authorised as an alternative method for acidifying wines.

In the cation exchange method, a powerful acid solution, such as sulfuric or hydrochloric acid, is used to activate the resin beads during standard wine treatment. The beads are then rinsed with soft water and loaded with the wine. As the wine passes through the column, the hydrogen ions (H⁺) on the resins are exchanged for the wine cations, such as potassium K⁺. This exchange leads to a reduction in cation concentration and wine pH, and also reduces the risk of tartaric acid precipitation via the formation of tartrate salts (KTH) (Benítez *et al.*, 2002; Ibeas *et al.*, 2015; Lasanta *et al.*, 2013; Mira *et al.*, 2006; Palacios *et al.*, 2001; Walker *et al.*, 2004).

The use of cation exchange resins in red winemaking is a useful tool for decreasing pH, as well as for improving tartaric stability. However, its use can be problematic due to the strong affinity of the styrene-divinylbenzene matrix for anthocyanins and polyphenols (Ibeas *et al.*, 2015; Lasanta *et al.*, 2013; Mira *et al.*, 2006). The cation exchange treatment of red wine has been found to result in a slight decrease in anthocyanins and tannins, as well as a decrease in colour hue and an increase in colour intensity, likely due to the decrease in wine pH. However, the treatment was found to have little impact on volatile compounds, and the wines processed using cation exchangers were perceived to have a higher overall quality in the sensory evaluation (Lasanta *et al.*, 2013).

Cation exchange resins have been studied for their potential to acidify white wines. In the study of Just-Borràs *et al.* (2022), the base wine used for sparkling white wine was treated with cation exchange resin, resulting in a significant

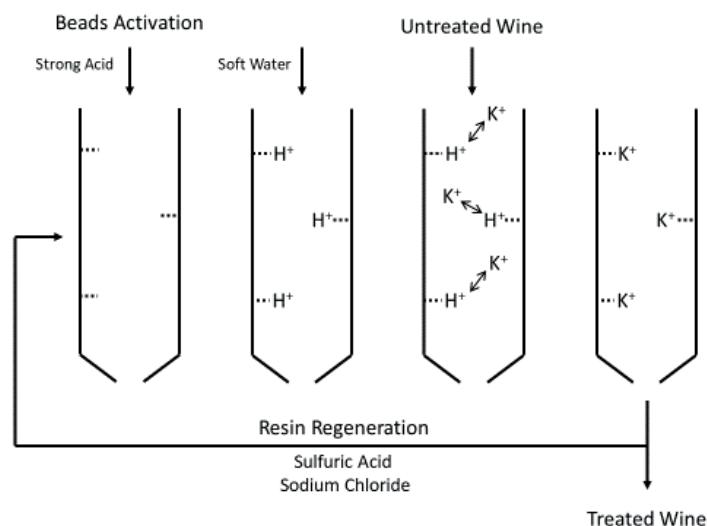


FIGURE 7. Diagram of cation exchanger resin conditions for wine acidification.

decrease in pH and an increase in titratable acidity. Cisilotto *et al.* (2019) focused on Chardonnay must, which was treated with cation exchange resin and fermented to produce wines with a lower pH of 3.15 (control) to 2.97. The results showed an improvement in oxidative stability of the treated wines and significant changes in the concentration of several volatile compounds, which could affect the wines’ sensory properties.

After the treatment, cation exchange resins can be regenerated using concentrated solutions of sulfuric acid and sodium chloride (Ribéreau-Gayon *et al.*, 2012). However, this process requires a significant amount of water, and the resulting effluents must be treated and recycled as special waste, meaning the technology is quite costly to the environment and unsustainable.

2. Electrodialysis

Electrodialysis is an electrochemical process that moves dissolved ions from one solution to another with the help of an electrical potential. The difference between electrodialysis and a classical exchange process is that electrodialysis uses ion-selective semi-permeable membranes to segregate ions based on their charge. There are three types of ion-exchanged membranes used in electrodialysis: anion-exchange membranes, cation-exchange membranes and bipolar membranes. Ion-exchange membranes are thin and dense, and they have insoluble walls composed of polymeric material permeable to ions, whereas cationic and anionic membranes are only permeable to cations and anions respectively (El Rayess and Mietton-Peuchot, 2016). Cationic membranes are composed of styrene-divinylbenzene copolymers with sulfonic functional groups (SO_3^-), while anionic membranes are made of styrene-divinylbenzene copolymers that are functionalised with quaternary ammonium (NR_4^+) (International Organization of Vine and Wine, 2017). Bipolar membranes comprise a cation exchange membrane laminated with an anion exchange membrane, through which cations or anions cannot permeate.

Electrodialysis was first used in oenology for wine tartaric stabilisation (Escudier *et al.*, 1993). The principle of electrodialysis for tartrate stabilisation is based on the migration of anions (such as TH^- and T^-) towards the positive electrodes (anode), while cations (K^+) are attracted to the negative electrode (cathode) under the influence of an electric field.

Electrodialysis with bipolar membranes (EBM) can precisely adjust the pH of must or wine with a precision of 0.05 units, irrespective of the initial pH, without altering the tartaric and malic acid contents and matrix wine compounds (such as alcohol, aromas and polyphenols). EBM can be used for both acidification and deacidification; when used for acidification, the bipolar membranes are coupled with cationic membranes (Figure 7). The process of wine acidification via EDM involves the circulation of wine within a membrane pack, allowing it to flow between cationic membranes and the cationic side of bipolar membranes. Water flows in an adjacent compartment. Upon application of an electric field, the potassium ions move towards the cathode, cross the cationic membranes and are extracted from the wine, becoming concentrated in the water, which is transformed into brine. Within the wine compartment, potassium is replaced by protons (H^+) formed at the bipolar membrane junction. Similarly, bitartrate ions tend to move towards the anode, but are prevented from crossing the cationic layer of the bipolar membrane and hence remain in the wine. As a result, the wine preserves bitartrate (and the conjugate bases of other organic acids) and is enriched with H^+ ions, leading to a decrease in pH and an increase in total acidity.

EBM treatments have been reported to have positive effects on wine composition, such as improving the balance of the acidic fraction, particularly in relation to tartaric acid addition. Wines treated with EBM are perceived to be fresher and lighter on the palate compared to untreated products. In addition, treated wines do not exhibit the harsh mouthfeel that is commonly associated with the addition of tartaric acid (Moutounet *et al.*, 2005). Other classical parameters, such as

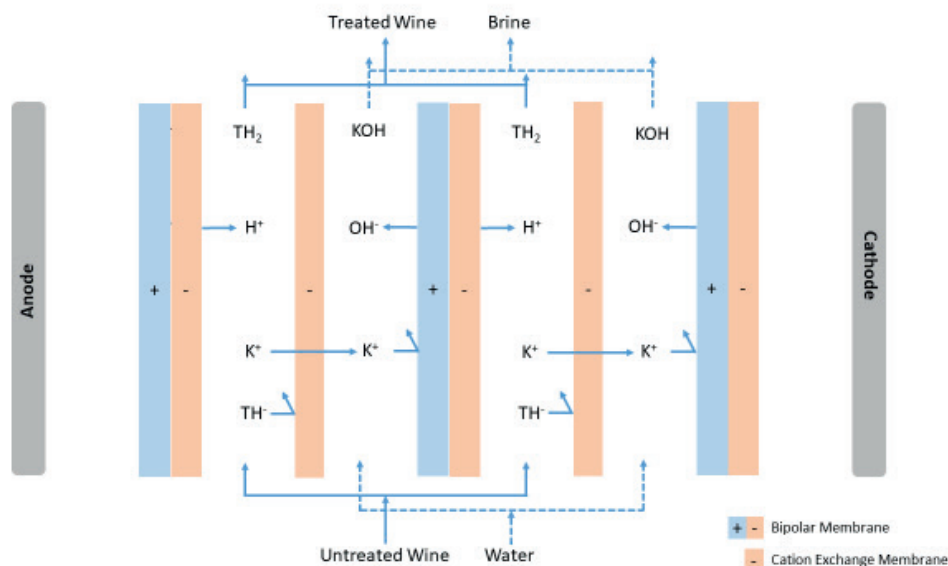


FIGURE 8. Diagram of membrane assembly and operating conditions for wine acidification by electromembrane techniques.

must sugars, alcohol content and polyphenolic compounds, are not impacted by EBM treatment (Granès *et al.*, 2008). However, this technique is associated with significant water consumption (Halama *et al.*, 2015).

MICROBIOLOGICAL ACIDIFICATION

Microbiological approaches can be used to deal with low acidity in wine. The use of *S. cerevisiae* strains is one such option, as they can produce small quantities of malic, lactic or succinic acid. Another approach is the use of *L. thermotolerans*, which can increase lactic acid levels by several grams per liter and lower the pH by several decimal units. *S. bacillaris*, on the other hand, can generate α -ketoglutaric acid and pyruvic acid, while *C. stellata* is known for producing significant amounts of succinic acid.

1. *Saccharomyces*

Multiple biological methods can enhance wine acidity. The *Saccharomyces* genus is capable of augmenting acidity by elevating the concentration of malic or lactic acid, typically below 1 g/L for natural strains. Although genetically modified *Saccharomyces* strains can substantially affect acidity, its use is restricted by regulations in most countries (Benito, 2019; Maicas, 2021).

Some strains of *S. cerevisiae* can produce small amounts of malic acid (less than 1 g/L) during alcoholic fermentation (Su *et al.*, 2014; Yéramian *et al.*, 2007). Typically, *S. cerevisiae* strains that originate from warm regions maintain or even increase malic acid levels during fermentation, while those from cool regions tend to consume it. Malic acid production is also influenced by various fermentation conditions, including low temperature, high pH and low sugar. Additionally, higher concentrations of pyruvate and fumarate can lead to increased levels of malic acid at the end of fermentation. Recently, a selection of *S. cerevisiae* strains was able to produce up to 3 g/L of malic acid (Vion *et al.*, 2023). Conversely, some

selected strains of *Saccharomyces*, such as *S. paradoxus* and *S. pombe*, were able to degrade up to 40% of malic acid (Redzepovic *et al.*, 2003)

S. cerevisiae can generate small quantities of lactic acid during alcoholic fermentation (Dequin *et al.*, 1999). Nonetheless, the production of lactic acid by *S. cerevisiae* strains is very limited and has a negligible impact on overall total acidity, unless genetically modified strains are used.

The *Saccharomyces* genus has the potential to produce various organic acids, apart from malic, acetic and lactic acids, which can impact the total acidity of wine (Volschenk *et al.*, 2017). Succinic, α -ketoglutaric, pyruvic and fumaric acids are among the main organic acids released by the *Saccharomyces* genus during wine production. These acids are either intermediates or by-products of the TCA cycle or glycolysis (Chidi *et al.*, 2015).

2. *Lachancea thermotolerans*

Lachancea thermotolerans, formerly known as *Kluyveromyces thermotolerans*, is a non-*Saccharomyces* yeast that is commonly used in winemaking, especially for producing white wines. *L. thermotolerans* has gained attention in recent years as a potential tool for acidification in winemaking (Morata *et al.*, 2018; Porter *et al.*, 2019). Recent study highlighted the genetic diversity and adaptability of *L. thermotolerans*, suggesting that it could be used in a variety of winemaking contexts (Hranilovic *et al.*, 2017).

Early studies on wine acidification using *L. thermotolerans* have been carried out in the Mediterranean countries of Greece and Italy, which are wine regions affected by climate change (Benito, 2018b; Vicente *et al.*, 2021). A study on white wines from warm areas shows that the use of *L. thermotolerans* can effectively reduce pH while also improving the freshness and aromatic profiles of the wines (Vaquero *et al.*, 2020). Due to its ability to raise the final concentration of lactic acid in wine by several grams per liter,

significantly influencing its acidity and pH, *L. thermotolerans* has become the most trustworthy biological approach for acidifying wine (Vicente *et al.*, 2021). *L. thermotolerans* has an acidifying capacity of 1 to 9 g/L in lactic acid and 1 to 6 g/L in total acidity (Benito, 2018b). The L-lactic acid produced during alcoholic fermentation induces a reduction in pH, which can vary from 0.1 to 0.5 units. Various scientific studies have reported that *L. thermotolerans* produces lower amounts of acetic acid than control wines fermented with *S. cerevisiae*. Typically, the concentration of acetic acid generated by *L. thermotolerans* is below 0.2 g/L (Benito, 2018b; Vilela, 2018).

Although *L. thermotolerans* has a significant competitive advantage in that it increases wine acidity, there are also limitations to its use. *L. thermotolerans* has moderate fermentative power, being unable to ferment ethanol in concentrations higher than 9-10 % (v/v). Therefore, it needs to be combined with a more fermentative yeast. In one of the first studies on the subject, the use of *L. thermotolerans* and *S. cerevisiae* in co-fermentation was investigated as a strategy for enhancing acidity and improving the overall quality of wine (Gobbi *et al.*, 2013). To ensure the total fermentation of sugars in the must, genera such as *Saccharomyces* or *Schizosaccharomyces* can be used in combination with *L. thermotolerans* (Benito, 2020). Another study has found that *L. thermotolerans* can significantly influence the progression of MLF in both white and red wines, depending on the lactic acid production (Snyder *et al.*, 2021). *L. thermotolerans* is limited in its resistance to sulfur dioxide, usually only tolerating up to 20 mg/L of free sulfur dioxide, although some strains can tolerate up to 40 mg/L (Benito, 2018a, 2018b; Vicente *et al.*, 2021). Therefore, it is most suitable for grapes with good sanitary conditions and low requirements for SO₂ additions.

3. Other non-*Saccharomyces*

In addition to conventional yeasts, certain non-conventional strains can also be useful for acidifying wine. *Candida zemplinina*, formerly known as *Starmerella Bacillaris*, is a yeast that was initially discovered in Tokaj wine grapes and is often found in overripe or botrytised grapes (Ciani *et al.*, 2016).

C. Zemplinina is known for its ability to produce pyruvic acid in anaerobic conditions, as it prefers the glycerol-pyruvic pathway. Some strains of *C. Zemplinina* can generate up to 100 mg/L of pyruvic acid, while controls of *S. Cerevisiae* typically produce only around 20 mg/L. This suggests that under low-oxygen conditions *C. Zemplinina* generates various organic acids via the TCA cycle (Goold *et al.*, 2017; Magyar *et al.*, 2014).

Because of the associated reduction in pH, the use of *C. Zemplinina* can also influence wine colour. Additionally, pyruvic acid can react with anthocyanins to produce pyroanthocyaninVitisin, a highly stable colouring pigment resulting from wine oxidation (Romboli *et al.*, 2015). Moreover, *C. Zemplinina* is known for producing low levels of acetic acid. In a co-inoculation approach with *S. Cerevisiae*,

the resulting acetic acid content was 0.3 g/L lower than in the pure *S. Cerevisiae* control (Rantsiou *et al.*, 2012).

According to Englezos *et al.* (2018), when *C. Zemplinina* and *S. Cerevisiae* were used in combined fermentations of four different white grape musts, the resulting total acidity concentrations were higher than those obtained with the pure *S. Saccharomyces* controls. A sequential fermentation approach involving *C. Zemplinina* and *Saccharomyces Cerevisiae* resulted in the lowest alcohol content and the highest total acidity out of all the pure *Saccharomyces* fermentations, as well as sequential fermentations that used *L. thermotolerans*, *Torulaspota delbrueckii* and *Metschnikowia fructicola* (Castrillo *et al.*, 2019; Comitini *et al.*, 2011).

CONCLUSION

The most commonly used acidification technique, chemical acidification is well known and multiple options are available. Tartaric acid is widely used by winemakers to reduce pH and increase total acidity. However, the use of tartaric acid can lead to the precipitation of tartrate salt and, if added in too high concentrations, its use can result in imbalanced and harsh wine. When added to wine, malic acid increases the sourness and freshness of young wines; however, as malic acid is a good substrate for LAB, it should not be considered for pH adjustment. Lactic acid, on the other hand, produces rounder and smoother wines. Being the weakest acid permitted by the OIV for wine acidification, it needs to be added in higher quantities to obtain the same pH decrease as malic or tartaric acids. While citric acid enhances the taste of white wines, it is not microbiologically stable and its use entails the high risk of producing unwanted compounds such as acetic acid. Fumaric acid is a promising acidifying agent, as it has the greatest acidifying power of all the aforementioned organic acids. Its use is already permitted by the OIV for preventing MLF.

While the addition of organic acids is a common practice for reducing wine pH, physical acidification methods such as ion exchange resins and electro dialysis with bipolar membranes have been developed as alternatives to traditional oenological practices. Cation exchange resins are used to treat wine by exchanging ions to lower the pH, decrease cation concentration and restrict the formation of tartrate salts. However, the use of cation exchange resins can lead to some problems, such as reduced colour and tannins in red wines. Electro dialysis with a bipolar membrane, on the other hand, uses ion-selective semi-permeable membranes to segregate ions based on their charge. Electro dialysis has been shown to be efficient in reducing the pH of wine without impacting classical oenological parameters and in improving its organoleptic properties, making it fresher and lighter on the palate. However, both of these techniques have environmental impacts resulting from the regeneration of resins and high water consumption.

Finally, there are several microbiological approaches to dealing with low acidity in wine. *Saccharomyces* strains, such as *S. Cerevisiae*, can produce low quantities of organic

acids, including malic, lactic and succinic acid, while *Lachancea thermotolerans* is a non-*Saccharomyces* yeast that can increase lactic acid levels and lower pH by several decimal units. Other non-*Saccharomyces* strains, such as *Candida zemplinina*, can also produce organic acids that can impact the total acidity of wine. The choice of yeast strains for wine fermentation depends on various factors, including grape variety, climate, and desired wine style. Nonetheless, these microbiological approaches can provide winemakers with useful tools for controlling wine acidity and improving wine quality.

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