Chapter

Strategies to Improve the Freshness in Wines from Warm Areas

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Abstract

Trends in wine consumption are continuously changing. The latest in style is fresh wine with moderate alcohol content, high acidity, and primary aromas reminiscent of grapes, whereas certain fermentative volatiles may also influence the freshness of the wine. In addition, the effects of climate change on the composition of the grapes (high sugar content and low acidity) are adverse for the quality of the wine, also considering the microbiological stability. Herein, different strategies aiming at improving wine freshness are presented, and their performance in winemaking is discussed: among them, the addition of organic acids able to inhibit malolactic fermentation such as fumaric acid; the use of acidifying yeasts for alcoholic fermentation, such as *Lachancea thermotolerans*; and the selection of non-*Saccharomyces* yeasts with β-glucosidase activity in order to release terpene glycosides present in the must.

Keywords: wine freshness, organic acids, *Lachancea thermotolerans*, high acidity, climate change

1. Wine freshness

Wine freshness is an unspecific concept which includes parameters concerning acidity, aroma, alcohol content, and even color. It is also strongly correlated with fruit maturity, but the grapes from warm areas frequently have excessive sugar content that produces high alcoholic degree (>13%v/v) and low acidity (pH > 3.8). Wines produced with these grapes are normally winey, with unpleasant taste, scarce aromaticity mainly supported by higher alcohols with low levels of fruity esters, and a lack of sourness being usually less appreciated by the consumers. Moreover, these wines have a complex management during production and storage, because the low acidity produces higher sensibility to microbial spoilage and also because of the oxidation due to the low contents of molecular and free SO_2. For a better management and preservation, these wines are frequently dosed with tartaric acid,

thus favoring a more suitable management which counteracts both oxidative and spoilage processes but at the same time produces a typical excessive and over-perceived sourness.

2. Wine acidity

Types of acidity in wine: wine acidity is due to the organic acids from grapes, mainly tartaric, malic, and citric acids. There are also other acids that are formed during alcoholic and malolactic fermentations (e.g., acetic, fumaric, succinic, and lactic acids) [1]. Among the grape acids, the most stable and with higher repercussion in pH is the tartaric acid. Malic acid is metabolized by lactic acid bacteria (LAB) during malolactic fermentation (MLF), and its influence in pH is not too relevant. Moreover, potassium contents in soil affect the levels of tartaric acid in grape and must, forming potassium tartrates that are highly insoluble, especially in a polar condition. The precipitation of these salts, especially when ethanol level increases during the alcoholic fermentation, produces the reduction of tartaric acid contents with a subsequent pH augmentation.

Harvesting time is another strongly influential parameter; the sooner the grape is harvested, the higher the acidity. However, acidity decreases significantly when the collection is retarded beyond the normal harvesting conditions because the enologist looks out for the optimum skin phenolic ripeness and also a good seed maturity especially in red varieties. Some alternatives have been proposed to keep acidity using non-matured grapes; one interesting proposal is the use of unripe bunches coming from cluster thinning. These grapes are pressed obtaining a high-acidity must which later is cleaned of astringency and excessive vegetal taints by using adsorbents, such as activated charcoal or other products. The juice is mixed with the matured and well-balanced grape to both reduce the pH and improve the acidity [2].

3. Wine aroma: influence of both winemaking practices and biotechnologies in freshness

The lack of freshness in the aroma fraction is produced by a relative excess of higher alcohols regarding the fruity esters (especially acetate esters) and varietal aromatic compounds (terpenes, thiols, etc.). It makes the smell simple, warm, and flat. The approach to improve this shortcoming in wines is variable according to the type of wine. Wines made with terpenic varieties can be improved by physical techniques such as cryomacerations, to enhance the extraction of varietal aromatic compounds; however, significant differences in aroma cannot always be perceived when cold soak is used to make prefermentative macerations in red wines [3, 4]. Conversely, color extraction is usually increased when cold soak is used [4, 5]. On the other hand, the use of cold soak can influence the yeast populations that can be developed in wine. It has been observed that macerations at 14°C favor the development and growth of *Hanseniaspora uvarum* and *Candida zemplinina*, but when temperature is kept at 8°C, the predominant yeast specie is *Saccharomyces cerevisiae* (Sc) (**Figure 1a**) [6]. In addition, fermentation at low temperature, 15°C instead of 28°C, has also proven the formation of higher flowery aroma [7], thus enhancing the freshness. Finally, the optimization of harvesting time, delaying or alternatively advancing the time window to collect the grapes, can help to optimize the concentration of aromatic compounds.

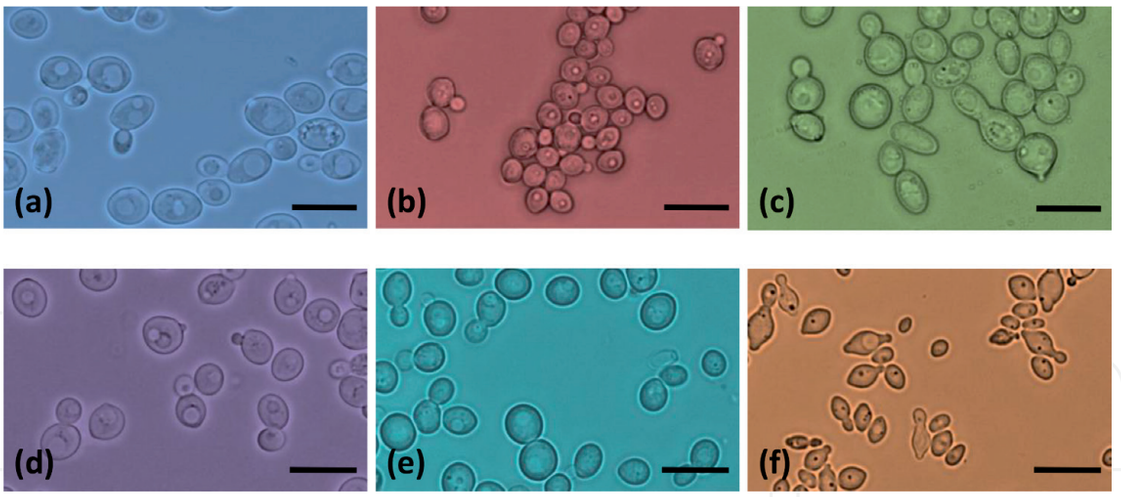

Figure 1.
Yeast morphology and asexual reproduction by budding. (a) Saccharomyces cerevisiae, (b) Torulaspora delbrueckii, (c) Wickerhamomyces anomalus. (d) Lachancea thermotolerans, (e) Metschnikowia pulcherrima, and (f) Kloeckera apiculata. Scale = 10 μm.

High contents of aldehydes have been related to oxidative off-flavors and reduced freshness in wines [8, 9]. Methional is an especially defective compound with a typical smell of boiled potato [9]. Moreover, other compounds like phenylacetaldehyde, with a typical honey smell, may increase the heaviness and sweetness, thereby reducing the wine freshness.

Conversely, several aromatic compounds have been described as enhancers of freshness; among them furaneol together with homofuraneol enhance red wine quality and fruitiness [10, 11] and ethyl 2-hydroxy-4-methylpentanoate contributes with the smell of fresh blackberries [12]. High contents of ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate have also been correlated with blackberry aromas, and ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate conferred redberry aromas [13]. Moreover, the formation of fruity (isoamyl acetate, ethyl butyrate, etc.) or floral esters (2-phenylethyl acetate) increases the sensation of fresh complexity in white wines, especially when accompanied by suitable acidity.

In the last years, the use of non-*Saccharomyces* yeasts has been described as an efficient tool to promote the formation of esters during fermentation. Species such as *Torulaspora delbrueckii* (**Figure 1b**) in sequential and mixed fermentations have been used extensively to promote the formation of fruity esters like isoamyl and isobutyl acetate [14] and floral esters such as 2-phenylethyl acetate [15]. Moreover, 3-ethoxy propanol is formed during the fermentation with *T. delbrueckii*, and it is not found in *S. cerevisiae* single fermentations [15]. The presence of this later compound is correlated with blackcurrant nuances in red wines [16].

Wickerhamomyces anomalus (formerly *Pichia anomala*, **Figure 1c**) has also been described as a good producer of isoamyl acetate and, in general, several acetate and ethyl esters [17–21]. Sequential fermentations in which *W. anomalus* is involved have a more complex aroma and an increased fruitiness that can help to improve the freshness of wines from warm areas. Concerning terpenic varieties, the expression of several enzymes, β-D-glucosidase, α-L-arabinofuranosidase, α-L-rhamnosidase, and β-D-xylosidase, can help to hydrolyze bonded terpenes to free aglycones enhancing varietal aroma [21, 22]. Nevertheless, β-glucosidase activity can be detrimental for the processing of red grape varieties since this enzyme may degrade anthocyanins, affecting their stability and causing an unwanted color loss in red wines [23].

Fermentation of Syrah and Sauvignon blanc musts by *Lachancea thermotolerans* (Lt) increased the formation of 2-phenylethanol, phenethyl propionate, ethyl salicylate, methyl salicylate, and 3-methylthio-1-propanol [24]. The release of varietal terpenes and volatile thiols can be promoted by Lt because the β-D-glucosidase [25] and carbon-sulfur lyase [26] enzymatic activities have been described in some strains.

Metschnikowia pulcherrima (Mp) in single fermentations has shown an excessive production of ethyl acetate with negative sensory repercussion [27]. However, the mixed use of *M. pulcherrima* with *S. uvarum* diminishes the production of ethyl acetate simultaneously increasing the formation of 2-phenyl ethanol and 2-phenyl-ethyl acetate [27]. Furthermore, the use of mixed fermentations Mp/Sc produces high content of acetate esters and β-damascenone with reduced levels of C6 alcohols in ice wines made from Vidal blanc grape variety [28]. The β-glucosidase and β-lyase enzymatic activities have also been described in Mp [29, 30].

Most of the acetate esters can be enhanced by using *Hanseniaspora/Kloeckera* (**Figure 1f**) species [31, 32]. Several works with *H. vineae* in lab assays, but also industrial wines made in sequential fermentations with *S. cerevisiae*, have demonstrated a fruitier aroma with increased concentrations of both 2-phenylethyl acetate and ethyl acetate [31–33]. Moreover, the de novo formation of several aromatic compounds such as benzyl alcohol, benzaldehyde, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzyl alcohol in the absence of precursors has been verified during the fermentation with *H. vineae* [34, 35]. Concerning enzymes, it has been observed that β-glucosidase activity, which facilitates the release of free terpenes increasing the varietal aroma, can be 6.6-fold higher in *H. vineae* than *S. cerevisiae* [36].

4. Yeast to improve acidity

The fermentation with *Saccharomyces cerevisiae* (*S. cerevisiae*) strains usually does not affect significantly the pH values. Some strains are able to degrade (more commonly) or produce malic acid. However, concerning malic acid production, even when the amount can reach up to 1 g/L [37], this happens in musts with low acidity, where this amount is inefficient to produce a suitable pH reduction. Under enological conditions, most of the malic acid producing *S. cerevisiae* strains (4%) are able to release 0.3–1 g/L of malic acid. It should also be considered that in red wines and some white and rose wines, malic acid is usually transformed into lactic acid during the MLF. It makes the effect of this natural acidification under enological conditions even lower.

Acidification by the use of non-*Saccharomyces* yeasts: In the last years, the species *Lachancea thermotolerans* (formerly *Kluyveromyces thermotolerans*) has been used for acidification purposes in several beverages as wine [38, 39] and beer [40–42]. The maximum alcoholic degree reached by *L. thermotolerans* ranges 5–9% v/v during fermentation [38, 43, 44], so it must be used mixed or sequentially with *S. cerevisiae* or *S. pombe* to completely ferment the sugars [45]. *L. thermotolerans* has shown the ability to modify significantly the pH in grape musts even at industrial level in crushed red grape [39], decreasing the initial value in 0.5 pH units. Indeed, a higher decrease in pH may be obtained (up to 1 pH unit) when Lt is used for the malt fermentation in beer production, due to the lower buffer effect of this matrix [46]. The acidification produced by *L. thermotolerans* is a consequence of the metabolization of sugars to lactic acid. Moreover, metabolic properties, physiology, nutritional requirements, and enological applications of this yeast have been recently reviewed [45]. Some strains can produce extremely high concentrations of lactic acid, higher than 16 g/L [47]. This acidification is produced not only with some

sugar degradation and a slight effect in the alcoholic degree [39] but also with a low production of volatile acidity [38, 48]. What is especially interesting is that lactic acid is stable under enological conditions; it does not degrade during processing or storage, so it can affect permanently the pH values. Moreover, in some situations, a synergistic effect in the production of lactic acid when *L. thermotolerans* is used in co-inoculation together with *Oenococcus oeni* has been observed [39].

Most of the acidification occurs at the beginning, during the first 3–4 days of fermentation. This facilitates the production of lactic acid even under enological conditions because it is just at the beginning of the fermentation when the wild population is lower and the implantation of *L. thermotolerans* can succeed (**Figure 2**). The typical industrial acidification with *L. thermotolerans* includes a subsequent inoculation with *S. cerevisiae* to completely ferment the sugars in a sequential fermentation (**Figure 2**). This is necessary because the fermentative power of *L. thermotolerans* is always lower than 9% v/v.

In warm areas, the acidification by *L. thermotolerans* may increase the microbial stability of wines, especially during barrel aging, and it also increases the effectivity of sulfur dioxide because the contents of free and molecular SO_2 are much higher at pH 3.5 than at 3.9. This pH reduction is feasible under enological conditions as it was previously seen.

Yeasts can influence wine color by affecting the production of stable pigments, such as pyranoanthocyanins or polymeric pigments. In addition, yeast strains with low ability to adsorb grape anthocyanins in their cell walls are suitable to decrease color loss during fermentation, and, finally, yeasts can affect color stability and intensity by pH reduction [49]. The effect of *L. thermotolerans* on color stability and the formation of stable pigments have been studied recently [50]. However, this study revealed that a low effect in the formation of these pigments can be promoted with the *S. cerevisiae* when it is used in either mixed or sequential fermentation to completely ferment the sugars. Concerning color stability, acidity is a main parameter to protect anthocyanins in wine and to increase color intensity by a

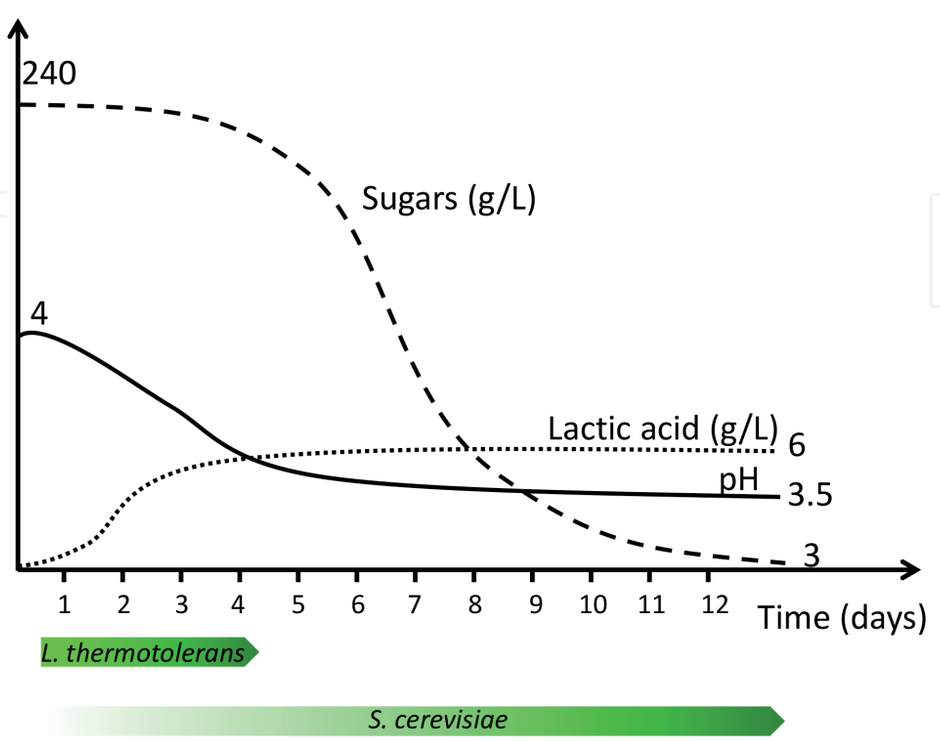

Figure 2.
Evolution of the pH, lactic acid level, and sugar content during the sequential fermentation with L. thermotolerans and S. cerevisiae.

hyperchromic effect. Indirectly, as pH affects the levels of both molecular and free sulfur dioxide, it may also promote a protective effect on color.

From a sensory perspective, the biological acidification with *L. thermotolerans* produces a good and perceptible sourness, thus increasing wine freshness [39]. Usually, no unpleasant nuances of dairy foods are found, even when higher levels of ethyl lactate are produced, but the levels of acetoin and diacetyl in the sequential fermentations with *S. cerevisiae* are quite controlled and similar to single *S. cerevisiae* fermentations [39].

5. Yeast selection to improve acidity, aromatic profile, or color

The selection of yeast strains to obtain non-*Saccharomyces* able to improve the wine freshness in terms of acidity, aromatic profile, or color starts with the isolation of a yeast collection from a vine environment, mainly grapes, and also leaves, wood, or soil. After that, the yeast can be initially classified by using both selective and differential agar media. Later, the pre-identified yeasts can be confirmed by PCR amplification of the ribosomal region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene using as primers the ITS1 and ITS4 [51], the subsequent sequencing and the comparison of the sequence in a genomic database that facilitates the proper identification of genus and species [45]. Microfermentations in triplicate can be performed in order to select specific yeast strains with improved properties, e.g., a *L. thermotolerans* strain with suitable production of lactic acid, during spontaneous fresh must fermentation. Later, the production of lactic acid and whatever other metabolites with repercussion in wine sensory quality can be evaluated by instrumental analysis (**Figure 3**).

Yeast selection can be focused on the identification of strains with specific properties of technological, fermentative, or sensory repercussion during wine

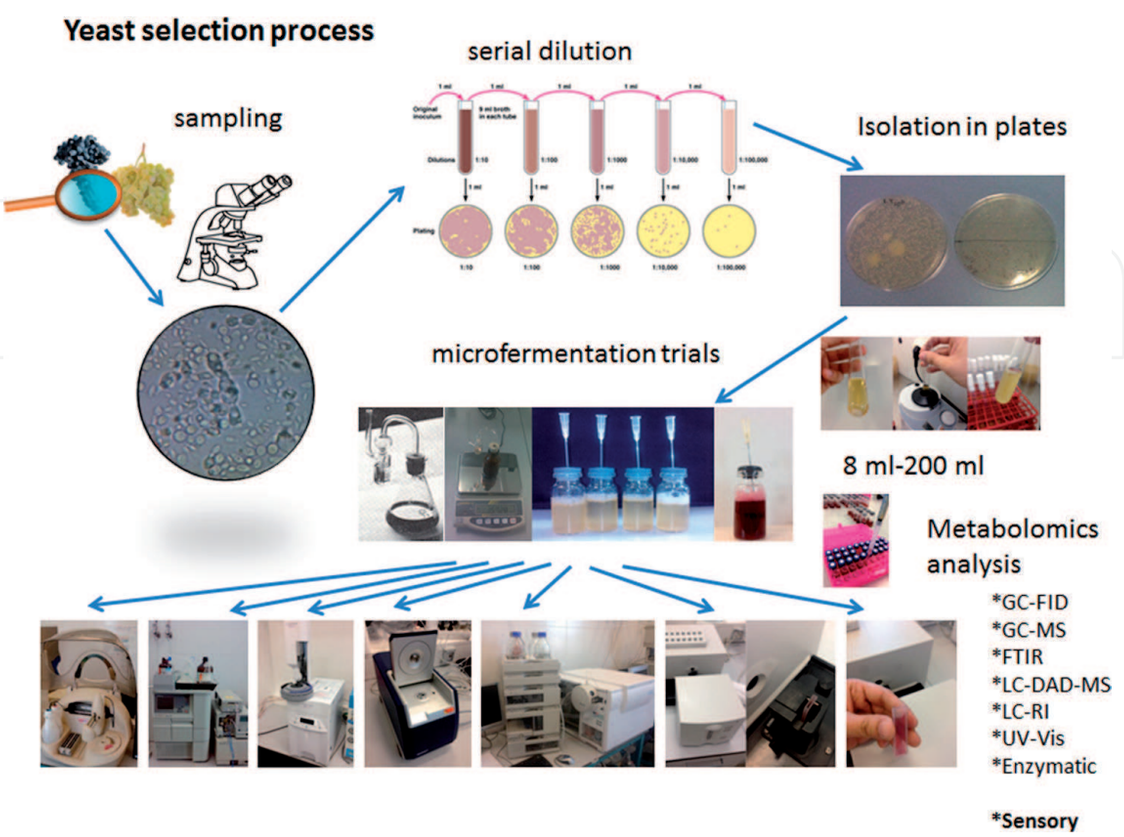

Figure 3.
Isolation of wild yeast and selection protocol under a metabolic approach.

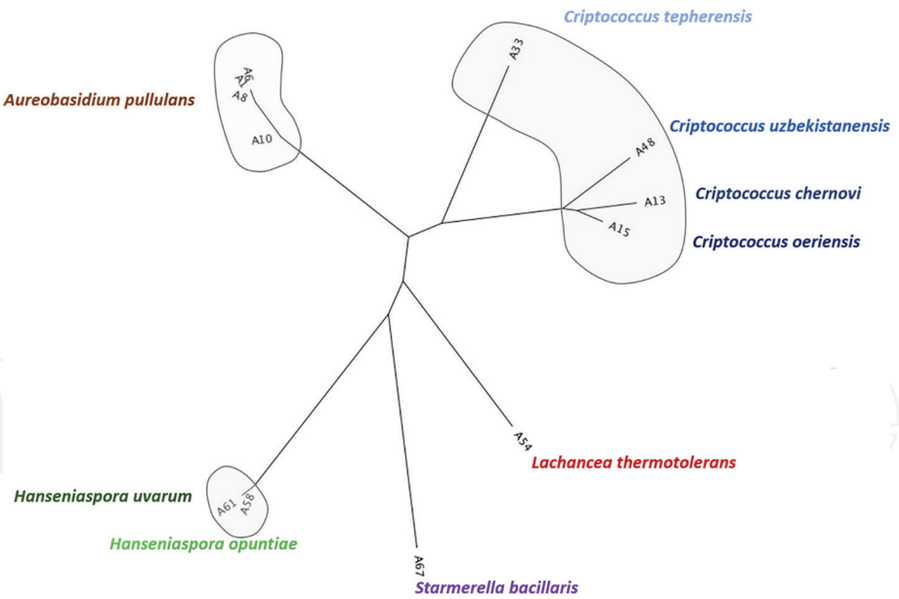

Figure 4.
*Phylogenetic tree of the wild non-*Saccharomyces *yeast species that were found in the grapes of a vineyard from a warm region.*

fermentation [52–54]. These properties can be targeted to improve color by the formation of stable pigments as vitisins [55, 56], vinylphenolic pyranoanthocyanins [57], and polymeric pigments [50, 58], the enhancement of aroma by the production of esters or enzymatic activities able to release varietal aroma [59, 60], or the improvement of the mouthfeel and flavor by the production/release of polyalcohols, polysaccharides [61, 62], acids [39, 45], etc.

The isolation of wild yeasts and the subsequent sequencing and comparison of the rDNA can help to elucidate the yeast microbioma from a vineyard (**Figure 4**). Normally, when the wild yeast populations are evaluated at the grape maturity stage, several mold species are frequently found together with apiculate yeasts such as those which belong to the genus *Kloeckera* or *Hanseniaspora*, making difficult to isolate and identify *S. cerevisiae* strains. Apiculate yeast can reach populations of 2–4 log CFU/mL.

6. Ternary sequential inoculations in warm areas: biotechnological approach to improve freshness

The use of sequential fermentations with non-*Saccharomyces* species has been used to improve wine acidity, aromatic and flavor complexity, and freshness. As reviewed in Section 3, non-*Saccharomyces* yeasts such as *H. vineae*, *T. delbrueckii*, *W. anomalus*, *M. pulcherrima*, *K. apiculata*, *S. bombicola*, and *C. stellata* improve aroma by either the increased production of acetate esters or the development of enzymatic activities that enhance the varietal aroma. Some of them can also increase sweetness and body by the production of polyalcohols such as glycerol or 2,3-butanediol. Moreover, it is currently possible to control pH in fermentation by the formation of suitable amounts of lactic acid with *L. thermotolerans*. The use of sequential combinations of two yeasts is already used at industrial level, but the combination of three yeast species (**Table 1**), namely, ternary inoculations, is less explored as a biotechnology to improve freshness in warm areas. In this case, it is more similar to what happens in a spontaneous fermentation according to the principle of succession: the fermentation is started by an apiculate yeast, followed by a medium fermentative power yeast like *T. delbrueckii*, *L. thermotolerans*, or

Aroma and flavor improvement	pH and acidity	To completely deplete sugars
Hanseniaspora vineae *Torulaspora delbrueckii* *Wickerhamomyces anomalus* *Metschnikowia pulcherrima* *Kloeckera apiculata* *Starmerella bombicola* *Candida stellata*	*Lachancea thermotolerans*	*Saccharomyces cerevisiae* *Schizosaccharomyces pombe*

Table 1.
Potential combinations of three yeasts to improve freshness.

M. pulcherrima, and finally the sugars are completely depleted by *S. cerevisiae* to obtain a dry wine. In ternary fermentations, the use of several non-*Saccharomyces* species to improve aroma and flavor must be completed with *L. thermotolerans* to decrease pH, improve the acidity, and, therefore, enhance the wine freshness. Lastly, the sugars are finished by *S. cerevisiae* or alternatively *S. pombe*. Using the latter species, it would be possible to make interesting wines in the absence of *S. cerevisiae*.

7. Conclusions

The use of fermentation biotechnologies such as sequential ternary fermentations with non-*Saccharomyces* emerges as a natural and useful bio-tool to improve freshness in warm areas. The use of *L. thermotolerans* favors a powerful pH modulation by the production of a stable acid without the production of off-flavors. Yeast selection to obtain appropriate non-*Saccharomyces* strains facilitates the development of safer and sensory-improved fermentation, with the added advantage of protecting the wine typicity, compared to the traditional fermentation driven by a single yeast, especially when only *S. cerevisiae* is used.

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