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Review

The Influence of Non-*Saccharomyces* Species on Wine Fermentation Quality Parameters

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Abstract: In the past, some microbiological studies have considered most non-*Saccharomyces* species to be undesirable spoilage microorganisms. For several decades, that belief made the *Saccharomyces* genus the only option considered by winemakers for achieving the best possible wine quality. Nevertheless, in recent decades, some strains of non-*Saccharomyces* species have been proven to improve the quality of wine. Non-*Saccharomyces* species can positively influence quality parameters such as aroma, acidity, color, and food safety. These quality improvements allow winemakers to produce innovative and differentiated wines. For that reason, the yeast strains *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe*, and *Pichia kluyveri* are now available on the market. Other interesting species, such as *Starmerella bacillaris*, *Meyerozyma guilliermondii*, *Hanseniospora* spp., and others, will probably be available in the near future.

Keywords: *Torulaspora delbrueckii*; *Lachancea thermotolerans*; *Metschnikowia pulcherrima*; *Schizosaccharomyces pombe*; *Pichia kluyveri*; *non-Saccharomyces*

1. Introduction

Over the last decades, the dry yeast market based on *Saccharomyces cerevisiae* has allowed alcoholic fermentation to start faster than the regular spontaneous methods, reducing the production times. In contrast, non-*Saccharomyces* species have often been inhibited by *S. cerevisiae* inoculations at the industrial level, despite being the predominant species in grapes before fermentation starts to take place [1]. The inoculation of *S. cerevisiae* in large populations exceeding 10^6 cfu/mL and the inhibition of non-*Saccharomyces* species such as *Hanseniorsopara, Kloeckera*, or *Candida* by initial sulfur dioxide addition make it difficult for those non-*Saccharomyces* species to influence alcoholic fermentation. However, first-phase non-*Saccharomyces* species play an important role in spontaneous fermentations until the alcohol level reaches 4 % (*v/v*), when most non-*Saccharomyces* species can no longer survive. Temperatures below 30 °C increase the ethanol resistance of species such as *Starmerella stellata* and *Kloeckera apiculate* [2].

The populations of yeast during alcoholic fermentation change over time. During the first stage, yeast from genera with a low resistance to ethanol, such as *Hanseniaspora*, *Candida*, *Rodotorula*, and *Pichia*, predominate [3–5]. Later, some genera with a moderate resistance to ethanol, such as *Lachancea* [6] or *Torulaspora* [1], may persist for longer. In the last stages of fermentation, most authors report that the *Saccharomyces* genus dominates the medium until fermentable sugars are completely metabolized into ethanol [3–5].

Several studies have reported that non-*Saccharomyces* species show advantages that can improve specific parameters of wine quality [7–15], depending on the specific yeast species and strains used (Table 1). Because of these advantages, the most important manufacturers are now commercializing strains of non-*Saccharomyces* [16] from *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Metschnikowia*

pulcherrima, *Schizosaccharomyces pombe*, and *Pichia kluyveri* (Table 2). However, most of these species also show disadvantages, which must be taken into account during their use. The main disadvantages are their low capacity to metabolize sugar into ethanol and their low resistance to additives such as sulfur dioxide in most cases, although some specific genera, such as *Schizosaccharomyces* [17,18], can withstand those disadvantages. The low fermentative activity of some non-*Saccharomyces* species is usually corrected by combining them with a high fermentative commercial *S. cerevisiae* strain able to metabolize all the sugar into ethanol [19]. This combination usually assumes a fermentation delay of a few days compared to pure fermentation inoculation by *S. cerevisiae*. The main positive influences of non-*Saccharomyces* species in modern winemaking are explained in the following paragraphs.

Table 1. Influence of non-*Saccharomyces* species on winemaking quality parameters.

Starmerella bacillaris	Glycerol ↑
Hanseniaspora spp.	Acetate esters ↑, terpenes ↑, Biogenic amines ↓
Hansenula anomala	C6 alcohols ↓
Lachancea thermotolerans	L-lactic acid ↑, Acidification ↑
Metschnikowia pulcherrima	Esters ↑, Terpenes ↑, Thiols ↑, Aroma complexity ↑,
Pichia guillermondii	Color Stability ↑
Pichia kluyveri	Thiols ↑, Esters ↑
Schizosaccharomyces pombe	L-Malic acid ↓, Deacidification ↑
Torulospora delbrueckii	Acetic acid ↓, Esters ↑, Thiols ↑,
Zygosaccharomyces bailii	Polysaccharides ↑

↑, higher activity; ↓, lower activity≈

Table 2. Main commercial products that contain non-*Saccharomyces* strains.

Product Name	Manufacturer	Species
Biodiva™	Lallemand www.lallemandwine.com (access on 29/06/2019).	*T. delbrueckii*
Concerto™	Chr. Hansen www.chr-hansen.com (access on 29/06/2019).	*L. thermotolerans*
Flavia®	Lallemand www.lallemandwine.com	*M. pulcherrima*
Frootzen®	Chr. Hansen www.chr-hansen.com	*P. kluyveri*
Prelude™	Chr. Hansen www.chr-hansen.com	*T. delbrueckii*
Primaflora® VB BIO	CENOLIA www.sud-et-bio.com	*T. delbrueckii*
ProMalic	Proenol https://www.proenol.com	*S. pombe*
Viniferm NS TD	Agrovin www.agrovin.com	*T. delbrueckii*
Zymaflore® Alpha	Laffort www.laffort.com (access on 29/06/2019).	*T. delbrueckii*

Modern enology looks for strategies to reduce the final content of ethanol in wine. The main causes of this trend are the consumer demand for products with a lower content of ethanol. High polyphenolic maturity usually increases grape sugar due to the delay in harvest. This effect is common in warm viticulture areas where the over-ripening risk is high. There are some alternative methodologies that can be used to reduce the content of ethanol in wine, such as enzyme or osmotic filtration [20]. Non-*Saccharomyces* species allow us to reduce the initial ethanol content by about 1–2% (*v/v*), depending on the yeast species and fermentation conditions [21–23].

Chemical methods based on food quality acid additions, such as tartaric acid, were the classical solution to acidity imbalances in over-ripe grape juices. On the other hand, for excess acidity, which is commonly found in cold areas, the most common solution was the use of calcium carbonate, potassium bicarbonate, or potassium carbonate to deacidify to regular levels. The main inconveniences of these solutions are the costs of these chemical products, which must be certified as being of food-quality. Nevertheless, over the last decade, some microbial alternatives have been proposed. The first alternatives involved some strains of *S cerevisiae* that are able to influence wine acidity [24,25]; however, the influence on the pH was not significant. The use of some non-*Saccharomyces* species has been proven to be able to reduce the pH by 0.5 units [6], while other species are able to increase the pH by up to 0.5 units [26].

Acetic acid is the main acid responsible for the wine fault termed volatile acidity. Although acetic acid influences the total acidity, it is usually considered separately, as it can negatively influence the wine quality. The fault threshold of volatile acidity is considered to be about 0.8 g/L; above this, most consumers can easily identify the negative vinegar characteristic. The main acetic acid ester, ethyl acetate, in concentrations higher than 12 mg/L produces undesired odor faults [27], which are of even more concern than acetic acid. Some non-*Saccharomyces* species, such as *T. delbrueckii* [1] or *L. thermotolerans* [6], can produce wines with lower contents of acetic acid than *S. cerevisiae*, while other species, such as *Schizosaccharomyces* sp. [18], tend to produce wines with concentrations higher than the fault limit. Nevertheless, large strain variability is reported in most cases [7,28–31].

Some studies have shown that specific non-*Saccharomyces* species are able to produce higher concentrations of fruity esters than *S. cerevisiae* (control) [32]. Specific non-*Saccharomyces* strains can increase the release of varietal aromas, such as terpenes or thiols, which are responsible for the quality of some grape varieties, such as Muscat, Gewurztraminer, Sauvignon blanc, and Verdejo [19,33].

The main strategies for increasing the color of red wines are based on obtaining higher final concentrations of total anthocyanins or higher levels of the most stable anthocyanins, such as vitisins or pyranoanthocyanins [34]. pH reduction is another strategy used to increase the color perception [6,35]. The latest studies have developed strategies to avoid malolactic fermentation [6,36], whose effect on wine quality is the reduction of color due to increases in pH and lactic bacteria enzymatic activity [36,37]. However, the necessity of producing stable wines that will not re-ferment in the bottle means that the vast majority of red wines go through malolactic fermentation. The first microbiological approaches used *S. cerevisiae* strains that absorbed reduced amounts of anthocyanins. Later approaches selected *S. cerevisiae* strains in order to obtain higher contents of acetaldehyde and pyruvic acid, which slightly increase the contents of vitisin A and B. Some non-*Saccharomyces* species may produce up to four times higher concentrations of pyruvic acid or acetaldehyde than *S. cerevisiae*. The combination of specific non-*Saccharomyces* species allows the stabilization of wines from a microbiological point of view, avoiding malolactic fermentation and additionally increasing the acidity and color perception [34,35].

Although wine is a safe product from a microbiological health hazard point of view, as no pathological microorganisms such as *Salmonella* or *E. coli* can withstand the wine ethanol concentrations [38], modern enology has discovered toxic compounds that can appear in wine such as biogenic amines, ethyl carbamate, and ochratoxin A. The main strategy employed to avoid biogenic amines in wine is based on the use of selected lactic bacteria from *Oenococcus oeni* species without decarboxylase activity able to convert specific amino acids into biogenic amines. Regarding this fact, some non-*Saccharomyces* species have been reported to produce higher concentrations of amino acids such as histidine that can evolve to histamine if bacteria decarboxylase activity takes place [30]. Other non-*Saccharomyces* species prevent the malolactic fermentation process, where the production of biogenic amines takes place [36]. Ochratoxin A is produced prior to harvest by spoilage fungal attacks. There are several methods that can be used to reduce the ochratoxin A concentration during the winemaking process, such as that which is conducted through the use of amicrobic filtrations of about 0.45 μm that allow the initial concentration to be reduced by up to 80% [39]. A promising biotechnology method is the use of yeast lees to remove ochratoxin A. This method was first tested using *S. cerevisiae*,

although newer studies have shown that some non-*Saccharomyces* species are more efficient at removing ochratoxin A, with rates of up to 70% [38,40–42]. Ethyl carbamate is mainly produced by lactic acid bacteria and through the chemical combination of urea with ethanol during wine ageing. The most common type of management in the wine industry is based on the use of a commercial urease enzyme which is able to remove all the urea that can evolve into ethyl carbamate [38]. Nevertheless, the use of non-*Saccharomyces* species with urease activity allows the removal of the main ethyl carbamate precursor from wine, making it virtually impossible for ethyl carbamate to appear during wine ageing.

Polysaccharides have been proven to improve the mouthfeel properties of wine [43–45]. The improvements in wine quality are mainly related to softening the wine astringency [45] or increasing positive aromatic compounds [46]. The most abundant group of polysaccharides is the arabinogalactan proteins, which originate in grapes [43]. Mannoproteins represent the second most abundant group; however, these polysaccharides are formed during alcoholic fermentation or ageing during lees processes [26,47]. Although the first microbiological applications for increasing the content of mannoproteins in wines were based on the use of *S. cerevisiae* strains, later studies showed that some non-*Saccharomyces* species release higher concentrations of mannoproteins than *S. cerevisiae* [47–50]. Other polysaccharides of a different nature than mannoproteins are also reported for some non-*Saccharomyces* species [26,51–53].

All yeast species inevitably produce acetaldehyde during alcoholic fermentation. The highest concentration is reached during the tumultuous phase of alcoholic fermentation. It usually takes place within 48–72 h of alcoholic fermentation, depending on the fermentation power of the yeast species. Concentrations higher than 125 mg/L usually negatively influence the flavor of wine [54,55], and wines are usually described as being oxidized. Some of the descriptors used for wines where acetaldehyde predominates in the aroma are green apples and fresh-cut grass [55]. Such aromas are easy to identify in white wines. Newer studies on red wines have proven that concentrations below the fault threshold of 125 mg/L increase the valuable stable color forms, such as vitisin B [35], which improves wine color, while the aroma of acetaldehyde cannot be identified in a sensory analysis. Some non-*Saccharomyces* species produce lower concentrations of acetaldehyde than *S. cerevisiae* [56], while others produce higher levels [57].

Glycerol can increase the softness and body of wine. *S. cerevisiae* synthesizes glycerol from glucose through glycolysis, where dihydroxyacetone phosphate is reduced to glycerol-3-phosphate and later oxidized to glycerol [58,59]. One of the first reported advantages of using non-*Saccharomyces* species was the increased glycerol concentration in wine and its influence on wine quality [9,48,60]. Depending on the non-*Saccharomyces* species employed, it is possible to achieve increases from a few decimals to 4 g/L compared to *S. cerevisiae* [9,60]. From a biochemical point of view, species other than those of the *Saccharomyces* genus possess less developed alcohol dehydrogenase enzymatic activity, but more developed glycerol-3-phosphate dehydrogenase enzymatic activity. This metabolism deviates to produce higher final concentrations of glycerol during alcoholic fermentation [59].

Several studies attribute the properties of some non-*Saccharomyces* species to improved wine quality. Nevertheless, recent studies have shown large differences, depending on the non-Saccharomyces strain used [31,61,62]. This oenological phenotypical variability is based on the huge number of different populations and the genomic diversity of those species [63–69]. These results suggest the importance of performing selective processes, such as those that were conducted for *S. cerevisiae* strains in the past.

2. *Torulaspora Delbrueckii*

Torulaspora delbrueckii (Figure 1(1)) is the most studied and commercialized of the non-*Saccharomyces* species in winemaking [1]. The management of *T. delbrueckii* is relatively easy compared to other non-*Saccharomyces* species due to its relatively high fermentative power of up to 9–10% (*v/v*) [70], while several non-*Saccharomyces* species, such as *M. pulcherrima, P. guillermondii, P. kluyveri, S. stellata*, and *Hanseniaspora vinae*, do not tolerate ethanol concentrations higher than 4% (*v/v*). Due to this ethanol resistance, this species can notably influence the final wine product during most of the

alcoholic fermentation period, although in most wines, a second more fermentative species such as *S. cerevisiae* [48] or *S. pombe* [71] is required to properly end the alcoholic fermentation. Nevertheless, some industries other than conventional winemaking have started to exclusively use *T. delbrueckii* for fermentation; some examples are for the production of beer or sparkling base wine [1].

One of the first advantages attributed to *T. delbrueckii* was the reduction of the volatile acidity concentration in wines. Some authors have reported reductions in the final acetic acid concentration of about 0.14 to 0.28 g/L compared to *S. cerevisiae* [1,70]. The application of *T. delbrueckii* can decrease the final ethanol concentration in wines by up to 1% (*v/v*) [22], while increasing the glycerol concentration from 0.2 to 0.9 g/L [1,72–74]. Several authors report *T. delbrueckii* as being a greater mannoprotein releaser than *S. cerevisiae* and other non-*Saccharomyces* species [47,48]. Moderate malic acid consumption by *T. delbrueckii* has been commonly observed in sequential fermentations in quantities varying from 20% to 25% [43,48].

T. delbrueckii can improve the intensity and quality of wine aroma, increasing the overall impression and the varietal and fruity characters [72]. *T. delbrueckii* is able to diminish the concentrations of higher alcohols when it is used in sequential fermentations with *S. cerevisiae* [48]. This effect contributes to an increase in the varietal character perception. However, an increase in alcohol production has also been reported [75,76]. Several authors have reported the production of higher final concentrations of fruity esters [72,77]. In contrast, other studies have reported the opposite effect [73,76]. These differences in higher alcohols and ester formation have been explained by the high strain variability in these parameters shown by the species [31,62]. *T. delbrueckii* is reported to release conjugated terpenes in some wine varieties characterized by these varietal compounds [78]. In addition, proper *T. delbrueckii* strain selection allows for the release of higher concentrations of thiols, which increase the varietal character of varieties such as Sauvignon blanc or Verdejo [72,79].

A moderate undesirable effect reported by most authors is a delay in sequential fermentation involving *T. delbrueckii* and *S. cerevisiae* compared with the *S. cerevisiae* control.

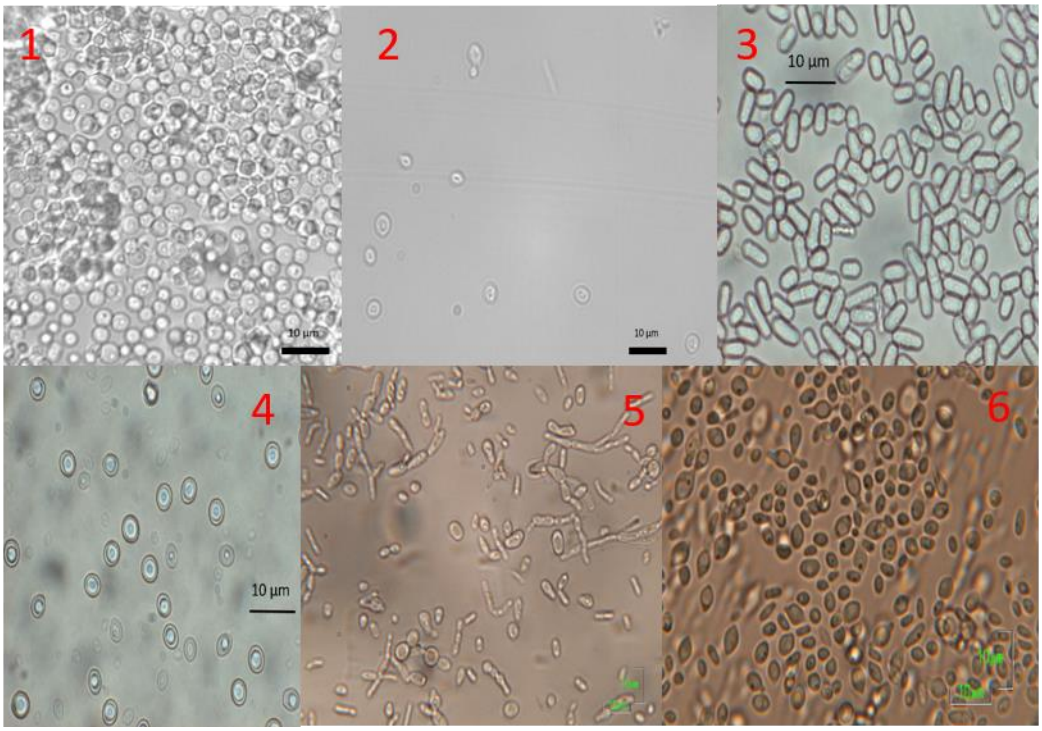

Figure 1. Microscopic observation of Torulaspora delbrueckii (**1**), Lachancea thermotolerans (**2**), Schizosaccharomyces pombe (**3**), Metschnikowia pulcherrima (**4**), Meyerozyma guilliermondii (**5**) and Hanseniospora uvarum (**6**) cells.

3. *Lachancea Thermotolerans*

Lachancea thermotolerans (Figure 1(2)) is the most recommended of the non-*Saccharomyces* species used to acidify grape juices that suffer from a lack of acidity [80]. This ability is very useful in viticultural areas in the south of Europe or any other warm viticulture region [6,81]. *L. thermotolerans* can acidify wines due to its unique ability among yeasts to produce lactic acid during its fermentative metabolism [7,82]. Hranilovic et al. (2018) have shown the pathway of lactate formation from pyruvate through the enzyme lactate dehydrogenase enzyme [83]. The production of lactic acid can vary from a few decimals of g/L to up to almost 10 g/L, depending on factors such as the strain or fermentative temperature used [6,84]. The production of lactic acid without degrading any malic acid directly influences the titratable acidity quality parameter. The production of lactic acid is reported to increase the titratable acidity by up to about 9 g/L [84] compared with the regular *S. cerevisiae* control. Some studies have reported a reduction in pH from about pH 4 to pH 3.5 in low-acid grape juice, which would be considered an acidic wine in most warm viticulture regions [6]. The reduction of pH also positively influences the color of red wine due to the increase in the color intensity of anthocyanins such as the flavylium ion [35,36].

L. thermotolerans also has other interesting properties. Some authors have described *L. thermotolerans* as an interesting resource that can be employed to reduce the final concentration of volatile acidity in wine [85]. Some studies have reported that *L. thermotolerans* fermentations produce lower concentrations of acetic acid than *S. cerevisiae,* by about 0.24 g/L [82,84], while other authors have reported smaller differences in sequential fermentations of about 0.05 g/L [36,86]. Recent studies reported *L. thermotolerans* strains to show a strain variability of up to 49% in acetic acid production [31]. Later studies supported the idea that strain variability shows great biodiversity around the world [69], which translates into large differences in the phenotypic fermentative performance for the different strains [31]. Although the first studies showed lower final concentrations in glycerol than *S. cerevisiae* for single pure fermentations of about 1.5 g/L [84], later studies showed that, in sequential fermentations, those including *L. thermotolerans* often reach higher final levels of glycerol of up to 1 g/L [7,86]. These results, combined with those related to ethanol production, indicate that although *L. thermotolerans* is less fermentative than *S. cerevisiae*, it possesses a more developed glycerol–pyruvic pathway. Nevertheless, the production of glycerol by *L. thermotolerans* also depends on other factors, such as temperature [82], as it produces higher contents at 20 °C than at 30 °C. Later studies reported that the injection of oxygen during *L. thermotolerans* fermentations increases the production of glycerol while reducing the production of ethanol [87].

Some studies have reported that *L. thermotolerans* sequential fermentations produce lower final concentrations of higher alcohols than *S. cerevisiae*—from 13 to 55 mg/L, depending on the study [81,82,88]. Nevertheless, other authors have reported the opposite effect, with fermentations involving *L. thermotolerans* increasing higher alcohol concentrations by up to 100 mg/L [7]. These discrepancies are explained by the great variability in *L. thermotolerans* strains, in terms of higher alcohol production (up to 40%) [31,62] and oxygen availability [87].

Some authors have reported increases in the total ester content of up to 33% [69], while other studies have not observed important differences [7]. Nevertheless, all studies have reported increases in the specific ester ethyl lactate, which is related to lactic acid metabolism [32,34]. *L. thermotolerans* produces lower concentrations of fatty acids than *S. cerevisiae* in pure fermentations, although specific strains of *L. thermotolerans* tend to produce higher concentrations of the specific fatty acid isovaleric acid [62]. Specific *L. thermotolerans* strains are able to release higher amounts of terpenes, depending on their glucosidase activity [7,32].

Some authors have reported higher total final anthocyanin concentrations in sequential fermentations involving *L. thermotolerans* than in the *S. cerevisiae* controls. The differences varied from 8% to 10% [34,89]. Additionally, the higher coloration of anthocyanins produced by lactic acid acidification at a low pH notably influences the final color intensity, which is higher than that of *S. cerevisiae* [6,18,35,36].

Some authors have reported that some specific *L. thermotolerans* strains release up to 100 mg/L higher concentrations of polysaccharides than *S. cerevisiae* [7]. Nevertheless, this ability is strain-dependent, as the observed strain variability between the studied *L. thermotolerans* strains is close to 40% [7].

Studies that perform sensory analyses usually describe the wines fermented by *L. thermotolerans* as being more acidic than the controls fermented by *S. cerevisiae* [36,57]. This perception is not as obvious in highly acidic wines from northern Germany [32], but is very evident in wines fermented from low acidic grape juices from warm areas in southern Europe, where the pH is reduced by about 0.4 units and the total acidity increases by about 3 g/L [81]. The color intensity perception is usually higher due to the increase of visible red and purple colors at lower pH values [6].

Other modern applications of *L. thermotolerans* facilitate the management of ochratoxin A. Some *L. thermotolerans* strains are able to efficiently inhibit the development of ochratoxigenic fungi in the vineyard [90,91]. As the legislation trend is to reduce the applications of pesticides, *L. thermotolerans* appears to be an interesting alternative to the management of ochratoxin A.

The main problems in the industry management of *L. thermotolerans* are its sensitivity to sulfur dioxide and its moderate ethanol tolerance. Among the non-*Saccharomyces* species, *L. thermotolerans* is considered a fermentative species that is able to ferment wines at up to levels slightly higher than 10% (*v/v*) in ethanol [28,82], but it must be combined with a *S. cerevisiae* [86] strain for the production of regular dry wines or with another more fermentative genus, such as *Schizosaccharomyces* [36], to ensure proper alcoholic fermentation cessation. In other fermentative industries such as beer, sweet wine, or sparkling base wines, the fermentative power of *L. thermotolerans* is sufficient to achieve the desired final ethanol concentration [6,51]. Additionally, some studies have observed that sequential fermentations between *L. thermotolerans* and *S. cerevisiae* produce lower final ethanol concentrations varying from 0.2% to 0.4% (*v/v*) [36,69,86].

Another reported problem is the release of higher concentrations of biogenic amine amino acid precursors such as lysine, ornithine, and tyrosine during alcoholic fermentation compared with *S. cerevisiae* [32,57]. Although there is no direct correlation between biogenic amine formation and the presence of amino acid precursors, this fact must be taken into account, especially for wines that will perform malolactic fermentation or barrel ageing [38]. Nevertheless, the acidification performed by *L. thermotolerans* can partially inhibit the capacity of unselected lactic bacterial strains to produce biogenic amines, as the development of lactic bacterial genera is limited at low pH values. This potential food safety problem is also present when *L. thermotolerans* is used in ageing over lees for histidine, tyrosine, ornithine, and lysine amino acids [49].

Although most studies regarding the *Lachancea* genus and oenology have focused on *L. thermotolerans*, other species have started to show promising potential. For example, *L. fermentati* is a higher fermenter than *L. thermotolerans*. Pure inoculations of *L. fermentati* produce wines with lower concentrations of acetaldehyde, SO_2, and H_2S compared to the *S. cerevisiae* controls [80,92].

4. *Schizosaccharomyces* Species

Schizosaccharomyces pombe is the most recommended of the non-*Saccharomyces* species to de-acidify excessively acidic wines from cool areas, such as those from the north of Europe. Indeed, modern studies also employ this species to stabilize wines from a microbiological point of view, for example, in red wines from warm viticulture areas, where the performance of a proper malolactic fermentation process is complicated due to the low levels of malic acid and the high pH [36]. *S. pombe* is able to metabolize malic acid into ethanol and CO_2, consequently reducing the total wine acidity [18]. Benito et al. (2014) have shown the biochemical pathway used to degrade malic acid into ethanol through pyruvate decarboxylase and alcohol dehydrogenase enzymes [93]. In wines with malic acid contents higher than 5 g/L, which are considered very acidic by regular consumers, *S. pombe* can completely remove any malic acid present, decreasing the total acidity by about 4 g/L and the pH by about 0.4 units [94]. Figure 1(3) shows a microscopic observation of *S. pombe* cells during pure alcoholic fermentation.

Recently, the use of *S. pombe* has been suggested in warm viticulture areas where grape juices contain high levels of sugar, pH values are close to 4, and malic acid concentrations are usually less than 1 g/L [26,48]. Under these circumstances, to try to perform malolactic fermentation is dangerous, with a high risk of deviations, such as the production of undesired high levels of volatile acidity or biogenic amines. Nevertheless, if malolactic fermentation is not performed before bottling, it often takes place in the bottle, generating undesired turbidity. In these scenarios, the use of *S. pombe* alone or in small percentages in combined inoculums with *S. cerevisiae* allows the achievement of microbiological stability so that wine can be bottled without the risk of bottle refermentation.

Specific strains of *S. pombe* are the most effective option to remove gluconic acid from wine during alcoholic fermentation, with a removal percentage of up to 91% [95–99]. Gluconic acid can negatively influence the quality of wine, generating microbial instability, as it can be used by lactic acid bacteria to increase volatile acidity, reducing the protective effect of sulfur dioxide.

One of the main problems of using *S. pombe* is that it tends to generate high levels of acetic acid [18,30]. This acid usually produces a quality-detrimental vinegar character, which is not tolerated by consumers of quality wines. This undesirable effect has been solved with different strategies, such as the combined use with *S. cerevisiae* [94], *L. thermotolerans* [6,36], or *T. delbrueckii* [71]; the addition of magnesium [100]; or the use of alginate cells [101] and fed-batch fermentation [102]. These alternatives allow the production of wines with lower acetic acid contents than those produced with *S. cerevisiae*. Another undesirable effect of the use of *S. pombe* is an increase in the ethanol concentration, as the degradation of 2.33 g/L of malic acid produces about 0.1% (*v/v*) of additional ethanol [103]. Although no malolactic fermentation is needed after *S. pombe* alcoholic fermentation, the concentration of amino acids that can evolve to biogenic amines usually increases [30].

The malolactic fermentation process usually reduces the anthocyanin content and color intensity from 10% to 23% [36,37]. This phenomenon takes place due to the cell absorption and glycosidase enzymatic activity of lactic bacteria [35,104]. *S. pombe*-fermented wines show higher contents of total anthocyanins and consequently higher color intensities as malolactic fermentation is not needed. Additionally, *S. pombe* is able to produce up to five times more pyruvic acid than *S. cerevisiae* [105], which translates to the formation of a consequently higher concentration of the stable anthocyanin vitisin A, which contains pyruvic acid [35]. Additionally, the combined use with *L. thermotolerans* increases the color intensity due to the additional reduction of pH that increases the color intensity of flavylium ions [6,18,35] *S. pombe* releases higher amounts of polysaccharides than any other *Saccharomyces* or non-*Saccharomyces* yeast [26,52], consequently improving the wine structure. The nature of these polysaccharides is different than that reported for *S. cerevisie*, including the presence of α-galactomannose and β-glucans in their compositions [53].

S. pombe is characterized by producing significantly lower concentrations of higher alcohols and esters than *S. cerevisiae* and other yeast species [34,57]. This is very interesting when retention of the varietal aroma of grapes is desired more than the fermentative aroma [19,48,72].

Regarding food safety, the use of *S. pombe* allows the control of biogenic amines, as no malolactic fermentation, which is able to produce this toxic compound, is required [38]. Additionally, the urease enzymatic activity developed by *S. pombe* eliminates the main precursor of ethyl carbamate: urea. Indeed, *S. pombe* can remove 70% of the initial concentration of the carcinogen ochratoxin A during alcoholic fermentation [40].

In recent years, other industries have started to use *Schizosaccharomyces* species in products and processes other than the production of grape wine, such as ginger fermentation [106,107], apple wine [108], kei-apple fermentation [109], sparkling wine [110], bioethanol [111], bilberry fermentation [71], plum wine [112], and water purification [18].

Although *S. pombe* is the most studied yeast from the genus *Schizosacchromyces*, *Schizosaccharomyces japonicus* shows similar properties to *S. pombe* and a better performance in specific quality parameters such as glycerol production and polysaccharide release [53].

5. *Metschnikowia Pulcherrima*

Metschnikowia pulcherrima (Figure 1(4)) influences wine quality parameters. It can increase the glycerol concentration by a few decimals in combined fermentations compared to single *S. cerevisiae* controls. It is also able to reduce the malic acid content by about 10% and the acetaldehyde concentration by about 10 mg/L [19]. One modern application is the reduction of the final ethanol concentration. For that purpose, *M. pulcherrima* can be used in order to achieve ethanol reductions down to 1% (*v/v*) [22,113,114].

Following the comparison of sequential fermentations of *M. pulcherrima* and *S. cerevisiae*, some studies have described *M. pulcherrima* as a producer of low higher alcohol concentrations compared to *S. cerevisiae* that vary from 20% to 30% [32]. On some occasions, this effect means that varietal aroma compounds such as terpenes or thiols that are not masked by concentrations of higher alcohols that are higher than the perception threshold have a greater effect on wine aroma [19]. On the other hand, most studies have reported that *M. pulcherrima* is a higher producer of fruity esters [32]. Most studies have reported significant differences, especially for ethyl octanoate, which is produced in higher concentrations varying from 20% to 25% in sequential fermentations involving *M. pulcherrima* than in *S. cerevisiae* [19]. This specific ester increases fruity aromas related to pineapple, which are usually considered pleasant and very positive, in neutral grape varieties that do not possess varietal aroma compounds such as terpenes or thiols.

The most relevant influence on wine quality related to the use of *M. pulcherrima* is the ability of the cystathionine-β-lyase activity of selected strains to cause the release of varietal thiols such as 4-methyl-4-sulfanylpentan-2-one in concentrations six times higher than those in *S. cerevisiae* [19]. This aromatic compound is the most important quality indicator in thiolic wine varieties such as Sauvignon blanc or Verdejo. Figure 1(4) shows a microscopic observation of alcoholic fermentation performed by a pure culture of *M. pulcherrima* and sterilized grape juice.

6. *Meyerozyma Guilliermondii*

The use of *Meyerozyma guilliermondii* (Figure 1(5)) focuses on wine color improvements. *M. guilliermondii* is reported to be the yeast species with the highest hydroxycinnamate decarboxylase enzymatic activity [115]. This enzymatic activity allows the production of pyranoanthocyanin adducts, which condensate with grape anthocyanins to produce highly stable colored compounds that remain for a longer period of time than other anthocyanins. This biological enzymatic activity was first investigated in *S. cerevisiae*; however, although the enzymatic activity improved the color intensity, color stability, and removed ethyl phenol precursors, a maximum activity level of 16% was reached and there was a great dependency on the studied strain [116]. *M. guilliermondii* has been reported to increase hydroxycinnamate decarboxylase enzymatic activity by up to 90%. This type of biotechnology allows us to produce modern wines that contain up to 11-times higher concentrations of vinylphenolic pyranoanthocyanin adducts, which are the most stable color forms reported in winemaking [115].

7. *Pichia Kluyveri*

Some studies have reported the use of *Pichia kluyveri* (Figure 2) in sequential fermentations to produce higher levels of esters than *S. cerevisiae*, such as 2-phenylethyl acetate, by about 20%, or ethyl octanoate, by about 10% [32]. The total terpene concentration was also shown to increase by about 20%; this fact contributed to an increase in the grape variety typicity.

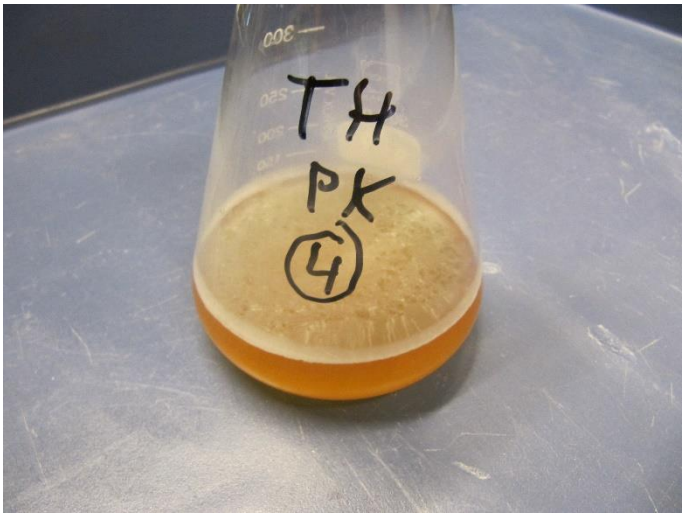

Figure 2. Film produced by *Pichia kluyveri* over grape juice.

8. *Starmerella Bacillaris*

Starmerella bacillaris, formerly *Candida stellata* [59], produces the highest glycerol concentration (up to 14 g/L) of the non-*Saccharomyces* yeasts during alcoholic fermentation [9,117,118], while most *S. cerevisiae* strains have been shown to produce final concentrations that vary from 5 to 8 g/L. These concentrations can improve the mouthfeel sensation and flavor of wine [9]. Another interesting property is its fructophilic character [119,120], in contrast with the glucophlilic character of *S. cerevisiae*.

9. *Hanseniaspora*

Species from the *Hanseniospora* genus possess a characteristic apiculate shape (Figure 1(6)). Most of the yeasts present at the beginning of spontaneous fermentation belong to this genus [121–125]. Although no manufacturer has offered a commercial strain to date, the genus is supposed to make up an important percentage of the yeasts that are in grapes. This indicates that, in traditional fermentations, the *Hanseniospora* genus notably influences alcoholic fermentations during the first phase until alcohol levels of about 4% are reached. At these levels, most *Hanseniospora* strains cannot survive due to their low tolerance to ethanol [126]. In combination with *S. cerevisiae*, which properly ends alcoholic fermentation, strains of the *Hanseniospora* genus can positively influence wine quality [9,125]. The *Hanseniaspora* genus is an interesting source of enzymes for modern winemaking challenges [125]. The most remarkable enzymatic activity is reported for β-glucosidase [127], β-xylosidase [128], glycolytic, and protease [123,129].

From a sensory point of view, the improvements are based on more intense wine flavor and aroma complexity. At an industrial scale, *Hanseniospora guilliermondii*, *Hanseniospora uvarum*, and *Hanseniospora vinae* [130] are the most appropriate species to achieve these purposes [125]. The aroma improvements are explained from a chemical point of view due to the production of higher concentrations of 2-phenylethyl acetate [131,132], acetate esters such as isoamyl acetate [124,127,130,132,133], medium-chain fatty acid ethyl esters [134], benzenoids [135,136], and terpenes [125,127] and reductions in the final concentration of higher alcohols [124,130,133]. Martin et al. 2018 [125] have explained the main metabolic pathways responsible for the ability of some species of *Hanseniaspora/Kloeckera* genera to produce benzenoids, diacetyl-acetoin, lactones, higher alcohols, acetate esters, fatty acids, and isoprenoids.

The most appropriate species to improve the color and polyphenolic composition in red wines from the *Hanseniaspora* genus are *Hanseniaspora clermontiae*, *Hanseniaspora opuntiae*, *H. guilliermondii*, and *H. vinae* [125]. These species can improve quality parameters such as color intensity and total anthocyanins [124]. These color improvements are based on the ability of *Hanseniaspora* species to produce vitisin A [137], vitisin B [138], and malvidin-3-O-glucoside-4-vinylguaiacol [137].

10. Conclusions

Non-*Saccharomyces* species can play an important role in winemaking. Depending on the specific type of wine or the enological problem to solve, different non-*Saccharomyces* strains should be selected to attain the desired objective. The combination of non-*Saccharomyces* species with *Saccharomyces* species or even with another high fermentative non-*Saccharomyces* species can also lead to the best solution. At this time, the most commonly used strains in industry are *Torulaspora delbrueckii, Lachancea thermotolerans, Schizosaccharomyces pombe, Metschnikowia pulcherrima,* and *Pichia kluyveri,* which are present in available products. It is likely that over the next few years new species will start to be available on the market, and products that contain combinations of non-*Saccharomyces* species will also be available in order to simulate spontaneous alcoholic fermentations.

Conflicts of Interest: The authors declare no conflict of interest.

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