Microbial interactions in winemaking: Ecological aspects and effect on wine quality

Microbial interactions in winemaking: ecological aspects and effect on wine quality

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ABSTRACT

Background: Wine microbiota is a dense and diverse ecosystem that is directly involved in the production and synthesis of many metabolites of oenological interest thereby directly affecting wine composition. The biodiversity and successional evolution of yeast and lactic acid bacteria (LAB) species and strains within species during alcoholic (AF) and malolactic fermentation (MLF) is greatly influenced by the complexity of the wine environment. Consequently, the successful prediction of wine characteristics is limited.

Scope and approach: The use of starter cultures has allowed better control of the fermentation process and the production of wines with desired characteristics. Mixed culture fermentations with selected non-*Saccharomyces* and *Saccharomyces* yeasts has regained attention in recent years due to their potential to modulate a wide range of metabolites of oenological interest. In this context, interactions among yeast species and LAB throughout the AF and MLF are known to influence the main enological parameters and aromatic profile of the wines. Studies have been conducted to uncover the nature of these interactions, with the aim to better control the AF and MLF.

Key findings and conclusions: This review provides an overview of microorganism interactions during the different steps of the winemaking process. This gives wine producers the ability to control and fine-tune microorganism population dynamics and therefore the fermentation process and finally wine quality.

Keywords: Wine yeasts; Lactic acid bacteria; Interactions; Selection; Fermentation

1. Introduction

Grapes and fermenting must for wine production represent a complex ecological niche that determines the presence and activity of specific yeast and bacteria species (Ciani et al., 2016). Despite the frequent dominance of *Saccharomyces cerevisiae*, it is generally accepted that a wide variety of non-*Saccharomyces* yeasts and lactic acid bacteria (LAB) are also present during spontaneous and inoculated wine fermentations. The non-*Saccharomyces* yeast and LAB also contribute significantly to the transformation of grape sugars into ethanol, carbon dioxide and other secondary metabolites essential to the flavour profile of wine (Dzialo, Park, Steensels, Lievens, & Verstrepen, 2017).

Currently, the use of inoculated mixed-cultures, based on the incorporation of multiple *S. cerevisiae* strains or specifically the addition of non-*Saccharomyces* yeasts and/or LAB (either in co-inoculation and sequential inoculation strategy), has been proposed as a solution to achieve the benefits of spontaneous fermentation while reducing the risks of spoilage and/or stuck fermentation (Fig. 1) (Padilla, Gil, & Manzanares, 2016). The benefits include improved wine complexity by increasing the diversity of chemical compounds present. Generally, wines with increased complexity are more preferred by consumers, and in mixed-culture fermentations yeasts produce aromas and flavours in way that cannot be reached with a single pure starter culture of *S. cerevisiae* (Jolly et al., 2014). Despite these positive factors, the fermentation conditions in which yeasts are subjected to need to be carefully controlled to achieve the desired results (Albergaria et al., 2016).

Successful mixed-culture fermentations can be achieved by increasing the contribution of the non-*Saccharomyces* yeasts by enhancing their metabolic activity and survival time (Morrison-Whittle, Lee, Fedrizzi, & Goddard, 2020). However, several scientific publications have reported contrasting results, even when the same species were studied (Albertin et al., 2017; Benito, 2019). Until recently, scientists generally believed that non-*Saccharomyces* yeasts "die off" and disappear during the early stages of AF, due to their low capacity to resist the changes in fermenting must composition (increasing ethanol levels, nutrient depletion). However, detailed studies have shown that the survival time and the reason for the disappearance of non-*Saccharomyces* yeast and bacteria includes several types of antagonistic interactions among the microorganisms (Liu et al., 2017).

These interactions can be passive (nutrients, oxygen and space competition) or active (antimicrobial compounds, volatile organic compounds, organic acids, cell-to-cell contact) (Di Gianvito et al., 2022). More recently it was demonstrated that some wine-related strains such

as *S. cerevisiae* (Legras et al., 2018), *Lachancea thermotolerans* (Hranilovic et al., 2018) and *Torulaspora delbrueckii* (Albertin et al., 2014a), were able to survive until the end of wine fermentation because they underwent a domestication event that made them highly adapted to this man-made environment. Furthermore, it was demonstrated that in a wine environment positive interactions also took place through the formation of mixed-species biofilms, aggregation and/or cross-feeding (the product of one strain's metabolism may be utilised in the nutrition of another). These interactive phenotypes were observed between *S. cerevisiae* and *Lactobacillus* sp. (Xu et al., 2021) and between yeasts such as *Hanseniaspora vineae* (Bagheri et al., 2017), *Saccharomyces uvarum* (Cheraiti et al., 2005), *Metschnikowia pulcherrima* (Seguinot et al., 2020) or *Torulaspora delbrueckii* (Renault et al., 2016).

Scientific publications reporting on the impact of different non-*Saccharomyces* yeasts with selected *S. cerevisiae* strains in mixed culture fermentations has increased significantly in the last years. In both co-inoculation and sequential inoculation approaches it has been shown that there are numerous chemical and physical interactions that influence compatibility and the success of fermentation. The *S. cerevisiae*/non-*Saccharomyces* fermentation process presents a new environment in which malolactic fermentation (MLF) needs to take place. Although the effects of population dynamics during non-*Saccharomyces*/*S. cerevisiae* and LAB//*S. cerevisiae* mixed-culture fermentations have received extensive attention, little is known about the ability of LAB to perform MLF during or at the end of the fermentation of the wines produced by mixed-yeast cultures. This review summarizes the current knowledge on microbial interactions during wine making, with a focus on yeast-yeast and yeast-bacteria interactions during alcoholic and MLF. The impact of mixed culture fermentations on LAB involved in MLF and the most important factors that modulate these interactions, as well as their impact on wine production, are also considered.

2. Impact of non-*Saccharomyces* species on wine quality

The impact that non-*Saccharomyces* yeasts have on wine quality largely depends on the initial population (microbial numbers and species diversity) in the fermenting juice, albeit from a natural population or inoculated strain (commercial or other) (Table 1). Must characteristics such as osmotic pressure (sugar level), ratio of glucose to fructose, yeast assimilable nitrogen (YAN), presence of sulfur dioxide (SO_2), temperature, degree of clarification (for white musts) and presence/absence of inoculated *S. cerevisiae* all affects the activity of the initial non-*Saccharomyces* population (Padilla et al., 2016). The degree of non-

Saccharomyces activity in turn determines the concentrations of metabolites formed. The impact of more robust and ethanol tolerant non-*Saccharomyces* species can be expected to be greater than more sensitive ones. However, as large strain diversity exists within species (Liu et al., 2017) conclusions on the contribution after investigating a single strain, cannot necessary be extrapolated to the entire species.

Wine flavour (aroma and taste) is made up of primary flavours derived from compounds in the grapes themselves, secondary flavours due to yeast and LAB metabolites and yeast mediated aromas from non-volatile precursors (Dzialo et al., 2017; Sumby et al., 2019). Depending on concentration, these compounds can contribute either positively or negatively to wine flavour. The range of flavour compounds produced or mediated by non-*Saccharomyces* yeasts includes esters, higher alcohols, glycerol, terpenoids, acetic acid, succinic acid, volatile fatty acids, carbonyl and sulfur compounds (Dzialo et al., 2017).

More than 160 esters have been identified with a positive effect on wine quality, especially in wines produced from neutral grape varieties (Dzialo et al., 2017). Non-*Saccharomyces* yeasts form varying levels of esters. Yeasts known to produce higher levels of esters include *Hansenula anomala* (*Pichia anomala*), *Hanseniaspora uvarum* (*Kloeckera apiculata*) and *Metschnikowia pulcherrima* (*Candida pulcherrima*) being regarded as higher producers (Jolly et al., 2014). Higher alcohols produced from amino acid catabolism through the Erlich pathway are generally not desired in wine, since high levels are strongly correlated with unpleasant sensory attributes. However, low levels of higher alcohols can impart fruity characters to wine and contribute to the wine's overall complexity (multiple identifiable sensory elements). Although there is a large strain variability, non-*Saccharomyces* yeasts often form lower levels of higher alcohols than *S. cerevisiae* (Jolly et al., 2014).

After ethanol, glycerol is the next major metabolite produced by yeast during wine fermentation. Glycerol is important for regulating redox potential in the yeast cell but can also contribute to mouth-feel, sweetness and complexity in wines (Dzialo et al., 2017). Extrinsic factors such as grape variety and wine style determines the extent to which increased glycerol levels impact on the wines' quality. Spontaneously fermented and non-*Saccharomyces* inoculated wines often have higher glycerol levels than *S. cerevisiae* inoculated wines, indicating a contribution by non-*Saccharomyces* yeasts (Jolly et al., 2014). Several non-*Saccharomyces* yeasts, such as *Lachancea thermotolerans* and *Starmerella bacillaris* (also known in older literature as *Candida zemplinina* or *Candida stellata*), consistently produce high glycerol concentrations (up to 14 g/L) during wine fermentation (Table 1; Fig. 2). However, increased glycerol production is linked to increased acetic acid (volatile acidity)

production (Dzialo et al., 2017). Volatile acidity is generally not desired in wine. However, decreased volatile acidity and acetic acid concentration can be obtained when using some non-*Saccharomyces* yeast in mixed fermentations with *S. cerevisiae* (Table 2). Volatile acidity is especially a problem during production of wines from botrytized and/or high-sugar musts using *S. cerevisiae* (Benito, 2019). A non-*Saccharomyces* yeast solution has been proposed whereby *Torulaspora delbrueckii* and *Starm. bacillaris* could be used, in combination with *S. cerevisiae*, to obtain wines with decreased levels of volatile acidity (Table 1).

Some non-*Saccharomyces* yeasts are linked to increased total acidity, a useful characteristic where natural acidity in wine is lacking due to variances in temperatures during grape ripening (Vilela 2019). *L. thermotolerans* is well known for its ability to produce lactic acid that can be beneficial to wines produced in geographical regions affected by global warming where grapes are characterized by low natural acidity (Binati et al. 2020; Balicki et al. 2016). Increases in acidity due to the metabolism of *T. delbrueckii* is a result of the production of succinic acid (Benito, 2019). However, as succinic acid is a harsher acid than lactic acid and has a 'salt-bitter-acid' taste, excessive levels could be detrimental to wine quality.

Wine aroma can also be affected when glycosylated flavourless precursors, present in grapes, are hydrolysed by β-glucosidase enzymes to form free flavour-active volatiles (Dzialo et al., 2017). These enzymes are not encoded by the *S. cerevisiae* genome (Maicas & Mateo, 2016). However, non-*Saccharomyces* yeasts belonging to the genera *Debaryomyces, Hansenula*, *Candida, Pichia, Starmerella* and *Hanseniaspora* variably possess β-glucosidase activity (Maicas & Mateo, 2016) so can play a role in the expression of wine aroma.

Wines from some grape varieties are more amenable to improvement by the contribution of non-*Saccharomyces* yeasts than other varieties. For Chardonnay, co-fermentation with *Debaryomyces pseudopolymorphus* and *S. cerevisiae* led to increased concentrations of terpenols (citronellol, nerol and geraniol) in wine (Mateo & Maicas, 2016), although the effect on wine aroma was not investigated. Similarly, co-fermentation with *Debaryomyces vanriji* and *S. cerevisiae* produced Muscat wines with increased concentrations of several terpenols (Mateo & Maicas, 2016), that could make a positive contribution to the Muscat wine aroma. It was also shown that mixed cultures of *Starm. bacillaris* and *S. cerevisiae* or *T. delbrueckii* and *S. cerevisiae* produced Sauvignon Blanc wines with high concentrations of terpenols compared to reference wines fermented with only *S. cerevisiae* (Jolly et al., 2014). Varieties such as Sauvignon Blanc and Chenin Blanc depend on volatile thiols to contribute to the varietal character of the wine. It has been shown that non-*Saccharomyces* yeasts such as

Starm. bacillaris and *Pichia kluyveri* can produce significant amounts of the volatile thiols 3-sulfanyl hexanol (3SH) and 3-sulfanyl hexyl acetate (3SHA), respectively, in Sauvignon Blanc wines (Anfang, Brajkovich, & Goddard, 2009). Similarly, *T. delbrueckii*, *M. pulcherrima* and *L. thermotolerans* have also been described as being able to produce significant amounts of 3SH during Sauvignon Blanc fermentation (Fig. 2, Table 1).

Ethanol, although the main product of alcoholic fermentation, is a cause for concern for modern consumers, who now demand wines containing low to moderate alcohol levels. The use of non-*Saccharomyces* yeasts in fermentation can lead to lower ethanol yields due to lower sugar-ethanol transformation efficiencies when compared to *S. cerevisiae.* A possible counter effect is a high residual sugar concentration. Another natural approach to decease wine ethanol levels is to take advantage of the respiratory metabolism found in some non-*Saccharomyces* species (Gonzalez, Quirós, & Morales, 2013). It has been shown that using an aeration regime, alcohol content could be lowered by 1.5, 2.0 and 3.8% by *T. delbrueckii, Zygosaccharomyces bailii* and *M. pulcherrima,* respectively (Contreras et al., 2015). The trials were done in a chemically defined medium, so the effect of aeration on wine aroma was not established. With more intensive aeration, the use of *Williopsis saturnus* in a laboratory-scale protocol could produce a 3.0 % (v/v) ethanol wine from a 15% (w/v) total sugar grape juice that was judged to have an interesting, but acceptable estery and fruity sensory profile (Jolly et al., 2014).

Non-*Saccharomyces* yeasts have also been reported to affect the mouth-feel properties (texture or body) and colour of wine (Table 1; Fig. 1) by increasing polysaccharides concentrations (Domizio et al., 2011;) and affecting phenolic composition, respectively (Escribano-Viano et al., 2019). Polysaccharides can affect wine taste and mouth-feel positively by increasing the perception of wine viscosity and fullness on the palate. Specifically strains of *Hanseniaspora osmophila*, *Pichia fermentans*, *Saccharomycodes ludwigii*, Z. *bailii* and/or *Zygosaccharomyces florentinus* as mixed cultures with *S. cerevisiae* were found to produce wines with increased polysaccharides concentrations (Domizio et al., 2011).

Wine astringency, bitterness and colour is determined by phenolic content. Yeast cell walls can adsorb anthocyanins during fermentation. These anthocyanisn can then interact with mannoproteins and arabinogalactans in the wine. The degree of adsorption is generally dependent on the yeast species and strain Non-*Saccharomyces* yeasts therefore affect the composition of polyphenols (Escribano-Viano et al., 2019).

Sequential fermentation of grape juice enriched with anthocyanins using *Pichia guilliermondii* and *S. cerevisiae* lead to increased formation of vinylphenolic pyranoanthocyanins molecules, which showed greater wine colour stability (Benito, Morata,

Palomero, Gonzalez, & Suarez-Lepe, 2011). *T. delbrueckii* has also been shown to improve colour (anthocyanins) and mouthfeel (flavanols) of red wine, but this was dependent on grape variety and as already mentioned, the specific yeast strain (Escribano Viana et al., 2019).

Some non-*Saccharomyces* can also play a non-fermentative role in the wine production process by producing extracellular proteolytic and pectolytic (polygalacturonase) enzymes. These enzymes could potentially play a role in reducing wine protein levels with the accompanying increase in wine protein stability (Belda et al., 2016). Therefore, lower doses of extraneous enzymes would be needed bringing about cost savings to the producer. Non-*Saccharomyces* yeast can also deplete essential nutrients in the fermenting must adversely affecting the ability for *S. cerevisiae* to complete a sequential fermentation. However, contrary to this is the death and lysis of weaker non-*Saccharomyces* yeast cells during the earlier phases of fermentation that can in turn be a source of nutrients, especially nitrogen, for *S. cerevisiae* (Prior, Bauer, & Divol, 2019).

Non-*Saccharomyces* yeast metabolites acting against spoilage organisms e.g. *Brettanomyces bruxellensis,* is another area receiving attention (Mewa Ngongang et al., 2019; Di Gianvito et al., 2022). This has potential for application during wine production, maturation and storage to preserve the wine quality. The success of the use of non-*Saccharomyces* yeast in research wines has led to the commercialisation of a number of species. The phenotypic traits of the commercial yeast available in the market are shown in Fig. 2.

3. Mixed yeast alcoholic fermentations and their effect on LAB and malolactic fermentation

MLF is a secondary fermentation process that plays an important role in the production of many red and full-bodied white wines. During this secondary fermentation LAB are responsible for the enzymatic decarboxylation of L-malic to L-lactic acid thereby providing deacidification, with a concomitant increase in pH (Sumby, Bartle, Grbin, & Jiranek, 2019). Other benefits are the enhancement of microbial stability through removal of nutrients from the medium, and contributing to the flavour profile of the wine (Sumby et al., 2019). The LAB responsible for MLF, include the genera *Oenococcus*, *Lactobacillus sensu lato*, *Lactiplantibacillus*, *Pediococcus* and *Leuconostoc*. Over recent years, various reviews have been published giving increasing amounts of information on bacterial metabolism during MLF and the coexistence and compatibility of the LAB with yeast starter cultures (Krieger-Weber, Heras, & Suarez, 2020; Sumby et al., 2019). The phenotypic traits of the commercial LAB

available in the marker are presented in Fig. 2. *Oenococcus oeni* is best adapted to the harsh conditions found during fermentation, which includes high ethanol, low pH and the presence of SO_2. Concomitantly, the majority of the commercial LAB starter cultures belong to this species. In recent years, *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) has also been considered to be a promising LAB to be used as a malolactic starter culture (Krieger-Weber et al., 2020). This is mainly due to its ability to conduct MLF and produce a wide range of extracellular enzymes like glucosidases, b-glucosidases, esterases, phenolic acid decarboxylase (PAD) and citrate lyases able to enhance the sensorial properties of the wines to higher levels than that achieved by *O. oeni* strains. The glucosidase activity of *L. plantarum* strains are greatly affected by the environmental factors such as pH, ethanol and temperature of the medium, while it was found to be strain-dependent (Kriger-Weber et al., 2020). Previous studies on *L. plantarum* isolated from grape and wine samples demonstrated that 60% of the overall isolates possess genes encoding for esterases.

The selection of LAB species and strains within species, as well as the inoculation protocol (co-inoculation or sequential inoculation), is crucial to ensure a fast and successful MLF. This is due the interactions between LAB and yeasts having a direct effect on LAB growth and malolactic activity (Bartle, Sumby, Sundstrom, & Jiranek, 2019). Table 2 reports a summary of the main outcomes of these interactions on wine composition. Wine is considered a selective medium for LAB, especially when they are inoculated at the end of the alcoholic fermentation, due mainly to the presence of high levels of inhibitory compounds such as ethanol, SO_2 and organic acids (Sumby et al., 2019). Conducting MLF by controlled co-inoculation of yeasts together with LAB starter cultures has gained attention in recent years, due to the potential reduction of the duration of MLF. The selection of compatible yeast and LAB strains is fundamental in order to ensure a successful AF and MLF (Liu et al., 2017), as yeast species have been found to have either stimulatory, inhibitory or neutral effect on LAB and vice versa. These interactions are mainly associated with the ability of the yeast to consume or release nitrogen compounds and/or to produce metabolites that affect LAB metabolism (Liu et al., 2017). Most of the studies evaluated the interactions between S. *cerevisiae* and LAB, mainly *O. oeni*. Using different *S. cerevisiae* strains and two LAB species (*O. oeni* and *L. plantarum*), Englezos et al. (2019a) and Lucio, Pardo, Krieger-Weber, Heras, & Ferrer (2016) concluded that co-inoculation of *S. cerevisiae* with the above-mentioned microorganisms clearly affect lactic acid and titratable acidity in a LAB species-dependent manner. More specifically, wines that underwent MLF with *L. plantarum* completed MLF faster and contained higher levels of lactic acid compared to the respective wines inoculated with *O. oeni*.

However, the amount of lactic acid formed, was also dependant on the *S. cerevisiae* strain used to conduct AF.

The use of mixed starter cultures with non-*Saccharomyces* and *S. cerevisiae* can result in wines with chemical compositions that differ in ways that cannot be attained by *S. cerevisiae* in pure culture fermentations (Table 1). This concept is not new. However, the focus of interest has now moved to the specific phenotypic characteristics of the non-*Saccharomyces* yeasts aligned to consumption of nitrogen compounds and production of metabolites that positively or negatively affects the LAB starter culture (Gobert, Tourdot-Maréchal, Sparrow, Morge, & Alexandre, 2019). To date only a few studies have investigated how non-*Saccharomyces* species (*Starm. bacillaris*, *H. uvarum*, *M. pulcherrima*, *L. thermotolerans,* and *T. delbrueckii*) affect the growth and malolactic activity of LAB in MLF performed by *O. oeni* (Capozzi, Berbegal, Tufariello, Grieco, & Spano, 2019; Du Plessis et al., 2017) and *L. plantarum* (Du Plessis et al., 2019; Russo et al., 2020).

In general, it was found that co-inoculation with LAB does not affect yeasts behaviour during alcoholic fermentation (Russo et al., 2020). In contrast, non-*Saccharomyces* yeast influence LAB development and consequently, the MLF in terms of both technological i.e. fermentation time and compositional aspects i.e. primary and secondary metabolite production, in a species and strain dependent manner (du Plessis et al., 2017; Russo et al., 2020). In particular, it was observed that in pure culture fermentation with *Starmerella stellata* (previously *Candida stellata*) the MLF took longer to complete due to the yeast inhibiting the bacteria and reducing their cell numbers (du Plessis et al., 2017). Divergently, other non-*Saccharomyces* yeasts (*L. thermotolerans*, *M. pulcherrima* and *Starm. bacillaris*) had a beneficial effect on MLF duration in pure and mixed fermentations with *S. cerevisiae*, leading to wines with improved quality parameters, such as improved body (du Plessis 2019; Russo et al., 2020). A particular case is represented by *H. uvarum.* The fermentation of grape must with this non-*Saccharomyces* yeast in pure culture led to a slight inhibitory effect on MLF, possibly due to depletion of essential nutrients for the LAB, or the production of toxic metabolites against the LAB. In mixed fermentation with *S. cerevisiae*, *H. uvarum* had a positive effect on the growth of inoculated and naturally occurring LAB in comparison to *S. cerevisiae* only (du Plessis et al., 2019). This further illustrates how different yeast/bacteria interactions varyingly affect fermentation processes.

4. Microbial interactions during alcoholic and malolactic fermentations

As in several natural environments, wine microorganisms often form complex ecological ecosystems that result in the dominance of a specific species or a strain within a species, which then determines the final quality of wine (Knight, Klaere, Fedrizzi, & Goddard, 2015). These interactions are presented in Fig. 3 and may mediate one-way, two-way, and multi-way communications, which in turn could be intra-species, inter-species or inter-kingdom interactions (Arneborg, Appels, & Howell, 2019). Starting from the surface of the grapes in the vineyard, these interactions continue during both the primary AF and the secondary MLF leading to the hegemonic role of *S. cerevisiae* and *O. oeni*, respectively (Knight, Karon, & Goddard, 2020; Liu et al., 2018). Yeasts and LAB interactions are strongly influenced by several factors that will be discussed below and in Fig. 1.

4.1 Environmental conditions

One of the most important factors that should be considered in the study of interactions among different wine microorganisms is the role of environmental conditions. Furthermore, it is also important to remember that the wine ecosystem is continually changing due to the utilisation of compounds e.g. sugar and the production of alcohol, organic compounds, fatty acids, peptides and antimicrobial compounds by the microorganism involved (Branco, Viana, Albergaria, & Arneborg, 2015). As a result, compatibility between yeasts and LAB is affected by chemical and physical parameters that are strain and cultivar specific (Bartle et al., 2019). Several studies have investigated the effect of grape variety and vineyard management practices (organic, bio-dynamic or conventional) on the composition, number and biodiversity of indigenous yeasts and bacteria on grape berries (Martins et al., 2012). Although these studies all showed an effect on the diversity of yeasts and bacteria, the results cannot be generalized and are often contradictory.

Another important factor that can influence population dynamics is must composition. It was demonstrated that even small changes in must composition results in a critical affect on the growth and metabolism of wine yeasts and LAB, and thus affects the formation of aroma compounds. For example, Brou Taillandier, Beaufort, & Brandam, (2018) showed that a modification of nutrient concentration completely reversed the domination of *S. cerevisiae* in a mixed fermentation with *T. delbrueckii* and *S. cerevisiae*. In particular, they found that an increase in lipids affected growth and fermentation performance that was dependant on the nature of the lipid mixture, the yeast genus and the medium composition. Fatty acid content, as well as an increase in SO_2 addition, as part of winemaking, and a decrease in pH also

influence LAB ethanol tolerance (Bartle et al., 2019). Additionally, pH directly affects the growth and fermentation rate of yeasts and LAB, and the constitution of fermentation products (Bartle et al., 2019; Ciani et al., 2016). Consequently, this parameter is a determinant factor when choosing *O. oeni* or *L. plantarum* to conduct MLF. *O. oeni* is well adapted to low pH fermentations (pH below 3.5), while *L. plantarum* shows the best performances at higher pH values (pH above 3.5) (Krieger-Weber et al., 2020).

A decisive variable of microrganisms interactions is the nitrogen content of the must. Nitrogen depletion can lead to slow or sluggish alcoholic fermentations. Therefore, the addition of exogenous nitrogen sources is a common practice in wineries. Grape musts contain a wide range of YAN (yeast assimilable nitrogen) sources, including, not only amino acids and ammonium, but also urea and small peptides (Gobert et al., 2019). The YAN content is dependent on many factors including rootstock, irrigation, grape variety, climate, vine growing conditions and grape processing. During fermentation, a diverse pattern of nitrogen consumption has been observed for different yeasts species and strains (Englezos et al., 2018b; Su et al., 2019). Such diverse behaviour is related to both the nature of the nitrogen source (amino acids or ammonium) (Englezos et al., 2018b; Kemsawasd, Viana, Ardö, & Arneborg, 2015), as well as the type of amino acids required (Englezos et al., 2018b; Medina, Boido, Dellacassa, & Carrau, 2012; Su et al., 2020). Su et al. (2020) found that proline, generally considered an unassimilable nitrogen source for *S. cerevisiae* under anaerobic conditions, was consumed by non–*Saccharomyces* yeasts. Furthermore, in mixed fermentations with sequential inoculums, the non-*Saccharomyces* yeast species release significant amounts of nitrogen (and probably other nutrients) supporting the growth and fermentation of *S. cerevisiae* (Englezos et al., 2018b; Su et al., 2019) and LAB (Bartle et al., 2019).

4.2 Winemaking practices

Wine production involves numerous practices that affect the dynamics of microbial populations during fermentation. The most important are: harvesting (hand-picked or machine harvested grapes), manner of transportation to the winery, pre-fermentation operations such as method of crushing and/or juice extraction (pressing), juice clarification and SO_2 addition, and yeast/LAB inoculation. In winemaking, pre-fermentation operations comprise the time between grape harvest until the start of AF. This phase can last from a few hours to several days and leads to substantial changes in the indigenous biota (Albertin et al., 2014b). Albertin et al. (2014b) showed that pre-fermentation operations had a great impact on species with high

initial population in a Chardonnay grape must, such as *Hanseniaspora* spp. and *Starm. bacillaris*. In contrast, these two yeasts were less affected by cold settling of white grape juice than *H. anomala*, *Issatchenkia terricola* and *S. cerevisiae* (Grangeteau et al., 2017).

Maceration may also affect the grape must microbiota. In general, the dominance of yeasts and LAB starter cultures is easier to achieve in white musts than in red. This is probably due to contact with grape skins in red wine maceration that increase the quantity of yeasts naturally present that are able to compete with the starter culture. In fact, some authors found that the duration of inoculated MLF in sterile-filtered red wine samples was reduced, in comparison to the non-sterile must, due to reduction or complete elimination of competing microorganisms (Cinquanta, De Stefano, Formato, Niro, & Panfili, 2018). Furthermore, Guzzon Malacarne, Larcher, Franciosi, & Toffanin, (2020) found that carbonic maceration (delayed crushing for some days while grapes are anaerobically stored in fermentation vats), used in some wine regions like Beaujolais and the Rhone Valley in France or Rioja in Spain, had a strong impact on the evolution of the microbiota during fermentation. In that study, carbonic maceration, and consequently the unavailability of oxygen, affected the biodiversity and the development of the microbial groups usually found during fermentation. It was especially *Saccharomyces* spp. that were characterized by a slow development. Other researchers studied the effect of grape juice saturation with CO_2 and highlighted that growth of *H. uvarum* and *Starm. bacillaris* was strongly inhibited, while *Metschnikowia* spp., *P. kluyveri* and *T. delbrueckii* species were promoted (Chasseriaud, Coulon, Marullo, Albertin, & Bely, 2018).

Oxygen concentration is one of the main forces driving microbial growth during fermentation (Guzzon et al., 2020) and consequently, yeast and bacteria interactions. During fermentation the decrease in levels of oxygen are dependent on the shape and size of the vats, as well CO_2 released. However, oxygen can be supplied to fermenting must to facilitate yeast biomass accumulation and to promote colour extraction in red wines (Gonzalez et al., 2013). Several authors demonstrated that during wine fermentation, changes in the initial aeration regime had a strong impact on the growth of non-*Saccharomyces* yeasts in mixed culture fermentations. In particular, *M. pulcherrima* (Morales, Rojas, Quirós, & Gonzalez, 2015), *Starm. bacillaris* (Englezos et al., 2019), *Hanseniaspora vinae*, *T. delbrueckii*, *L. thermotolerans* (Yan, Zhang, Joseph, & Waterhouse, 2020) and *Saccharomyces kudriavzevii* (Arroyo-López, Pérez-Través, Querol, & Barrio, 2011) were able to survive and coexist for longer period with *S. cerevisiae* when oxygen was added to the fermentation medium. Recently, oxygen addition to fermenters, under a controlled flowrate, was applied to promote

441 the respiratory consumption of sugars by non-*Saccharomyces* yeasts in order to reduce alcohol
442 content in the wines (Gonzalez et al., 2013; Alonso-del-Real, Contreras-Ruiz, Castiglioni,
443 Barrio, & Querol, 2017a). Judicious addition of oxygen could help increase the overall impact
444 of non-*Saccharomyces* yeasts on wine quality, accelerate transformation of phenols to reduce
445 astringency and avoid the excessive production of unpleasant metabolites, such as acetic acid.
446 Another oenological practice that can influence interactions between microorganisms
447 is the fermentation temperature, due to its effect on microbial performance. This evidence was
448 widely reported for yeasts in pure and mixed fermentations (Arroyo-López, Orlić, Querol, &
449 Barrio, 2009). During wine fermentation, temperatures naturally increase mainly due to *S.*
450 *cerevisiae* fermentative activity. Although the fermentation temperature is usually controlled
451 in modern wineries, any increase represents an inhibition factor for temperature sensitive
452 species (Liu et al., 2017). However, at lower temperatures e.g. 10°C and 15°C, ethanol
453 tolerance of non-*Saccharomyces* yeasts is higher enabling a stronger contribution in low-
454 temperature fermentations (Jolly et al., 2014). This phenomenon is also evident within the
455 *Saccharomyces* genus. Alonso- del- Real, Lairón-Peris, Barrio, & Querol (2017b) evaluated
456 the performance of *S. cerevisiae* and *Saccharomyces* non-*cerevisiae* strains in mixed culture
457 fermentations at different temperatures. These authors revealed that cryotolerant
458 *Saccharomyces* non-*cerevisiae* particularly *S. uvarum*, has a notable effect on *S. cerevisiae*
459 dominance at low and intermediate temperatures (8, 12 and 20°C). This clarifies why *S. uvarum*
460 can replace *S. cerevisiae* during wine fermentations in European regions with oceanic and
461 continental climates (Alonso- del- Real et al., 2017b), where *S. uvarum* can be found naturally
462 on grapes.
463 The use of SO_2 as an antioxidant and antimicrobial agent is known since Roman times
464 where it was used to prevent food and beverage spoilage. In winemaking, SO_2 is often added
465 at the end of the fermentation process or before bottling to act as a preservative agent, however,
466 it is mostly used before the start of the fermentation. At this stage, it promotes the establishment
467 of *S. cerevisiae* as the dominant yeast because generally non-*Saccharomyces* yeasts (*Candida*,
468 *Cryptococcus*, *Hanseniaspora* and *Metschnikowia*), LAB and acetic acid bacteria are more
469 sensitive to SO_2 (Albertin et al., 2014b). In this context, Cinquanta et al. (2018) found that SO_2
470 has a major effect against LAB at low pH where there is a high percentage of SO_2 in the
471 molecular form. Additionally, during wine fermentation yeasts can release SO_2 due to their
472 metabolism. Generally, *S. cerevisiae* strains can produce more than 100 mg/L SO_2. Information
473 regarding non-*Saccharomyces* yeasts is lacking.

Given the importance of SO_2 and the synergic effect of pH together with ethanol on the survival of specific microorganisms, knowledge of the tolerance of this metabolite by the microorganisms present during the fermentation process is necessary. This can lead to the desired reduction of added SO_2 levels in wine (to satisfy consumers) while avoiding the inhibition of the microorganisms necessary during the winemaking process.

The development of large-scale fermentations, as often required in commercial wineries, highlighted the unpredictability and complexity of spontaneous fermentations due to the interactions among microorganisms. Therefore, to maintain repeatable results, the use of selected cultures tailored to complete AF and MLF has become the norm in commercial wineries. However, the dominance of a specific starter culture depends on factors such as the species/strain used, the yeast/yeast or yeast/bacteria combination chosen, the inoculum size and ratio, and the rehydration conditions. The species that inhabit the must ecosystem have different responses to wine fermentation parameters and the behaviour of the non-*Saccharomyces* yeast is influenced by *S. cerevisiae* and vice versa (Bagheri, Bauer, & Setati, 2017). Generally, in the presence of *S. cerevisiae*, populations of *Wickerhamomyces anomalus*, *M. pulcherrima*, *Pichia terricola*, and *Candida parapsilosis* decrease in the early stages of the fermentation, while *L. thermotolerans, T. delbrueckii* and *Starm. bacillaris* survive until late stages of fermentation. The presence of non-*Saccharomyces* yeasts in the initial stages of the alcoholic fermentation could limit the growth of *S. cerevisiae* yeasts by utilizing large quantities of nitrogen and oxygen from the must (Liu et al., 2017). However, in contrast, growth of *H. vineae* is promoted by the presence *S. cerevisiae* suggesting a positive interaction between these two yeasts (Bagheri et al., 2017).

The diversity of yeasts involved in the AF affects the growth of LAB and their capacity to conduct MLF (Du Plessis et al., 2017, 2019; Capozzi et al., 2019). Du Plessis et al. (2017) found that *S. cerevisiae*, *T. delbrueckii* and *M. pulcherrima* possessed a larger inhibitory effect on the levels of the naturally occurring LAB than *Starm. bacillaris* and *H. uvarum.* The reduced MLF duration in mixed fermentations using *Starm. bacillaris* co-inoculated with *S. cerevisiae* was probably due to the chemical composition of the medium. Firstly, *Starm. bacillaris* was found to produce less ethanol compared to sugar consumed, implying that *O. oeni* had more favourable environmental conditions for growth and consumption of malic acid. Secondly, *Starm. bacillaris* consumed less nitrogen compounds, compared to *S. cerevisiae,* further benefiting the growth of the LAB. Results from *L. thermotolerans* trials were conflicting thereby highlighting that interactions are also strain-specific and not only species-specific (Bagheri et al., 2017; Du Plessis et al., 2017). The MLF inoculation strategy is also important

and Capozzi et al. (2019) found that some *O. oeni* strains showed better malolactic activity when co-inoculated with the selected yeasts at 0% (v/v) ethanol or added up to 4% (v/v) of ethanol.

The size and ratio of the yeast inoculum is a key parameter for a successful pure and mixed (multistarter) fermentation (Comitini et al., 2011). In a multistarter fermentation, inoculum ratios of 10:1, 100:1 and 10,000:1 (non-*Saccharomyces*:*S. cerevisiae*) caused a reduced or delayed growth of *S. cerevisiae*. In contrast, an inoculum ratio of 1:1 between non-*Saccharomyces* yeasts (*C. zemplinina*, *L. thermotolerans*, *M. pulcherrima* and *T. delbrueckii*) and *S. cerevisiae* did not affect the performance of the second yeast (Comitini et al., 2011; Medina et al., 2012). However, inhibition was not observed between *S. kudriavzevii* and *S. cerevisiae* in low temperature fermentation (Alonso-del-Real et al., 2017a) demonstrating the synergistic effect of temperature and inoculum sizes.

Co- or sequential inoculation of *S. cerevisiae* has a great impact on the performance of the non-*Saccharomyces* yeasts. In general, when simultaneously inoculated, *S. cerevisiae* shows a highly antagonistic behaviour and reduces the other population in comparison to sequential inoculations (Table 1). The chemical composition of the wine produced from simultaneously inoculated fermentations is very similar to the respective pure fermented wine with *S. cerevisiae* only. On the contrary, in sequential fermentations the initial growth of the non-*Saccharomyces* yeasts enables further modulation of metabolites of oenological interest due the ability of this group of species to achieve higher population levels and be present for a longer time, in comparison to the respective co-inoculated fermentations.

4.3 Interaction mechanisms

In the wine ecosystem, microorganisms interact at different levels. Firstly, they are driven by the need to consume nutrients. Secondly, their existence necessarily leads to physical contact with each other as well as the production of metabolites that can affect other populations, either as a source of nutrients, or by producing inhibitory factors. In the following two sections the mechanisms responsible for the above-mentioned interactions will be further discussed in relation to the various steps of the winemaking process.

4.3.1 Interactions concerning substrate

During wine fermentation, all microorganisms must consume nutrients from the same source, so competition between different populations takes place. Starting from the grape crushing, yeasts consume oxygen, sugars, nitrogen, vitamins and lipids (Brou et al., 2018) thereby determining the inhibition level for other species. In general, spontaneous and co-inoculated wine fermentations end with the dominance of the glucophilic *S. cerevisiae,* due to its extensively reprogrammed gene expression during the first phases of the fermentation. This change results in an enhanced nutrient uptake and an up-regulation of genes involved in amino acids, vitamins and lipids uptake. This behaviour was observed in competition against bacteria, non-*Saccharomyces* and *Saccharomyces* non-*cerevisiae* yeasts (Bartle et al., 2019). Yeasts such as *Starm. bacillaris* and *H. uvarum* can probably survive during fermentation due to their fructophilic nature (ability to consume fructose preferentially to glucose as a carbon source) enabling them to compete against *S. cerevisiae* (Fig. 1). Divergently, when a sequential inoculation is followed, *S. cerevisiae* performs poorly in comparison to a pure fermentation equivalent. This is independent from the non-*Saccharomyces* used (Medina et al., 2012; Lleixà, Manzano, Mas, & Portillo, 2016). A sluggish or stuck fermentation has been attributed to nutrient unavailability (Gobert et al., 2019). This was observed for S. *cerevisiae* in mixed fermentations with *Hanseniaspora* spp., *M. pulcherrima* and *T. delbrueckii*, and was probably due to nitrogen and vitamin depletion (Medina et al., 2012). In a mixed fermentation between *L. thermotolerans* and *S. cerevisiae*, Petigonnet et al. (2019) showed that the non-*Saccharomyces* yeast consumed most of the oxygen and approximately 68% of the *β*-sitosterol, 14% of the stigmasterol and all the campesterol content present in the must in only 24 h of fermentation. Consequently, *S. cerevisiae* growth was slow, as ergosterol and unsaturated fatty acids biosynthesis were inhibited due to the oxygen unavailability (enzymes for their formation are oxygen-dependent) and because phytosterols needed to replace ergosterol in the membrane had been consumed (Petigonnet et al., 2019).

Competition for nutrients have differing outcomes dependant on the yeast species involved. Yeasts with complex nutrient requirements show an increased antagonistic behaviour with LAB (Bartle et al., 2019). Some *S. cerevisiae* strains and non-*Saccharomyces* yeasts (*T. delbrueckii*, *Starm. bacillaris*, *M. pulcherrima*, *I. orientalis* and *Schizosaccharomyces* spp.) are able to consume L-malic acid that then becomes unavailable for LAB during MLF (Balmaseda, Bordons, Reguant, & Bautista-Gallego, 2018). Nutrient depletion has an essential role in promoting wine shelf life. During fermentation LAB consume L-malic acid and other nutrients. This impoverishes the wine and prevents the development of contaminant microorganisms (Balmaseda et al., 2018).

During wine fermentation, nutrients may also lead to mutualism (positive interactions between species). Some yeasts are able to produce or release amino acids and vitamins that can stimulate LAB growth (Ivey, Massel, & Phister, 2013). This cross feeding was observed between *S. cerevisiae* and *L. plantarum* in grape juice (Ponomarova et al., 2017). It was highlighted that the yeast released amino acids and other metabolites able to stimulate the growth of the LAB strain. Furthermore, it was demonstrated that this metabolic dependency of *L. plantarum* was unidirectional and was conserved among diverse yeast isolates. Another nutrient source is a consequence of yeast autolysis when weaker yeast cells die off during fermentation. This phenomenon is characterized by the release of extra nitrogen sources that can be used by LAB as nutrient source during MLF, and by *S. cerevisiae* when it is added in the wine towards the end of fermentation (Lleixà et al., 2016).

4.3.2 Chemical-physical interactions

Throughout the last decade, many studies demonstrated that the reduction in the numbers of non-*Saccharomyces* yeasts during early to late stages of mixed culture wine fermentations involves physical cell-to-cell contact. In non-*Saccharomyces/S. cerevisiae* mixed culture fermentations conducted in double compartment fermentors (in which a membrane separates the cells of the two species), the disappearance of non-*Saccharomyces* yeasts was not associated with nutrient limitation or the presence of inhibitory compounds. It was concluded that the reduction was induced by direct physical contact through receptor/ligand like interactions. This phenomenon was observed when *S. cerevisiae* populations reached high cell densities in fermentation with *Starm. bacillaris* (Englezos et al., 2019b), *L. thermotolerans* (Petitgonnet et al., 2019), *T. delbrueckii* (Branco et al., 2017a), *Hanseniaspora* spp. (Rossouw et al., 2015), *K. marxianus* (Lopez, Beaufort, Brandam, & Taillandier, 2014), *H. uvarum*, *M. fructicola*, *P. kudriavzevii* or *Cr. flavescens* (Borded et al., 2020; Rossouw et al., 2015). Other authors observed that a contact-dependent mechanism also occurs in intra-species competition, highlighting that physical contact is a prerequisite for dominance (Pérez-Torrado et al., 2017). It was also shown by Kemsawasd et al. (2015) that the association between cell-to-cell contact and other inhibitory factors (antimicrobial peptides [AMPs]) was responsible for *L. thermotolerans* death during mixed-culture fermentation with *S. cerevisiae*.

Antimicrobial compounds (AMCs) such as fatty acids, peptides, proteins, SO_2 and other molecules are produced by yeasts (Liu et al., 2017). Additionally, LAB in wine are able

609 to excrete carboxylic acids, proteases, glucanases and bacteriocins (Balmaseda et al., 2018;
610 Bartle et al, 2019). The aforementioned can all have an effect on the yeast and bacterial
611 population in the wine. The use of antimicrobial compounds is an attractive topic for many
612 researchers, due to consumer demands for safer alternatives to SO_2. However, sometimes it is
613 difficult to understand which molecules are responsible for the inhibition. Simonin et al. (2018)
614 found that the inoculation of *T. delbrueckii* at the start of AF induced a decrease in must
615 biodiversity, spoilage microorganisms included. However, they could not explain the cause of
616 this observation. In addition, Mewa-Ngongang et al. (2019) demonstrated that *C. pyralidae* and
617 *P. kluyveri* showed growth inhibition activity against spoilage yeasts and fungi namely *D.*
618 *bruxellensis*, *D. anomala*, *Z. bailii*, *Botrytis cinerea*, *C. acutatum* and *Rhizopus stolonifera in*
619 *vitro* and on fruits (grapes and apples). These authors found that both direct contact and
620 extracellular volatile organic compounds (VOCs) were two of the mechanisms of inhibition.
621 VOCs include alcohols, organic acids and esters previously described with antimicrobial
622 properties. However, it was not clear which compound, or combinations were responsible for
623 the growth inhibition activity.

624 Antimicrobial compounds, and specifically AMPs have been proposed for use in the
625 biocontrol of undesired microorganisms during winemaking. Peptides are generally used as
626 host defence molecules, but some microorganisms are able to produce AMPs with the purpose
627 of ensuring survival (Mahlapuu, Håkansson, Ringstad, & Björn, 2016). However, this
628 biocontrol strategy has not been thoroughly investigated against wine-related spoilage
629 microorganisms (Di Gianvito et al., 2022).

630 *S. cerevisiae* is able to release an AMP called "Saccharomycin" (Kemsawasd et al.,
631 2015; Branco et al. 2017a). This is a natural biocide (2–10 kDa) active against several wine-
632 related non-*Saccharomyces* yeasts and LAB (Branco et al., 2014, Kemsawasd et al. 2015;
633 Branco et al., 2017a, 2019). Branco et al. (2017a) demonstrated that "Saccharomycin" is a
634 fragment of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an
635 energy metabolism-related enzyme. These authors revealed that during wine fermentation this
636 peptide is involved in the death of non-*Saccharomyces* yeasts by cell-to-cell contact, because
637 GAPDH-derived AMPs accumulate on the *S. cerevisiae* cell surface at the end of the growth
638 phase (24 - 48 h). With reference to non-*Saccharomyces* yeasts, a recent study reported the
639 release of an AMP by the *C. intermedia* strain LAMAP1790. This peptide affected the growth
640 of several strains of the spoilage yeast, *B. bruxellensis*, without influencing the *S. cerevisiae*
641 performance during fermentation (Peña & Ganga, 2019).

The production of yeast killer toxins, a characteristic first observed in *S. cerevisiae*, is well distributed in several yeast genera such as *Candida*, *Hansenula*, *Pichia*, *Williopsis*, *Tetrapisispora*, *Schwanniomyces*, *Debaryomyces*, *Ustilago*, *Cryptococcus*, *Metschnikowia*, *Williopsis*, *Kluyveromyces* and *Zygosaccharomyces* (Liu et al., 2017). Yeast killer toxins are effective under wine conditions and for this reason the production of killer toxins are often a sought after characteristic for wine yeast starter culture selections. Killer toxins are able to inhibit *S. cerevisiae* as well as spoilage yeast species in the presence of reduced SO_2 concentrations (Di Gianvito et al., 2022). Oro, Ciani, Bizzaro, & Comitini (2016) found that Kwkt and Pikt, two killer toxins produced by *K. wickerhamii* and *W. anomalus*, respectively, had an antimicrobial activity against *B. bruxellensis*. Furthermore, Mehlomakulu, Prior, Setati, & Divol (2017) exposed this spoilage yeast to the killer toxin CpKT1 produced by *C. pyralidae* and revealed that the loss of viability was due to damages to the cell membrane and cell wall. Mazzucco, Ganga, & Sangorrín (2019), also observed an inhibition against *B. bruxellensis*. These authors studied the killer toxin SeKT, produced by *Saccharomyces eubayanus,* in wine, demonstrating that this protein could be used for the biocontrol of four common spoilage wine yeasts (*B. bruxellensis*, *Pichia membranifaciens*, *P. guilliermondii* and *Pichia manshurica*). However, further studies are necessary to understand the efficacy against undesired microorganisms under real winemaking conditions. A factor missing in some investigations is the determination whether populations die off or if they enter in a viable but not culturable (VBNC) state. In this context, Branco et al. (2015) demonstrated that interactions through excreted compounds determined the VBNC status of *Hanseniaspora guilliermondii* during fermentation.

5. Conclusion and future perspectives

Currently, mixed fermentations with selected non-*Saccharomyces* and *S. cerevisiae* starter cultures are considered a state of art strategy to modulate the production of target metabolites. Investigations on how these species interact with each other and/or with LAB are developing fast, highlighting the potential future directions in this research area. However, more comprehensive data is needed to further uncover the nature of these interactions. This will permit the management of starter cultures in specific inoculation protocols and winemaking conditions in order to increase their metabolic activity and survival time (Morrison-Whittle, Lee, Fedrizzi, & Goddard, 2020). This will ensure their dominance and enhance their contribution to the final wine.

A relatively unexplored field that requires more detailed investigation is the impact of mixed culture fermentations on the LAB responsible for MLF. Recent studies demonstrated that certain non-*Saccharomyces* yeast caused a strong inhibition or stimulated the growth and malolactic activity of *O. oeni* and *L. plantarum*, but more clarification is required before a practical application can be devised.

In recent years, there has been a rapid increase in omics-methodology based studies, with the aim to extract more information from the wine microbiome. The integration of multiple-omics approaches has revealed molecular based information and enhanced the existing knowledge regarding microbial diversity during the various steps of wine production. This knowledge will help to further understand the complex interactions between microorganisms, the substrate and physical fermentations conditions. However, the overall potential of combining the different omics approaches remains underexploited and there are significant challenges to be addressed before any of these techniques become a routine procedure (Siren et al., 2019). Among the different challenges is the difficulty to extract RNA from grape must and wine, due to the increased levels of inhibitors, such as polyphenols and polysaccharides. Furthermore, since most of the studies are performed in a synthetic grape must medium, the optimization of omics-analysis in samples of natural origin need attention. This could help reveal the effect of specific stress conditions during the fermentation process and identify metabolic pathways that lead to the formation of metabolites responsible for wine quality. Such approaches could help to predict the population dynamics and biochemical activities of yeasts and bacteria and allow better control of their growth during the fermentation process. This will have a positive impact on wine quality, according to the needs of wine producers.

Continued advances in the knowledge of microbial interactions could provide many opportunities for innovation and adaptation to a changing market, as recently proposed by Di Gianvito et al. (2022). This will enable the development of new products based on the ability of the starter cultures to control the growth of spoilage microorganisms. More research is required to identify the mechanisms of action exerted by wine yeasts and LAB during the different steps of wine production. Mainstream consumer's demand for diverse wine styles and their increasing concern of the effects of chemical preservatives (such as SO_2) on human health present new challenges for innovation in wine industries. Legislation regarding permitted additives to wine and the continuous search for wines without, or reduced levels of SO_2, are likely to have a cascading effect on microbial community dynamics during wine production. Consequently, a comprehensive understanding of microbial interactions during wine

fermentation will be a key factor for the future elaboration of quality wines. This will assist in addressing the challenges and opportunities (Table 3) that lie ahead in winemaking industries.

References

1. Aplin, J. J., Paup, V. D., Ross, C. F., & Edwards, C. G. (2021). Chemical and Sensory Profiles of Merlot Wines Produced by Sequential Inoculation of *Metschnikowia pulcherrima* or Meyerzyma guilliermondii. *Fermentation*, *7(3)*, 126. https://doi.org/10.3390/fermentation7030126

2. Albertin, W., Chasseriaud, L., Comte, G., Panfili, A., Delcamp, A., Salin, F., et al. (2014a). Winemaking and bioprocesses strongly shaped the genetic diversity of the ubiquitous yeast *Torulaspora delbrueckii*. *PLoS One*, *9(4)*, e94246. https://doi.org/10.1371/journal.pone.0094246.

3. Albertin, W., Zimmer, A., Miot-Sertier, C., Bernard, M., Coulon, J., Moine, V., et al. (2017). Combined effect of the *Saccharomyces cerevisiae* lag phase and the non-*Saccharomyces* consortium to enhance wine fruitiness and complexity. *Applied Microbiology and Biotechnology*, *101*, 7603-7620. https://doi.org/10.1007/s00253-017-8492-1

4. Alonso-del-Real, J., Contreras-Ruiz, A., Castiglioni, G. L., Barrio, E., & Querol, A. (2017a). The use of mixed populations of *Saccharomyces cerevisiae* and *S. kudriavzevii* to reduce ethanol content in wine: limited aeration, inoculum proportions, and sequential inoculation. *Frontiers in Microbiology*, *8*, 2087. https://doi.org/10.3389/fmicb.2017.02087.

5. Alonso-del-Real, J., Lairón-Peris, M., Barrio, E., & Querol, A. (2017b). Effect of temperature on the prevalence of *Saccharomyces* non *cerevisiae* species against a *S.*

cerevisiae wine strain in wine fermentation: competition, physiological fitness, and influence in final wine composition. *Frontiers in Microbiology*, *8*, 150. https://doi.org/10.3389/fmicb.2017.00150.

6. Anfang, N., Brajkovich, M., & Goddard, M. R. (2009). Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc. *Australian Journal of Grape and Wine Research*, 15, 1-8. https://doi.org/10.1111/j.1755-0238.2008.00031.x

7. Arroyo-López, F. N., Pérez-Través, L., Querol, A., & Barrio, E. (2011). Exclusion of *Saccharomyces kudriavzevii* from a wine model system mediated by *Saccharomyces cerevisiae*. *Yeast*, *28*, 423–435. https://doi.org/10.1002/yea.1848.

8. Bagheri, B., Bauer, F. F., & Setati, M. E. (2017). The impact of *Saccharomyces cerevisiae* on a wine yeast consortium in natural and inoculated fermentations. *Frontiers in Microbiology*, *8*, 1988. https://doi.org/10.3389/fmicb.2017.01988.

9. Balmaseda, A., Bordons, A., Reguant, C., & Bautista-Gallego, J. (2018). Non-*Saccharomyces* in wine: effect upon *Oenococcus oeni* and malolactic fermentation. *Frontiers in Microbiology*, *9*, 534. https://doi.org/10.3389/fmicb.2018.00534.

10. Balikci, E. K., Tanguler, H., Jolly, N. P., & Erten, H. (2016). Influence of *Lachancea thermotolerans* on cv. Emir wine fermentation. *Yeast*, *33(7)*, 313-321. https://doi.org/10.1002/yea.3166.

11. Bartle, L., Sumby, K., Sundstrom, J., & Jiranek, V. (2019). The microbial challenge of winemaking: yeast-bacteria compatibility. *FEMS Yeast Research*, *19*, foz040. https://doi.org/10.1093/femsyr/foz040.

12. Benito, S., Morata, A., Palomero, F., Gonzalez, M. C., & Suarez-Lepe, J. A. (2011). Formation of vinylphenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *Pichia guillermondii* in red wines produced following different fermentation strategies. *Food Chemistry,* 124, 15-23. https://doi.org/10.1016/j.foodchem.2010.05.096.

13. Benito, S. (2018). The impact of *Torulaspora delbrueckii* yeast in winemaking. *Applied Microbiology and Biotechnology*, *102*, 3081–3094. https://doi.org/10.1007/s00253-018-8849-0.

14. Belda, I., Navascués, E., Marquina, D., Santos, A., Calderon, F., & Benito, S. (2015). Dynamic analysis of physiological properties of *Torulaspora delbrueckii* in wine fermentations and its incidence on wine quality. *Applied Microbiology and Biotechnology*, *99(4)*, 1911-1922. https://doi.org/10.1007/s00253-014-6197-2.

15. Belda, I., Ruiz, J., Alastruey-Izquierdo, A., Navascués, E., Marquina, D., & Santos, A. (2016). Unraveling the Enzymatic Basis of Wine "Flavorome": A Phylo-Functional Study of Wine Related Yeast Species. *Frontiers in Microbiology*, *7*, 12. https://doi.org/10.3389/fmicb.2016.00012

16. Belda, I., Ruiz, J., Beisert, B., Navascués, E., Marquina, D., Calderón, F., et al. (2017). Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. *International Journal of Food Microbiology*, *257*, 183-191. https://doi.org/10.1016/j.ijfoodmicro.2017.06.028.

17. Binati, R.L., Lemos Junior, W.J.F., Luzzini, G., Slaghenaufi, D., Ugliano, M., Torriani, S.. (2019). Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on *Lachancea thermotolerans, Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. *International Journal of Food Microbiology*, *318*, 108470. https://doi.org/10.1016/j.ijfoodmicro.2019.108470

18. Branco, P., Viana, T., Albergaria, H. & Arneborg, N. (2015). Antimicrobial peptides (AMPs). produced by *Saccharomyces cerevisiae* induce alterations in the intracellular pH, membrane permeability and culturability of *Hanseniaspora guilliermondii* cells. *International Journal of Food Microbiology, 205*, 112–118. https://doi.org/10.1016/j.ijfoodmicro.2015.04.015.

19. Branco, P., Francisco, D., Monteiro, M., Almeida, M. G., Caldeira J, Arneborg, N., et al. (2017a). Antimicrobial properties and death-inducing mechanisms of saccharomycin, a biocide secreted by *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology, 101*, 159 – 171. https://doi.org/10.1007/s00253-016-7755-6.

20. Branco, P., Kemsawasd, V., Santos, L., Diniz, M., Caldeira, J., Almeida, M. G., et al. (2017b) *Saccharomyces cerevisiae* accumulates GAPDH-derived peptides on its cell surface that induce death of non-*Saccharomyces* yeasts by cell-to-cell contact. *FEMS Microbiology Ecology, 93*, fix055. https://doi.org/10.1093/femsec/fix055.

21. Branco, P., Sabir, F., Diniz, M., Carvalho, L., Albergaria, H., & Prista, C. (2019). Biocontrol of *Brettanomyces*/*Dekkera bruxellensis* in alcoholic fermentations using saccharomycin-overproducing *Saccharomyces cerevisiae* strains. *Applied*

Microbiology and Biotechnology, *103*, 3073-3083. https://doi.org/10.1007/s00253-019-09657-7.

22. Brizuela, N. S., Franco-Luesma, E., Bravo-Ferrada, B. M., Pérez-Jiménez, M., Semorile, L., Tymczyszyn, E. E., et al. (2021). Influence of Patagonian *Lactiplantibacillus plantarum* and *Oenococcus oeni* strains on sensory perception of Pinot Noir wine after malolactic fermentation. *Australian Journal of Grape and Wine Research*, *27(1)*, 118-127. https://doi.org/10.1111/ajgw.12460.

23. Brou, P., Taillandier, P., Beaufort, S., & Brandam, C. (2018). Mixed culture fermentation using *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* with direct and indirect contact: impact of anaerobic growth factors. *European Food Research and Technology*, *244*, 1699-1710. https://doi.org/10.1007/s00217-018-3095-3

24. Capozzi, V., Berbegal, C., Tufariello, M., Grieco, F., & Spano, G. (2019). Impact of co-inoculation of *Saccharomyces cerevisiae*, *Hanseniaspora uvarum* and *Oenococcus oeni* autochthonous strains in controlled multi starter grape must fermentations. LWT—Food Science and Technology, *109*, 241-249. https://doi.org/10.1016/j.lwt.2019.04.045

25. Chasseriaud, L., Coulon, J., Marullo, P., Albertin, W., *&* Bely, M. (2018). New oenological practice to promote non-*Saccharomyces* species of interest: saturating grape juice with carbon dioxide. *Applied Microbiology and Biotechnology*, *102*, 3779-3791. https://doi.org/10.1007/s00253-018-8861-4.

26. Cheraiti, N., Guezenec, S., & Salmon, J. M. (2005). Redox interactions between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in mixed culture under

oenological conditions. *Applied and Environmental Microbiology*, *71*, 255–260. https://doi.org/10.1128/AEM.71.1.255-260.2005

27. Ciani, M., Capece, A., Comitini, F., Canonico, L., Siesto, G., & Romano, P. (2016). Yeast Interactions in Inoculated Wine Fermentation. *Frontiers in Microbiology*, 7, 555. https://doi.org/10.3389/fmicb.2016.00555

28. Cinquanta, L., De Stefano, G., Formato, D., Niro, S., & Panfili, G. (2018). Effect of pH on malolactic fermentation in southern Italian wines. *European Food Research and Technology*, *244*, 1261-1268. https://doi.org/10.1007/s00217-018-3041-4.

29. Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., & Ciani, M. (2011). Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiology*, *28*, 873-882. https://doi.org/10.1016/j.fm.2010.12.001.

30. Contreras, A., Hidalgo, C., Schmidt, S., Henschke P. A. Curtun, C. & Varela, C. (2015). The application of non-*Saccharomyces* yeast in fermentations with limited aeration as a strategy for the production of wine with reduced alcohol content. *International Journal of Food Microbiology, 205*, 7-15. https://doi.org/10.1016/j.ijfoodmicro.2015.03.027.

31. Di Gianvito, P., Englezos, V., Rantsiou, K., & Cocolin, L. (2022). Bioprotection strategies in winemaking. *International Journal of Food Microbiology*, 109532. https://doi.org/10.1016/j.ijfoodmicro.2022.109532

32. Dìez, L., Rojo-Bezares, B., Zarazaga, M., Rodrıguez, J. M., Torres, C., & Ruiz-Larrea, F. (2012). Antimicrobial activity of pediocin PA-1 against *Oenococcus oeni* and other wine bacteria. *Food Microbiology, 31*, 167–172. https://doi.org/10.1016/j.fm.2012.03.006

33. Diez-Ozaeta, I., Lavilla, M., & Amárita, F. (2021). Wine aroma profile modification by Oenococcus oeni strains from Rioja Alavesa region: selection of potential malolactic starters. *International Journal of Food Microbiology*, *356*, 109324. https://doi.org/10.1016/j.ijfoodmicro.2021.109324.

34. Domizio, P., Romani, C., Lencioni, L., Comitini, F., Gobbi, M., Mannazzu, I., et al. (2011). Outlining a future for non-*Saccharomyces* yeasts: Selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *International Journal of Food Microbiology*, *147*, 170-180. https://doi.org/10.1016/j.ijfoodmicro.2011.03.020.

35. Domizio, P., Liu, Y., Bisson, L.F., & Barille, D. (2014). Use of non-*Saccharomyces* wine yeasts as novel sources of mannoproteins in wine. *Food Microbiology*, *43*, 5-15. https://doi.org/10.1016/j.fm.2014.04.005.

36. Du Plessis, H. W., Du Toit, M., Hoff, J. W., Hart, R. S., Ndimba, B. K., & Jolly, N. P. (2017). Characterisation of non-*Saccharomyces* yeasts using different methodologies and evaluation of their compatibility with malolactic fermentation. *South African Journal of Enology and Viticulture*, *38*, 46-63. https://doi.org/10.21548/38-1-819.

37. Du Plessis, H., Du Toit, M., Nieuwoudt, H., Van der Rijst, M., Hoff, J., & Jolly, N. (2019). Modulation of wine flavor using *Hanseniaspora uvarum* in combination with

different *Saccharomyces cerevisiae*, lactic acid bacteria strains and malolactic fermentation strategies. *Fermentation*, *5*(3), 64. https://doi.org/10.3390/fermentation5030064.

38. Dutraive, O., Benito, S., Fritsch, S., Beisert, B., Patz, C. D., & Rauhut, D. (2019). Effect of sequential inoculation with non-*Saccharomyces* and *Saccharomyces* yeasts on Riesling wine chemical composition. *Fermentation*, *5(3)*, 79. https://doi.org/10.3390/fermentation5030079

39. Dzialo, M. C., Park, R., Steensels, J., Lievens, B., & Verstrepen, K. J. (2017). Physiology, ecology and industrial applications of aroma formation in yeast. *FEMS Microbiology Reviews*, *41*, S95-S128. https://doi.org/10.1093/femsre/fux031.

40. Englezos, V., Rantsiou, K., Cravero, F., Torchio, F., Pollon, M., Fracassetti, D., et al. (2018a). Volatile profile of white wines fermented with sequential inoculation of *Starmerella bacillaris* and *Saccharomyces cerevisiae*. *Food chemistry*, *257*, 350-360. https://doi.org/10.1016/j.foodchem.2018.03.018.

41. Englezos, V., Cocolin, L., Rantsiou, K., Ortiz-Julien, A., Bloem, A., Dequin, S., et al. (2018b). Specific phenotypic traits of *Starmerella bacillaris* related to nitrogen source consumption and central carbon metabolite production during wine fermentation. *Applied and Environmental Microbiology, 84*, e00797-18. https://doi.org/10.1128/AEM.00797-18.

42. Englezos, V., Torchio, F., Vagnoli, P., Krieger-Weber, S., Rantsiou, K., & Cocolin, L. (2019a). Impact of *Saccharomyces cerevisiae* Strain Selection on Malolactic

Fermentation by *Lactobacillus plantarum* and *Oenococcus oeni. American Journal of Enology and Viticulture,* 71, 157-165.10.5344/ajev.2019.19061

43. Englezos, V., Rantsiou, K., Giacosa, S., Segade, S. R., Rolle, L., & Cocolin, L. (2019b). Cell-to-cell contact mechanism modulates *Starmerella bacillaris* death in mixed culture fermentations with *Saccharomyces cerevisiae. International journal of food microbiology*, *289*, 106-114. https://doi.org/10.1016/j.ijfoodmicro.2018.09.009.

44. Escribano-Viana, R., Portu, J., Garijo, P., López, R., Santamaría, P., López-Alfaro, I., et al. (2019). Effect of the Sequential Inoculation of Non-*Saccharomyces/Saccharomyces* on the Anthocyans and Stilbenes Composition of Tempranillo Wines. *Frontiers in Microbiololgy*, 9;10:773. https://doi.org/10.3389/fmicb.2019.00773.

45. Gobert, A., Tourdot-Maréchal, R., Sparrow, C., Morge, C., & Alexandre, H. (2019). Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiology, 83*, 71-85. https://doi.org/10.1016/j.fm.2019.04.008.

46. Gonzalez, R., Quirós, M., & Morales, P. (2013). Yeast respiration of sugars by non-*Saccharomyces* yeast species: a promising and barely explored approach to lowering alcohol content of wines. *Trends in Food Science & Technology*, *29*(1), 55-61. https://doi.org/10.1016/j.tifs.2012.06.015.

47. Grangeteau, C., Roullier-Gall, C., Rousseaux, S., Gougeon, R. D., Schmitt-Kopplin, P., Alexandre, H., & Guilloux-Benatier, M. (2017). Wine microbiology is driven by vineyard and winery anthropogenic factors. *Microbial Biotechnology*, *10*, 354-370. https://doi.org/10.1111/1751-7915.12428.

48. Guzzon, R., Malacarne, M., Larcher, R., Franciosi, E., & Toffanin, A. (2020). The impact of grape processing and carbonic maceration on the microbiota of early stages of winemaking. *Journal of Applied Microbiology*, *128*(1), 209-224. https://doi.org/10.1111/jam.14462.

49. Hranilovic, A., Gambetta, J. M., Schmidtke, L., Boss, P. K., Grbin, P. R., Masneuf-Pomarede, I., et al. (2018). Oenological traits of *Lachancea thermotolerans* show signs of domestication and allopatric differentiation. *Scientific reports*, *8(1)*, 1-13. https://doi.org/10.1038/s41598-018-33105-7

50. Hranilovic, A., Gambetta, J. M., Jeffery, D. W., Grbin, P. R., & Jiranek, V. (2020). Lower-alcohol wines produced by *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* co-fermentations: The effect of sequential inoculation timing. *International Journal of Food Microbiology*, *329*, 108651. https://doi.org/10.1016/j.ijfoodmicro.2020.108651.

51. Hu, K., Zhao, H., Edwards, N., Peyer, L., Tao, Y., & Arneborg, N. (2022). The effects of cell-cell contact between *Pichia kluyveri* and *Saccharomyces cerevisiae* on amino acids and volatiles in mixed culture alcoholic fermentations. *Food Microbiology*, *103*, 103960. https://doi.org/10.1016/j.fm.2021.103960.

52. Ivey, M., Massel, M., & Phister, T. G. (2013). Microbial interactions in food fermentations. *Annual Review of Food Science and Technology*, *4*, 141-162. https://doi.org/10.1146/annurev-food-022811-101219.

53. Jolly, N P., Varela, C., & Pretorius, I. S. (2014). Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Research*, *14*, 215-237. https://doi.org/10.1111/1567-1364.12111

54. Kemsawasd, V., Viana, T., Ardö, Y., & Arneborg, N. (2015). Influence of nitrogen sources on growth and fermentation performance of different wine yeast species during alcoholic fermentation. *Applied Microbiology and Biotechnology*, *99*, 10191-10207. https://doi.org/10.1007/s00253-015-6835-3.

55. Knight, S., Klaere, S., Fedrizzi, B., & Goddard, M.R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Scientific Reports*, *5*, 14233. https://doi.org/10.1038/srep14233

56. Knight, S.J., Karon, O., & Goddard, M.R. (2020). Small scale fungal community differentiation in a vineyard system. *Food Microbiology*, *87*, 103358. https://doi.org/10.1016/j.fm.2019.103358.

57. Krieger-Weber, S., Heras, J. M., & Suarez, C. (2020). *Lactobacillus plantarum*, a new biological tool to control malolactic fermentation: a review and an outlook. *Beverages*, *6*, 23. https://doi.org/10.3390/beverages6020023.

58. Legras, J. L., Galeote, V., Bigey, F., Camarasa, C., Marsit, S., Nidelet, T., et al. (2018). Adaptation of *S. cerevisiae* to fermented food environments reveals remarkable genome plasticity and the footprints of domestication. *Molecular Biology and Evolution*, *35(7)*, 1712-1727. https://doi.org/10.1093/molbev/msy066

59. Liu, Y., Rousseaux, S., Tourdot-Maréchal, R., Sadoudi, M., Gougeon, R., Schmitt-Kopplin, P., et al. (2017). Wine microbiome: a dynamic world of microbial interactions. *Critical Reviews in Food Science and Nutrition*, *57*, 856-873. https://doi.org/10.1080/10408398.2014.983591.

60. Lleixà, J., Manzano, M., Mas, A., & Portillo, M. D. C. (2016). *Saccharomyces* and non-*Saccharomyces* competition during microvinification under different sugar and nitrogen conditions. *Frontiers in Microbiology*, *7*, 1959. https://doi.org/10.3389/fmicb.2016.01959.

61. Lopez, C. L. F., Beaufort, S., Brandam, C., & Taillandier, P. (2014). Interactions between *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* in tequila must type medium fermentation. *World Journal of Microbiology and Biotechnology*, *30*, 2223-2229. https://doi.org/10.1007/s11274-014-1643-y.

62. Lucio, O., Pardo, I., Krieger-Weber, S., Heras, J.M. & Ferrer, S. (2016) Selection of *Lactobacillus* strains to induce biological acidification in low acidity wines. *LWT—Food Science and Technology*, 73, 334–341. https://doi.org/10.1016/j.lwt.2016.06.031

63. Mahlapuu, M., Håkansson, J., Ringstad, L., Björn, C. (2016). Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Frontiers in Cellular and Infection Microbiol*ogy, *6*, 1–12. https://doi.org/10.3389/fcimb.2016.00194.

64. Maicas, S., Mateo, J.J. (2016). Microbial Glycosidases for Wine Production. *Beverages*, *2*, 20. https://doi.org/10.3390/beverages2030020.

65. Mangani, S., Buscioni, G., Guerrini, S., & Granchi, L. (2020). Influence of sequential inoculum of *Starmerella bacillaris* and *Saccharomyces cerevisiae* on flavonoid composition of monovarietal Sangiovese wines. *Yeast, 37(9-10)*, 549-557. https://doi.org/10.1002/yea.3474.

66. Marcon, A. R., Schwarz, L. V., Dutra, S. V., Moura, S., Agostini, F., Delamare, A. P. L., et al. (2018). Contribution of a Brazilian *Torulaspora delbrueckii* isolate and a commercial *Saccharomyces cerevisiae* to the aroma profile and sensory characteristics of Moscato Branco wines. *Australian Journal of Grape and Wine Research, 24(4)*, 461-468. https://doi.org/10.1111/ajgw.12347

67. Martins, G., Miot-Sertier, C., Lauga, B., Claisse, O., Lonvaud-Funel, A., Soulas, G., et al. (2012). Grape berry bacterial microbiota: Impact of the ripening process and the farming system. *International Journal of Food Microbiology*, *158*, 93-100. https://doi.org/ 10.1016/j.ijfoodmicro.2012.06.013.

68. Mateo, J. J., & Maicas, S. (2016). Application of Non-*Saccharomyces* Yeasts to Wine-Making Process. *Fermentation*, *2*, 14. https://doi.org/10.3390/fermentation2030014

69. Mazzucco, M. B., Ganga, M. A., & Sangorrín, M. P. (2019). Production of a novel killer toxin from *Saccharomyces eubayanus* using agro-industrial waste and its application against wine spoilage yeasts. *Antonie van Leeuwenhoek*, 1-9. https://doi.org/10.1007/s10482-019-01231-5

70. Medina, K., Boido, E., Dellacassa, E., & Carrau, F. (2012). Growth of non-*Saccharomyces* yeasts affects nutrient availability for *Saccharomyces cerevisiae* during

wine fermentation. *International Journal of Food Microbiology*, *157*, 245-250. https://doi.org/10.1016/j.ijfoodmicro.2012.05.012.

71. Mehlomakulu, N. N., Prior, K. J., Setati, M. E., & Divol, B. (2017). *Candida pyralidae* killer toxin disrupts the cell wall of *Brettanomyces bruxellensis* in red grape juice. *Journal of Applied Microbiology*, *122*, 747-758. https://doi.org/10.1111/jam.13383.

72. Mewa-Ngongang, M., du Plessis, H. W., Ntwampe, S. K. O., Chidi, B. S., Hutchinson, U. F., Mekuto, L., & Jolly, N. P. (2019). The Use of *Candida pyralidae* and *Pichia kluyveri* to Control Spoilage Microorganisms of Raw Fruits Used for Beverage Production. *Foods*, *8*, 454. https://doi.org/10.3390/foods8100454.

73. Morales, P., Rojas, V., Quirós, M., & Gonzalez, R. (2015). The impact of oxygen on the final alcohol content of wine fermented by a mixed starter culture. *Applied Microbiology and Biotechnology*, *99*, 3993-4003. https://doi.org/10.1007/s00253-014-6321-3

74. Morrison-Whittle, P., & Goddard, M.R. (2018). From vineyard to winery: a source map of microbial diversity driving wine fermentation. *Environmental Microbiology*, *20*, 75-84. https://doi.org/10.1111/1462-2920.13960

75. Nisiotou, A., Sgouros, G., Mallouchos, A., Nisiotis, C. S., Michaelidis, C., Tassou, C., et al. (2018). The use of indigenous *Saccharomyces cerevisiae* and *Starmerella bacillaris* strains as a tool to create chemical complexity in local wines. *Food Research International*, *111*, 498-508. https://doi.org/10.1016/j.foodres.2018.05.035.

76. Oro, L., Ciani, M., Bizzaro, D., & Comitini, F. (2016). Evaluation of damage induced by Kwkt and Pikt zymocins against *Brettanomyces/Dekkera* spoilage yeast, as compared to sulphur dioxide. *Journal of Applied Microbiology*, *121*, 207-214. https://doi.org/10.1111/jam.13121.

77. Padilla, B., Gil, J. V., & Manzanares, P. (2016). Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Frontiers in Microbiology*, *7*, 411. https://doi.org/10.3389/fmicb.2016.00411.

78. Peña, R., & Ganga, M. A. (2019). Novel antimicrobial peptides produced by *Candida intermedia* LAMAP1790 active against the wine-spoilage yeast *Brettanomyces bruxellensis*. *Antonie van Leeuwenhoek*, *112*, 297-304. https://doi.org/10.1007/s10482-018-1159-9.

79. Pérez-Torrado, R., Rantsiou, K., Perrone, B., Navarro-Tapia, E., Querol, A., & Cocolin, L. (2017). Ecological interactions among *Saccharomyces cerevisiae* strains: insight into the dominance phenomenon. *Scientific reports*, *7*, 43603. https://doi.org/10.1038/srep43603.

80. Pérez-Magariño, S., Cano-Mozo, E., Albors, C., Santos, A., & Navascués, E. (2021). Autochthonous *Oenococcus oeni* Strain to Avoid Histamine Formation in Red Wines: A Study in Real Winemaking Conditions. *American Journal of Enology and Viticulture*, 72(2), 170-180. https://doi.org/10.5344/ajev.2020.20010.

81. Petitgonnet, C., Klein, G. L., Roullier-Gall, C., Schmitt-Kopplin, P., Quintanilla-Casas, B., Vichi, S., et al. (2019). Influence of cell-cell contact between *L. thermotolerans* and

S. cerevisiae on yeast interactions and the exo-metabolome. *Food Microbiology*, *83*, 122-133. https://doi.org/10.1016/j.fm.2019.05.005.

82. Ponomarova, O., Gabrielli, N., Sévin, D. C., Mülleder, M., Zirngibl, K., Bulyha, K., et al. (2017). Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. *Cell systems*, *5*, 345-357. https://doi.org/10.1016/j.cels.2017.09.002.

83. Renault, P., Coulon, J., Moine, V., Thibon, C., & Bely, M. (2016) Enhanced 3-sulfanylhexan-1-ol production in sequential mixed fermentation with *Torulaspora delbrueckii/Saccharomyces cerevisiae* reveals a situation of synergistic interaction between two industrial strains. *Frontiers in Microbiology*, *7*, 293. https://doi.org/10.3389/fmicb.2016.00293

84. Ruiz, J., Belda, I., Beisert, B., Navascués, E., Marquina, D., Calderón, F., et al. (2018). Analytical impact of *Metschnikowia pulcherrima* in the volatile profile of Verdejo white wines. *Applied Microbiology and Biotechnology*, *102(19)*, 8501-8509. https://doi.org/10.1007/s00253-018-9255-3.

85. Russo, P., Englezos, V., Capozzi, V., Pollon, M., Segade, S. R., Rantsiou, K., et al. (2020). Effect of mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* on management of malolactic fermentation. *Food Research International*, *134*, 109246. https://doi.org/10.1016/j.foodres.2020.109246

86. Seguinot, P., Ortiz-Julien, A., & Camarasa, C. (2020). Impact of nutrient availability on the fermentation and production of aroma compounds under sequential inoculation with *M. pulcherrima* and *S. cerevisiae*. *Frontiers in Microbiology*, *11*, 305. https://doi.org/10.3389/fmicb.2020.00305

87. Simonin, S., Alexandre, H., Nikolantonaki, M., Coelho, C., & Tourdot-Maréchal, R. (2018). Inoculation of *Torulaspora delbrueckii* as a bio-protection agent in winemaking. *Food Research International*, *107*, 451-461. https://doi.org/10.1016/j.foodres.2018.02.034

88. Su, Y., Seguinot, P., Sanchez, I., Ortiz-Julien, A., Heras, J. M., Querol, A., et al. (2020). Nitrogen sources preferences of non-*Saccharomyces* yeasts to sustain growth and fermentation under winemaking conditions. *Food Microbiology*, *85*, 103287. https://doi.org/10.1016/j.fm.2019.103287.

89. Sumby, K. M., Bartle, L., Grbin, P. R., & Jiranek, V. (2019). Measures to improve wine malolactic fermentation. *Applied Microbiology and Biotechnology*, *103*, 2033-2051. https://doi.org/10.1007/s00253-018-09608-8.

90. Tufariello, M., Capozzi, V., Spano, G., Cantele, G., Venerito, P., Mita, G., et al. (2020). Effect of co-inoculation of *Candida zemplinina*, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* for the industrial production of Negroamaro wine in Apulia (Southern Italy). *Microorganisms*, 8(5), 726. https://doi.org/10.3390/microorganisms8050726.

91. Varela, C., Bartel, C., Espinase Nandorfy, D., Bilogrevic, E., Tran, T., Heinrich, T., et al. (2021). Volatile aroma composition and sensory profile of Shiraz and Cabernet Sauvignon wines produced with novel *Metschnikowia pulcherrima* yeast starter cultures. *Australian Journal of Grape and Wine Research*, *27(3)*, 406-418. https://doi.org/10.1111/ajgw.12484.

92. Vilela, A. (2019). Use of Nonconventional Yeasts for Modulating Wine Acidity. *Fermentation*, *5*, 27. https://doi.org/10.3390/fermentation5010027.

93. Wang, S., Li, S., Zhao, H., Gu, P., Chen, Y., Zhang, B., et al., (2018). Acetaldehyde released by *Lactobacillus plantarum* enhances accumulation of pyranoanthocyanins in wine during malolactic fermentation. *Food Research International*, *108*, 254–263. https://doi.org/10.1016/j.foodres.2018.03.032.

94. Wang, S. Y., Zhu, H. Z., Lan, Y. B., Liu, R. J., Liu, Y. R., Zhang, B. L., et al., (2020). Modifications of phenolic compounds, biogenic amines, and volatile compounds in Cabernet Gernishct wine through malolactic fermentation by *Lactobacillus plantarum* and *Oenococcus oeni*. *Fermentation*, *6(1)*, 15. https://doi.org/10.3390/fermentation6010015.

95. Whitener, M. B., Stanstrup, J., Carlin, S., Divol, B., Du Toit, M., & Vrhovsek, U. (2017). Effect of non-*Saccharomyces* yeasts on the volatile chemical profile of Shiraz wine. *Australian Journal of Grape and Wine Research*, *23(2)*, 179-192. http://dx.doi.org/10.1111/ajgw.12269.

96. Yan, G., Zhang, B., Joseph, L. & Qaterhouse, A. (2020). Effects of initial oxygenation on chemical and aromatic composition of wine in mixed starters of *Hanseniaspora vineae* and *Saccharomyces cerevisiae*. *Food Microbiology*, *90*, 10340. https://doi.org/10.1016/j.fm.2020.103460.

97. Ženišová, K., Cabicarova, T., Sidari, R., Kolek, E., Pangallo, D., Szemes, T., et al. (2021). Effects of co-fermentation with *Lachancea thermotolerans* or *Metschnikowia pulcherrima* on concentration of aroma compounds in Pinot Blanc wine. *Journal of Food & Nutrition Research*, *60(1)*, 87-91.

98. Zhang, B., Ivanova-Petropulos, V., Duan, C., & Yan, G. (2021). Distinctive chemical and aromatic composition of red wines produced by *Saccharomyces cerevisiae* co-fermentation with indigenous and commercial non-*Saccharomyces* strains. *Food Bioscience*, *41*, 100925. https://doi.org/10.1016/j.fbio.2021.100925.

99. Zhang, B. Q., Luan, Y., Duan, C. Q., & Yan, G. L. (2018a). Use of *Torulaspora delbrueckii* co-fermentation with two *Saccharomyces cerevisiae* strains with different aromatic characteristic to improve the diversity of red wine aroma profile. *Frontiers in Microbiology*, *9*, 606. https://doi.org/10.3389/fmicb.2018.00606.

100. Zhang, B. Q., Shen, J. Y., Duan, C. Q., & Yan, G. L. (2018b). Use of indigenous *Hanseniaspora vineae* and *Metschnikowia pulcherrima* co-fermentation with *Saccharomyces cerevisiae* to improve the aroma diversity of Vidal Blanc icewine. *Frontiers in Microbiology*, 9, 2303. https://doi.org/10.3389/fmicb.2018.02303.

Figure Captions

Fig. 1 Wine mycobiota and their evolution during the various steps of wine production

Fig. 2 Phenotypic traits of the commercial yeasts and lactic acid bacteria available for wine production (except *Starmerella bacillaris*)

Fig. 3 Factors affecting microrganisms interactions in wine

Table 1. Summary of recent studies evaluating the influence of mixed fermentations with different non-*Saccharomyces* yeasts and *Saccharomyces cerevisiae* on wine composition

Species	Inoculation protocol	Trial conditions	Grape *cultivar*	Impact on chemical composition or sensory attributes	Reference
Lachancea thermotolerans	Sequential	- Fermentation temperature: 20-23°C - pH: 3.23 - Total SO_2: 60 mg/L - Inoculation size: *L. thermotolerans*: 1×10^7 cells/mL, *S. cerevisiae* 1×10^6 cells/mL	Cabernet Sauvignon	- Increase in lactic acid Increase in terpenes (linalool and geraniol)	Zhang et al., 2021
	Sequential	- Fermentation temperature: 22 °C - pH: not reported - No SO_2 addition - Inoculation size: 1×10^6 CFU/mL	Pinot Grigio	- Reduction in ethanol content - L-lactic acid production ranged from 0.53 to 4.42 g/L -- Decrease in fatty acids	Binati et al., 2020
	Co-inoculation and sequential	- Fermentation temperature: 18 °C - pH: 3.37 - Total SO2: not reported - Inoculation size: 5×10^6 cells/mL	Emir	- Increase in total acidity Sequential fermentations: - Increase in n-propanol, acetaldehyde and decrease in esters and higher alcohols (except n-propanol) Co-inoculation: - Increase in isoamyl acetate	Balikci et al., 2016
	Sequential	- Fermentation temperature: 25° C - pH: 3.8 - Total SO_2: 60 mg/L - Inoculation size: 1×10^6 CFU/mL Malolactic fermentation: YES	Shiraz	- Increase in 2-methyl propanoic acid and some of its esters - Increase in esters (isoamyl lactate, acetic acid, butyl ester, butanoic acid, pentylester, 3-nonenoic acid, ethyl ester, propanoic acid, and 2-hydroxy-, ethyl ester)	Whitener et al., 2017
	Sequential	- Fermentation temperature: 20-23 °C - pH: 3.23 - Total SO_2: 60 mg/L - Inoculation size: *T. delbrueckii*: 1×10^7 cells/mL, *S. cerevisiae* 1×10^6 cells/mL	Cabernet Sauvignon	- Decrease in acetic acid - Increase in 2-phenylethyl alcohol and esters	Zhang et al., 2021
	Co-inoculation and sequential	- Fermentation temperature: 18 °C - pH: 3.16 - Total SO_2: 60 mg/L - Inoculation size: *T. delbrueckii*: 1×10^7 cells/mL, *S. cerevisiae* 1×10^6 cells/mL	Cabernet Sauvignon	- Decrease in acetic acid - Reduction in ethanol content from 0.05% to 0.82% (v/v) - Decrease in succinic acid - Decrease in fatty acids - Increase in Phenylethyl alcohol - Decrease in volatile phenols	Zhang et al., 2018a
	Sequential	- Fermentation temperature: 20 °C	Verdejo	- Ethanol reduction content (0.52% (v/v))	Belda et al., 2017

		- pH: 3.42 - No SO_2 addition - Inoculation size: 1×10^6 cells/mL		- Increase in glycerol - Increase in pyruvic acid (from 27 to 52 mg/L) - Decrease in acetic acid - Decrease in higher alcohols - Increase in 2- phenylethyl acetate and 2-phenyl-ethanol - Volatile thiols release (3-sulfanylhexan-1-ol and methyl-4-sulfanylpentan-2-one)	
Torulaspora delbrueckii	Co-inoculation and sequential	- Fermentation temperature: 20 °C - pH: 3.42 - Total SO_2: 40 mg/kg - Inoculation size: 1×10^6 cells/mL	Tempranillo	- Decrease in total acidity - Increase in pH (higher malic acid consumption) - Decrease in ethanol content - Increase in glycerol - Mannoproteins release - Decrease in higher alcohols	Belda et al., 2015
	Co-inoculation and sequential	- Fermentation temperature: 24 °C - pH: 3.15 - Total SO_2: 60 mg/L -Inoculation size: *T. delbrueckii*: 2×10^7 cells/mL, *S. cerevisiae* 1×10^6 cells/mL	Sauvignon Blanc	- Volatile thiols release (3-Sulfanylhexan-1-ol and its acetate, 3-sulfanylhexyl acetate increase)	Renault et al., 2016
	Co-inoculation	- Fermentation temperature: 22 °C - pH: 3.5 -Total SO_2: 10 mg/L - Inoculum ratio: 1:1, 3:1 T.d/S.c, 1:3 T.d/S.c (5×10^6 cells/mL)	Moscato Branco	- Increase in 2-Phenylethanol and ethyl esters (Ratio 3:1 NS/S) - Increase in acetaldehyde - Release of Linalyl acetate (Ratio 3:1 NS/S and 1:1 NS/S)	Marcon et al., 2018
	Sequential	- Fermentation temperature: 16°C - pH: 3.0 - No SO_2 addition - Inoculation size: 10^6-10^7 cells/mL	Pinot blank	- Decrease in ethanol content - Increase in2-phenylethanol, diethyl succinate, phenylethyl acetate and 3-methylbutanoic acid	Ženišová et al., 2021
	Co-inoculation and sequential	- Fermentation temperature: 16° C - pH: 3.35 - High sugar content (400 g/L) - SO_2: 80 mg/L - Inoculation size: 5×10^6 cells/mL	Vidal	- Ethanol reduction - Decrease in fatty acid ethyl esters	Zhang et al., 2018b
	Sequential	- Fermentation temperature: 20 °C - pH: 3.31 - No SO_2 addition - Inoculation size: 1×10^6 CFU/mL	Verdejo	- Decrease in ethanol content (0.6% v/v) - Decrease in acetaldehyde - Decrease in higher alcohols (increase on for phenylethanol) - Varietal thiols release (4-methyl ulfanylpentan-2-one)	Ruiz et al., 2018

	Sequential	- Fermentation temperature: 22 °C - pH: not reported - No SO_2 addition - Inoculation size: 1×10^6 CFU/mL	Pinot Grigio	- Increase in the esters and higher alcohols - Decrease in volatile phenols	Binati et al., 2020
Metschnikowia pulcherrima	Sequential	- Fermentation temperature: 22.5 °C - pH: not reported - No SO_2 addition - Inoculation size: 5×10^6 cells/mL	Chardonnay / Semillon blend	- Decrease in acetic and ethanol - Increase in ethanol and fumarate - Increase in acetate esters (mainly ethyl acetate, isoamyl acetate and phenylethyl acetate) - Increase in higher alcohols and monoterpenoids	Hranilovic et al., 2020
	Sequential	- Fermentation temperature: 22.5 °C - pH: 3.5 -No SO_2 addition -Inoculation size: 5×10^6 cells/mL	Merlot	- Reduction in ethanol content from 1.0 to 1.1% (v/v) - Increase in total acidity - Decrease in pH - Increase in higher alcohols (1-Propanol, 2-Phenylethanol)	Aplin et al., 2021
	Sequential	- Fermentation temperature: 16°C - pH: 3.0 - No SO_2 addition - Inoculation size: 10^6-10^7 CFU/mL	Pinot blank	- Increase in 2-phenylethanol and diethyl succinate - Decrease in acetaldehyde	Ženišová et al., 2021
	Sequential	- Fermentation temperature: 20° C - pH: 3.90 (Shiraz), 3.88 (Cabernet Sauvignon) - Total SO_2: 50 mg/L - Inoculation size: 5×10^6 cells/mL - Malolactic fermentation: YES	Shiraz, Cabernet Sauvignon	Shiraz: - Increase in total esters carbon disulphide Cabernet Sauvignon: - Decrease in volatile acids and dimethyl sulphide - Increase in total esters	Varela et al., 2021
	Sequential	- Fermentation temperature: 25° C - pH: 3.8 - SO_2: 60 mg/L - Inoculation size: 1×10^6 cells/mL - Malolactic fermentation: YES	Shiraz	- Increase in esters (butyl octanoate, isobutyl acetate, pentanoic acid, 4-methyl-, ethyl ester, hexanoic acid, 2-methylpropyl ester, 6-octen-1-ol, 3,7-dimethyl-, acetate, acetic acid, methyl ester, and cis-3-hexen-1-ol)	Whitener et al., 2017
	Sequential	- Fermentation temperature: 20 °C - pH: 2.9 - No SO_2 addition - Inoculation size: 10^6 cells/mL	Riesling	- Malic acid degradation - Reduction in ethanol content - Increase in glycerol - Increase in ethyl hexanoate and ethyl octanoate	Dutraive et al., 2019
	Sequential	-Fermentation temperature: 25° C -pH: 3.8 -SO_2: 60 mg/L -Inoculation size: 1×10^6 cells /mL Malolactic fermentation: YES	Shiraz	- Increase in acetic acid -Increase in acetaldehyde, ester and butyl octanoate	Whitener et al., 2017

	Sequential	- Fermentation temperature: 20 °C - pH: 2.9 - No SO_2 addition - Inoculation size: 10^6 cells/mL	Riesling	- Reduction in ethanol content - Increase in glycerol content - Increase in total esters (ethyl acetate, isoamyl acetate and 2-methyl butyl acetate, 2-Phenyl-ethyl acetate) - Increase in higher alcohols (2-phenylethanol, 3-methyl-butanol and 2-methylbutanol) - Increase in valeric acid	Dutraive et al., 2019
	Simultaneous	- Fermentation temperature: 24 °C - pH: 3.5 - No SO_2 addition - Inoculation size: 2 x 10^6 cells/mL	Pinot noir	- Increase in esters (ethyl acetate, isobutyl acetate, isoamyl acetate, hexyl acetate, benzyl acetate, 2-phenylethyl acetate, ethyl hexanoate and ethyl octanoate) - Increase in higher alcohols (isobutyl alcohol, isoamyl alcohol, benzyl alcohol) - Increase in volatile fatty acids (isobutyric acid, hexanoic acid)	Hu et al., 2022
Pichia kluyveri	Sequential	- Fermentation temperature: 22 °C - pH: not reported - No SO_2 addition - Inoculation size: 1.0 × 10^6 CFU/mL	Pinot Grigio	- Reduction in ethanol content - Increase in glycerol (average value 5.83 g/L) - Decrease in acetaldehyde - Decrease in fatty acids - Increase in production of nerol, benzyl alcohol and (E)-3-hexen-1-ol	Binati et al., 2020
	Sequential	- Fermentation temperature: 25° C - pH: 3.8 - SO_2: 60 mg/L - Inoculation size: 1 × 10^6 cells/mL Malolactic fermentation: YES	Shiraz	- Terpens release (linalool and geraniol amount increase) - Increase in δ -valerolactone and pentolactone as well as 2-hexenoic acid and 2-hexanoic acid, ethyl ester	Whitener et al., 2017
	Co-inoculation and sequential	- Fermentation temperature: 25° C - pH: 3.44 - Total SO_2: 30 mg/L - Inoculation size: 10^6- 10^7 cells/mL	Kotsifali and Mandilar (3:1)	- Reduction in ethanol content - Increase in glycerol - Increase in ethyl esters (ethyl octanoate, 2-Phenylethyl acetate, Hexyl acetate) - Decrease in higher alcohols	Nisiotou et al., 2018
Starmerella bacillaris	Sequential	- Fermentation temperature: 20° C - pH: Chardonnay 3.99, Muscat 3.81, Riesling 3.82, Sauvignon blanc 3.56 - No SO_2: addition -Inoculation size: 5 x 10^6- 10^7 cells/mL	Chardonnay, Muscat, Riesling and Sauvignon blanc	- Reduction in ethanol content - Increase in glycerol - Decrease in acetic acid - Increase in higher alcohols and esters in Sauvignon blanc (ethyl octanoate and ethyl decanoate) - Increase 2-Phenylethanol in Riesling and Sauvignon blanc	Englezos et al., 2018

				- Decrease in esters in Chardonnay and Muscat Increase - Decrease in terpenes - Increase in 3-mercapto-1-hexanol (3MH)	
	Co-inoculation	- Fermentation temperature: 25° C - pH: 3.52 - No SO_2 addition -Inoculation size: *Starm. bacillaris* 1 x 10^6 cells/mL, *S. cerevisiae*: 1 x 10^4 cells/mL	Negroamaro	- Increase in glycerol - Increase in terpenes	Truffariello et al., 2020
	Sequential	-Fermentation temperature: 28° C -pH: 3.4 -No SO_2 addition -Inoculation size: *Starm. bacillaris* 1 x 10^6 cells/mL, *S. cerevisiae*: 1 x 10^4 CFU/mL	Sangiovese	- Reduction in ethanol content - Increase in glycerol content - Decrease in anthocyanins and flavan-3-ols - Increase in Vitisin A and Vitisin B	Mangani et al., 2020

1259 **Table 2.** Summary of recent studies evaluating the influence of lactic acid bacteria on wine composition

Species	Inoculation protocol	Trial conditions	Must/wine	Quality advantages	References
Oenococcus oeni	After AF	- Fermentation temperature: 21° C - pH: 3.73 - No SO_2 addition - Inoculation size: 5 x 10^7 CFU/mL - Malic acid: 2 g/L - Ethanol: 14.5% (v/v) - Malic acid: 2.00 g/L	Pinot noir	- Increase in colour intensity and redness - Increase in procyanidin - Increase in esters (ethyl hexanoate, ethyl octanoate and ethyl cinnamate) - Increase in octanoic and n-decanoic fatty acids - Increase in 4-ethyl phenol - Increase in vanillin	Brizuela et al., 2021
	After AF	- Fermentation temperature: 21° C - pH: 3.52 - No SO_2 addition - Inoculation size: 3 × 10^7 CFU/mL - Ethanol: 13.3 % (v/v) - L-malic acid: 3.17 g/L	Tempranillo	- Increase in acetate and ethyl esters (isoamyl acetate, phenylethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate) - Increase in ethyl succinate and ethyl lactate - Increase in terpenes (linalool, a-Terpineol, citronellol and nerolidol)	Diez-Ozaeta et al., 2021
	After AF	- Fermentation temperature: 23° C - pH: 3.46 - No SO_2 addition - Inoculation size: 10^8 CFU/mL - Ethanol: 12.5 % (v/v) - L-malic acid: 2.51 g/L	Cabernet Gernischt	- Decrease in phenolic compounds - Production of caffeic acid and 4-hydroxycinnamic acid - Production of ethyl lactate and isoamyl lactate - Accumulation of 3,4-dimethylbenzaldehyde - Accumulation of linalool and α-terpineol accumulation	Wang et al., 2020
	Co-inoculation	- Fermentation temperature: 22° C - pH: 3.7 - No SO_2 addition - Inoculation size: 1 x 10^6 CFU/mL - Ethanol: 15.0 % (v/v) - L-malic acid: 1.91 g/L	Tinto Fino (Tempranillo)	- Prevention of the increase of histamine values during wine aging	Pérez-Magariño et al., 2021
	After 24h from the beginning of AF	- Fermentation temperature: 23° C - pH: 3.32 - SO_2 addition: 30 mg/L - Inoculation size: 1 x 10^6 CFU/mL - Malic acid: 1.85 g/L	Barbera	- Decrease of yellow/blue coordinate (b*) and increase of red/green coordinate (a*)	Englezos et al., 2019a
Lactiplantibacillus plantarum	Co-inoculation	- Fermentation temperature: 25° C - pH: 3.52 - No SO_2 addition - Inoculation size: 10^6 CFU/mL - Malic acid: 2.17 g/L	Negroamaro	- Increase in higher alcohols (1-Hexanol, phenylethanol, benzyl alcohol) - Production of ethyl lactate and diethyl succinate	Truffariello et al., 2020

		- Ethanol: 12.1 % (v/v)			
	After AF	- Fermentation temperature: 21° C - pH: 3.73 - No SO_2 addition - Inoculation size: 5 x 10^7 CFU/mL - Malic acid: 2 g/L - Ethanol: 14.5 % (v/v)	Pinot noir	- Increase in neutral polysaccharides - Increase in procyanidin - Increase in esters (diethyl succinate and ethyl cinnamate) - Increase in β -citronellol - Increase in 2-phenylethyl alcohol - Increase in vanillin	Brizuela et al., 2021
	After AF	- Fermentation temperature: 23° C - pH: 3.46 - No SO_2 addition - Inoculation size: 1 x 10^8 CFU/mL - Malic acid:3.5 g/L - Ethanol: 12.5% (v/v)	Cabernet Gernischt	wine color stabilization: - Increase in pyranoanthocyanins increase - Decrease in total anthocyanins - Vitisin B release - b* and H* values decrease	Wang et al., 2018
	After AF	- Fermentation temperature: 23° C - pH: 3.46 - No SO_2 addition - Inoculation size: 10^8 CFU/mL - Ethanol: 12.5 % (v/v) - L-malic acid: 2.51 g/L	Cabernet Gernischt	- Decrease in phenolic compounds - Decrease in biogenic amines reduction (strain dependent) - Release of 2-hydroxyisovaleric acid ethyl ester - Increase in esters (isoamyl hexanoate) - Production of ethyl lactate and isoamyl lactate - Accumulation of ((E)-3-hexen-1-ol, 2-nonanol and 2,3-butanediol) - Release of 4-ethylphenol	Wang et al., 2020

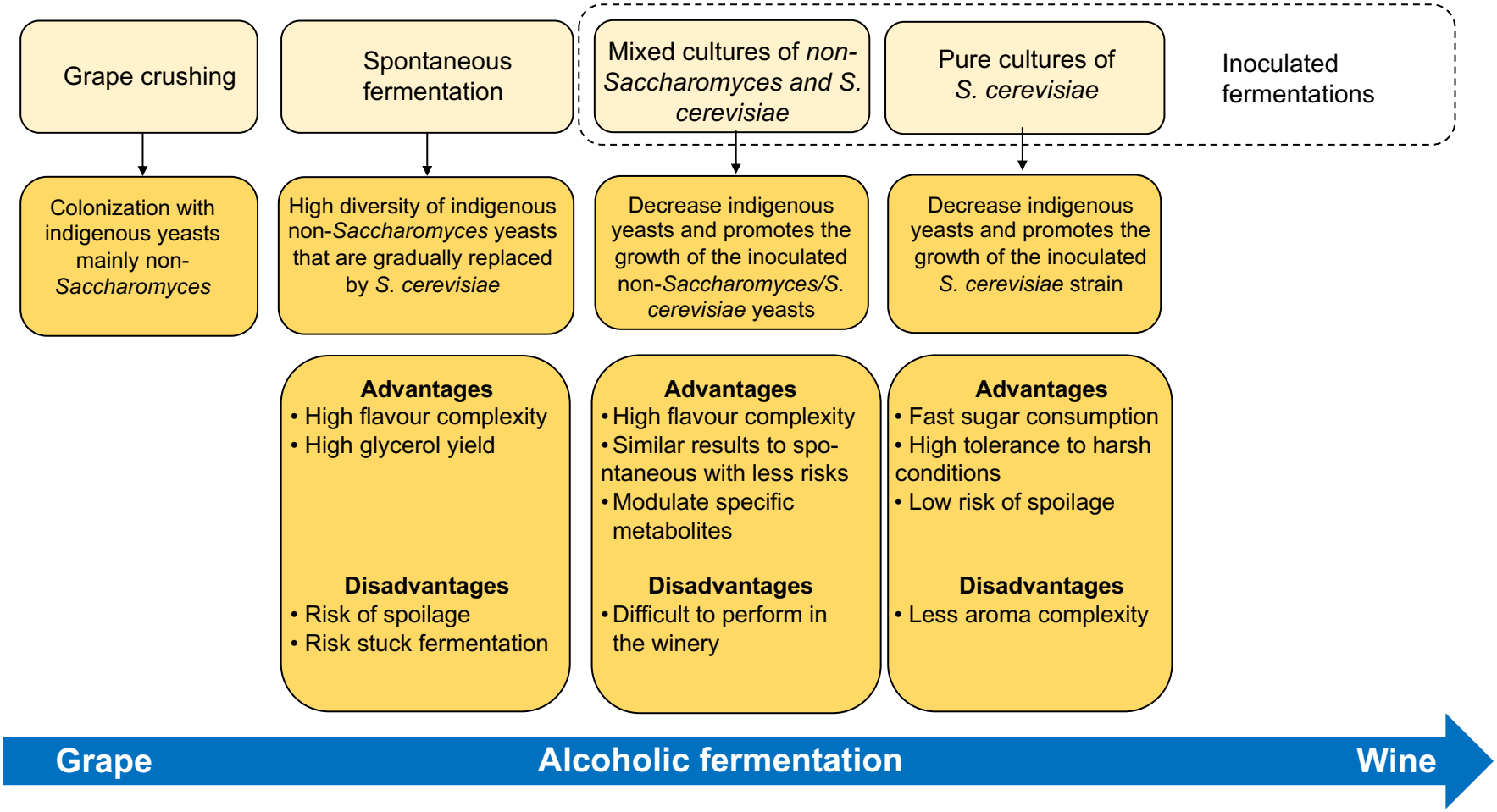
Table 3. Factors affecting microrganisms interactions in wine

Challenges[a]	**Opportunities**[a]
Further investigate the interaction mechanisms among wine microorganisms	Control the fermentation process and greater management of specific microorganisms
Integrate the knowledge of microbial dynamics and their impact on wine	Modulate specific metabolites concentration
Explore the potential of omics-based technologies in wine production	Omics could help to better predict the behavior of microorganisms during fermentation
Produce wines with less SO_2 by using bioprotective microorganisms	Fulfil consumer demands for wines free of chemical additives which are considered negative for health
Accessing low SO_2 addition to microbial interactions	

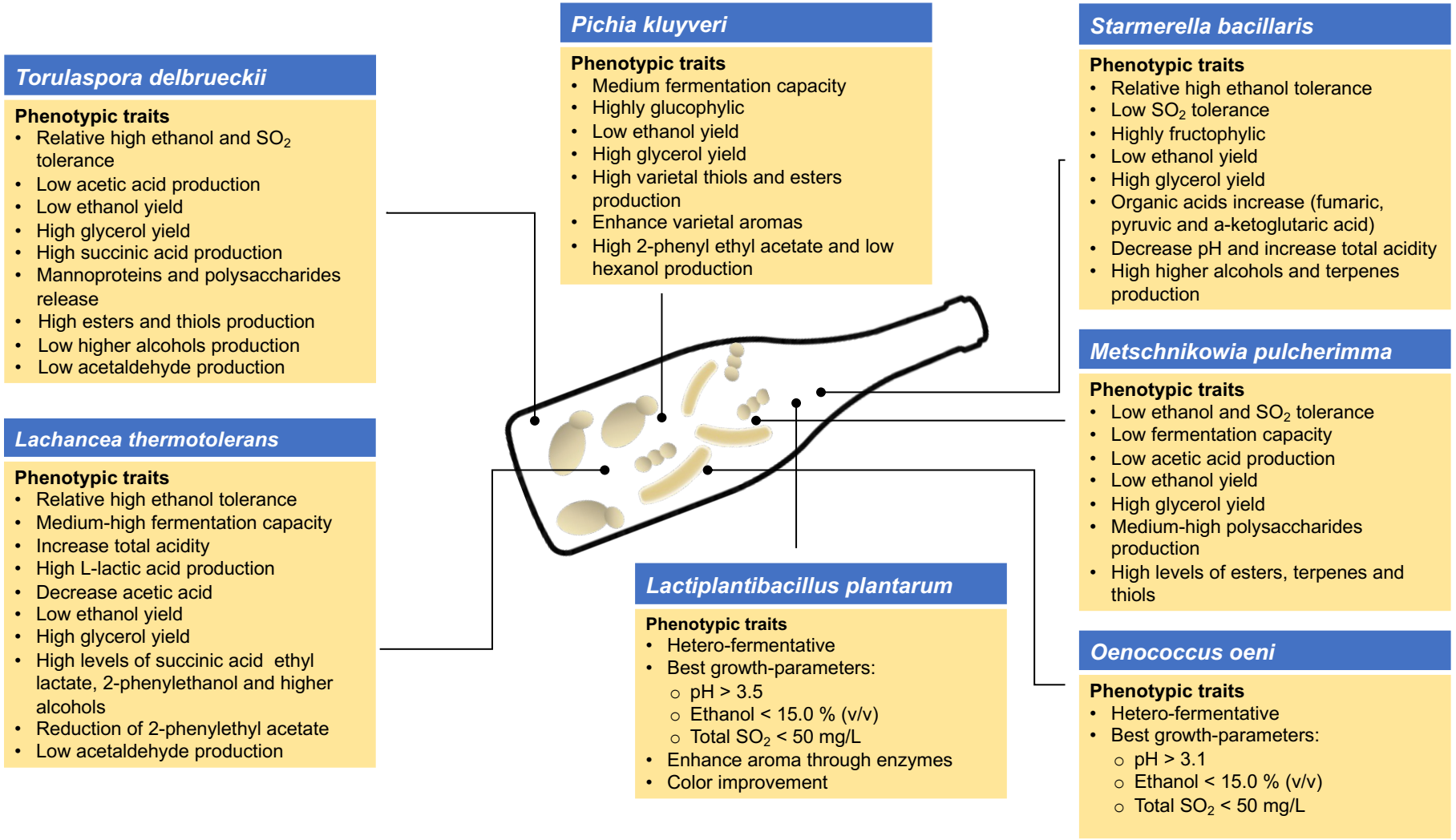
[a] Ciani et al., 2016, Di Gianvito et al., 2022, Liu et al., 2017, Siren et al., 2019.

Figures

Fig. 1



1293 **Fig. 2**



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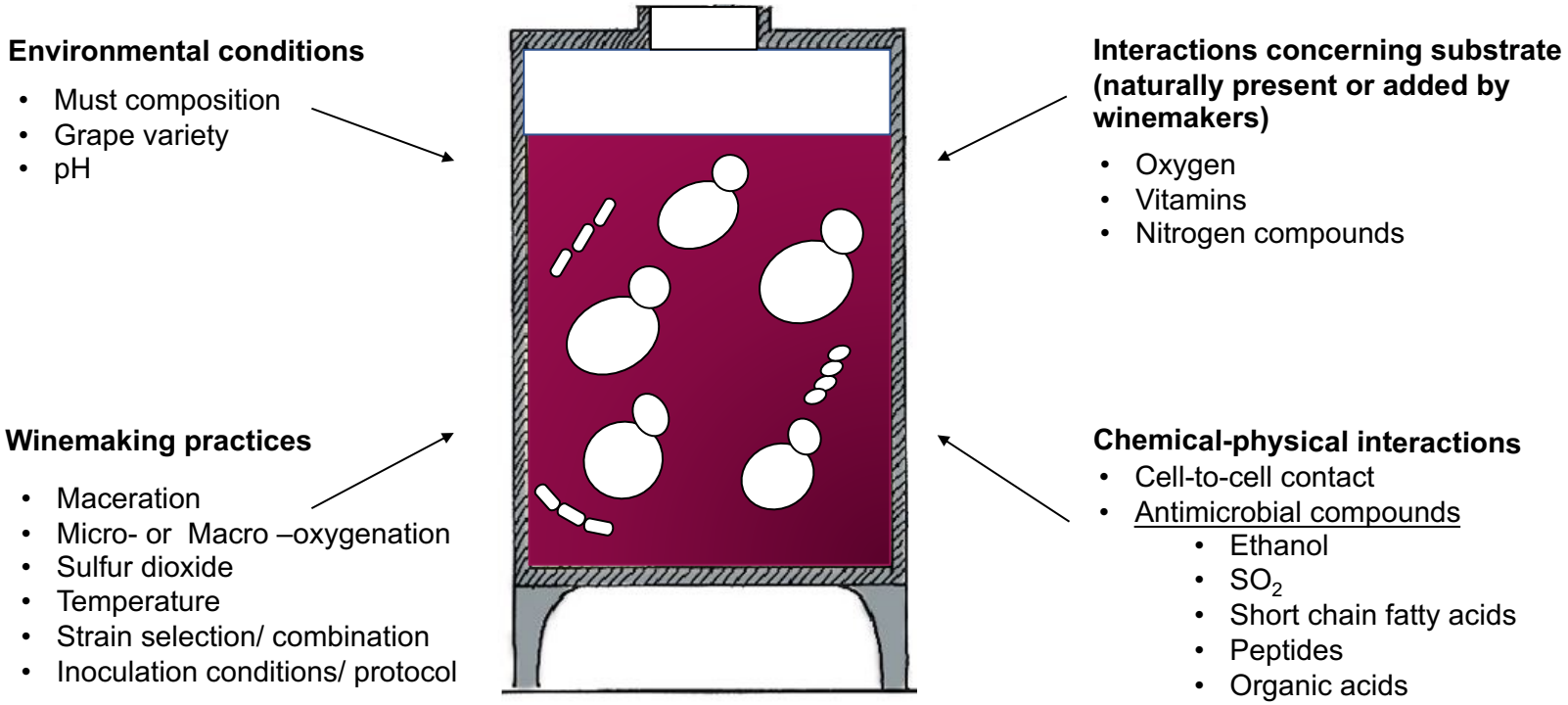
1296 **Fig. 3**



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