

A Review Article: With Advantages and Disadvantages The Role of Non-*Saccharomyces* Yeast in the Wine Industry

Metehan Gunhan  ^{1*}

¹ Manisa Viticulture Research Institute, Manisa-Türkiye

ABSTRACT

This article aims to describe non-*Saccharomyces* yeast and their effects on wine composition, fermentation, chemistry and organoleptic characters. The use of non-*Saccharomyces* yeast is on the rise in the wine industry despite the negative perception from previous research. It is known that higher levels of non-*Saccharomyces* yeast could cause implications during winemaking practices. On the contrary, non-*Saccharomyces* yeast provides complexity, richer aroma and flavour and decreases ethanol content. If the main goal is using indigenous yeast and having a starter culture, use of non-*Saccharomyces* yeast collected from winery environment could be an option, yet again a risky option. However, previous studies indicated the relation between acetic acid production and the use of non-*Saccharomyces* yeast. In brief, it is important to increase sanitation in the winery environment and personal awareness to maximize cleanliness and to reduce any unwanted yeast activity. More importantly, in the recent years, the use of non-*Saccharomyces* yeast is attracting winemakers to achieve unique wine styles, and it is an important topic that should be taken under consideration, particularly on a research basis, specifically for targeting consumer liking-perceptions of the wine. In addition to their positive effect on sensory characters on wines, non-*Saccharomyces* yeasts as bio-control agents (BCAs) is also charming researchers around the globe.

1. Introduction

In the late 1800's Louis Pasteur have studied yeasts and their roles in the winemaking (Jolly et al., 2014). Winemaking is a process that requires many steps and decision making to reach the best quality as final product. One of the most important of them is fermentation step and the choice of yeast. The role of the yeast is to transform sugar into alcohol, and it is depending on multiple factors such as N availability, temperature, aimed style, grape quality etc. The expectation from yeast is short lagging phase, minimum residual sugar, complexity of aroma and flavour. In addition, activity at low temperatures and less foaming are also important aspects. Besides, low temperatures could favour the growth phase of non-*Saccharomyces* yeast at the beginning of fermentation. In general, active dried yeast inoculation is the common method industry wise (Aranda et al., 2011). Fermentation usually begins with non-*Saccharomyces* yeast group, present on grape must or on winery environment and activity stops due to high ethanol levels approximately in 2-4 days. Moreover, *Saccharomyces cerevisiae* continues and ends the fermentation after this initial process. Whereas activity of non-*Saccharomyces* yeast for couple of days at the beginning they produce important elements that effects the quality of wine (Manzanares et al., 2011).

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metehangunhan@gmail.com

Following research displayed that the role of *Saccharomyces cerevisiae* is not only taking part in the fermentation process also affecting the aroma precursors of wine by manipulating the secondary metabolites. *Saccharomyces cerevisiae* were the main specie and both indigenous and exogenous forms were desired. Therefore, grape juice contains many other yeast species known as non-*Saccharomyces* yeasts. Latest technology helped the researchers to isolate good characteristics of non-*Saccharomyces* yeasts. Approximately 40 non-*Saccharomyces* species discovered and have been cultured in-vitro conditions derived from grapes and grape juices. Grape berries are the main foundation of these yeasts whilst non-*Saccharomyces* yeast existence in the vineyard is not a common situation (Hranilovic, 2018). Some of the important non-*Saccharomyces* yeasts found in vineyards and wineries can be listed as; *Aureobasidium*, *Brettanomyces*, *Debaryomyces*, *Hanseniaspora*, *Metschnikowia*, *Lachancea*, *Torulaspora*, *Pichia*, *Rhodotorula*, *Starmerella* and *Zygosaccharomyces*. Also, non-*Saccharomyces* yeasts *Torulaspora delbrueckii*, *Metschnikowia pulcherrima* and *Pichia kluveri* is preferred for sparkling wine production recently (Ivit and Kemp, 2018). Compared to *Saccharomyces cerevisiae*, these species usually present in grape juice naturally and abundant in population which

makes them viable against *Saccharomyces cerevisiae* dominance (Jolly et al., 2014). As a winemaking term, non-*Saccharomyces* yeast designates multiple yeast types other than *Saccharomyces cerevisiae* which they are present in wineries and around.

2. Advantages and Disadvantages

There are negative aspects of using non-*Saccharomyces* yeasts as well as positive aspects (Ivit and Kemp, 2018). Previous research was claiming non-*Saccharomyces* yeast negatively affected by added SO₂ and yeast growth controlled by the addition (Jolly et al., 2014). Presence of oxygen is critical for the lifespan and durability of some species such as *Lachancea thermotolerans* and *Torulospora delbreuckii* as well as cell viability of non-*Saccharomyces* species (Hranilovic, 2018). Therefore, late publications revealed that their intolerance were due to dual toxic effect between SO₂ and fermentation derived alcohol. However, spontaneous ferments have led unique, fine textured and more complex wines however with the threat of microbial spoilage (Jolly et al., 2014).

2.1. Alcohol

The modern research technique called cell counting arose the re-questioning of non-*Saccharomyces* yeasts' role. In winemaking higher alcohol levels are not desirable and non-*Saccharomyces* yeasts generally decreases the level of high alcohols however there is also a major problem of variability between species (Jolly et al., 2014). In addition, each non-*Saccharomyces* yeast produce diverse levels of alcohol during fermentation. A non-*Saccharomyces* yeast *Metshnikowia pulcherrima* has a key prospect for producing lower alcohol wines (Hranilovic et al., 2020) and some strains of *M. pulcherrima* are now available for wine industry (Aplin et al., 2021).

Decline of alcohol content could range between 0.6-1.2% (v/v) (Hranilovic et al., 2020). *M. pulcherrima* and sequential inoculation of *M. pulcherrima* and non-

Saccharomyces did not alter sensory properties but provided lower alcohol levels on final wines (Aplin et al., 2021).

Glycerol is another important yeast metabolite occurs during alcoholic fermentation in wine. It has positive effect on smooth mouth feel, sweetness and enhances the complexity of the wine. While this is the case, there is a positive correlation between glycerol and acetic acid production, which could be unfavourable for final wine quality (Jolly et al., 2014).

2.2. Flavour, aroma, mouthfeel and colour

There is a wide choice of flavour compounds by non-*Saccharomyces* yeast according to past publications. Some metabolites affected by non-*Saccharomyces* yeast activity, such as terpenes, esters, alcohols, glycerol, acetaldehyde, acetic and succinic acid. Some specific aroma and flavour compounds are existing in grape berries as non-flavoured or non-aromatic precursors. These compounds require a specific enzyme β-glucosidase to hydrolyze and form free volatile compounds (Jolly et al., 2014). Although, there are non-*Saccharomyces* species such as *Debaryomyces*, *Hansenula*, *Candida*, *Pichia* and *Kloeckera* that displays different levels of β-glucosidase activity and improve aroma and flavour of wine by freeing volatile compounds. In another trial, addition of an enzyme taken out from *Debaryomyces pseudopolymorphus* has enhanced the volatile terpenes in Riesling, Muscat and Arien varieties (Jolly et al., 2014). Interactions between *Saccharomyces* and non-*Saccharomyces* yeasts, regardless of positive and/or negative, significantly effects the foundation of aroma compounds. Consequently, co-inoculation with multiple non-*Saccharomyces* species is a likely way to enhance the aroma multiformity and the quality of the final product. Therefore, this can enthuse winemakers to create optimum multiple ferment starters to enhance the volatile compounds and organoleptic attributes of local wine industry (Zhang et al., 2022).

Table 1. Sensory characters and their technical effects of some non-*Saccharomyces* species

Non- <i>Saccharomyces</i> Species	Sensory Character	Technical Effect
<i>Hanseniaspora/Kloeckera</i>	Floral, rose petal notes	Increases floral notes 2-10x compared to <i>S. cerevisiae</i>
<i>Hanseniaspora vineae</i>	Floral jasmine	Floral
<i>Lachancea thermotolerans</i>	Floral, rose petal, strawberry, citrus hints	Slight alcohol reduction
<i>Metschnikowia pulcherrima</i>	Rose, Floral aromas	Enhanced varietal aromas
<i>Pichia kluveri</i>	Grapefruit, passionfruit	Fruit aromas
<i>S. pombe</i>	Stable pigments and colour, vitisin A precursor, silky astringency	Improve of vitisin A activity
<i>Torulospora delbreuckii</i>	Floral, honey, apple, black currant	Dark fruit aromas
<i>Wickerhamomyces anomalus</i>	Floral, honey, banana, fruity	Increases fruity aromas

(Adapted from Morata et al., 2019)

The bacteria *Oenococcus oeni* is the key factor for a successful malolactic fermentation (MLF) in wine

(Lonvaud-Funel, 1999; Balmaseda et al., 2021). However, physicochemical attributes of wine make it a rough

environment for *Oenococcus oeni* to dodge a couple of stress factors (Bech-Terkilsen et al., 2020). Therefore, specific loci identified in *Oenococcus oeni*, then again dependent on strain, due to the metabolic abilities of the yeasts, has a major effect on *Oenococcus oeni* to acclimatize to wine environment. For instance, study of Balmaseda et al., 2021, revealed linkage of yeast-bacteria interaction and their harmony during Malo-lactic fermentation (MLF) in which resulted as the use of non-*Saccharomyces* yeast triggered a faster paced MLF.

Even if there are not enough studies for colour effect of non-*Saccharomyces* yeast, it is known that yeast has an impact on wine colour (Jolly et al., 2014). A non-*Saccharomyces* species *Pichia guilliermondii* has provided better colour stability and well-formed anthocyanin molecules as well as *S. cerevisiae* (Benito et al., 2011). Mostly, flavour compounds primarily derived from grape berries and secondarily from esters by yeast activity throughout fermentation. There are some strains of *S. cerevisiae* which are varying by their tannin binding attraction (Mazauric and Salmon 2006; Sidari et al., 2007; Hranilovic et al., 2018). The ability of tannin binding of a yeast can be sturdily affected by the structure of the fermentation environment (Rinaldi et al., 2016; Hranilovic et al., 2018). Božić et al. (2021) have investigated the effect of indigenous yeast on colour of Pinot Noir wines. According to their study, the use of indigenous yeast had an effect on chemical attributes of wines, along with sensory characters. Even though more intense colour is achievable via indigenous strains, factors such yeast interaction, unwanted metabolite secretion needs to be taken into consideration when choosing fitting starter cultures since they might decrease the wine quality.

2.3. Risks and benefits

New inoculation methods which involve non-*Saccharomyces* yeasts often leads to slow paced fermentations, changes in wine structure and quality enhancement (Hranilovic et al., 2018). According to previous publications, as an alternative to *S. cerevisiae* yeast, there are several roles of non-*Saccharomyces* yeast in winemaking which are increase of complexity, flavour, aroma, decrease of alcohol levels, reaching a better colour. The sequential inoculation of *Starmerella bacillaris* with *S. cerevisiae* declines the acetic acid levels in sweet style wines owing to the growth performance of the *S. bacillaris* even at peak sugar levels (Rantsiou et al., 2012). Moreover, invertase activity of *S. bacillaris* strains revealed that, it can consume sugars other than glucose during the fermentation. However, hydrolytic enzyme activities of non-*Saccharomyces* yeast such as glucosidases, lipases and proteases which are the causes of arose of volatile and non-volatile by-products (Genc, 2022). Moreover, Barbosa et al. (2022) have investigated the supervised and unsupervised machine learning to modulate fermentation environment. According to their study, it was achievable to improve aromatic and fermentation prospect of *H. guilliermondii* UTAD222 strain by temperature, nitrogen (N) and/or sugar modifications which then directly involved in production of specific volatiles. Due to use of non-*Saccharomyces* yeast such as Brett or high amounts of volatile aroma compounds may cause reductions in wine. In addition, malic acid in wine

is linked to tough mouthfeel characteristics and decrease towards ripening stage (Ribéreau-Gayon et al., 2006; Su et al., 2014; Hranilovic et al., 2018). However, *Schizosaccharomyces pombe* can completely break down malic acid throughout the fermentation whereas other species takes part of its fractional break down or an incline in concentration, reliant on the strain and environment (Kapsopoulou et al., 2007; Jolly et al., 2014; Su et al., 2014; Benito et al., 2015; Hranilovic et al., 2018). Sidari et al. (2021) have tested the fermentation performances of eleven yeast strains. Non-*Saccharomyces* yeasts have indicated lower fermentation vigour compared to *S. cerevisiae* yeasts which were performed the peak performance. In the same study, alike observations were defined for *M. pulcherrima* 125/4 strain and worst performance defined for *D. hansenii* 5-1-6 strain.

Milestone studies about *S. cerevisiae* up to date is essential to illustrate the identified and/or potentially unique pathways of aromatic compound metabolism in non-*Saccharomyces* yeasts (Hazelwood et al., 2008; Sumby et al., 2010; Hranilovic et al., 2018). Research from Italy have shown 58 samples isolated from various winery environments including winery equipment, grapes and musts. These winery surfaces hold the microorganisms which starts fermentations (Ciani et al., 2004). In a perfect world, *S. cerevisiae* is enough to start and end fermentation but research shows that it causes uniformity. In addition, lack of non-*Saccharomyces* yeasts may cause less complexity, flavour and aroma characteristics (Ivit and Kemp, 2018). A traditional method known as pied de-cuve is used to acquaint a chosen yeast or start a fermentation from an already fermenting grape must. Non inoculated pied de-cuve, which can be obtained started from early-stage fermenting grape musts, could be a potential method if the aim is to trigger the native microflora. This method could enhance the microbiological control during ongoing alcoholic fermentation, in comparison to spontaneous fermentation whilst conserving the typicity and terroir of the final product (Mas and Portillo, 2022)

Ocón et al. (2010) have discovered yeasts on winery environment nearly 40% of it were *S. cerevisiae* and suggested that it is more tolerant to use of SO₂ compared to non-*Saccharomyces* yeasts. In addition, yeast colony presence is also depending on the season, grape cleanliness as well as winery cleanliness. Non-*Saccharomyces* yeasts are much stronger compared to *S. cerevisiae* under general methods of sanitation and as a result population of non-*Saccharomyces* yeasts were higher than *S. cerevisiae* (Ocón et al., 2010).

The organic acids, as an outcome of some non-*Saccharomyces* yeast activity, aids to obtain sounder and more stable wines even during the process of ageing both in barrel and bottle. Moreover, the biocontrol attributes of these yeasts provide a harmless production line for wines by the decreasing the levels of wine preservatives, e.g. sulphites in different forms (Morata et al., 2019). Equally important, non-*Saccharomyces* strains consist of a major variety of species that have indicated effectual antagonism against pathogenic fungi on grapevines. Several studies have revealed the capabilities by further increasing its act by linking it with other components or organisms. Furthermore,

endophytic yeasts could shortly become a supply source as bio-control agents (BCAs). In fact, *Metschnikowia*, *Pichia*, and *Hanseniaspora* have recently been discovered in grapes (Di Canito et al., 2021). In a similar study, according to Agarbat et al. (2022), *A. pullulans* and *M. pulcherrima* have shown the most favourable performances against *Botrytis cinerea* (grey mould). This method is closely linked to consumer expectancies while taken advance of bio-control applications into account in agriculture and food industry, based on ecological welcoming and chemical free treatments.

3. Conclusion

Regardless of growing numbers of research focusing on identification of various non-*Saccharomyces* yeast species and strains, their prospective effects on wine quality widely undiscovered (Hranilovic, 2018). Non-*Saccharomyces* yeast known to be vulnerable against some stress factors and not able to metabolize all sugars during fermentation contrasting *S. cerevisiae* (Jolly et al., 2014; Hranilovic, 2018) However, to overcome inconsistency and declined complexity while avoiding the risks of naturally inoculated fermentation, mixed fermentations could be an option in vinification (Ciani et al., 2010; Hranilovic, 2018). Therefore, use of mixed starter culture with non-*Saccharomyces* is becoming a new trend recently (Aranda et al., 2011).

To summarize, factors such as sanitation practices in the winery environment, tools used in the vineyard, transport sanitation including equipment operations and personal awareness has a vital role to avoid unwanted yeasts. Sanitation frequency and quality of sanitation products are equally important facts. Equally important, *S. cerevisiae* and non-*Saccharomyces* species found in the wineries could be a source for indigenous yeast cultures for both scientific purposes and small batch winemaking for further research to prove their effectiveness and investigate quality alterations in the world but most specifically in Türkiye.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Metehan Gunhan: Validation, Writing - Original draft, Visualization, Investigation, Review

Ethical approval

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Data availability

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