



## **The role of non-*Saccharomyces* microorganisms and their technological importance in winemaking: a review**

**Olga Soldatenco\*, Nicolae Taran, Victoria Adajuc**

*Public Institution Scientific-practical Institute of Horticulture and Food Technologies  
Vierul street, 59, Republic of Moldova, Chisinau, Codru, MD-2070*

*\*Corresponding author: soldatencoolga1987@gmail.com*

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### **Abstract**

In recent decades, the role of non-*Saccharomyces* microorganisms in winemaking has gained significant attention due to their potential impact on wine quality and sensory characteristics. This review provides a comprehensive analysis of the contribution of non-*Saccharomyces* yeasts to various aspects of winemaking, including fermentation kinetics, aroma and flavor development, and overall wine quality. The review also explores the technological importance of non-*Saccharomyces* microorganisms in the context of modern winemaking practices, highlighting their potential applications in improving wine complexity, enhancing aroma profiles, and promoting sustainability in the wine industry. Through an extensive examination of recent research findings and technological advancements, this review aims to provide insights into the diverse roles and promising applications of non-*Saccharomyces* microorganisms in contemporary winemaking.

**Key words:** non-*Saccharomyces* yeasts, winemaking, fermentation kinetics, aroma development, flavor complexity, sustainability

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### **1. Introduction**

In recent years, the use of non-*Saccharomyces* yeasts in fermentation and wine production has increasingly preoccupied experts in countries with winemaking traditions (France, Spain, Italy, Portugal, Greece), as well as in other countries (Australia, Chile, Argentina, South Africa). Some authors consider their impact on wine quality to be negative, while others discover some favorable technological capacities of this group of yeasts. Their ability to enhance the complexity of the final product, produce various aromas and enzymes is reported in numerous scientific publications [1-7].

Thus, the oenological usefulness of non-*Saccharomyces* yeasts is reported by K. Zott *et al.*, who, through the analysis of volatile thiols, demonstrated that unconventional yeasts - non-*Saccharomyces* - transform cysteine precursors into aromas even during partial fermentation [8]. In the Department of Agricultural Biotechnologies at the University of Florence, as well as in the Department of Food, Industrial and

Environmental Microbiology at the Polytechnic University of Ancona, Italy, researchers P. Domizio, C. Romani, L. Lencioni *et al.*, through screening of 55 strains of non-*Saccharomyces* yeasts of the genera *Hanseniaspora*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces*, have established their usefulness for winemaking, expressed through the production of valuable secondary fermentation metabolites, demonstrating that these strains are useful microbiological tools in the wine industry [5].

F. Comitini, M. Gobbi found that mixed fermentation by inoculating conventional species - *Saccharomyces* and unconventional species - non-*Saccharomyces*, allows for a significant improvement in the organoleptic and analytical characteristics of wines, but it is necessary to monitor the toxic compounds of fermentation [4].

Research on the enzymatic activity of non-*Saccharomyces* yeast strains has established that strains of the *Hansenula* genus possess

high  $\beta$ -glucosidase activity. Zarosso, B. et al. established the proteolytic, esterolytic, and pectinolytic activity of about 60 strains of unconventional yeasts and demonstrated the capacity of indigenous strains to produce extracellular enzymes with technological significance that impart specific sensory properties to the wine grape variety [6].

In Greece, at the Laboratory of Biotechnologies of the Fermentative Industry of the Department of Oenology of the Institute of Educational Technologies in Athens, P.Tataridis, A. Kanellis et al. studied the possibility of obtaining wines and beer using the *Torulaspora delbrueckii* strain. It was found that this strain ferments slowly, and to complete the process, a *Saccharomyces* strain is needed. However, the strain has the ability to produce larger quantities of organic acids, glycerol, and 2-phenylethanol compared to yeasts of the *Saccharomyces* genus. Sensory analysis revealed that wine fermented with the *Torulaspora delbrueckii* strain has higher acidity, giving it freshness, a complex floral-fruity aroma, and is appreciated with a high organoleptic score [10].

At the Institute of Wine Biotechnology, Department of Viticulture and Oenology in Matieland (Stellenbosch) in South Africa, N. P. Joly, O.P. Augustin, in collaboration with I.S. Pretorius from the Wine Research Institute in Glen Osmond, Australia, studied the separate influence of non-*Saccharomyces* yeasts and combined with *Saccharomyces* yeasts on the wine production process. The fermentation kinetics, analytical and biochemical indices of musts from different grape varieties were analyzed. The quality of wines in samples fermented with combined yeasts was improved, imparting specific taste and aroma characteristics to the wine. Thus, this practice allows for the discovery of new types of wine with specific terroir characteristics, compared to most wines obtained by classical technology. In this way, countries with ancient winemaking traditions have conquered global markets [11,12].

Mendoza L. et al. studied the possibility of obtaining Argentine wines with special organoleptic characteristics using conventional and non-*Saccharomyces* strains. As a result, a new technology for obtaining wines with specific characteristics of Argentine wines based on initial fermentation with the *Kloeckera apiculata* strain, followed by

*Saccharomyces*, and at the end of alcoholic fermentation, inoculation with *O. oeni* lactic bacteria was proposed to reduce the harsh acidity of the wines [13].

Producers of active dry yeasts have also achieved performance in this field. Until recently, there was no non-*Saccharomyces* species in the form of active dry yeast efficient enough from a technological point of view to promote their production and use in winemaking. Winemakers could not rely on the spontaneous development of indigenous yeasts to benefit from their positive effects due to the risk of fermentation failure and inherent sensory deviations. However, such active dry strains from the *T. delbrueckii* genus are currently available for winemakers. This genus must be used in combination with the *Saccharomyces* genus to efficiently and rapidly carry out alcoholic fermentation. Consequently, combinations of *Saccharomyces* and non-*Saccharomyces* for wine fermentation are now available in active dry form and contribute to improving the sensory qualities of wines, revealing specific organoleptic properties. Thus, a bibliographic study of the literature on the use of non-*Saccharomyces* yeasts in alcoholic fermentation in winemaking highlighted several factors:

- *Torulaspora delbrueckii* yeasts produce specific aromatic profiles during fermentation, improve the organoleptic properties of wines, and their use is recommended alongside *Saccharomyces* strains;
- The use of *Torulaspora delbrueckii* yeasts in winemaking is possible in combination with (one or more) *Saccharomyces cerevisiae* strains, yeasts that ensure the completion of alcoholic fermentation in a reasonable time. However, it is necessary to study, from an applicative point of view, the inoculum quantity, as well as to model the timing or strategy of inoculation (co-inoculation, sequential inoculation). Studies on the synergy of the interaction between these two species are relatively few, especially in terms of quantifying the effect of this interaction.
- The influence of the nutrient medium on the development of these two different strains is also poorly studied. However, we can assume that there is competition between the two species for certain nutrition components such as nitrogen source,

vitamins, and trace elements.

The intricate art of winemaking has long been intertwined with the microbial world, where yeasts play a pivotal role in fermenting grape must into the exquisite elixir known as wine. While *Saccharomyces cerevisiae* has traditionally been regarded as the primary workhorse responsible for alcohol fermentation in winemaking, recent decades have witnessed a resurgence of interest in the diverse community of non-*Saccharomyces* microorganisms inhabiting vineyard ecosystems and contributing to the winemaking process [1].

In this review, we delve into the fascinating realm of non-*Saccharomyces* microorganisms and their technological importance in winemaking. These microorganisms, often overshadowed by the dominance of *Saccharomyces cerevisiae*, offer a wealth of metabolic capabilities and sensory contributions that can profoundly influence wine quality, aroma, and complexity. Through an exploration of their taxonomic diversity, ecological niches, metabolic versatility, and practical applications, this review aims to provide insights into the multifaceted roles of non-*Saccharomyces* yeasts in contemporary winemaking practices [2].

As we navigate through the intricate tapestry of microbial interactions within the winery environment, we uncover the potential of non-*Saccharomyces* microorganisms to enhance fermentation kinetics, modulate wine composition, and promote sustainability in the wine industry. By harnessing the metabolic diversity and functional traits of these microorganisms, winemakers can embark on a journey of innovation, embracing alternative fermentation strategies and exploring new avenues for crafting wines of exceptional quality and distinction [3].

Through a comprehensive analysis of recent research findings and technological advancements, this review seeks to illuminate the promising applications of non-*Saccharomyces* microorganisms in shaping the future landscape of winemaking. By shedding light on their contributions to fermentation dynamics, aroma development, and overall wine quality, we aim to inspire further exploration and experimentation in the realm of wine microbiology, fostering a deeper understanding of the intricate interplay between microorganisms and grape-derived substrates [10].

## **2. Material and methods**

### **2.1. Literature Review Approach**

A literature review was conducted to study the role of non-*Saccharomyces* microorganisms in winemaking. The focus was on research addressing the taxonomic diversity, ecological niches, metabolic capabilities, and practical applications of non-*Saccharomyces* yeasts during wine fermentation.

### **2.2. Data Sources**

The analysis was based on scientific publications from peer-reviewed journals, reviews, and experimental studies published between the 1990s and 2022. Key sources included articles from specialized journals and scientific databases, covering works in English and other accessible languages.

### **2.3. Data Analysis**

The collected data were categorized according to the following criteria:

Taxonomic classification of microorganisms.

Impact of non-*Saccharomyces* yeasts on the chemical composition and organoleptic properties of wine.

Biotechnological potential and practical use in mixed fermentations with *Saccharomyces cerevisiae*.

Each study was evaluated in terms of experimental conditions, analytical methods, and conclusions drawn by the authors.

### **2.4. Limitations**

The review included sources available in open-access databases or through subscriptions.

## **3. Results and Discussion**

### **3.1. Diversity of Non-*Saccharomyces* Microorganisms**

Non-*Saccharomyces* yeasts constitute a diverse group of microorganisms with significant roles throughout various stages of winemaking, including grape must fermentation. Unlike *Saccharomyces cerevisiae*, the predominant yeast species traditionally linked with wine fermentation, non-*Saccharomyces* yeasts offer a broad spectrum of metabolic capabilities and contribute distinct sensory characteristics to wines.

**Taxonomic Diversity:** The taxonomic landscape of non-*Saccharomyces* yeasts encompasses numerous genera and species, each endowed with unique physiological traits and metabolic activities. Commonly found

genera within vineyard and winery environments include *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Candida*, and *Zygosaccharomyces*, among others. The genetic and phenotypic diversity within these genera leads to variations in fermentation kinetics, stress tolerance, and metabolic pathways among different yeast strains [1, 11].

**Ecological Niches and Distribution:** Non-*Saccharomyces* yeasts thrive in diverse ecological niches within vineyard ecosystems, ranging from grape surfaces and grapevine tissues to winemaking equipment. Their distribution is influenced by factors such as grape variety, geographical location, climate, vineyard management practices, and seasonal variations. Studies have reported variations in the prevalence and abundance of non-*Saccharomyces* yeasts across different wine regions and vineyard sites, with certain species displaying preferences for specific climatic conditions [2].

**Metabolic Diversity and Functional Traits:** Non-*Saccharomyces* yeasts demonstrate a wide range of metabolic capabilities, enabling them to metabolize various carbon sources and produce a diverse array of secondary metabolites. These metabolites, including esters, higher alcohols, fatty acids, and volatile sulfur compounds, significantly influence wine aroma, flavor, and mouthfeel. Additionally, non-*Saccharomyces* yeasts contribute to glycerol production, acidity modulation, and polysaccharide release, further impacting wine sensory attributes [10].

**Genetic and Phenotypic Variation:** Genetic studies have unveiled substantial genetic diversity among non-*Saccharomyces* yeast strains, manifesting in variations in gene content, regulatory networks, and metabolic pathways. Phenotypic variations among yeast strains contribute to differences in fermentation kinetics, stress tolerance, and aroma production. For instance, certain strains may exhibit heightened ester-producing capabilities, enhancing fruity aromas in wines, while others may contribute to acidity modulation or glycerol production [8, 11].

**Interactions with *Saccharomyces cerevisiae*:** Non-*Saccharomyces* yeasts frequently coexist with *Saccharomyces cerevisiae* during wine fermentation, influencing fermentation kinetics, wine composition, and sensory characteristics. While some species exhibit synergistic interactions, enhancing

fermentation efficiency and aroma complexity, others may compete for nutrients and substrates, potentially affecting fermentation outcomes [11, 14, 15].

In summary, the diversity of non-*Saccharomyces* yeasts is a pivotal determinant of their metabolic activities and contributions to wine quality and diversity. Understanding

### 3.2. Fermentation kinetics

Fermentation kinetics stand as a cornerstone in winemaking, dictating the duration and intricacies of alcoholic fermentation. Historically, *Saccharomyces cerevisiae* has held sway as the primary fermenter, celebrated for its vigorous fermentation and adept sugar metabolism. However, recent studies have illuminated the significant contributions of non-*Saccharomyces* yeasts in molding fermentation kinetics and ultimately shaping wine composition.

In stark contrast to *Saccharomyces cerevisiae*, non-*Saccharomyces* yeasts often exhibit a more leisurely pace in fermentation, potentially extending the duration of fermentation processes. This prolonged exposure of grape must to yeast metabolites offers a prolonged interaction period, which could significantly impact the sensory attributes of the resultant wine. Moreover, the cohabitation of non-*Saccharomyces* yeasts alongside *Saccharomyces cerevisiae* introduces layers of complexity to the fermentation milieu.

Numerous investigations have delved into the influence of non-*Saccharomyces* yeasts on fermentation kinetics, revealing intriguing insights into their modulatory role. For instance, Medina et al. [16] conducted a study showcasing that co-fermentation of Chardonnay must with *Hanseniaspora vineae* and *Saccharomyces cerevisiae* led to heightened flavor diversity compared to singular *Saccharomyces cerevisiae* fermentation. Similarly, Sadoudi et al. [17] observed that the co-culturing of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* influenced the aromatic profile of Sauvignon Blanc wine, underscoring the capacity of non-*Saccharomyces* yeasts to influence fermentation kinetics and flavor evolution.

Moreover, the presence of non-*Saccharomyces* yeasts during fermentation has been associated with notable alterations in wine composition. Kręgiel et al. [18] provided a comprehensive

review of the potential of *Metschnikowia pulcherrima* in winemaking, emphasizing its prowess in generating unique aroma compounds and enriching wine complexity. Additionally, Renouf *et al.* [19] explored the impact of oxygen on the production of volatile phenols by *Brettanomyces*, highlighting the pivotal role of environmental factors in shaping fermentation kinetics and ultimately influencing wine quality.

In conclusion, the involvement of non-*Saccharomyces* yeasts in winemaking significantly influences fermentation kinetics and the ultimate composition of wine. Their leisurely fermentation pace, coupled with their ability to coexist harmoniously with *Saccharomyces cerevisiae*, introduces layers of complexity to the winemaking process, yielding wines endowed with diverse flavor profiles and heightened sensory characteristics.

### 3.3. Technological Importance and Practical Applications

The integration of non-*Saccharomyces* microorganisms into winemaking practices represents a paradigm shift, offering a myriad of opportunities for innovation and product diversification. These microorganisms, often overshadowed by the dominance of *Saccharomyces cerevisiae*, bring forth a wealth of metabolic capabilities and sensory contributions that can be harnessed to craft wines of exceptional quality and complexity. One of the primary avenues through which non-*Saccharomyces* microorganisms exert their influence is through the formation of mixed microbial consortia. By combining non-*Saccharomyces* yeasts and bacteria with *Saccharomyces cerevisiae*, winemakers can orchestrate fermentation processes that transcend the limitations of monoculture fermentations. This approach allows for the exploration of synergistic interactions among different microbial species, leading to the development of wines with enhanced aromatic complexity, structural nuances, and overall sensory richness [4].

Moreover, the utilization of non-*Saccharomyces* microorganisms enables winemakers to implement alternative fermentation strategies, such as sequential or simultaneous inoculation techniques. Through strategic manipulation of microbial populations, winemakers can tailor fermentation kinetics, control the release of

volatile compounds, and modulate the expression of desirable sensory attributes. This flexibility empowers winemakers to adapt their practices to suit specific grape varieties, regional characteristics, and market demands, thereby expanding the scope of possibilities in winemaking [6].

Furthermore, non-*Saccharomyces* microorganisms offer a toolbox of biotechnological tools that can be employed to address various challenges encountered during winemaking. These microorganisms possess enzymatic activities that contribute to the breakdown of undesirable compounds, such as acetic acid and ethyl acetate, thus mitigating the risk of off-flavor development. Additionally, certain non-*Saccharomyces* yeasts exhibit enzymatic pathways capable of enhancing the production of glycerol, polysaccharides, and other mouthfeel-enhancing compounds, thereby improving wine texture and mouthfeel [6].

The integration of non-*Saccharomyces* microorganisms into winemaking practices underscores the dynamic interplay between microbial ecology and technological innovation. By embracing the diversity of microorganisms present in the winery environment, winemakers can unlock new dimensions of creativity and quality in wine production. These advancements not only enrich the sensory experience of consumers but also contribute to the preservation of regional identity and the sustainability of the wine industry as a whole.

### 3.4. Research Findings on Co-Fermentation

The co-fermentation of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* has emerged as a promising strategy to enhance wine flavor diversity and aromatic complexity. Several key studies have shed light on the intricate interactions between different microbial species during fermentation and their collective impact on wine quality. By integrating findings from multiple sources, including Medina *et al.* [16] and Sadoudi *et al.* [17], we can elucidate the dynamics of co-culturing non-*Saccharomyces* yeasts and their effects on fermentation kinetics and flavor development.

Medina *et al.* [16] conducted a seminal study that demonstrated the influential role of non-*Saccharomyces* yeasts in shaping wine flavor diversity. Through co-fermentation experiments with Chardonnay must, the researchers

observed a significant increase in flavor complexity when *Hanseniaspora vineae* was co-cultured with *Saccharomyces cerevisiae*, compared to single *Saccharomyces cerevisiae* fermentation. This finding highlights the potential of non-*Saccharomyces* yeasts to augment the sensory attributes of wines and underscores their importance in modern winemaking practices.

Furthermore, Sadoudi *et al.* [17] explored the impact of co-culturing non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* on the aromatic profile of Sauvignon Blanc wine. The researchers found that the presence of non-*Saccharomyces* yeasts significantly influenced the volatile composition of the wine, leading to distinctive aroma profiles not typically observed in wines produced through monoculture fermentation. This study underscores the capacity of non-*Saccharomyces* yeasts to modulate fermentation kinetics and flavor development, thereby enriching the sensory experience of wine consumers.

Moreover, recent research has highlighted the importance of understanding the metabolic interactions between different microbial species during co-fermentation. By employing advanced analytical techniques such as metabolomics and transcriptomics, researchers have been able to unravel the intricate metabolic pathways involved in flavor formation and aroma release during fermentation. These studies have provided valuable insights into the mechanisms underlying the synergistic effects of co-culturing non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae*, paving the way for more targeted fermentation strategies aimed at optimizing wine quality.

In addition to the aforementioned studies, several other researchers have contributed to our understanding of co-fermentation dynamics and its impact on wine quality. Gobbi *et al.* [9] studied the co-fermentation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae*, revealing its potential to enhance acidity and improve overall wine quality.

Table 1 depicts the interaction between yeast species and indicates the purpose of their use, mode of administration, and the authors of these studies.

### 3.5. Influence on Wine Composition

The composition of wine is a complex interplay of various factors, including grape

variety, terroir, winemaking techniques, and importantly, the microbial community involved in fermentation. Non-*Saccharomyces* yeasts, once considered mere contaminants or spoilers, are now recognized for their significant contributions to wine complexity and aroma profile. This discussion will delve into the research conducted by Milanović *et al.* [41], Renouf *et al.* [19], as well as other researchers, to illustrate how non-*Saccharomyces* yeasts shape wine composition through the production of unique aroma compounds and volatile phenols. Milanović *et al.* [41] explored the potential of *Metschnikowia pulcherrima*, a common non-*Saccharomyces* yeast species, in winemaking. Their research revealed that *M. pulcherrima* possesses enzymatic capabilities to produce a diverse array of aroma compounds, thereby enhancing wine complexity. Through co-fermentation experiments, Milanović *et al.* demonstrated that *M. pulcherrima* contributes to the synthesis of volatile compounds such as esters, terpenes, and thiols, which are known for their impact on wine aroma. These findings underscore the importance of non-*Saccharomyces* yeasts in enriching the sensory attributes of wines and highlight their potential as valuable contributors to wine quality. Furthermore, Renouf *et al.* [19] investigated the impact of non-*Saccharomyces* yeasts, specifically *Brettanomyces/Dekkera*, on wine composition, focusing on the production of volatile phenols. *Brettanomyces / Dekkera* species are notorious for their ability to produce volatile phenols, such as 4-ethylphenol and 4-ethylguaiacol, which contribute distinct aroma characteristics often described as "barnyard" or "spicy." Renouf *et al.* elucidated the enzymatic pathways involved in volatile phenol production by *Brettanomyces/Dekkera* and demonstrated the influence of environmental factors, such as oxygen exposure, on phenol synthesis during fermentation. Their findings underscore the dynamic nature of microbial interactions in shaping wine composition and highlight the importance of managing microbial populations to control undesirable flavor compounds. Moreover, recent research by Padilla *et al.* [42] has provided insights into the role of non-*Saccharomyces* yeasts in modulating wine aroma complexity. Their study investigated the impact of various non-*Saccharomyces* yeast species, including *Hanseniaspora*, *Metschnikowia*, and *Pichia*, on the volatile profile of wines.

**Table 1.** Overview of Saccharomyces and Non-Saccharomyces Species, Objectives, and Administration Methods for Wine Fermentation Studies.

<i>The species used</i>	<i>The aim</i>	<i>The method of administration</i>	<i>References</i>
<i>S. cerevisiae</i> / <i>T. delbrueckii</i>	The reduction of acetic acid content and the increase in aromatic complexity of wines	Sequential strains	[20,21]
<i>S. cerevisiae</i> / <i>S. pombe</i> și <i>Schizosaccharomyces spp</i>	Malic acid degradation	Immobilized yeasts (batch and continuous processes)	[22-24]
<i>S. cerevisiae</i> / <i>C. stellata</i>	Increasing glycerol concentration	Immobilized yeasts (sequential strains)	[25,26]
<i>S. cerevisiae</i> / <i>C. cantarellii</i>	Increasing glycerol concentration	Mixed or sequential strains	[27]
<i>S. cerevisiae</i> / <i>C. stellata</i>	Increasing the aromatic profile	Mixed or sequential strains	[28]
<i>S. cerevisiae</i> / <i>H. uvarum</i> ( <i>K. apiculata</i> )	Enhancing natural AF (improving aromatic complexity)	Mixed or sequential strains	[29-31]
<i>S. cerevisiae</i> / <i>K. thermotolerans</i>	Reducing titratable acids by decreasing acetic acid concentration	Sequential strains	[32, 33]
<i>S. cerevisiae</i> / <i>I. orientalis</i>	Malic acid degradation	Mixed alcoholic fermentation	[34]
<i>S. cerevisiae</i> / <i>P. fermentans</i>	Increasing aromatic complexity	Sequential strains	[35]
<i>S. cerevisiae</i> / <i>P. kluyveri</i>	Increasing varietal thiols	Mixed alcoholic fermentation	[36]
<i>S. cerevisiae</i> / <i>C. pulcherrima</i>	Improvement of the aromatic profile	Mixed alcoholic fermentation	[37, 38]
<i>S. cerevisiae</i> / <i>D. vanriji</i>	Improvement of geraniol concentration	Mixed alcoholic fermentation	[39]
<i>S.cerevisiae</i> / <i>Schizosaccharomyces et Pichia</i>	Influence on the organoleptic and physico-chemical properties of wine	Reproduction during wine maturation	[40]

Padilla *et al.* demonstrated that co-fermentation with specific non-*Saccharomyces* yeasts led to the production of unique aroma compounds not typically associated with *Saccharomyces cerevisiae* fermentation, thus enhancing overall wine complexity.

Additionally, Ciani *et al.* [3] explored controlled mixed-culture fermentation as a strategy to improve wine quality using non-*Saccharomyces* yeasts. Through simultaneous or sequential inoculation of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae*, they demonstrated the potential to enhance aroma diversity, acidity modulation, and overall sensory characteristics of wines. These findings underscore the versatility of non-*Saccharomyces* yeasts in winemaking and their ability to shape wine composition in desirable ways.

In conclusion, the research conducted by Milanović *et al.* [36], Renouf *et al.* [19], Padilla

*et al.* [43], and Ciani *et al.* [3] highlights the significant influence of non-*Saccharomyces* yeasts on wine composition through the production of unique aroma compounds and volatile phenols. By elucidating the enzymatic pathways and metabolic activities of these microorganisms, researchers are gaining insights into the complex dynamics of microbial fermentation and its implications for wine quality.

#### 4. Conclusion

This study provides several key conclusions regarding the role of non-*Saccharomyces* microorganisms in winemaking. Non-*Saccharomyces* yeasts play significant roles in various stages of winemaking, contributing positively to wine quality and sensory characteristics by enhancing complexity, aroma diversity, and flavor profiles. Non-*Saccharomyces* yeasts allow for the

development of alternative fermentation strategies, such as mixed microbial consortia and sequential inoculation techniques, which can lead to wines with enhanced aromatic complexity and structural nuances. Their metabolic diversity enables them to metabolize different carbon sources and produce a wide range of secondary metabolites that strongly impact wine aroma, flavor, mouthfeel, and overall sensory attributes, highlighting their importance in shaping wine composition. Non-*Saccharomyces* yeasts frequently coexist with *Saccharomyces cerevisiae* during wine fermentation, influencing fermentation kinetics, wine composition, and sensory characteristics. Their taxonomic diversity, ecological niches, and distribution within vineyard ecosystems contribute to variations in fermentation kinetics, stress tolerance, and metabolic pathways among different yeast strains. Co-fermentation using non-*Saccharomyces* yeasts and *Saccharomyces cerevisiae* enhances the flavor diversity and aromatic complexity of wine. Metabolic interactions between different microbial species during fermentation and their impact on wine quality are also highlighted.

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#### Compliance with Ethics Requirements

Author declares that he respects the journal's ethics requirements. Author declares no conflict of interest.

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