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Enhancing the Acidity and Sensory Profile of Two Wines from the Stefan Voda PGI Wine Region Using Native Grape Microflora

Ecaterina Covaci*, Aliona Sclifos, Natalia Vladei

Department of Oenology and Chemistry, Faculty of Food Technology, Technical University of Moldova, Chisinau, Republic of Moldova
Email: *ecaterina.covaci@enl.utm.md

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Abstract

This study focuses on identifying, selecting, and multiplying indigenous yeast species of technological interest in the Stefan Voda Protected Geographical Indication grape region. Based on microbiological studies, the multiplication of non-*Saccharomyces* yeast species: *Hanseniaspora*, *Kloeckera*, and *Torulaspora*, was performed under sterile conditions, with inoculum prepared from sterile, fresh grape must. To assess how the inoculum prepared from indigenous yeasts affects the wine's physicochemical properties, aromatic profile, and sensory qualities, two grape varieties, Muscat and Traminer Rose (Vitis vinifera), were used. Standard and microbiological analyses of the wines produced by two established processes showed that the presence of non-*Saccharomyces* yeasts during the initial fermentation stage (days 1 to 3), followed by inoculation with *S. cerevisiae*, enhances wine complexity and increases glycerol and 2,3-butanediol levels. The results from yeast microbiome correlation and PCA analysis of the two fermentation methods clearly distinguished the wines produced with mixed-sequencing fermentation (sample II) from the control samples. This indicates that the process improves microbiological stability, develops a more complex aromatic profile, and aligns with tasters' preferences while maintaining the wine's authenticity linked to its specific geographical origin.

Keywords

Authenticity, Grape Microbiome, Indigenous Flora, Wine Technology

1. Introduction

The winemaking industry in the Republic of Moldova has a rich historical and cultural heritage, with white wines constituting a significant part of the country's

production. Traditional winemaking mainly relies on the yeast *Saccharomyces cerevisiae*, which ensures a complete and effective alcoholic fermentation. However, this yeast alone often produces wines with similar aromatic profiles and less complexity [1]. In recent decades, commercial *Saccharomyces cerevisiae* strains have been used as starter cultures, whereas non-*Saccharomyces* yeasts have largely been overlooked and considered of little technological importance.

In recent years, the study and use of non-*Saccharomyces* yeasts have gained significant attention as tools for diversifying and improving wine sensory qualities. These yeasts, naturally found on grape skins and in winery environments, possess unique metabolic activities that can enhance the aromatic and flavor profiles of white wines [2]. Studies conducted by Taran, N., on the native Codrinschii grape variety selected nine yeast strains with high biotechnological potential for producing dry red wines. These local yeast strains can adapt to specific environmental conditions, ferment carbohydrates from the must, and contribute to wines with characteristic qualities and high organoleptic standards typical of the wine-growing region [3]. Researchers Roudil L. and Russo P. (2005) noted that non-*Saccharomyces* yeasts can enhance wine aroma, quality, and food safety by producing various metabolites during the alcoholic fermentation of must samples [4].

As is known, the definition of vitivinicultural "terroir" according to resolution OIV/VITI 333/2010 refers to an area where collective knowledge of the interactions between the physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for products originating from the PGI area (PGI—noted protected geographical indication). The concept of "terroir" includes specific soil, topography, climate, landscape features, and biodiversity [5]. Based on the principles of sustainable vitiviniculture adopted by the OIV-CST 518-2016 RESOLUTION (OIV, 2016) [6], and the guidelines for their implementation adopted by the OIV-VITI 641-2020 RESOLUTION (OIV, 2020) [7], protecting soils, water, air, biodiversity, and landscapes is especially important in the vitivinicultural sector. Therefore, careful planning is essential before establishing new vineyards or other vitivinicultural facilities, using proven ecological principles and optimal management of both existing and new assets.

Biodiversity of living organisms is essential to implementing the principles of sustainable vitiviniculture. In this context, the OIV-VITI 655-2021 RESOLUTION (OIV, 2021) presents recommendations regarding the valuation and significance of microbial biodiversity in sustainable vitiviniculture [8].

In this context, research over the past 5 - 10 years in microbiology and biotechnology related to wine products has begun to focus on isolating and utilizing local yeast strains or species to produce natural, organic, sustainable wines with organoleptic qualities typical of the vineyards where the grapes are grown [9]. The use of microbial resources in wine production is essential for driving innovation and improving wine quality. Ongoing research on *Saccharomyces cerevisiae* and non-*Saccharomyces* species to enhance wine characteristics and accommodate changing consumer preferences is promoting a competitive, sustainable wine industry [10].

In this context, non-*Saccharomyces* yeast species such as *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe*, and *Pichia kluyveri* are already commercially available as cultures suitable for various winemaking applications. However, they do not preserve the authenticity of the "terroir" microbiome, which imparts aromatic complexity and a unique regional expression to wines produced using them. Another benefit of species from the indigenous grape microbiome is that they can limit the growth of unwanted microorganisms during the early stages of alcoholic fermentation through competitive inhibition mechanisms and the secretion of antimicrobial substances. Recent studies by Rubio-Breton, P., have shown that yeasts such as *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, and *Lachancea thermotolerans* contribute to ensuring the microbiological stability of wine and contribute to aromatic complexity by producing unique metabolites, including aromatic esters and organic acids [11]. Non-*Saccharomyces* yeasts include a diverse range of genera and species that were once considered spoilage microorganisms. However, recent research has highlighted their beneficial role in controlled alcoholic fermentation. Some of the most important species are listed in **Table 1**.

Table 1. The primary impact of non-*Saccharomyces* on the sensory attributes of wines.

Non-*Saccharomyces* Species	Expected Technological Effect of Wine Technology Application	Responsible Component/ Produced Metabolite	Reference
Torulaspora delbrueckii	Reducing the mass concentration of volatile acidity and harshness and improving mouthfeel (flower, honey, red apple), and contributes to high glycerol production.	3-Phenylethyl acetate, Ethyl hexanoate, 3-Ethoxy-1-propanol	[12]
Lachancea thermotolerans	Modulating wine acidity by producing lactic acid, increasing freshness (floral, strawberry, citric hints) and acidity in white wines.	2-Phenylethyl acetate, Ethyl lactate, Lactic acid	[13]
Metschnikowia pulcherrima	Reducing the alcoholic strength by high β-glucosidase activity, releasing bound terpenes and enhancing floral aromas.	2-Phenylethanol, Monoterpenes	[14]
Hanseniaspora uvarum/ *vineae*	Increasing varietal aromatic complexity by fruity and floral esters in early fermentation stages.	Mannas, Benzyl acetate	[15] [16]
Pichia kluyveri	Enhancing wine aroma with fruity and floral esters and releasing thiols, which contribute to scents like passion fruit, rose, and grapefruit.	Ethyl acetate, Isoamyl acetate, 2-Phenethyl acetate	[17]

Each species has specific enzymatic and metabolic abilities that can be strategically combined with *Saccharomyces cerevisiae* to produce more balanced, aromatic wines unique to the wine-growing Protected Geographical Indication (PGI) region.

The purpose of the study was to use microbiological methods and techniques to observe, isolate, and identify microorganisms in the examined grapes from the Stefan Voda PGI region. The aim was to determine whether harmful microorgan-

isms were present or absent, with a particular focus on the native microflora that has technological importance in white wine production.

2. Materials and Methods

The practical study methods focused on identifying, isolating, and multiplying native flora, including both non-*Saccharomyces* yeast species and *Saccharomyces* yeast species of Muscat grape varieties from Javgur, Cimislia district (Stefan Voda PGI viticultural region), to select indigenous yeast species with technological interest.

2.1. Yeast Strains

The technological stages for quantifying the microbiota in Muscat grape varieties involved sampling the surface of the grape berries, starting the alcoholic fermentation process, monitoring the active phase of fermentation, concluding fermentation, and analyzing the raw wine material. Samples collected at these five stages of the dry white wine production were tested as microbial suspensions, serially diluted, and plated on Petri dishes containing various microbiological media: Potato Dextrose Agar (PDA), MRS sterilized, Broth, Bretanomyces Agar, and Yeast Extract Peptone Dextrose (YEPD) for culturing. Individual colonies developed on Petri dishes incubated at 25˚C and 30˚C over 5 - 7 days [18]. The microbiological colonies identified were classified based on criteria such as colony morphology (including color, shape, edge characteristics, surface texture, etc.), size, and growth traits on different media to ensure the selection of pure colonies, which would later be used to produce two experimental wine batches from the selected species of interest, specific to the Stefan Voda PGI grapes region.

2.2. Preparation of Inoculum

To promote positive microbial activity from native yeasts that can improve aromatic complexity, acid balance, and mouthfeel while ensuring the safe use of isolated yeast strains in white wine production, sterile multiplication was performed in a fresh, sterile must medium. The isolation of local yeast cells began with a single-cell colony, followed by successive dilutions and pure culture isolation via the sector method, using loop exhaustion. The indigenous yeast species (*Torulopsis*, *Hanseniaspora*, *Kloeckera*, and *Saccharomyces*) listed in compartment 1, with technological interest in wine production, were selected from Petri dishes as starter yeasts (method described in Section 2.1.), including both non-*Saccharomyces* and *Saccharomyces* species, using the "Exhausted Loop" method [19].

The experimental inoculum of strains *Torulopsis*, *Hanseniaspora*, *Kloeckera*, and separated *Saccharomyces* was prepared to a final concentration of 1.2 and 1.8×10^7 cells/mL (7.08 and 7.26 log CFU/mL) with a viability of 92.7%, as determined by plate count (serial dilutions) and vital strain cells (methylene blue—to distinguish live/dead cells). These were used in the production of two wine batches at TUM's micro winery section of the Department of Oenology and Chemistry.

2.3. Preparation of Must Samples and Fermentation Conditions

The Muscat and Traminer Rose grape varieties, harvested in 2024 and shown in Figure 1, were de-stemmed and pressed. The resulting juice was treated with potassium metabisulfite at 50 mg/L, pectinolytic enzyme at 4 g/hL (Enartis Zym AROM MP), and then stored at 5˚C for 3 days for clarification. Then, the clear must was divided into two 25-liter vessels.

▪ Sample I (control samples) was inoculated with *Saccharomyces cerevisiae* (Enartis Ferm Q Citrus), an industrial oenological dry yeast, at a dose of 0.3 g/L.

▪ Sample II was initially inoculated with indigenous non-*Saccharomyces* yeasts (*Hanseniaspora*, *Kloeckera*, and *Torulaspora*), and *Saccharomyces cerevisiae* yeast was added on the third day to complete alcoholic fermentation.

Figure 1. Experimentally processed grapes of ampelographic varieties: a) Traminer Rose and b) Muscat.

2.4. Physicochemical and Organoleptic Analysis

At the Oenological Research Center of TUM, the physicochemical and quality indices of grapes and wine raw materials were measured using modern analytical methods recommended by the OIV (Compendium of International Methods of Wine and Must Analysis, 2023) [20]. The spectrophotometric analysis was performed using a single-beam spectrophotometer PG T80 (PG Instruments, UK) at TUM's Oenological Research Center.

The sensory analysis was conducted in TUM's specialized tasting room to evaluate the quality of four experimental wine samples. The samples were presented simultaneously in two tasting glasses at 18˚C, each containing 35 ml of wine. Each sample was coded and assessed by 10 professional tasters (7 women and 3 men, with an average age of 30).

2.5. Statistical Analysis

Experimental data were analyzed in Microsoft Excel 2009 to determine the mean and standard error. With a significant level of $p < 0.05$, ANOVA and PCA were applied to assess variance using Pearson's correlation coefficient [21].

3. Results and Discussion

The presence of *Saccharomyces* yeasts, acetic bacteria, *Torulopsis*, *Metschnikowia*,

*Hanseniaspor*a, and *Bretanomyces* species was detected in experimental samples during microbiological examination and is shown in Figure 2.

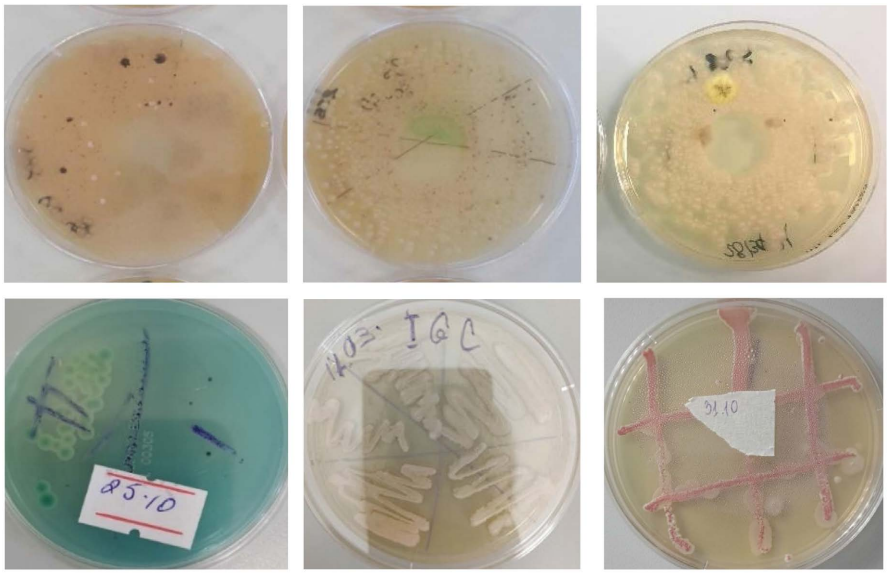

Figure 2. Photographs of Petri dishes with thermostatic culture media of experimental samples.

Table 2. The dynamics of microflora during white wine production.

Genus and Taxonomic Species	CFU	Time of Isolation of Microflora				
		Surface of Grape Berry	Beginning of Alcoholic Fermentation	Active Phase of Alcoholic Fermentation	End of Alcoholic Fermentation	Wine Raw Material
Deuromycotina	**35**					
Candida mycoderma	8	4	1	1	1	1
Kloeckera apiculata	27	16	10	1		
Ascomycotina	**110**					
Saccharomyces bailii	3		2	1		
Saccharomyces bayanus	12	5	2	2	1	2
Saccharomyces cerevisiae	42	17	12	3	4	6
Saccharomyces oviformis	33	4	5	8	12	4
Saccharomyces uvarum	5		3	1		1
Pichia membranefaciens	3	3				
Hanseniaspora	2	1	1			
Dekkera bruxelensis	1				1	
Torulopsis stelleta	9	2	6	1		
Total	**145**	**52**	**42**	**18**	**19**	**14**

Based on morphological classification of the indigenous microbiome, fermen-

tation yeasts of the genus *Saccharomyces* have round or ellipsoidal, white-colored cell morphology. In contrast, microorganisms of the genus *Torulopsis* have beige, spherical morphology. The white, lemon-shaped, or cylindrical morphology is characteristic of microorganisms in the genera *Hanseniaspora* and *Kloeckera*. *Metschnikowia* sp. is ovoid to ellipsoidal in shape, reproduces by budding, with cell colonies of pink color and lactic bacteria of *Lactobacillus* forming large colonies of gray bacilli [22].

The results showed that the studied yeasts do not form true mycelium and reproduce vegetatively through multilateral budding and sexually via spores, confirming that these strains belong to the genus *Saccharomyces*. Based on assessments of morphological, cultural, and reproductive features, isolated yeast cultures from the indigenous microflora of grape PGI Stefan Voda were found to comprise uniform, viable cells, as shown in **Table 2**.

Out of the 145 identified strains, 65 were microbiologically characterized. Based on the evaluation of morphological, cultural, and reproductive traits observed in the developed Petri dish cultures, it was determined that yeast cultures isolated from the indigenous microflora are uniform and viable cell strains, with potential for use in winemaking.

The practical analysis examined 145 individual colonies of dominant fungi, with Ascomycotina accounting for 75.86% ± 2.08% and the Deuteromycotina genus representing 24.14% ± 2.68%, according to **Figure 3**. Over time, the microbiota population in the alcoholic fermentation medium decreases from 52 colonies during the must stage to 14 colonies in the raw material wine.

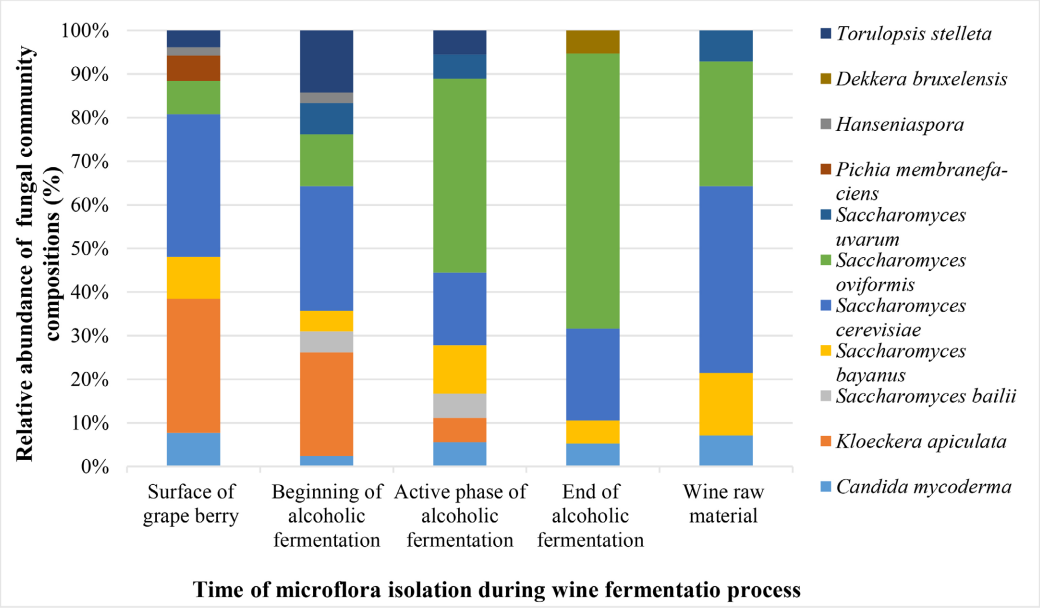

Figure 3. The structure of the microbial community during the wine production process.

In the alcoholic fermentation process of the studied Muscat must, species of *Saccharomyces*, *Kloeckera*, and *Torulopsis* are present at moderate levels. How-

ever, as non-*Saccharomyces* species are converted into alcohol, they become inactive, and in the raw material wine, these species account for 9.7% - 13.1% of the initial microbiota population.

Experimental grape samples and dry white wines from the study were subjected to physicochemical analysis, and the results are presented in Table 3.

Table 3. Physicochemical indices of experimental must samples, harvest 2024.

Parameter	Type of Grapes	
	Muscat	Traminer Rose
Active Acidity, pH	3.71 ± 0.01	4.05 ± 0.01
Mass Concentration of Sugar, g/L	210 ± 5	223 ± 5
Mass Concentration of Titratable Acids, g/L Tartaric Acid	5.81 ± 0.22	6.62 ± 0.34
Mass Concentration of Nitrogen (Ammonia and Alpha-Amino Acids), mg/L (YAN)	172.84 ± 2.16	278.46 ± 3.10
Turbidity, Nephelometric Turbidity Units (NTU)	22.49 ± 1.02	28.68 ± 1.65

Comparing the physicochemical indices, the Traminer Rose must sample shows a higher total titratable acid concentration (6.62 g/L compared to 5.81 g/L for Muscat) and a significantly higher pH (0.34 pH units higher). The higher acidity in the Traminer Rose must does not indicate a sourer taste, as the must's buffer system maintains a high pH. Muscat must have a more balanced acidity profile and a safer pH from a microbiological perspective.

The Traminer Rose must sample stands out for its amino acid concentration of over 100 mg/L, which is higher than that of Muscat and is referred to as technological Yeast Assimilable Nitrogen (YAN). This provides an excellent nutrient medium for alcoholic fermentation, reducing the risk of slow or stalled fermentation. Both must show normal turbidity levels for freshly pressed must. The slightly higher level in Traminer Rose (at 28.68 NTU) indicates a greater concentration of suspended solids (e.g., pulp particles and pectin), which require an effective decantation or clarification step before fermentation to produce higher-quality wine. Both must demonstrate good quality, with high phenological and technological maturity.

As an experimental observation, the kinetics of alcoholic fermentation in the two technological wine study options depended on the inoculated yeast strains. During alcoholic fermentation of the control samples (Muscat and Traminer Rose) using *S. cerevisiae* industrial oenological dry yeast, the total sugar concentration was depleted within the first 5 days of fermentation. In comparison, the duration of alcoholic fermentation with non-*Saccharomyces* strains (samples II) ranged from 8 to 10 days for the sequential fermentation with yeasts of *Hanseniaspora*, *Kloeckera*, *Torulaspora*, and indigenous species *Saccharomyces cerevisiae*, which aligns with the literature data [23].

The physicochemical indices of the study samples show significant differences

for both Muscat and Traminer rose varieties, as shown in Table 4. Muscat wines are slightly more acidic (average pH ~3.28) than Traminer Rose wines (average pH ~3.44). A lower pH enhances the sensation of freshness. Variations among samples are minimal, indicating consistent winemaking. In terms of titratable acidity, Traminer Rose has a slightly higher concentration (~5.89 g/L) than Muscat (~5.30 g/L) due to higher acidity in the processed grapes. Traminer Rose samples have significantly higher alcohol content (12.66% v/v in both samples) than Muscat (~12.07% v/v). This is explained by the higher initial sugar content in Traminer Rose grapes, as shown in Table 3. Regarding residual sugar content, the wine samples are dry, with less than 4 g/L.

Table 4. Physicochemical indices of dry white wine samples.

Parameter	Muscat		Traminer Rose	
	Sample I	Sample II	Sample I	Sample II
Active Acidity, pH	3.27 ± 0.01	3.30 ± 0.01	3.42 ± 0.01	3.47 ± 0.01
Mass Concentration of Residual Sugar, g/L	3.24 ± 0.15	3.44 ± 0.25	4.10 ± 1.63	3.90 ± 0.33
Alcohol by Volume, % v/v	12.10 ± 0.01	12.05± 0.01	12.66 ± 0.01	12.58 ± 0.01
Mass Concentration of Volatile Acids, g/L Acetic Acid	0.48 ± 0.05	0.32 ± 0.05	0.60 ± 0.1	0.42 ± 0.08
Mass Concentration of Titratable Acids, g/L Tartaric Acid	5.21 ± 0.20	5.40 ± 0.18	5.97 ± 0.24	5.82 ± 0.37
Mass Concentration of Glycerol, g/L	5.45 ± 0.02	5.80 ± 0.02	6.04 ± 0.02	6.42 ± 0.02
Mass Concentration of 2,3 Butylene Glycol, mg/L	185.45 ± 2.14	108.62 ± 3.21	230.17 ± 3.66	247.07 ± 4.35
Content of SO_2, Free/Total Forms, mg/L	21/74 ± 5	30/80 ± 5	18/70 ± 5	25/62 ± 5
Total Phenolic Compounds, mg/L	142.5 ± 8.5	150.7 ± 6.3	162.8 ± 4.2	174.2 ± 6.5
Color Intensity (AU), A420	0.14 ± 0.02	0.15 ± 0.02	0.18 ± 0.02	0.19 ± 0.02
Organoleptic Characteristics	Clear dry white wine, without strange odors, citric fruits with floral and tree fruit nuances, complete taste, rich and full.		Clear dry wine, without strange odors, with yellow-green hues, lime-tree odour, and honey-like/dried fruit, complete taste, rich and full.	
Total Quality Score, Points	80	86	84	88

One of the main advantages attributed to *T. delbrueckii* was its ability to lower volatile acidity in experimental wines. For the Muscat sample II wine, the reduction in volatile acidity was 0.16 g/L compared to Sample I, and for the Traminer Rose variety, it was 0.18 g/L. These technological results match those reported by other researchers, who observed decreases in the final volatile acidity concentration to 0.14 - 0.28 g/L compared to *S. cerevisiae*, as noted in Mas's studies [24].

There may be a metabolic conflict between the inoculated species *T. delbrueckii* and *Hanseniaspora*, as evidenced by an increase in volatile acidity. In the present

study, during the early stages of fermentation (the first days after inoculation), varietal aromatic complexity, mediated by fruit and flower esters, is observed to be dynamic in response to the selected *Hanseniaspora* species. Technically, slowing alcoholic fermentation with Hanseniaspora species can increase volatile acidity; however, in the practical study, the *Saccharomyces* inoculum was added on the third day of fermentation, thereby avoiding the side effect of increased volatile acidity. Additionally, applying *T. delbrueckii* can reduce the final ethanol concentration in wines by up to 1%, while increasing glycerol levels from 0.2 to 0.9 g/L, as reported by Yao [2]. In wines made with the technological variant II, glycerol content was higher by 0.35 g/L (Muscat) and 0.38 g/L (Traminer Rose) compared to the control method (Sample I). These glycerol levels stay within the range of 0.2 - 0.9 g/L, as shown by Van Leeuwen's [25] and Martin's [26] studies.

Several authors (Di Canito, 2021, and Morata, 2020) report that *T. delbrueckii* releases more mannoproteins than *Saccharomyces* and other non-*Saccharomyces* species [1] [27]. In this study, however, this mannoprotein content was not measured.

The levels of secondary fermentation compounds, glycerol and 2,3-butylene glycol, indicate proper alcoholic fermentation, with concentrations ranging from 5.45 to 6.42 g/L in the samples. Traminer wines had an average 2,3-butylene glycol level of 238.62 mg/L, which is notably higher than that of Muscat wines (147.03 mg/L). These elevated levels suggest increased body, texture, and naturalness in the wine, aligning with Morata's research, as noted in the specialized literature [28].

The total phenolic compounds in wine samples are higher, ranging from 146.5 to 168.5 mg/L. These compounds contribute to the structure, taste, and stability of the wines. Both wine varieties, in terms of organoleptic characteristics and quality, are described as clear, dry, and free from defects.

In general, in both cases, Sample II was evaluated as having higher overall quality than Sample I in the study samples.

Among the many researchers mentioned in section 1 of the article, non-*Saccharomyces* yeasts that enhance the complexity and fruity characters of experimental Muscat and Traminer rose dry white wines are discussed in **Figure 4** below.

The aroma impact of Sample II's technological method influenced the wine's aromatic quality. The aroma of *T. delbrueckii* is often described as "fruitiness," which aligns well with the sensory analysis [29]. Muscat Sample II (orange) generally scores slightly higher than control Sample I (blue) across most attributes, including Persistence, Body, and Sweetness. Traminer Rose Sample II (yellow) stands out with the highest Sweetness score of all four samples (nearly 3.5). In contrast, the control sample (gray) exhibits more pronounced Astringency and Structure, as well as aroma characteristics detected by the panel, especially Field flowers.

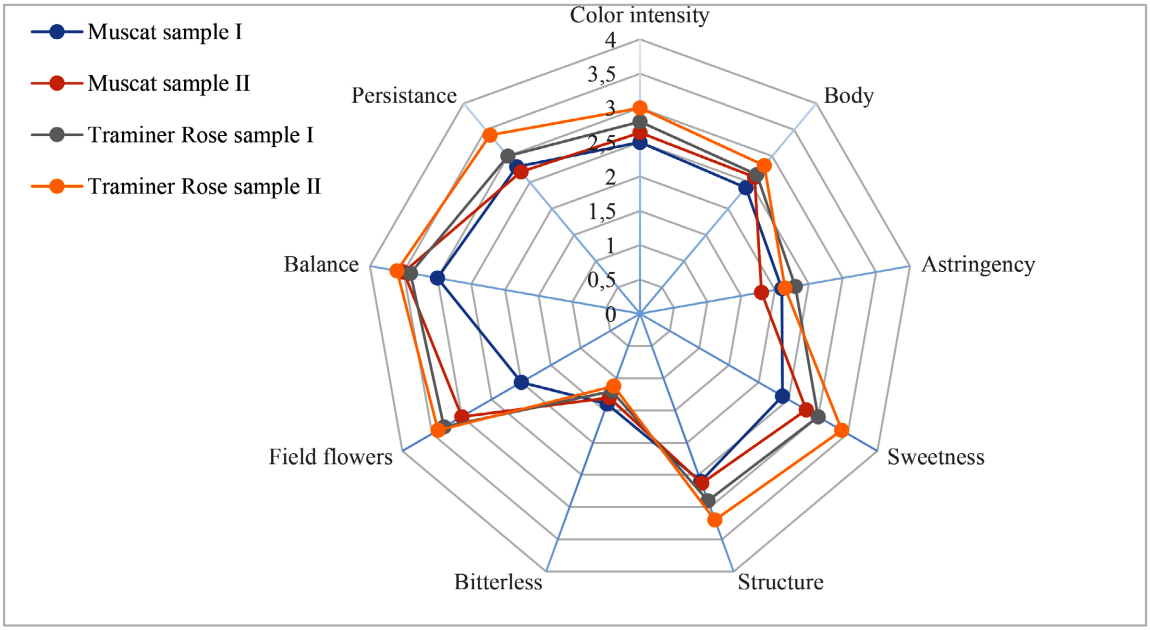

Figure 4. Radar chart of the sensory evaluation for the experimental dry white wine.

Another aspect to consider is the dominant attributes of each sample. Traminer Rose II (yellow) shows the highest sweetness and color intensity. In contrast, Muscat Sample I (blue) has the least structure and bitterness, indicating a light structure and a slight perception of bitterness. Balance scores are the highest for all samples except the control Muscat samples. These organoleptic analysis results align with those in **Table 4**, which explain why the Traminer Rose wines are, from both a chemical and sensory perspective, more intense and complex: they have higher alcohol content, titratable acidity, phenolic compounds, color, and 2,3-butylene glycol. These attributes contribute to their higher quality scores. Muscat wines are characterized by a fresher, smoother profile, lower pH, lower alcohol levels, and more delicate citrus and floral aromas.

The sensory analysis results of the wines (tasting sheets) were analyzed using principal component analysis (PCA). The PCA method helps visualize differences in organoleptic properties and panel preferences for wine samples produced by two methods (classic with selected industrial yeasts and sequential fermentation).

Figure 5 shows the compounds responsible for the most significant differences between the two samples. The first principal component (Factor 1) explained 71.63% of the total variation, while the second principal component (Factor 3) explained an additional 8.84% (totaling 80.47%).

Based on the PCA results, it was possible to distinguish the samples produced through both sequential fermentation and control studies [30].

In summary, the most organoleptically appreciated experimental samples were those produced by a mixture of non-*Saccharomyces* and *Saccharomyces* indigenous yeast species. They featured a complex aroma profile specific to the grape variety, a harmonious balance, and a blend of fruit and floral notes, with slight freshness from the acidity level.

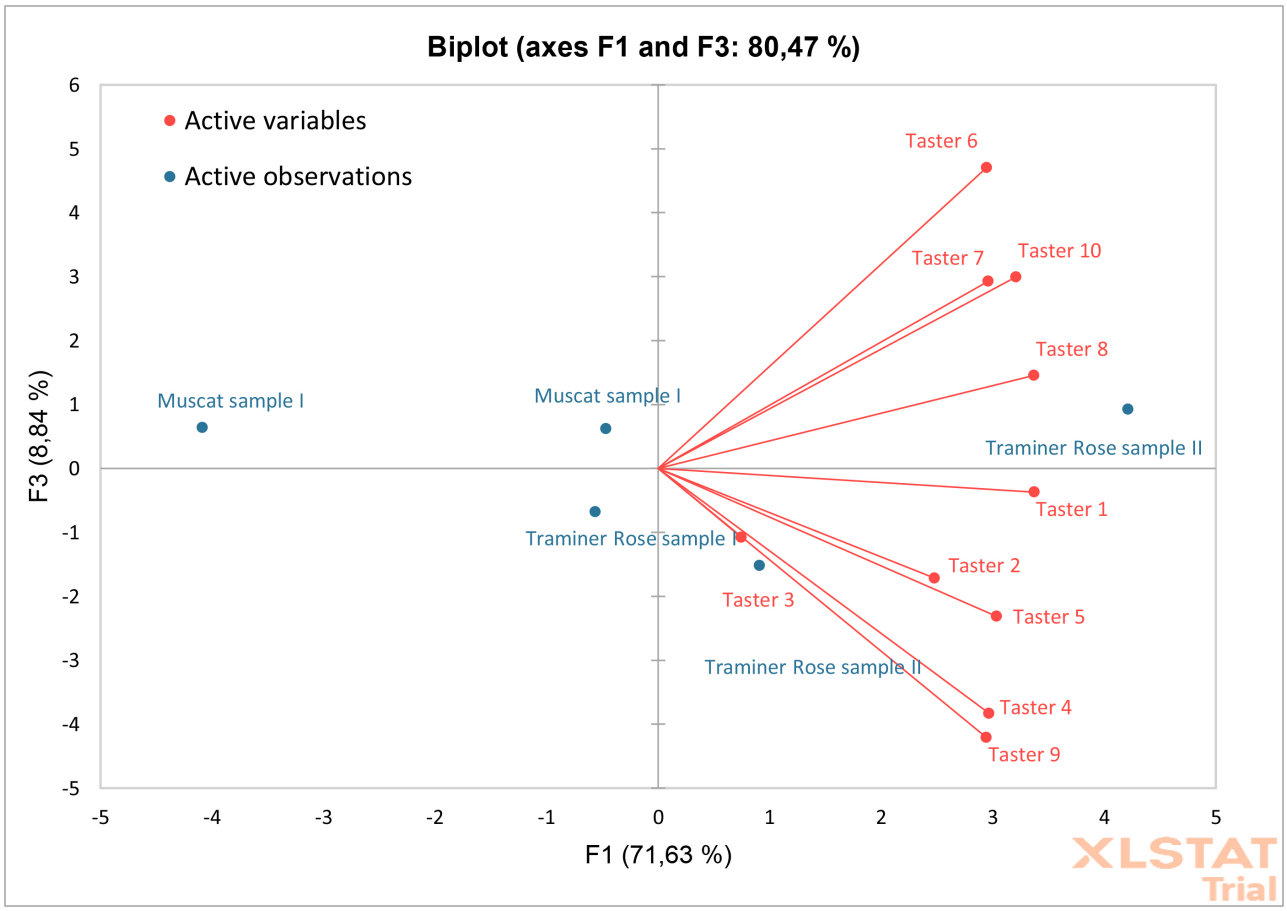

Figure 5. Principal component analysis (PCA) of wine aroma features evaluated by panel tasters.

The evaluation of the correlation level of indigenous grape yeasts, included in Table 5, likely involves coexistence, competition, or succession as a final step, which allowed us to establish the following aspects:

- Positive values close to "1" indicate a strong positive correlation, such as 0.974 between *Kloeckera apiculata* and *Saccharomyces cerevisiae*. This suggests that the two species tend to occur together or thrive under similar conditions.
- Negative values close to "−1" indicate a strong negative correlation, such as -0.652 between *Saccharomyces cerevisiae* and *Saccharomyces oviformis*. This could be due to a mutually exclusive or highly competitive relationship, where the presence of one species inhibits the other.
- Values close to "0" indicate a weak or no correlation (e.g., 0.085 between *Saccharomyces bailii* and *Saccharomyces cerevisiae*).
- Strong positive correlations (1.000) between species *Candida mycoderma*, *Saccharomyces bayanus*, and *Pichia membranefaciens*, indicating a close association.
- *Kloeckera apiculata*, a common wild yeast early in fermentation, shows strong correlations with most species of the genus *Saccharomyces* (the primary yeasts responsible for efficient alcoholic fermentation).

Table 5. Correlation levels of the microbial community at different stages of wine technology.

Species	*Candida*	*Kloekera*	*S. bailii*	*S. bayanus*	*S. cerevisiae*	*S. oviformis*	*S. uvarum*	*Pichia*	*Hansen.*	*Dekkera*	*T. stelleta*
Candida	1.000										
Kloeckera	0.815	1.000									
S. bailii	−0.375	0.184	1.000								
S. bayanus	0.958	0.844	−0.221	1.000							
S. cerevisiae	0.809	0.974	0.085	0.838	1.000						
S. oviformis	−0.423	−0.563	−0.146	−0.633	−0.652	1.000					
S. uvarum	−0.456	0.112	0.913	−0.269	0.103	−0.356	1.000				
Pichia	1.000	0.815	−0.375	0.958	0.809	−0.423	−0.456	1.000			
Hansen.	0.6124	0.955	0.408	0.662	0.937	−0.558	0.373	0.612	1.000		
Dekkera	−0.250	−0.415	−0.375	−0.516	−0.414	0.879	−0.456	−0.250	−0.408	1.000	
T. stelleta	0.045	0.613	0.853	0.159	0.564	−0.392	0.820	0.045	0.807	−0.404	1.000

- *Dekkera bruxellensis* (also known as *Brettanomyces*) and the genus *Saccharomyces* exhibit negative correlations with most other species, suggesting competitive interactions or different stages of development.
- The population dynamics show that *Kloeckera apiculata* (a "non-*Saccharomyces*" yeast) is the dominant species at the start of alcoholic fermentation. It multiplies rapidly in fresh must, consuming sugars and producing various aroma compounds, except alcohol.
- As fermentation progresses, *Saccharomyces cerevisiae* (the primary winemaking yeast) becomes dominant because of its traits, which allow it to tolerate higher sulfur dioxide (SO_2) and produce high levels of ethanol. These conditions quickly suppress *Kloeckera apiculata* and other non-*Saccharomyces* yeasts [31].

In terms of oenological impact, the *Kloeckera apiculata* species and other non-*Saccharomyces* yeasts initially contribute positive aromas and flavor precursors, as detected by the panelists, as illustrated in **Figure 4**.

Based on the experimental wine samples, various microflora and fermentation processes can significantly influence the chemical and sensory qualities of wines. Indigenous microflora contributes to a more balanced aroma, underscoring the importance of carefully selecting both viticultural and fermentation practices to shape the wine's local characteristics.

4. Conclusions and Recommendations

As noted above, plant-associated microbiomes are essential to viticulture and winemaking, where various fungi and bacteria can have positive, negative, or neutral effects on vine health and wine quality. Therefore, the sources and persistence of wine-related microbiota in vineyards are critical for the final product quality.

Additionally, it is well established that human intervention can influence the vineyard microbiome through multiple direct and indirect pathways [32], with potential impacts on microbial terroirs (OIV, resolution 2010) [33].

Fermentative yeasts are used industrially in wine production, primarily for their ability to ferment simple carbohydrates anaerobically, producing ethanol and carbon dioxide. Many microorganisms are present, especially during grape ripening. After harvest in autumn, yeasts on the leaves fall into the soil with their fallen leaves, where they remain until spring. This process allows natural selection to occur, resulting in the survival of the most resistant species.

The practical analysis reveals the presence of 145 individual colonies of dominant fungi, with Ascomycotina (75.86% ± 2.08%) and Deuromycotina genus (24.14% ± 2.68%) being predominant. During the alcoholic fermentation of the studied Muscat must varieties, *Saccharomyces*, *Kloeckera*, and *Torulopsis* species are present in moderate amounts. However, as monosaccharides are converted into alcohol, non-*Saccharomyces* species become inactivated, lowering their initial population in the raw material wine to a range of 9.7% - 13.1%.

One of the initial benefits linked to the *T. delbrueckii* species was a reduction in volatile acidity in the experimental wines: the volatile acidity decreased by 0.16 g/L in Muscat sample II compared to Sample I, and by 0.18 g/L in Traminer Rose wine. Consequently, the glycerol content was higher in samples II by 0.35 g/L in Muscat and 0.38 g/L in Traminer Rose than in the control (Sample I).

Another aspect was the organoleptic aroma-dominant attributes per sample: the Traminer Rose II sample had the most pronounced sweetness and color-intensity profile. At the same time, the Muscat Sample I presented a minor content in structure and bitterness-free (lack of bitterness), indicating a light structure and a slight perception of bitterness. The Traminer Rose wines analyzed, from a chemical and sensory perspective, are more intense and complex due to their higher levels of alcohol, titratable acidity, phenolic compounds, color, 2,3-butylene glycol, and higher organoleptic quality scores.

In terms of oenological impact, *Kloeckera apiculata* and other non-*Saccharomyces* yeasts initially contribute positive aromas and flavor precursors; however, if they remain dominant for too long, they can lead to the formation of undesirable volatile acidity (e.g., ethyl acetate) and reduced alcohol yield. The modern use of selected *Saccharomyces* yeasts helps control this transition, ensuring efficient fermentation and a balanced flavor profile [34].

This study shows that using starter cultures results in faster complete fermentation and produces more alcohol than spontaneous fermentation. The sensory characteristics are specific to the grape variety and terroir, and the grapes demonstrate high resistance to microbial changes. *T. delbrueckii* and *Kloeckera* enhance the intensity and quality of wine aroma, boosting the overall impression and highlighting the varietal and fruity qualities.

The results showed that using selected starter cultures can produce balanced wine and may also help develop wines that reflect their geographical origin. *Kloeckera*, *T. delbrueckii*, and *Torulaspora* species in the Republic of Moldova can nat-

urally occur on grapes, making them potential fermentation promoters, particularly for local wine-industry applications. However, their abilities need to be verified later, considering that they do not tolerate ethanol concentrations higher than 4% - 6% v/v.

This study demonstrated that by selecting and multiplying indigenous starter cultures and using them for fermentation, it is possible to preserve the wine's authenticity linked to a specific geographical area. A sustainable use of the grape microbiome involves this method, aligning with recent trends in microbiology and biotechnology, and results in natural, organic, sustainable wines with organoleptic qualities typical of the vineyards where the grapes are cultivated.

For a more detailed study of the influence of indigenous yeast species, we plan to investigate the mannoprotein content (a technological byproduct of *T. delbrueckii*) in the wine samples prepared, as well as to perform HPLS-DAD-MS analysis to quantify the aromatic compounds involved. This would complement the organoleptic analysis conducted in this article.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in designing the study; collecting, analyzing, or interpreting data; writing the manuscript; or deciding to publish the results.

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