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Comparative study of the dynamics of alcoholic fermentation and quality of white wines produced with selected and wild yeast

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Abstract

The quality of white wines is a complex and difficult to define category, which is described by many and diverse characteristics (color, aroma, freshness, taste, etc.). The main factors related to it, are the dynamics, duration and specificity of alcoholic fermentation. Some of these characteristics depend on technological parameters such as temperature (14°C), sugar content (220 g/dm³) and active acidity (pH). Improving certain elements of classical winemaking is challenging without incorporating innovative technologies or microbial biotechnologies. From this point of view, the proper selection of yeast strains is of paramount importance for achieving desired quality of white wines. The aim of the current study was to compare and evaluate the fermentation activity of selected Saccharomyces cerevisiae yeast strains, purchased from different producers, with wild yeasts, using two basic technologies for white wine production and two grape varieties. The results confirmed that there is no difference in the dynamics of alcoholic fermentation among the selected yeast strains; however, this does not apply to wild yeast.

***Keywords*:** yeast, alcoholic fermentation, dynamics, quality, sugars, pH

INTRODUCTION

The main process in winemaking is alcoholic fermentation, carried out primarily by yeasts (Carbonero-Pacheco et al., 2025), with *Saccharomyces cerevisiae* as the dominant species (Padilla et al., 2018; Wang et al., 2023). The advantages of using pure cultures of *S. cerevisiae* strains in wine fermentation are well-documented (Andorrà et al., 2010; Gardner et al., 2023; Gonzalez, 2022; Holešinský et al., 2020). Yeast efficiently utilize avaible fermentable sugars, ensure complete fermentation (Padilla et al., 2018), and exhibit high tolerance to ethanol and sulfur dioxide (Padilla et al., 2018; Wang et al., 2023). Their adaptability to challenging conditions such as high osmotic pressure, sugar concentration, and low nitrogen levels is also a key factor in the fermentation (Ashalou, 2019; Alexandre et al., 2001; Ortiz et al., 2013).

Other yeast species, generally referred to as non-*Saccharomyces*, typically dominate the early stages of fermentation but later are suppressed by increasing ethanol levels (Martín-García et al., 2023). While once considered undesirable (Padilla et al., 2018), recent studies recognize their positive contribution to wine aroma complexity (Padilla et al., 2016).

Terroir also plays a crucial role in shaping natural diversity of vineyard mycobiota, influenced by climate, topography, and seasonal factors (Gayevskiy et al., 2012; Pinto et al., 2014; Bokulich et al., 2016). On ripe grape surfaces, species like *Hanseniaspora/Kloeckera* and *Metschnikowia* predominate (Kačániová et al., 2020). In contrast, *S. cerevisiae* constitutes less than 0.00005% of the native grape yeast population (Taylor et al., 2014). Yeast genera such as *Candida*, *Pichia*, and *Hanseniaspora* significantly influence wine flavor during initial fermentation phases (Renouf et al., 2006; Carreto et al., 2008; Nisiotou et al., 2010).

Maceration of white aromatic grape varieties is an essential step in winemaking. It enhances the extraction of aromatic and phenolic compounds from grape skins into the must, thereby affecting wine aroma and antioxidant capacity (Casassa et al., 2021; Olejar et al., 2015; Olejar et al., 2016). Pre-fermentation maceration is widely applied to intensify varietal characteristics, resulting in more balanced and robust wines (Peinado et al., 2004; Radeka et al., 2008). Wines produced with maceration exhibit higher aromatic intensity, including floral and fruity notes derived from volatile compounds and their precursors (Bestulić et al., 2022; Buican et al., 2023; Darias-Martín et al., 2000; Salemnia et al., 2019). Additionally, compounds such as ethyl acetate and benzoic acid contribute to the wines' sensory complexity (Buican et al., 2023).

Fermentation temperature is a critical factor influencing both the fermentation dynamics and wine quality. Low-temperature fermentation (≤14°C) is globally accepted as the primary method for white wine production, as it preserves varietal and fermentation aromas while balancing fruit and herbal notes (Shi et al., 2022; Deet et al., 2017). It also promotes the production of terpene aromas and enhances the synthesis of volatile compounds by *S. cerevisiae* (Pérez et al., 2018; Molina et al., 2007; Ortiz-Tovar et al., 2019). Although fermentation proceeds more slowly at lower temperatures, yeast cell viability is improved (Shener et al., 2007).

The aim of this study is to compare and evaluate the dynamics of alcoholic fermentation and the quality of white wines produced with selected (commercial) *Saccharomyces cerevisiae* strains and wild yeasts.

MATERIALS AND METHODS

Two grape varieties were used – Muscat Ottonel and Dimyat, and a different technology was used to obtain the must from each variety, corresponding to the specifics of the variety. The grapes from the Muscat Ottonel variety were macerated for 6 hours, then pressed, and the grapes from the Dimyat variety were directly pressed. All other technological stages of obtaining and clarifying the musts were exactly the same.

Grape must

Two grape musts were obtained from grapes harvested in 2024. One was from the Muscat Ottonel variety (Grame nust 1), and the other from the Dimyat variety (Grape must 2). Both musts were used 24 hours after harvesting, and their turbidity was 50 NTU (Nephelometric Turbidity unit).

Yeast

The yeasts used in the study were Excellence® STR and Rhône 2056TM series, kindly provided by Lamothe-Abiet, France and Lallemand, Canada. The specific characteristics of the yeasts are shown in Table 1.

Table 1. The main characteristic of the yeast strains

Specific features	**Yeast**		
	Excellence® STR	**Rhône 2056™**	**Wild yeast**
Resistance to challenging fermentation conditions	Yes	Average	-
Fast integration into the must	Yes	Yes	-
Fast and clean fermentation	Yes	Yes	-
Alcohol tolerance (vol %)	up to 15	up to 16	-
Resistance to low temperature (°C)	up to 12	up to 15	-
Turbidity tolerance (NTU)	up to 50	up to 50	-
Need for nitrogen sources	Low	Medium	-
Profile of the produced wines	Esterene	Floral	-

Table 2. Type and quantity of yeast and starters

Specific features	Yeast line		
	Excellence® STR	Rhône 2056™	Wild yeast
Amount of used yeast, g/hl	20	20	-
Used yeast starter, g/hl			
OenoStim®	25	-	12.5
Go-Ferm Protect Evolution ™	-	25	12.5

To reduce contamination were used dry (lyophilized) yeast. The selected yeast strains, corresponding yeast starters and their quantity are shown in Table 2.

Initially, the yeast was rehydrated for 15 minutes in water at 36-40°C and a hydromodule of 1:10, resulting in 20 g/hl of sourdough, 25 g/hl of starter and 20 g/hl of sugar. Adaptation was carried out by dilution to a final hydromodule of 1:25. It was carried out for 2 hours, with gradual cooling to 14°C (the temperature of the grape must). The rehydrated and adapted yeast were added in quantities corresponding to the experimental variants.

Methods of analysis

The analysis of the samples was carried out in the physicochemical laboratory of the company "Vinar BG" Ltd - Haskovo. Samples for analyses were taken according to the requirements of the International Organisation of Vine and Wine (OIV). Sugar content was determined with a digital refractometer (ATAGO WM-7) and the method according to OIV-MA-AS311-01A. Other analyses were carried out according to the standard methods of OIV as follows: relative weight and density according to - OIV-MA-AS2-01; titratable acids according to - OIV-MA-AS313-01; volatile acids according to - OIV-MA-AS313-02; pH according to - OIV-MA-AS313-15; free sulfur dioxide according to - OIV-MA-AS323-04A1 and total sulfur dioxide according to - OIV-MA-AS323-04A2. The alcohol content was measured with an electronic ebulliometer (model AlkW001) according to OIV-MA-AS312-01.

In order to carry out a comparative study of the dynamics of alcoholic fermentation, two experimental series were developed for the two selected yeast types and the wild yeast. In each separate series, three variants were developed – one variant for each yeast type. The difference between the two series is the use of grape must from a different grape variety. The general scheme of all variants, temperature regimes and initial sugar content are presented in Table 3.

Table 3. General description of the experimental variants

Yeast	Variant	
	MO	D
Excellence® STR	STR-MO	STR-D
Rhône 2056™	R2056-MO	R2056-D
Wild yeast	WY-MO	WY- D
Fermentation temperature, °C	14	14
Initial sugar content, g/dm³	220	220

The experiment was conducted simultaneously in laboratory and industrial conditions. Two industrial batches of must were obtained, each with a volume of 2400 dm³, each batch of must was poured proportionally into one-ton capacity vessels of 800 dm³ in each vessel. Immediately after that, two of the vessels were inoculated with the corresponding strain of wine yeast.

The laboratory experiment per each variant used quantity of 1000 cm³ which was placed in the new PET (polyethylene terephthalate) bottles with a capacity of 1.5 dm³. Every day, until the end of the experiment, all samples were homogenized. The characteristics of the must/wine at the beginning and at the end of the experiment for all variants are shown in Tables 4 and 5.

Table 4. Characteristics of the Muscat Ottonel must/wine at the beginning and at the end of the experiment

Number	Parameter	Abbreviation	Unit	Initial values MO series	Final values MO Series		
					STR-MO	R2056-MO	WY-MO
1	Relative density	Dp	1	1.094	0.994	0.995	1.000
2	Common sugars	CS	g/dm³	220.0	3.61	4.17	10.43
3	Alcohol	A	vol%	0	13.30	13.21	12.2
4	Titratable acids	TA	g/dm³	4.74	5.73	5.66	5.04
5	Actual acidity	pH	1	3.15	3.19	3.21	3.16
6	Volatile acids	VA	g/dm³	0.27	0.36	0.36	0.54
7	Free SO_2	L(SO2)	mg/dm³	24	0	0	0
8	Total SO_2	T(SO2)	mg/dm³	33,6	8,0	6,4	11,2

Table 5. Characteristics of the Dimyat must/wine at the beginning and at the end of the experiment.

Number	Parameter	Abbreviation	Unit	Initial values D series	Final values D Series		
					STR-D	R2056-D	WY- D
1	Relative density	Dp	1	1.094	0.993	0.993	1.019
2	Common sugars	CS	g/dm³	220.0	2.92	2.95	46.84
3	Alcohol	A	vol%	0	13.34	13.37	10.18
4	Titratable acids	TA	g/dm³	5.53	6.56	6.47	5.84
5	Actual acidity	pH	1	3.23	3.29	3.28	3.22
6	Volatile acids	VA	g/dm³	0.27	0.36	0.36	0.66
7	Free SO_2	L(SO2)	mg/dm³	24	0	0	0
8	Total SO_2	T(SO2)	mg/dm³	33.6	6.4	4.8	8.0

Analyses were done in the physicochemical laboratory of "Vinar BG" Ltd. - Haskovo, and the industrial experiment was conducted in the winery of "Vinodar" Ltd., situated in Polyanovo village, Haskovo region. All presented results are the mean values of two experiments.

Sensory analysis

The organoleptic characteristics of the experimental variants were evaluated by a sensory panel comprised of professional enologists with extensive practical experience in wine production, along with one quality expert. In total, the panel included five men and four women, aged 30 to 59 years. All members of the sensory panel have substantial experience in wine production, tasting, and evaluation, and are well acquainted with the requirements and standards for organoleptic analysis (ISO 13299:2016, ISO 6658:2017, ISO 3972:2011, ISO 5496:2006). The sensory panel is highly proficient in recognizing aromas and flavors, including their intensity and balance. The panel evaluated the produced wines both descriptively and using a point-based system. Additionally, the wines were subjected to a triangle test to assess differences between samples fermented with different types of yeast (ISO 4120:2021 – Sensory analysis – Methodology – Triangle test).

RESULTS AND DISCUSSION

Changes in the sugar content (g/dm³) during alcoholic fermentation for all variants are shown in Figures 1 and 2. The experiment was terminated after 26 days, when a slowdown or cessation of fermentation was noted. The selected yeast strains fermented sugars to a level, necessary for the production of wine corresponding to the category of dry wines. Experimental variant WY-MO reached a sugar content of 5 g/dm³ on day 32, and the resulting wine has deteriorated technological and organoleptic characteristics. In the experimental variant WY-D, the alcoholic fermentation process was incomplete.

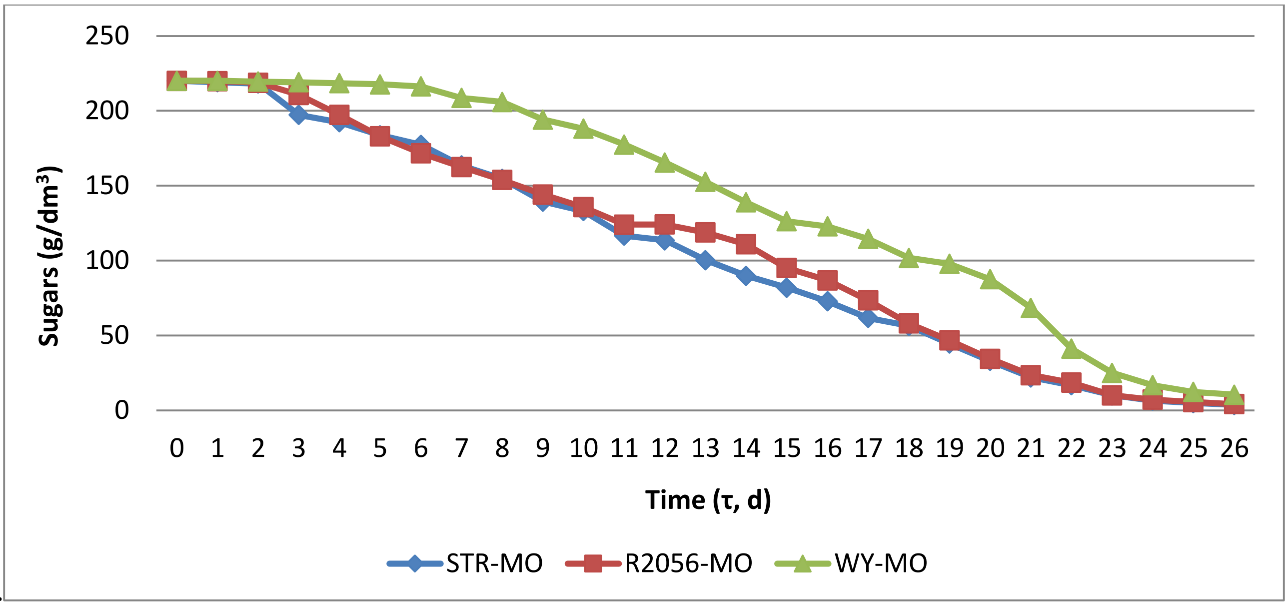

Figure 1. Changes in sugar content in g/dm³ during alcoholic fermentation of variants from the MO series

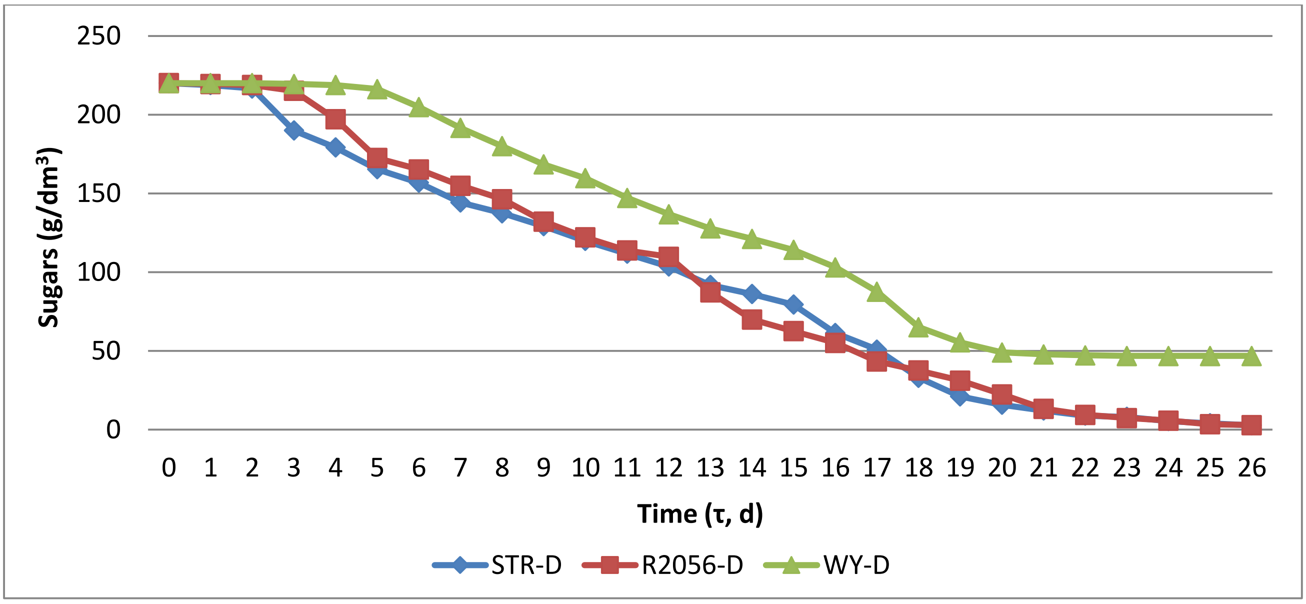

Figure 2. Changes in sugar content in g/dm³ during alcoholic fermentation of D series

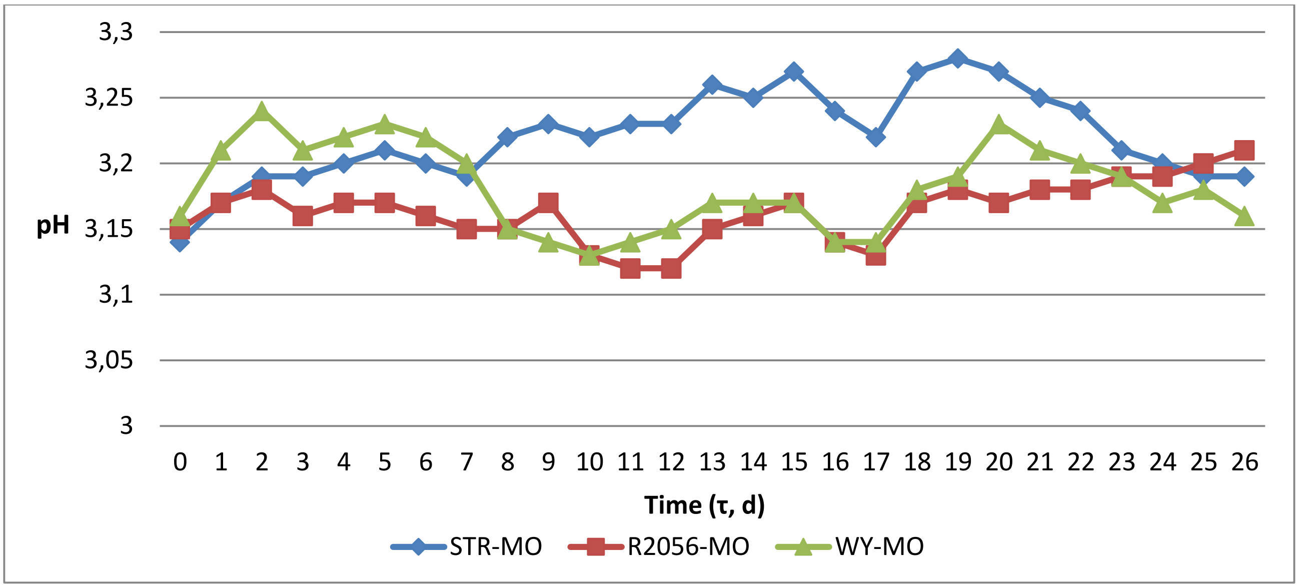

Figure 3. Changes in pH during alcoholic fermentation of MO series

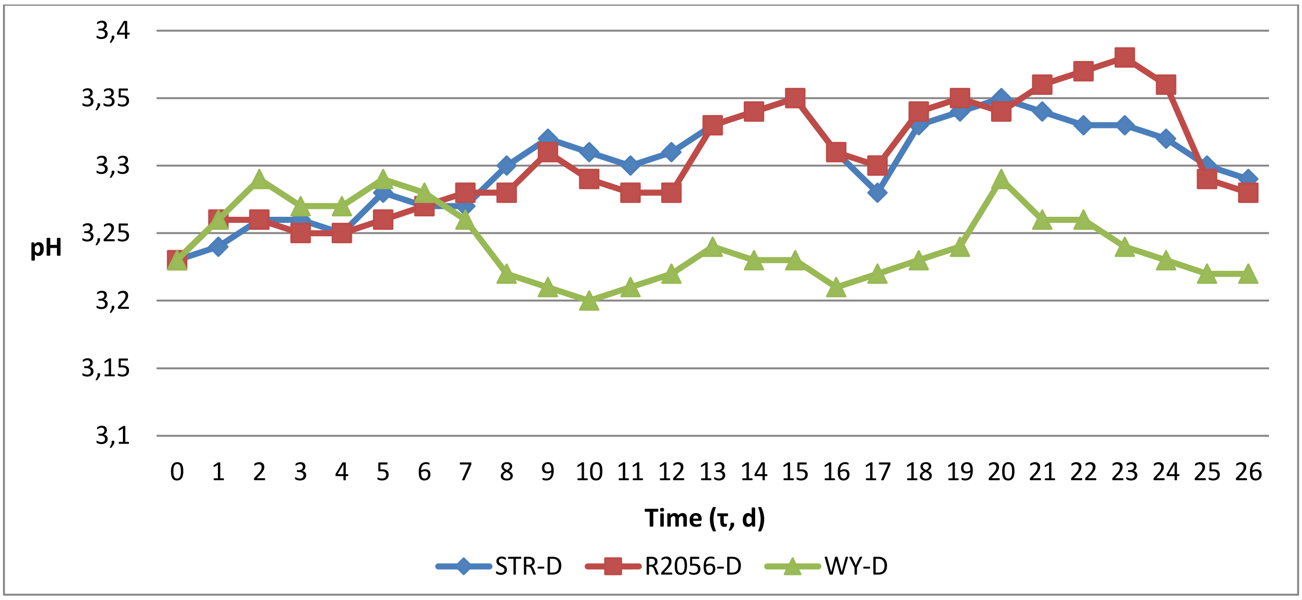

Figure 4. Changes in pH during alcoholic fermentation of D series

The dynamics of alcoholic fermentation is influenced by a number of factors, the main ones are the fermentation temperature and the initial sugar content in the grape must. Temperature is a technological factor that can be controlled while the sugar content cannot. Global warming and climate changes have led to changes in the terroir on a global scale. One of the challenges facing the wine industry is the increased sugar content in cultivated grape varieties. It is evident from the alcoholic fermentation process that the amount of alcohol depends primarily on the strain of yeast, and not on the primary sugar content. The high concentration of sugars (over 21.4%) in the grape must is a source of osmotic stress for yeast cells, which is partially alleviated during fermentation with to the conversion of sugars into ethanol and CO_2 (Marks et al., 2008). Yeast cells have a unique mechanism to respond to the presence of ethanol, regardless of glucose concentration (Marks et al., 2008). The selected yeast strains that were used, have an increased alcohol yield per sugar unit compared to wild yeast. Non-Saccharomyces yeasts can help to address the problem of high sugars by reducing the ethanol content of the wine (Ciani et al., 2016, Contreras et al., 2015, Englezos et al., 2016, Gonzalez et al., 2013).

Maceration also affects the species biodiversity of yeasts found in grape must (Regecová et al., 2024). A higher number of non-*Saccharomyces* yeasts was found in macerated must without added selected yeasts and a lower number of non-*Saccharomyces* yeasts when selected yeasts were added during the fermentation stage (Regecová et al., 2024). The results from the current study indicated that unmacerated grape must of the Dimyat variety (WY-D), fermented with wild yeast, did not complete alcoholic fermentation. Macerated grape must of the Muscat Ottonel variety (WY-MO) completed the alcoholic fermentation with some delay.

Yeasts other than *Saccharomyces* are not able to complete alcoholic fermentation (Regecová et al., 2024). For this reason, it would be advisable to carry out fermentation with two yeast species as an innovative technology for the production of white wines. The technological sequence of the processes could be as follows: yeasts other than *Saccharomyces* can be inoculated at the beginning of fermentation and, once the fermented must reaches about 10% vol. ethanol content, *Saccharomyces* yeasts should be added. In this way, the completion of alcoholic fermentation by *Saccharomyces* species can be guaranteed, relying on a smaller amount of alcohol produced per unit of sugar and the production of metabolites that positively influence the aroma of the wine by non-*Saccharomyces* yeasts. In summary, a sequential fermentations with *Saccharomyces* and non-*Saccharomyces* yeasts allow the development of local wines with low alcohol content (Nemcová et al., 2015). The other area where non-*Saccharomyces* yeasts could be useful is in the control of wine spoilage (Berbegal et al., 2016, Oro et al., 2014).

Considering the dynamics of alcoholic fermentation with selected yeasts regardless of the variety and the maceration of the grape must, there were no significant differences. In the last 1/5 of the process, their dynamics were analogous.

The initial and final values of the actual acidity (pH) for the wild yeast variants remained unchanged (the differences fall within the error limits of the analysis method). The final values the active acidity (pH) for the selected yeast variant were slightly higher. In the selected yeast variants, final titratable acids were higher with approximately 1 g/dm³ (as a difference from the initial titratable acids), while for the wild yeast variants this increase was only 0.25 g/dm³.

Sensory analysis

Wine is a complex, water-alcoholic, heterogeneous mixture of compounds, in which over of 1100 components have been identified (in terms of structure and concentration) (Georgiev, 2024). They determine wine appearance, aroma, taste and properties (Swiegers et al., 2005, Georgiev, 2024). Aroma is one of the most important characteristics that contribute to the quality of wine. It consists of hundreds of different compounds with concentrations that can vary between 10 -1 and 10 -10 g/kg (Rapp et al., 1986). The balance and interaction of compounds determine aromatic quality of wine (Padilla et al., 2016). The aroma of wine can be divided into three groups: primary (varietal) aroma, secondary (fermentation) aroma and tertiary (bouquet) aroma (Padilla et al., 2016, Georgiev, 2024). The compounds that form the primary aroma belong to a limited number of chemical groups, including methoxypyrazines, C13-norisoprenoids, volatile sulfur compounds, and terpenes (Ebeler et al., 2009). Methoxypyrazines are products of amino acid metabolism and are associated with vegetal, green, and herbaceous aromas in some grape varieties (Sidhu et al., 2015). C13-norisoprenoids are derived from carotenoids, and in particular β-ionone and β-damascenone are considered to be volatiles of non-floral grapes (Bindon et al., 2007, Fang et al., 2016). Some organic volatile sulfur compounds, such as volatile thiols, make an important contribution to wine aroma, but they are not found in the grape must before alcoholic fermentation (Kate et al., 2004), while terpenoids, although present in all grape varieties, are found in the highest amounts in the Muscat, Gewürztraminer and Rhine Riesling varieties (King et al., 2000). Approximately seventy terpenoid compounds have been identified in grapes and, consequently, in wines (Mateo et al., 2000). These compounds provide floral notes and have low odor thresholds (Zalacain et al., 2007). Interestingly, most primary aroma compounds are found in free or bound forms. The latter are not aromatic compounds whose hydrolysis can occur during fermentation by the action of wine yeasts (Padilla et al., 2016).

Secondary aromas are those that most significantly determine the aroma of wine. The number of chemical groups and their concentrations depend mainly on the predominant yeast and the fermentation conditions (Henick-Kling et al., 1998; Steger et al., 2000). Volatile fatty acids, higher alcohols, esters, and to a less extent, aldehydes have a greater contribution to secondary aroma (Rapp et al., 1991). The largest contribution to the secondary aroma of wine is made by esters and volatile thiols (Swiegers et al., 2005, Georgiev, 2024). It is worth noting that the biosynthesis of these compounds depends on the type and strain of used yeast. Furthermore, and depending on the concentration reached in the wine, these compounds resulting from yeast metabolism have a positive or negative impact on the aroma and quality of the wine. (Padilla et al., 2016).

In the conducted sensory difference tests, samples from each variety were found to be different (Parr et al., 2002). This also applies to different variants of the same grape variety. The intensity and balance of the aromas of all variants of wines from the two grape varieties are different. The main reason for this is the type of yeast, conducting the alcoholic fermentation (Georgiev, 2024, Zhang et al., 2024). The main aromas in wines are primarily esters, followed by thiol aromas. Fifteen predominant aromas were identified in the tasted wine samples: floral – ethyl octanoate (ethyl caprylate) and ethyl decanoate (ethyl caprate); rose – phenyl-ethyl acetate; hyacinth – phenyl-2-ethanol; pear – hexyl acetate; apple – ethyl octanoate (ethyl caprylate); peach – C6 compounds, lactones, aldehydes, alcohols, esters; apricot – 1-hexanol; boxwood – 4-mercapto-4-methylpentan-2-one; citrus fruits – 3-mercaptohexan-1-ol; pineapple – C4C2; orange; banana – isoamyl acetate; exotic fruits – A3SH (3-sulfanylhexyl acetate), passion fruit – 3-mercaptohexyl acetate; varietal – primary (Antalick et al., 2014, Avram et al., 2014, Marullo et al., 2021, Georgiev, 2024). The sensory panel used a five-point rating system, where 5 corresponds - to excellent, and 0 - to none. The results of sensory analysis of the variants of the Muscat Ottonel and Dimyat varieties are presented in Figure 5 and Figure 7.

It is mandatory to clarify that none of members of the sensotry panel were able to detect that they were tasting wines made from the same starting must (this applies to wines from both grape varieties).

Organoleptic profile of wines from the Muscat Ottonel variety.

The wines produced with selected yeasts have a balanced and intensive aroma and were different from each other in terms of aromatic profile. In the wine, produced with Excellence® STR yeast, the leading aromas were varietal, citrus, floral, exotic, rose, banana and pear. This wine has excellent color, aroma and balance, very good density and sweetness, excellent aftertaste, without defects.

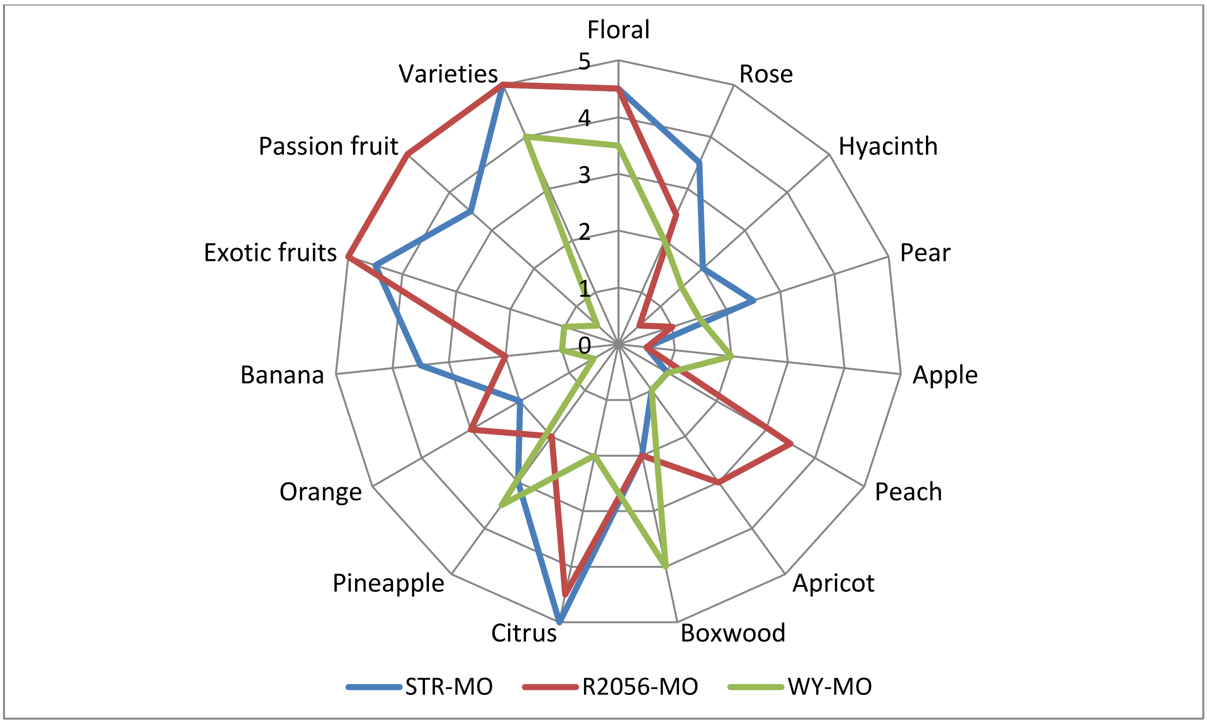

Figure 5. Main aromas in the tasted wines of the Muscat Ottonel variety

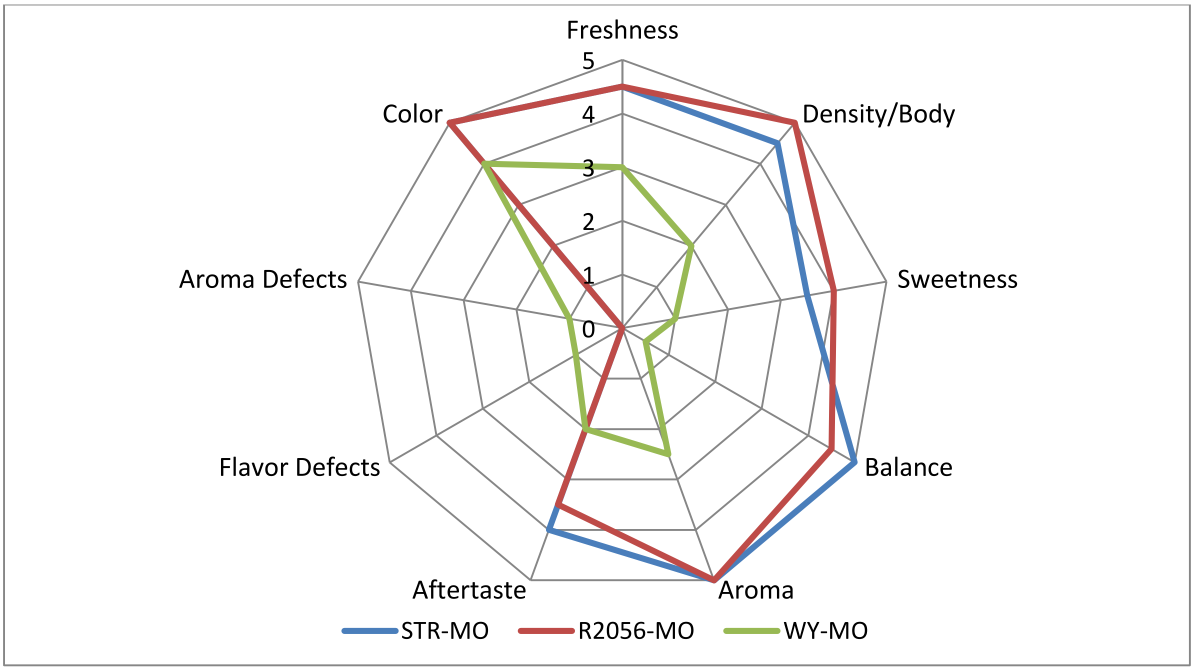

Figure 6. General characteristics of the tasted wines of the Muscat Ottonel variety

In the wine, produced with Rhône 2056TM yeast, the leading aromas were varietal, exotic, passion fruit, floral, citrus, peach and apricot. This wine has excellent color, aroma, density and sweetness, very good balance and aftertaste, without defects. In the wine with wild yeasts – varietal, floral, boxwood and pineapple (the aromatic intensity of this wine was significantly inferior to wines with selected yeasts). The wine has a very good color, fresh, with medium density and aftertaste, with minor defects in taste and aroma.

Organoleptic profile of Dimyat variety wines

Wines produced with selected yeasts have a balanced and medium aromatic intensity. They were different from each other in terms of aromatic profile. In the wine, produced with Excellence® STR yeast, the leading aromas are floral, citrus, exotic, hyacinth, banana and pear. This wine has excellent color, very good balance, density and sweetness, with good freshness and aftertaste, with medium aroma, without defects. In the wine, produced with Rhône 2056TM yeast, the leading aromas were floral, exotic, passion fruit, citrus and peach and apricot. This wine has excellent color and density, very good freshness, sweetness and balance, with medium aroma and aftertaste, without defects.

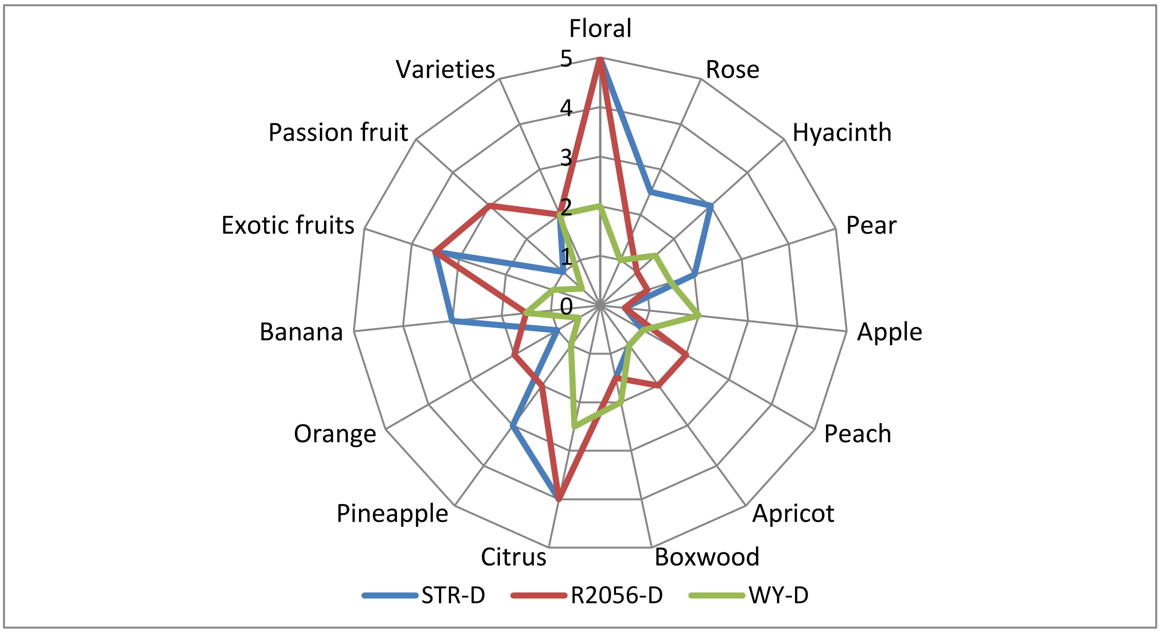

Figure 7. Leading aromas in the tasted wines of the Dimyat variety

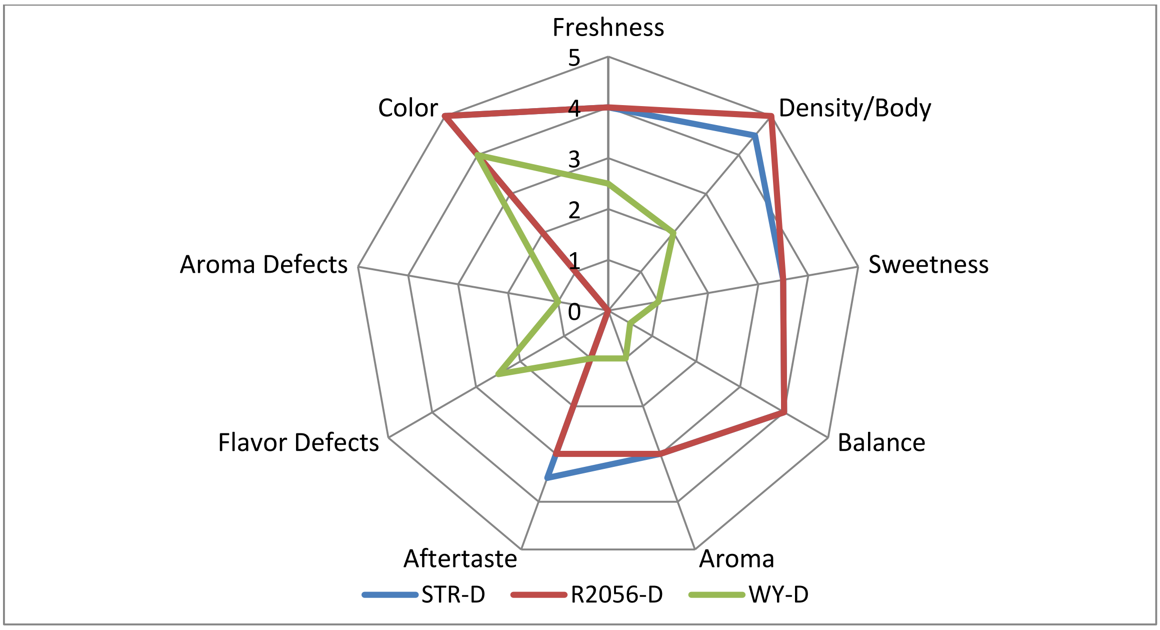

Figure 8. General characteristics of the tasted wines of the Dimyat variety

In the wine with wild yeasts – varietal, citrus, floral, apple and banana (the aromatic intensity of this wine was significantly inferior to wines with selected yeasts). The wine has a very good color, medium freshness and density, and significant flavor defects.

Ultimately, the selected yeasts are clearly distinguishable from other local strains (wild yeasts) (Zhang et al., 2024). Tracking the dynamics (kinetics) of alcoholic fermentation of selected yeast strains in different grape varieties shows a leveling of this indicator, whereas in wild yeasts the fermentation process is prolonged or does not reach completion. Global warming leads to the accumulation of high levels of fermentable sugars in grapes, which in turn results in excessive ethanol concentrations and insufficient acidity. A proposed solution is the use of mixed fermentations (Hranilovic et al., 2021).

CONCLUSIONS

The yeast strains Excellence® STR and Rhône 2056™ exhibited similar fermentation dynamics. However, from an aromatic perspective, they produced distinctly different wines. The results show that the selected yeasts have a strong influence on the levels of aromatic compounds. Significant differences in the dynamics and duration of fermentation were observed in wild yeasts. Fermentation of the macerated must from the Muscat Ottonel variety was longer and complete, whereas fermentation of the unmacerated must from the Dimyat variety ceased prematurely. The quality of the wines produced with wild yeasts was low. It is important to distinguish between white wines produced with selected Saccharomyces and/or non-Saccharomyces yeasts, and those obtained through spontaneous wild fermentation driven by the natural microflora inhabiting the grapes. Combining Saccharomyces and non-Saccharomyces yeasts may be justified when processing grapes with a higher content of fermentable sugars.

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