

STUDY ON THE OENOLOGICAL POTENTIAL OF SOME NATIVE YEASTS STRAINS ISOLATED FROM WINE CENTER ȘTEFĂNEȘTI

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Abstract

A study was conducted in 2023-2024 on the local yeast microflora in the Ștefănești wine center. Investigations led to the isolation of yeasts belonging to the families Saccharomycetaceae, Saccharomycodaceae, Sporidiobolaceae, and Metschnikowiaceae, including genera such as Saccharomyces and Metschnikowia, among others, morphologically characterized at the strain level. These were used to reproduce natural fermentation conditions in a controlled manner and to provide the possibility of studying the oenological parameters of wine. Yeast strains isolated from the native microbiome are the basis for the implementation of new biotechnologies in winemaking. The fermentation potential of native strains by inoculation in monoculture was studied. The results obtained confirm that native yeast strains can be a possible basic source for wine biotechnology. Ten types of native yeasts useful for wine production were selected and isolated, among which the following yeast strains stood out with useful characteristics for fermentation: 12St_G and 15St_F.

Key words: wine, fermentative potential, native yeast strains, yeasts.

INTRODUCTION

Wine production is an ancient tradition that was achieved through the spontaneous fermentation of grape juice (Rantsiou, 2017), which occurs due to the presence of indigenous yeasts of different genera and species (Nechita, 2022; Fleet, 2003; Romano, 2003). The number of species of yeasts during fermentation depends on several factors (Rantsiou, 2017; Pretorius, 1999), which lead to differences in the quality of the wine by geographical areas, but also from year to year (Pretorius, 2000). To avoid this situation many winemakers used pure commercial cultures *Saccharomyces cerevisiae* inoculated in must (Stojanovic, 2023; Pretorius, 2000). Commercial cultures selected for the fermentation of wines of different varieties, destroy the uniqueness of the bouquet, typical oenological characteristics of the wine and its originality from the micro-area in which it is produced (Capece, 2019; Cordero-Bueso, 2013). However, it has been suggested that native strains of *S. cerevisiae* are better suited to the climatic conditions of the micro-area in the wine production region (Lopez, 2002) and therefore can more easily dominate the natural biota

(Stojanovic, 2023; Nechita, 2022; La Jeune, 2006). In recent years, there has been a tendency to increase research on winemaking as closely related as possible to the terroir of the winemaking area. Current trends in scientific research as well as in the modern food and wine industry suggest continuous improvement of product quality (Stojanovic, 2023). This trend leads winemakers to adopt another technology of alcoholic fermentation, not using selected yeasts and using native yeasts, selected from the microflora naturally present in the area of origin of the wine (Lopez, 2007), existing in the vineyard, on the grapes, soil if the vineyard is old and on rachis or vegetable material of the vine. This approach might seem a step back from the scientific and technological stage of oenological science, but given its growing spread and economic relevance, it deserves scientific attention (Guzzon, 2018). The important wine regions have already made progress in adapting to the new requirements by introducing technological processes that will improve the complexity and quality of wine (Bisson, 2002).

The indigenous non-*Saccharomyces* yeast strains are now increasingly being brought to

the attention of winemakers (Mancic, 2022; Jolly, 2014), due to the good fermentative characteristics and production of aromatic compounds, specific to the geographical area of origin that give wine a recognized note (Mancic, 2022; Laroque, 2021; Rollero, 2021; Garcia, 2016). In addition, it is believed that specific strains of domestic yeast can be associated with specific regions and give wines a more typical, regional character. With unique and authentic wines gaining ground, the pursuit of new, untapped yeasts suitable for the wine industry is becoming inevitable. Many non-*Saccharomyces* yeasts have been characterized and applied in the wine industry (Mancic, 2022; Laroque, 2021; Rollero, 2021; Garcia, 2016). Among them, the genus *Hanseniaspora*, morphologically characterized as consisting of bee yeast with bipolar buds, has a special potential in wine production (Mancic, 2022; Testa, 2021; Cadez, 2002). This species of yeast has good oenological characteristics, with a positive effect on the color, taste, aroma and stability of the wine (Mancic, 2022; Testa, 2021).

Recent studies have shown that grape varieties and the climate of a geographical area influence native yeast strains (Mancic, 2022; Martin, 2018; Brilli, 2014). Recently, the correlation between native microbial strains and organoleptic characteristics of wine has seen an increasing increase in attention from winemakers (Dumonde-Neves, 2017; Bokulich, 2016; Knight, 2015). However, there is controversy about the origin of the native strains of *S. cerevisiae*. Several studies have shown that the direct isolation of *S. cerevisiae* strains from undamaged grapes and vineyards is very difficult (Agarbati, 2019; Agarbati, 2022; Mercado, 2007).

In this context, the present study investigates the diversity of yeasts from the *Saccharomyces* and non-*Saccharomyces* genera from the Stefanesti wine-growing area, by isolating and selecting new strains from soil, plant material (grape stalks and berries) and grape must. The study aimed at the oenological characterization of spontaneous fermentation performed by 10 yeast isolates, belonging to both the *Saccharomyces* genus and other genera, selected from the Stefanesti wine-growing area.

MATERIALS AND METHODS

For the oenological characterization of the isolated and selected native yeast strains, they were previously subjected to microscopic and molecular characterization, according to the method described by Manolescu et al. (2024). Aseptic soil samples were taken from the three distinct area from viticultural centres of INCDBH Stefanesti. Samples of plant materials (grape stalks and berry) and grape musts were collected. All juice grape samples were sterilized before they were used for fermentation with native yeast strains. The grape varieties from which musts resulted were: 'Riesling Italian' - RI, 'Fetească Regală' - FR, 'Muscat Ottonel' - MO, 'Sauvignon Blanc' - SB, 'Tămâioasă Românească' - TR, 'Merlot' - M, 'Fetească Neagră' - FN, 'Blauer Zweigelt' - BZ, 'Burgund Mare' - BM, and 'Cabernet Sauvignon' - CS (Table 1).

Table 1. Code strains of native yeasts and specific features

Yeast code	Source of Yeast insulation	Strains identification	Specific features
1\$ <u>t</u> _G	FR (washing water berry - Goleasca farm)	<i>Saccharomycetaceae</i>	Ovoidal, matte, slightly mucoid, cream
3\$ <u>t</u> _F	FR (washing water berry - Fitotron farm)	<i>Sporidiobolaceae</i>	Round, glossy, orange-pink, double cell wall
5\$ <u>t</u> _G	FR (soil washing water - Goleasca farm)	<i>Saccharomycetaceae</i>	Round, convex, slightly mucoid matte, cream
6\$ <u>t</u> _G	TR (fresh juice - Călinești farm)	<i>Saccharomycetaceae</i>	Round, convex, glossy, cream-white
7\$ <u>t</u> _G	FR (string washing water - Goleasca farm)	<i>Saccharomycetaceae</i>	Spherical-round, convex, glossy, cream-white
9\$ <u>t</u> _G	MO (Fresh juice - Goleasca farm)	<i>Saccharomycodaceae</i>	Ellipsoidal, convex, glossy, cream with yellow-orange pigments
11\$ <u>t</u> _G	FR (fresh juice - Goleasca farm)	<i>Saccharomycodaceae</i>	Oval-round, convex, glossy, cream-white
12\$ <u>t</u> _G	BZ (Fresh juice - Goleasca farm)	<i>Saccharomycetaceae</i>	Elliptical-apicular, convex, slightly mucoid matte, cream
14\$ <u>t</u> _G	MO (Fresh juice - Călinești farm)	<i>Saccharomycetaceae</i>	Spherical, slightly convex, slightly mucoid matte, cream-gray
15\$ <u>t</u> _F	FN washing water berry - Fitotron farm)	<i>Metschnikowiaceae</i>	Ovoidal-elongated, convex, cream-white, light pink pigmentation

The grape juice has been sterilized by autoclaving. Selected native strains were tested in fermentation processes with inoculation in monoculture (10^6 CFU/ml), *Saccharomyces* and non-*Saccharomyces* strains were used. The fermentation processes were dynamically monitored and were carried out at a temperature of $20 \pm 1^\circ\text{C}$. The main physico-chemical parameters were determined in the obtained wines: alcoholic strength (% vol); total acidity (g/L tartaric acid); volatile acidity (g/l acetic acid), unfermented sugars (g/L), and pH according to the methods OIV, 2025.

RESULTS AND DISCUSSIONS

The musts were characterized before being inoculated with native yeasts (Table 2).

Table 2. The characteristics of the musts used for experiments

Musts used	Sugars, g/L	Total acidity, g/l H ₂ SO ₄	pH
RI	232	1.7	3.64
FR	221	1.75	3.92
MO	209	1.95	3.73
TR	192	3.2	3.62
SB	220	2.65	3.64
BZ	198	4.1	3.62
CS	221	2.85	3.52
BM	205	4.8	3.6

After the isolation and selection of the native yeasts from the Stefanesti geographical area, experiments were made with them in the must varieties from Table 2. In the experiment, for each batch of must, a witness was used for which we used as a fermentation agent a commercial compound usually used in the production of white wine and red wine. Data obtained from monitoring fermentation processes carried out on strains of native yeast are graphically shown in Figures 1 and 2.

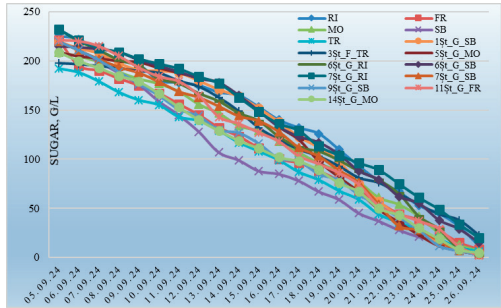


Figure 1. Evolution of yeast fermentation, native and commercial, to white varieties of must used

Figure 1 shows the evolution of fermentation for each native yeast and commercial yeast in white musts.

Figure 2 includes the fermentation of native and commercial yeasts in red musts.

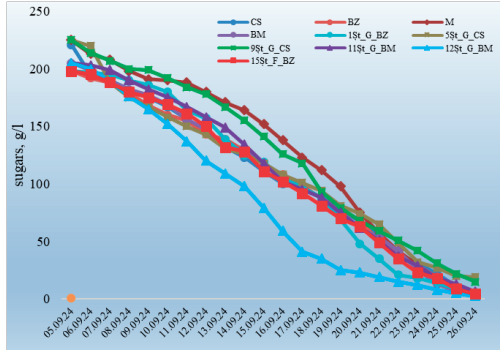


Figure 2. Evolution of yeast fermentation native and commercial to red varieties of must used

The fermentation processes in monoculture with native yeast strains were completed in 20 days, the average fermentation speed values being between 25 and 30 g/L sugars in 48 hours. Yeast strain *12St_G_BM* fermentation completed in 10-15 days, with a fermentation rate of 32.5 g sugars/48 hours.

The newly selected yeast strains began alcoholic fermentation 12-24 hours after introduction into the must. At this stage, the musts began to ferment tumultuously, and the temperature of the wort slowly increased by about 1°C . The relatively small duration of the period of preference is an advantage of the alcoholic fermentation process, it is always preferred by yeast strains that exhibit this feature. In the evolution of the fermentation curve, the sugar degradation is accentuated in the tanks considered control, in which commercial yeast was used for fermentation.

The phase of tumultuous fermentation began after 48 hours and continued for 10-15 days, when the temperature of the wort gradually increased, due to the increase in metabolic activity while increasing the number of yeasts. At this stage, the temperature was checked once a day, intervening to keep it between 15 and 16°C for white wines and $25-26^\circ\text{C}$ for red wines. In the case of native yeasts, an average sugar degradation was observed, which eventually resulted in wines with good oenological and sensory characteristics.

From the analysis of the compositional characteristics of the wines obtained presented in Table 3, it is noted that, regardless of the selected yeast strains, the alcohol concentrations were between 14.01% vol and 11.13% vol, obtaining mostly dry wines, which proves the alcoholic character of the selected strains of *Saccharomyces* and non-*Saccharomyces* isolated yeast from the Stefanesti wine-growing area. The total acidity values ranged from 4.44 g/L to 7.21 g/L tartaric acid, being considered normal for white wines and between 5.2 and 8.01 for red wines. Differences were found in the case of volatile acidity, it was between 0.28 g/l acetic acid in 'Fetească Regală' inoculated with native yeast *11St_G* and the highest of 0.88 g/l acetic acid in 'Burgund Mare' inoculated with *11St_G* and 'Blauer Zweigelt' inoculated with yeast *9St_G*.

Table 3. The physico-chemical properties of wines obtained by inoculation of native strains

Yeast code	Wine Type	AS, %vol	TA, g/L AT	VA, g/L AE	S, g/l	Ph	SC
<i>1St_G</i>	SB	12.91	5.35	0.44	2.9	3.75	+++
	BZ	12.10	6.8	0.45	2.9	3.32	+++
<i>3St_F</i>	TR	13.18	4.94	0.45	22.5	3.6	+++
	BZ	11.4	6.96	0.81	5.5	3.26	++
<i>5St_G</i>	MO	13.77	6.24	0.32	3	3.57	++
<i>6St_G</i>	RI	13.68	5.18	0.32	1.5	3.26	+++
	SB	11.85	5.35	0.56	11.5	3.65	+++
<i>7St_G</i>	RI	11.94	7.21	0.46	7.5	3.25	+++
	SB	12.22	6.4	0.54	3.5	3.69	+++
	CS	12.69	5.99	0.65	4.5	3.24	+++
<i>9St_G</i>	SB	13.94	6.21	0.81	3	3.83	+++
	CS	13.23	7.8	0.80	1.5	2.73	+++
	BZ	11.27	8.01	0.88	1.3	3.23	++
	CS	14.01	6.99	0.65	5.5	2.73	++
<i>11St_G</i>	FR	13.02	4.54	0.28	2	3.81	+++
	BM	11.13	7.8	0.88	6.5	3.29	+++
<i>12St_G</i>	BZ	11.28	6.5	0.38	2.5	3.27	+++
<i>14St_G</i>	MO	12.0	4.4	0.32	5	3.57	+++
<i>15St_F</i>	BM	11.8	5.2	0.42	4.5	3.29	+++

AC - alcoholic concentration; TA - total acidity, g/L tartaric acid; VA - volatile acidity g/L acetic acid; S - unfermented sugars, g/L; CS - sensory characteristics; ++ - good; +++ - very good

From a sensory point of view, the following native yeasts used for the production of wines were highlighted: *1St_G*, *5St_G*, *6St_G*, *7St_G*, *12St_G*, *13St_G*, *14St_G* and *15St_F*. They were rated as clear, bright, straw yellow with medium intensity. Flavourings were classified as normal in a simple mixture, with the overall flavour intensity being assessed as mean. Olfactory, a slight smell of lemon, pear and

quince, but also pineapple in white wines. In the case of red wines besides the intense color of bright red, the flavors were of blackcurrant at CS and with a smoky flavor at BM, blackberry flavors at BZ. The intensity of the basic aroma was balanced in the case of acidity and softness in the case of sweetness, without notes of bitterness and with a normal body for the type of wine. The mild astringency given by a low tannic intensity makes these wines to be appreciated as balanced, correct, with medium aromatic persistence.

CONCLUSIONS

After testing the oenological value of the newly selected yeast strains we determined that they can be appreciated as a useful biological material for the practice in micro- wine making of white and red wines. The fermentative processes, in the experimental variants realized, have highlighted the relevance of the native strains selected as possible sources for the early culture in micro-wine making. The compositional analysis of the wines obtained proved their alcoholic character, obtaining dry wines with a moderate volatile acidity.

The relatively small duration of the pre-fermentation period is an advantage of the alcoholic fermentation process, it is always preferred by yeast strains that exhibit this feature (*12St_G*, *15St_F*).

From the 10 native yeast strains tested: *12St_G* and *15St_F* are valuable in the fermentation process given their reduced foaming capacity.

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