Scientific Bulletin. Series F. Biotechnologies, Vol. XXVIII, No. 2, 2024
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

TESTING THE FERMENTATIVE POTENTIAL OF SOME LOCAL *Saccharomyces* AND non-*Saccharomyces* YEASTS

Raluca-Ștefania RĂDOI-ENCEA[1, 2], Florentina BADEA[1], Ana-Maria MANOLICĂ[1, 2], Luminița VIȘAN[1], Florentina MATEI[1, 2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnology, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Transilvania University of Brașov, Faculty of Food Industry and Tourism, 148 Castelului Street, Brașov, Romania

Corresponding author email: raluca.encea@unitbv.ro

Abstract

Considering that Starmerella bacillaris and Metschnikowia pulcherrima have repeatedly proved their desirable oenological properties, we have chosen to test the fermentative potential of local strains from our collection, in simple and sequential cultures with a local Saccharomyces cerevisiae. In order to prove their winemaking potential, the fermentative kinetics were observed, along with ethanol and sugar content, pH, total dry matter and wines colors. Local yeast S. cerevisiae BB06 finalized the fermentation with 10.5-11% ethanol and 9.5-9.3°Bx. The two sequential fermentations with S. bacillaris MI115 and M. pulcherrima MI109 led to an average ethanol content of 7.8%, respectively 7.8% and around 13.9°Bx, respectively 16.6°Bx. The pH levels were maintained between 3.0 and 4.0 throughout the experiments. The three tested strains confirmed they are suitable for winemaking in sequential steps, but several further investigations should be performed on biochemical and organoleptic level.

Key words*: autochthonous yeasts, Metschnikowia pulcherrima, oenological potential, Starmerella bacillaris.*

INTRODUCTION

Non-*Saccharomyces* (NS) yeasts show low fermentation performance, which rules out their exclusive use in the fermentation of grape must. They are not able to numerically dominate the entire fermentation process, nor can they bring the alcoholic fermentation to the end, due to their low tolerance to ethanol (Binati et al., 2020).

However, NS yeasts can be used in co-inoculations and in sequential inoculations with yeasts belonging to the *Saccharomyces* genus, because the metabolic impact of NS yeasts in the early stages of fermentation is sufficient to trigger significant changes in the wines' volatile profile (Carrau et al., 2020).

Considering that many of these non-conventional yeasts possess real winemaking properties, such as the production of higher alcohols, volatile esters, monoterpenes (Belda et al., 2017), wines can be obtained by inoculating non-*Saccharomyces* yeasts at the beginning, and by adding a *Saccharomyces* yeast after the fermented must reaches about 10% ethanol (Carrau et al., 2020).

Metschnikowia pulcherrima is a yeast which ferments glucose, but its fermentative power is low. It shows a low tolerance to ethanol, hardly withstanding concentrations that exceed 4-5% ethanol (*v/v*) (Vicente et al., 2020). *M. pulcherrima* can positively influence the content of esters, thiols and terpenes in wine and thus contribute to the aroma of the obtained wines (Benito et al., 2019).

Regarding the enzymatic activity of *M. pulcherrima*, it is known that it is represented by proteases, glucanases, pectinases, lipases (Canonico et al., 2023). In *S. cerevisiae* co-fermentation with *M. pulcherrima*, the obtained wine contains a volume of ethanol compared to simple fermentations with *S. cerevisiae*, a lower amount of malic acid and reduced total and volatile acidity (Canonico et al., 2023).

Starmerella bacillaris (also known as *Candida zemplinina*) is a non-*Saccharomyces* yeast with huge potential in winemaking, due to its strong fructophilic character and due to its low yield in the production of ethanol from consumed sugars (Magyar & Toth, 2011). Even if it shows a very close genetic relationship to *Candida stellata*, with which it was, until recently, confused,

C. zemplinina is a separate species, described as such in 2004, by Sipiczki.

According to studies carried out by several researchers, *S. bacillaris* presents the following desirable characteristics in winemaking: increased production of glycerol (Englezos et al., 2018 & 2019; Binati et al., 2020; Russo et al., 2020), low production of ethanol (Binati et al., 2019; Russo et al., 2020), tolerance to osmotic pressure (Vilela, 2019; Shen et al., 2022), pronounced fructophilic character (Wang et al., 2016), produces different compounds of aroma - linalool, geraniol, citronellal (Sadoudi et al., 2012). Due to the fact that *S. bacillaris* does not have the ability to complete the fermentation by itself, most of the characteristics described above are valid especially in co-cultures with *S. cerevisiae*, or in sequential cultures with the same wine yeast.

In order to develop an industrial winemaking process, it is necessary to go through several stages, as follows: the laboratory stage, the pilot station stage, the industrial production stage and the separation of the finished product. In the laboratory, the cultivation of yeasts is carried out on liquid media, in Erlenmeyer flasks, in static conditions (in the case of wine) or in continuous agitation, in small volumes (Lazăr et al., 2016). The next step is the cultivation in small volume bioreactors (up to 20L), where pH, temperature, aeration and agitation can be easily adjusted.

In the case of the present experiments, the volume was limited to 200 mL of grape must, to be able to carefully monitor the environmental conditions during the fermentative process using non-*Saccharomyces* yeasts, co-inoculated with *S. cerevisiae*.

MATERIALS AND METHODS

Yeast strains

The present study was conducted with the use of three yeast strains belonging to Faculty of Biotechnology Collection, two of them being non-*Saccharomyces* (*Starmerella bacillaris* and *Metschnikowia pulcherrima*) and one *Saccharomyces cerevisiae* strain (Table 1). All three yeast strains were isolated from Pietroasa vineyard, Buzău county and cryopreserved in Yeast Extract Peptone Dextrose (YEPD - yeast extract 10 g/L, peptone 20 g/L, dextrose 20 g/L, pH 6.5) containing 40% glycerol. Before initiating the experiments, the yeast strains were sub-cultured on YEPD and incubated at 20°C for 48 h. Subsequently, the strains were inoculated and maintained at 4°C on YEPD agar plates. For the analysis of the fermentation process at laboratory level, the experimental variants (Table 2) were inoculated on fresh white grape must from Pietroasa vineyard. All fermentations were conducted in triplicate.

Table 1. Tested yeast strains

No.	Species	Strain
1.	*Saccharomyces cerevisiae*	BB06
2.	*Starmerella bacillaris*	MI115
3.	*Metschnikowia pulcherrima*	MI109

Experimental variants were codified as stated in Table 2, depending on the inoculation modality (simple culture or co-inoculation). All of them were inoculated on sulfated must and sulfur-free must.

Table 2. Experimental variants

Sample	*S. cerevisiae* BB06	*S. bacillaris* MI115	*M. pulcherrima* MI109
V_1	100%	-	-
V_2	-	100%	-
V_3	-	-	100%
V_{12}	50%	50%	-
V_{13}	50%	-	50%

Grape must preparation

The must obtained from the fresh white grapes was divided in two sections, one without sulfur dioxide and the other with the addition of sulfur dioxide, according to the method used by Capece et al. (2020). The must (100 mL) was thus distributed into 250 mL Erlenmeyer flasks and sterilized by tyndallization.

For the preparation of the inoculum, a diluted must solution was obtained (50% must and 50% water) and then sterilized by autoclaving.

Primary and secondary fermentation

After cooling, the Erlenmeyer flasks containing the diluted must were inoculated with the yeast strains, according to the experimental variants presented previously (Table 2), adapting the protocol used by Dutraive et al. (2019). The 10 Erlenmeyer flasks with grape must (5 with sulfur dioxide and 5 without sulfur dioxide) were inoculated with a corresponding volume of

the pre-inoculum, to reach a level of 10^8 CFU/mL. V_{12} and V_{13} were inoculated at T_0 with the non-*Saccharomyces* strains and after 24 h with *S. cerevisiae* (protocol adapted after Englezos et al., 2019).

Primary fermentations were carried out in 250 mL Erlenmeyer flasks with silicone caps, in which a polyethylene tube was inserted, to allow the release of CO_2 and to prevent contamination. The flasks were kept at 20°C for 14 days. Throughout the two weeks, the wines were analyzed daily for weight loss, pH changes and sugar content (°Brix).

Secondary fermentations. Primary fermentations were evaluated as complete when there were no weight losses for three consecutive days. At the end of the fermentation process, the experimental wines were transferred into sterile corked bottles and left to decant for 24 h, at 4°C. The operation was repeated after another 24 h and the wines were stored at the same temperature. After this maturing period, the following analyzes were performed: weight loss, color, pH, residual sugars, total dry matter and ethanol content.

CO_2 losses

The fermentation kinetics was monitored daily by measuring the weight loss of the samples, due to CO_2 release, following a method adapted after Dutraive et al. (2019). The weight of each sample was checked regularly to track the fermentation progress. Loss was calculated by subtracting each day's weight from the initial weight (from T_0) and applying the following formula:

$$\text{Weight loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Sugar content determination

The amount of dissolved sugar in the grape must can be measured in °Brix or Brix units, which can show the potential ethanol content of the resulting wine. 1°Brix is equal to 1 g sucrose in 100 g solution, so 1°Brix is equal to 1% sucrose in must (Jaywant et al., 2022). At the laboratory level, Brix determinations were made with a Milwaukee MA-871 INR 9,800 digital refractometer. The sugar content value is obtained after converting the refractive index of the sample to % Brix.

pH determination

A Crison Basic 20+ pH meter (Barcelona, Spain) was used for these measurements, after calibration with standard solutions (pH 4.01, 7.00, 10.01) recommended by the manufacturer.

Ethanol content determination

A classic density meter was used to determine the ethanol content of the final wines. The working method involves inserting the hydrometer into a container with wine; when released, it will float freely in the liquid and allow the reading on the scale of the rod (to which the surface of the liquid will reach). The measurement is performed at 20°C, for accuracy. The result obtained represents the percentage volume of ethanol in the wine.

Total dry matter

Determination of dry matter using a thermo-balance is a fast and reliable method for determining dry matter content using the thermo-gravimetric principle. Thermo-gravimetry consists of weighing the sample before and after heating, to determine the moisture content by difference. 5 g of sample were taken, distributed homogeneously and in a thin layer on the weighing plate, in order to obtain correct results. After 105°C thermal balance treatment, total dry matter percentage was noted according to the thermo-balance display.

Color determination

A HunterLab MiniScan XE spectrocolorimeter was used to measure the color of the samples, with the following working conditions: Device geometry 45°/0°; LAV viewing area; Illuminant D65; Observatory 10°; The CIELAB 76 color system. CIELAB color system uses a rectangular three-dimensional color space that correlates its values with lightness, chroma and hue (Fairchild, 2018). The "L" axis is represented by luminance, with 0 being black and 100 being white. The "a" axis represents red-green values, with positive values being red, negative values being green, and 0 being neutral. The "b" axis represents blue-yellow values, with positive values being yellow, negative values blue, and 0 neutral.

RESULTS AND DISCUSSIONS

The three yeast strains *Saccharomyces cerevisiae* BB06, *Starmerella bacillaris* MI115 and *Metschnikowia pulcherrima* MI109 were tested for their oenological potential as follows. Ten fermentations (6 single cultures and 4 sequential cultures) were performed with and without sulfur dioxide (SO_2). All fermentations were successfully completed after 9 days, but were monitored until day 14.

Primary fermentation

Regarding the fermentation vigor displayed by the 10 cultures monitored (with and without SO_2), they all showed similar dynamics (Figures 1 and 2).

Figure 1. Primary fermentation of yeasts in must without SO_2 (left - simple cultures V_1, V_2 and V_3; right - co-fermentations V_{12} and V_{13})

Figure 2. Primary fermentation of yeasts in must with SO_2

Secondary fermentation

The secondary fermentation (Figure 3) took place after the wines obtained from the primary fermentation were transferred into sterile 250 mL bottles.

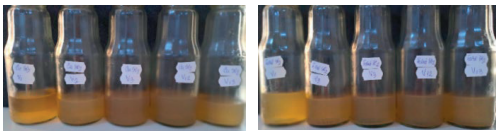

Figure 3. Obtained wines after secondary fermentation (left - with SO_2; right - without SO_2)

CO_2 losses

During the weight loss monitoring of the ten fermentations, significant differences were observed in the 4 sequentially inoculated fermentations, namely the experimental variants V_{12} and V_{13} (with and without SO_2). V_1, represented by *S. cerevisiae* BB06 followed a similar direction to V_2 (*S. bacillaris* MI115) in terms of weight loss, but V_2 maintained the same weight from day seven. V_3 had a constant weight loss during the 9 days of fermentation. V_{12} and V_{13}, on the other hand, showed significant day-to-day differences. It can be noticed in the Figures 4 and 5) that the presence of SO_2 significantly changes the CO_2 losses from the wines and, in the case of V_{13}, they stop on the seventh day (phenomenon observed in the wine without sulfur only on the ninth day.). In the case of V_{12}, the wine without SO_2 had constant and relatively low CO_2 losses from day to day, but the wine with added SO_2 followed a different trend.

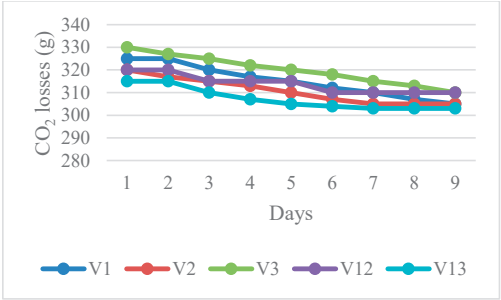

Figure 4. CO_2 release in sulfated wines

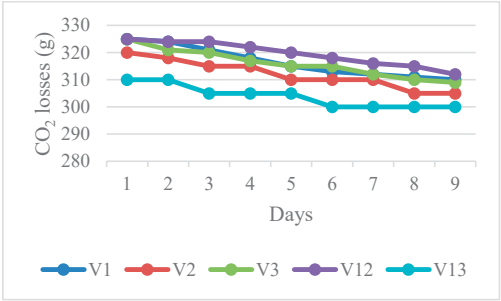

Figure 5. CO_2 release in sulfate-free wines

Sugar content

The °Brix levels were evaluated throughout the fermentation processes, in order to be able to compare the decrease in the amount of sugars in the fermented must.

On the first day of fermentation, all experimental variants had a similar sugar content, correlated with 22.6°Brix (approx. 216 g/L) in the must with SO_2, respectively with

22.3 °Brix (approx. 213 g/L) in the must without SO_2. Brix values on the ninth day of fermentation reached a minimum of 9.5°Brix and 9.3°Brix, respectively.

As presented in Figures 6 and 7) it can be seen that *S. cerevisiae* in monoculture has reached the lowest Brix level, with the previously mentioned values, and the graphs also show that the addition of SO_2 does not influence the Brix values from these experimental variants.

The other two monocultures also followed similar trends in must with SO_2 and in must without added SO_2. Also, the V_{12} and V_{13} variants had the same trend, without demonstrating any significant change in the progress of sugar consumption depending on the addition of SO_2. It should be noted that the experimental variants represented by the two sequential inoculations did not show any significant differences compared to the monocultures of each non-*Saccharomyces* strain tested in this study.

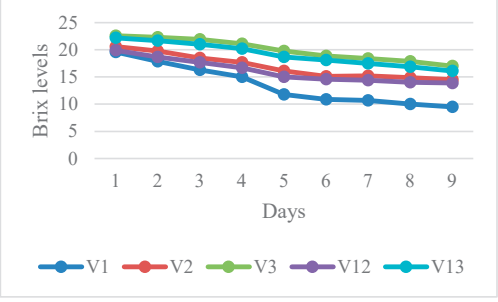

Figure 6. Brix levels of sulfated wines

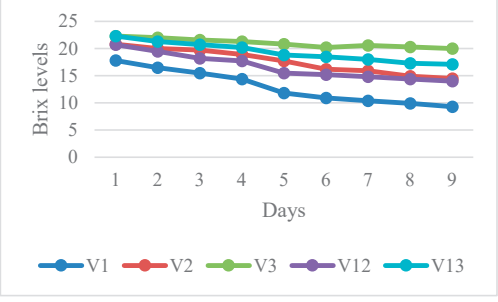

Figure 7. Brix levels of sulfate-free wines

pH values

Grape must usually has a total acidity of 6-6.5 g/L organic acids and a pH of 3.0-4.0, largely due to the malic and tartaric acids in its composition, but citric acid can also contribute to these values (Okafor, 2007). The pH is important in winemaking, because its' involvement in the microbiological stability of wine, in the start of malolactic fermentation and in the natural selection of fermentation microorganisms (Pastore et al., 2024). From the pH monitoring during 9 fermentation days, it was observed that the values of this parameter did not vary significantly neither from day 1 to day 9, nor from sulfated must to sulfate-free must. Thus, throughout the experiment and in all the experimental variants employed, the pH values were maintained between 3.0 and 4.0. Similar values were obtained by du Plessis et al. (2017), by testing simple fermentations of *S. cerevisiae* (pH 3.66) and sequential inoculations of *S. cerevisiae* with *S. bacillaris* (pH 3.70), respectively *S. cerevisiae* with *M. pulcherrima* (pH 3.77).

Ethanol content

Regarding the ethanol content of the experimental wines, the lowest value was recorded at V_3 in the wine without SO_2 (3.5%) and in the wine with SO_2 (5%). Obviously, the highest values were recorded in wines obtained only with *S. cerevisiae* (Figure 8).

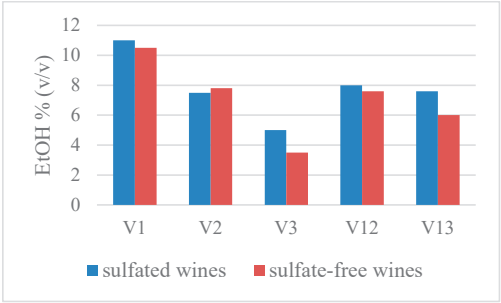

Figure 8. Ethanol content of final wines

From the values obtained, it can also be noted that the wines obtained from co-fermentations have a lower ethanol content compared to those obtained with *S. cerevisiae* in monoculture, which supports the possibility of using these experimental variants in future studies (for obtaining wines with lower percentage of alcohol).

Total dry matter

From the point of view of dry matter content (Table 3), sample V_1 presented the lowest

content, 4.97% in wine with SO_2 and 5.23% in wine without SO_2. At V_2, the same values of the dry matter and, implicitly, of the moisture were obtained, respectively 12.96% dry matter and 87.04% moisture (both in the wine with SO_2 and in the one without SO_2 addition). Sample V_{12} had a dry matter percentage similar to V_2, with 12.66% in the wine with SO_2 and 11.99% in the wine without SO_2.

V_3 showed the highest dry matter value, namely 16.96% in the wine sample with SO_2 and 20.37% in the wine sample without SO_2. Finally, V_{13} presented 14.84% dry matter (with added SO_2), respectively 16.70% dry matter (without SO_2).

Table 3. Total dry matter and moisture results

Sample	Yeast strains	Wine with SO_2 (%)		Wine without SO_2 (%)	
		Moisture (%)	Dry matter (%)	Moisture (%)	Dry matter (%)
V_1	*S. cerevisiae* BB06	95.03	4.97	94.77	5.23
V_2	*S. bacillaris* MI115	87.04	12.96	87.04	12.96
V_3	*M. pulcherrima* MI109	83.04	16.96	79.63	20.37
V_{12}	*S. cerevisiae* BB06 + *S. bacillaris* MI115	87.34	12.66	88.01	11.99
V_{13}	*S. cerevisiae* BB06 + *M. pulcherrima* MI109	85.16	14.84	83.30	16.70

Color determination

From the data presented in Tables 4 and 5, it can be seen that the luminance (L*) of the analyzed wine samples took values in the range of 8.61-17.27.

Table 4. Results of the colorimetric analyzes of the sulfated wines

Wine samples	Color indicators		
	L*	a*	b*
V_1 with SO_2	8.61	-0.07	2.31
V_2 with SO_2	16.63	-1.36	-0.24
V_3 with SO_2	16.19	-0.71	0.39
V_{12} with SO_2	16.80	-1.10	-1.43
V_{13} with SO_2	17.40	-1.13	-0.05

Table 5. Results of the colorimetric analyzes of the non-sulfated wines

Wine samples	Color indicators		
	L*	a*	b*
V_1 without SO_2	12.49	-1.06	-0.10
V_2 without SO_2	16.03	-1.51	0.60
V_3 without SO_2	17.27	-1.40	1.06
V_{12} without SO_2	16.96	-1.49	-0.43
V_{13} without SO_2	17.25	-1.49	-0.34

The color index a* (green-red) recorded negative values for all analyzed wine samples, they were in the range between -0.07 and -1.51, which places all the experimental variants in the green color zone (Figure 9).

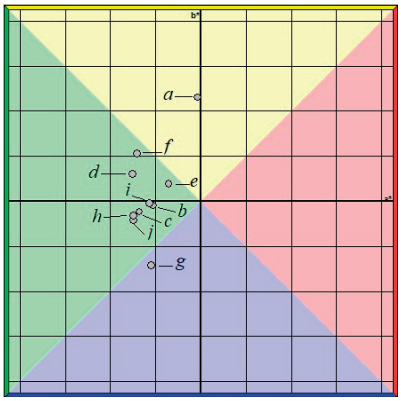

Figure 9. Graphic representation of colorimetric analyzes of the wines: (a) V_1 with SO_2; (b) V_1 without SO_2; (c) V_2 with SO_2; (d) V_2 without SO_2; (e) V_3 with SO_2; (f) V_3 without SO_2; (g) V_{12} with SO_2; (h) V_{12} without SO_2; (i) V_{13} with SO_2; (j) V_{14} without SO_2.

Regarding the b* index (blue-yellow), it can be seen that negative values predominate in the range between -0.05 and -1.43, but there are also four positive values.

The results listed below (luminance indicator column) show that the sulfated wines V_1 and V_{13} have a lighter shade than the wines obtained with the same yeasts, but without the addition of SO_2. In contrast, the sulfated wines V_2, V_3 and V_{12} showed a darker shade than the wines obtained with the same yeasts but without the addition of SO_2.

Also, sulfated V_1 (inoculated only with *S. cerevisiae*) showed a significantly lower luminosity value than the other sulfated wines (almost half less), and sulfate-free V_1 also showed a lower luminosity value, but only with few points. These values are correlated with the wines lightness as can be seen in Figure 3, where the wines obtained by *S. cerevisiae* simple culture are lighter and clearer than the rest of the wines.

It can be seen that most of the experimental variants are located on the graph in close color areas, but V_1 and V_{12} are divided into two color areas. V_1 with SO_2 is thus found on the yellow color and V_1 without SO_2 is found on the green color. V_{12} with SO_2 is found in blue and V_{12} without SO_2 is found in green.

CONCLUSIONS

In small-scale laboratory fermentations, no significant differences in fermentation dynamics were observed while using co-inoculation of *Saccharomyces* and non-conventional yeast like *Starmerella bacillaris* and *Metschnikowia pulcherrima*. The monitoring of CO_2 losses provided information on the speed of the fermentation activity, which was slower in the analyzed non-*Saccharomyces* strains compared to the *S. cerevisiae* strain, without major differences between sulfated and non-sulfated wines.

Sugar consumption followed similar curves for all experimental variants, with the mention that in simple fermentations with *S. cerevisiae* BB06 the lowest values of °Brix were reached, of 9.3 (wine without SO_2) and 9.5 (wine with SO_2). The ethanol content of the wines obtained with only *S. cerevisiae* was 11% (*v/v*) in the sulfated wine and 10.5% (*v/v*) in the non-sulfated wine. The other experimental variants contained an average volume of approx. 7.5%, with the exception of wines fermented by *M. pulcherrima*, in which 5% ethanol was produced in the sulfated wine, respectively 3.5% ethanol in the non-sulfated wine. The pH values were maintained throughout the experiment between 3.0 and 4.0. Among the results of the dry substance, a value of 4.97% (sulfated wine) and 5.23% (no sulfites wine) can be noted in the fermentations conducted only by *S. cerevisiae* BB06. The highest content of dry matter was recorded in the wine obtained from the fermentations conducted by *M. pulcherrima*, namely 16.96% in the sulfated wine, respectively 20.37% in the non-sulfated wine.

Regarding the results of the colorimetric analyses, the experimental variants represented by V_1 and V_{13} with SO_2 show a lighter shade than the wines obtained with the same yeasts, but without sulfur dioxide. V_2, V_3 and V_{12} showed a darker shade than the wines obtained with the same yeasts, but with the addition of sulfur dioxide.

This preliminary study indicates that the local non-*Saccharomyces* yeast strains isolated from Pietroasa vineyard, belonging to *Starmerella bacillaris* and *Metschnikowia pulcherrima* have fermentative potential to be used for the production of low alcohol wines, as requested nowadays on the market. Further research should be performed on biochemical and organoleptic levels to prove the oenological potential of their co-inoculation.

REFERENCES

Belda, I., Ruiz, J., Esteban-Fernandez, A., Navascues, E., Marquina, D., Santos, A., Moreno-Arribas, M.V. (2017) Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules, vol. 22* (2), 189

Binati, R.L., Lemos Junior, W.J.F., Luzzini, G., Slaghenaufi, D., Ugliano, M., Torriani, S. (2020) Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on *Lachancea thermotolerans*, *Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. *International Journal of Food Microbiology, vol. 318*, 108470

Canonico, L., Agarbati, A., Galli, E., Comitini, F., Ciani, M. (2023) *Metschnikowia pulcherrima* as biocontrol agent and wine aroma enhancer in combination with a native *Saccharomyces cerevisiae*. *LWT – Food Science and Technology, vol. 181*, 114758

Capece, A., Pietrafesa, R., Siesto, G., Romano, P. (2020) Biotechnological approach based on selected *Saccharomyces cerevisiae* starters for reducing the use of sulfur dioxide in wine. *Microorganisms, vol. 8* (5), 738

Carrau, F., Boido, E., Ramey, D. (2020) *Chapter Three* – Yeasts for low input winemaking: Microbial terroir and flavour differentiation. *Advanced in Applied Microbiology, vol. 111*, pp. 89-121

Dutraive, O., Benito, S., Fritsch, S., Beisert, B., Patz, C.D., Rauhut, D. (2019) Effect of sequential inoculation with non-*Saccharomyces* and *Saccharomyces* yeasts on Riesling wine chemical composition. *Fermentation, vol. 5* (3), 79

Englezos, V., Pollon, M., Rantsiou, K., Ortiz-Julien, A., Botto, R., Rio Segade, S., Giacosa, S., Rolle, L., Cocolin, L. (2019) *Saccharomyces cerevisiae-Starmerella bacillaris* strains interaction modulates chemical and volatile profile in red wine mixed fermentations. *Food Research International, vol. 122*, pp. 392-401

Englezos, V., Rantsiou, K., Cravero, F., Torchio, F., Pollon, M., Fracassetti, D., Ortiz-Julien, A., Gerbi, V., Rolle, L., Cocolin, L. (2018) Volatile profile of white wines fermented with sequential inoculation of *Starmerella bacillaris* and *Saccharomyces cerevisiae*. Food *Chemistry, vol. 257*, pp. 350-360

Fairchild, M.D. (2018) The colors of the wine. *International Journal of Wine Research, 10*, pp. 13-31

Jaywant, S.A., Singh, H., Arif, K.M. (2022) Sensors and instruments for Brix measurement: A review. *Sensors, vol. 22* (6), 2290

Magyar, I. & Toth, T. (2011) Comparative evaluation of some oenological properties in wine strains of *Candida stellata*, *Candida zemplinina*, *Saccharomyces uvarum* and *Saccharomyces cerevisiae. Food Microbiology, vol. 28*, pp. 94-100

Pastore, A., Badocco, D., Cappelin, L., Tubiana, M., Pastore, P. (2024) Real-time monitoring of the pH of white wine and beer with colorimetric sensor arrays (CSAs). *Food Chemistry, vol. 452*, 139513

Russo, P., Englezos, V., Capozzi, V., Pollon, M., Rio Segade, S., Rantsiou, K., Spano, G., Cocolin, L. (2020) Effect of mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* on management of malolactic. *Food Research International, vol. 134*, 109246

Sadoudi, M., Tourdot-Marechal, R., Rousseaux, S., Steyer, D., Gallardo-Chacon, J.J., Ballester, J., Vichi, S., Guerin-Schneider, R., Caixach, J., Alexandre, H. (2012) Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. *Food Microbiology, vol. 32*, pp. 243-253

Shen, Y., Bai, X., Zhou, X., Wang, J., Guo, N., Deng, Y (2022) Whole-genome analysis of *Starmerella bacillaris* CC-PT4 against MRSA, a non-*Saccharomyces* yeast isolated from grape. *Journal of Fungi, vol. 8* (12), 1255

Singleton, P. & Sainsbury, D. (2006) Dictionary of Microbiology and Molecular Biology, *Third Edition, Revised. John Wiley & Sons Ltd*.

Sipiczki, M. (2004) Species identification and comparative molecular and physiological analysis of *Candida zemplinina* and *Candida stellata*. *Journal of Basic Microbiology, vol. 44* (6), pp. 471-479

Vicente, J., Ruiz, J., Belda, I., Benito-Vazquez, I., Marquina, D., Calderon, F., Santos, A., Benito, S. (2020) The genus *Metschnikowia* in enology. *Microorganisms, vol. 8* (7), 1038

Vilela, A. (2019) Use of nonconventional yeasts for modulating wine acidity. *Fermentation, vol. 5* (1), 27

Wang, C., Mas, A., Esteve-Zarzoso, B. (2016) The interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. *Frontiers in Microbiology, vol. 7*, 502