THE STUDY OF THE FERMENTAL POTENTIAL OF SOME YEAST STRAINS ISOLATED FROM THE IASI VINEYARD

STUDIUL POTENȚIALULUI FERMENTATIV AL UNOR TULPINI DE LEVURI IZOLATE DIN AREALUL VITICOL AL PODGORIEI IAȘI

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Abstract. *The use of non-Saccharomyces / Saccharomyces cerevisiae starter cultures to reproduce the conditions of natural fermentation in a controlled manner, offers the possibility of improving the aroma profiles of wine and is the basis of strategies for the implementation of new winemaking technologies. In this context, the fermentation potential of the autochthonous strains Torulospora delbrueckii code 10 and Saccharomyces cerevisiae code 4.1.11, 4.3, 4.6 and 4.10 was studied, by inoculation in monoculture, mixed and sequential culture. The obtained results confirm the oenological characteristics of the selected autochthonous yeast strains, as possible basic sources for obtaining new starter cultures.*
Key words: yeasts, fermentation, monoculture, sequential, starter

Rezumat. *Utilizarea culturilor starter non Saccharomyces / Saccharomyces cerevisiae pentru a reproduce controlat condiţiile din fermentaţia naturală, oferă posibilitatea îmbunătăţirii profilelor de arome din vin și stă la baza strategiilor de implementare a unor noi tehnologii de vinificație. În acest context, s-a studiat potenţialul fermentativ al tulpinilor autohtone Torulospora delbrueckii cod 10 și Saccharomyces cerevisiae cod 4.1.11, 4.3, 4.6 și 4.10, prin inoculare în monocultură, cultură mixtă şi secvenţială. Rezultatele obținute confirmă caracteristicile oenologice ale tulpinilor de levuri autohtone selectate, ca surse posibile de bază pentru obținerea de noi culturi starter.*
Cuvinte cheie: levuri, fermentație, monocultură, secvențială, starter

INTRODUCTION

The use of *Saccharomyces cerevisiae* starter cultures at an industrial level has proven effective in controlling fermentation processes, thus avoiding the negative effects frequently encountered in naturally driven fermentations. The starter cultures, based on alcoholic *Saccharomyces cerevisiae* strains, trigger the fermentation processes in a short time, becoming dominant in the must, reduce the completion period and minimize the development of *non-Saccharomyces* weakly alcoholic yeast species with reduced fermentative power: *Candida*,

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Rodotorula, some *Hanseniospora* strains, *Pichia* etc. Over time it was found that this biotechnological practice with *Saccharomyces cerevisiae* starter monocultures led to wines that are less structurally complex and deficient in aroma compounds, compared to wines obtained in natural fermentation led by the biodiversity of yeasts present in the non-sterile must (Rainieri *et al*, 2000, Mannazzu *et al*., 2002, Rodriguez, 2010).

The diversification of both *non-Saccharomyces* and *Saccharomyces cerevisiae* starter cultures, through the isolation and selection of new strains from the natural must fermentations, based on wine grape varieties from different vineyards, will ensure new *non-Saccharomyces/Saccharomyces cerevisiae* combinations available for the purpose to simulate spontaneous alcoholic fermentations in new technologies in the wine industry (Benito *et al.*, 2018; Benito *et al.,* 2019; Dimitrios Kontagiannatos *et al.*, 2021). In this context, a number of 30 *Saccharomyces cerevisiae* strains and 11 *non-Saccharomyces* strains were screened according to their physiological characteristics (ethanol tolerance, SO_2, H_2S production) and oenological (extracellular enzyme activity: β-glucosidase, esterase, protease, lipase, polygalacturonase and pectinase). Thus, four *Saccharomyces cerevisiae* strains code 4.1.11, 4.3, 4.6 and 4.1 and one *non-Saccharomyces* strain, *Torulospora delbrueckii* code 10 (Nechita *et al.,* 2020), were selected, which met the criteria for starter cultures.

The aim pursued in the paper was to obtain information on the potential of the selected strains in fermentation processes, which will contribute to the foundation of new technologies in winemaking.

MATERIAL AND METHOD

The must used from the Fetească albă variety was obtained according to the grape processing operations for white wines, having a concentration of 181 g/L sugars, 6.7 g/L $C_4H_6O_6$ total acidity and pH 3.9. The must was sterilized by autoclaving. Selected autochthonous strains were tested in fermentation processes with inoculation in monoculture, mixed culture and sequential. In the experimental variant with monoculture inoculation (10^6 CFU /mL), four *Saccharomyces cerevisiae* strains 4.1.11, 4.3, 4.6 and 4.10 were used. In the mixed culture variants, *Torulospora delbrueckii* strain code 10 (10^5 CFU/mL) and each selected *Saccharomyces cerevisiae* strain (10^6 CFU/mL) were inoculated simultaneously at a ratio of 1/10. In the sequential culture fermentations, the above densities and ratio were kept, with the mention that the *Torulospora delbrueckii* 10 strain was inoculated first, and after 24 and 48 hours the *Saccharomyces cerevisia*e strains were inoculated. The fermentation processes were monitored dynamically and conducted at a temperature of 20 ± 1°C. The main physico-chemical parameters were determined in the obtained wines: alcohol (% vol); total acidity (g/L); unfermented sugars (g/L) according to OIV methods (2019); average values of sugars consumed / 24 hours (OIV, 2012). The total concentrations of polyphenols (PT), the polyphenolic indices (IPT) and the chromatic parameters were determined according to the methods of Singleton et. al., 1965 and Glories et. al., 1984.

RESULTS AND DISCUSSIONS

The dynamics of fermentations in the experimental variants is presented in figure 1. The fermentation processes in monoculture with yeast strains *Saccharomyces cerevisia*e 4.1.11, 4.3 and 4.10 were completed in 10 days, the average values of the fermentation speed being between 19.91 and 20.18 g/L sugars / 24 hours. Yeast strain 4.6 completed fermentation in 9 days with a fermentation rate of 22.15 g sugars / 24 hours. In the mixed cultures, the fermentation processes evolved similar to those in the monoculture, the fermentations being completed in 11 days, with average fermentation speeds between 18.10 and 18.20 g sugars / 24 hours. In the sequential cultures, the fermentations were completed in 13 days with average values of the fermentation rate between 13.23 and 15.36 g/L sugars/24 hours, regardless of the time of inoculation of the *S. cerevisiae* strains at 24 and 48 hours after inoculation of the *T. delbrueckii* strain.

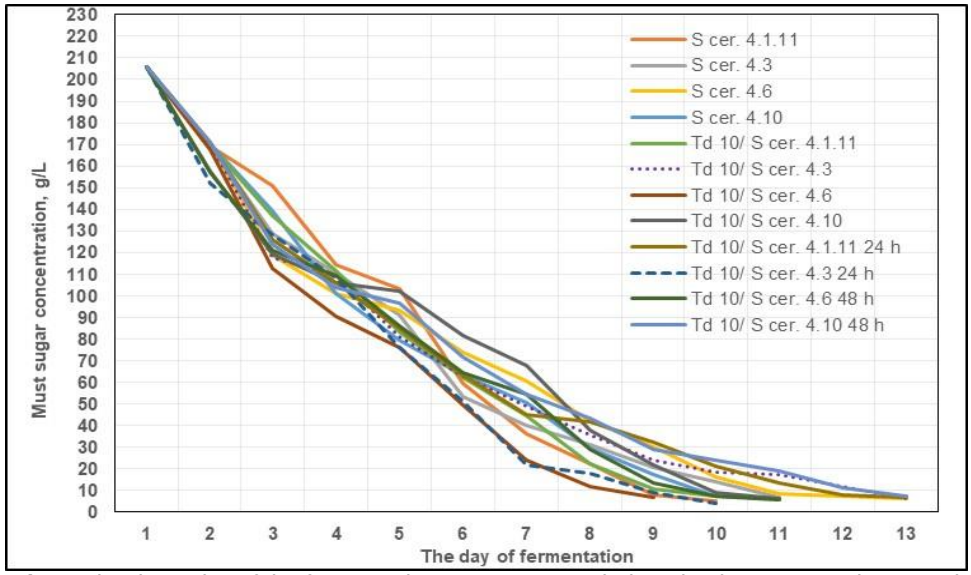

Fig. 1 The dynamics of the fermentation processes carried out by the yeast strains tested

From the analysis of the compositional characteristics of the obtained wines presented in table 1, it is noted that, regardless of the inoculation method and the selected yeast strains, the alcohol concentrations were between 10.30% vol and 10.80% vol, obtaining dry wines, which proves the alcohologenic character of the selected *S. cerevisiae* strains. Total acidity values were between 6.37 g/L and 7.42 g/L $C_6H_4O_4$, being considered normal for white wines. Differences were found in the case of volatile acidity. Thus, in wines obtained by conducting monoculture fermentations with yeast strains *S. cerevisiae* 4.6 and 4.10, lower values of 0.34 g/L and 0.37 g/L CH_3COOH were determined, compared to wines made by strains *S. cerevisiae* 4.1.11 and 4.3. In the wines from

the experimental variants in mixed culture, the values of volatile acidity were lower than in monoculture.

Table 1

The compositional characteristics of Fetească albă wines obtained in the fermentation processes in monoculture, mixed and sequential culture

	Alcohol concentration, % vol	Total acidity, g/L $C_6H_4O_4$	Volatile acidity, g/L CH_3COOH	Unfermented sugars, g/L
Monoculture				
S cer. 4.1.11	10,60 ± 0,09	6,41 ± 0,07	0,46 ± 0,16	0,60 ± 0,11
S cer. 4.3	10,45 ± 0,10	7,05 ± 0,10	0,48 ± 0,18	3,60 ± 0,14
S cer. 4.6	10,45 ± 0,08	7,05 ± 0,08	0,50 ± 0,12	2,00 ± 0,12
S cer. 4.10	10,80 ± 0,09	7,42 ± 0,11	0,37 ± 0,13	0,00 ± 0,02
Mixed culture				
Td 10/ S cer. 4.1.11	10,90 ± 0,12	6,37 ± 0,06	0,40 ± 0,15	1,00 ± 0,10
Td 10/ S cer. 4.3	10,45 ± 0,10	6,97 ± 0,11	0,38 ± 0,13	3,60 ± 0,11
Td 10/ S cer. 4.6	10,45 ± 0,10	6,97 ± 0,09	0,36 ± 0,18	2,00 ± 0,14
Td 10/ S cer. 4.10	10,80 ± 0,11	7,12 ± 0,10	0,28 ± 0,17	1,00 ± 0,10
Sequential culture				
Td 10/ S cer. 4.1.11 24 h	10,30 ± 0,09	7,14 ± 0,08	0,44 ± 0,16	1,68 ± 0,14
Td 10/ S cer. 4.3 24 h	10,40 ± 0,11	7,40 ± 0,12	0,46 ± 0,19	1,45 ± 0,11
Td 10/ S cer. 4.6 48 h	10,39 ± 0,10	7,16 ± 0,11	0,38 ± 0,15	1,30 ± 0,12
Td 10/ S cer. 4.10 48 h	10,38 ± 0,12	7,25 ± 0,11	0,32 ± 0,11	1,49 ± 0,10

The wines made by the association *Td. delbrueckii* 10/ *S. cerevisiae* 4.10 were noted both in the case of mixed and sequential fermentation, where the volatile acidity was 0.28 g/L, respectively 0.32 g/L CH_3COOH. The reduction in volatile acidity is due to the positive interrelationships between the two strains, *T. delbrueckii* being able to produce lower concentrations of acetic acid during fermentation, compared to *S. cerevisiae* strains (Renault *et. al.*, 2009).

The compositional profile of the wines was completed with information on the total concentration of polyphenols (PT mg GAE/L), the value of the total polyphenol index (IPT), as well as the chromatic characteristics: coloring intensity (IC), color hue (H) and participation (%) of yellow, red and blue pigments.

The phenolic and chromatic characteristics of the obtained wines are presented in table 2. From the analysis of the obtained data, it was found that the total concentration of polyphenols (PT) and the total index of polyphenols (IPT) were not influenced by the method of inoculation and the selected strains. Thus, the PT values (mg GAE/L) determined in the wines obtained in the experimental variants were close, being between 0.256 and 0.259 mg GAE/L. The same aspect was found in the case of the total index of polyphenols, the values obtained being between 5.30 and 5.40. The IPT values determined in the wines obtained in the experimental variants are within the limits mentioned in the literature for white wines, respectively between 3 and 15.

From the analysis of the chromatic parameters, it was noted that the color intensity values were influenced by the method of inoculation of the strains, being higher in the wines obtained in the mixed culture and lower in the wines from the

sequential variants. In the wines analyzed, yellow pigments predominate, a specific aspect of white wines. The highest values were determined in the wine obtained in the monoculture variant with strain 4.6 (60.57) and in the sequential variant *Td. delbrueckii* 10/ *S. cerevisiae*, 4.3 with inoculation at 24 hours (62.20).

Table 2

Phenolic and chromatic characteristics of wines obtained in fermentation processes in monoculture, mixed and sequential culture

Parameters	PT (mg GAE/L)	IPT	Coloring intensity (CI - 1 cm)	Tint of color	d 420% (yellow pigments)	d 520% (red pigments)	d 620% (blue pigments)
Monoculture							
S cer. 4.1.11	0.256	5.30	0.3171	2.1524	59.67	27.72	12.61
S cer. 4.3	0.258	5.35	0.4227	1.7383	51.86	29.83	18.31
S cer. 4.6	0.256	5.30	0.3320	2.1303	60.57	28.43	10.99
S cer. 4.10	0.259	5.50	0.4219	1.5204	45.84	30.15	24.01
Mixed culture							
Td 10/ S cer. 4.1.11	0.258	5.40	0.3820	1.6334	48.87	29.92	21.20
Td 10/ S cer. 4.3	0.258	5.40	0.4545	1.9398	53.91	27.79	18.31
Td 10/ S cer. 4.6	0.258	5.30	0.4070	1.6824	50.76	30.17	19.07
Td 10/ S cer. 4.10	0.259	5.40	0.4494	1.7276	51.94	30.06	18.00
Sequential culture							
Td 10/ S cer. 4.1.11 24 h	0.259	5.35	0.3290	1.8017	52.19	28.97	18.84
Td 10/ S cer. 4.3 24 h	0.256	5.40	0.3651	2.2986	62.20	27.06	10.74
Td 10/ S cer. 4.6 48 h	0.256	5.30	0.4728	1.7712	52.07	29.40	18.53
Td 10/ S cer. 4.10 48 h	0.258	5.40	0.3721	1.9724	57.62	29.21	13.17

From a sensory point of view, the wines resulting from the sequential fermentation stood out: *Td. delbrueckii* 10/ *S. cerevisiae*, 4.1.11, *Td. delbrueckii* 10/ *S. cerevisiae*, 4.3 and *Td. delbrueckii* 10/ *S. cerevisiae*, 4.10. They were rated as clear, bright, straw-yellow with medium intensity. The aromas were classified in the normal category, in a simple mixture, the overall intensity of the aromas being rated as average. Olfactory, a light smell of lemon, pear and quince was noted in the case of the wine obtained in the sequential fermentation with the strain *Td. delbrueckii* 10 / *S. cerevisiae* 4.1.11 and aromas of lemon, grapefruit and pineapple in the wines obtained with the strains *Td. delbrueckii* 10 / *S. cerevisiae* 4.3 , respectively *Td. delbrueckii* 10 / *S. cerevisiae* 4.10. The intensity of the basic flavor was balanced in the case of acidity and soft in the case of sweetness, without notes of bitterness and with a normal body for the type of wine. The light astringency given by a low tannic intensity makes these wines appreciated as balanced, correct, with medium aromatic persistence.

CONCLUSIONS

1. Fermentative processes, in the experimental variants carried out by inoculation in monoculture, highlighted the relevance of autochthonous Saccharomyces cerevisiae strains selected as possible sources for starter cultures. The compositional analysis of the obtained wines proved their alcoholic character, finally obtaining dry wines with a moderate volatile acidity.

2. In the experimental variants with *non Saccharomyces /Saccharomyces cerevisiae* mixed inoculation, dry wines were also obtained, in which the values of the main analyzed parameters were close compared to the wines obtained in monoculture.

3. In the mixed culture they stood out *Td. delbrueckii* 10/ *S. cerevisiae* 4.6, *T. delbrueckii* 10/ *S. cerevisiae* 4.10, and in sequential culture *Td. delbrueckii* 10/ *S. cerevisiae* 4.10, with inoculation at 48 hours. In both variants, a decrease in the value of volatile acidity was noted compared to the values determined in wines obtained in monoculture, as a result of the interrelationships between the two different strains of yeasts used.

4. The phenolic and chromatic composition of the wines obtained was not influenced by the method of inoculation, the values of the analyzed parameters being within the specific limits for white wines.

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