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Assessment of the technological performance of some *Saccharomyces* and *non-Saccharomyces* indigenous yeast strains

A. Nechita[1], R. Paşa[1], R.V. Filimon[1], F. Manolache[2], C.B. Nechita[3]*, R.M. Filimon[1], G. Zaldea[1] and D. Damian[1]

[1]Research and Development Station for Viticulture and Winemaking Iasi, Romania

[2]National Research and Development Institute for Food Bioresources – IBA Bucharest, Romania

[3]Research Center for Oenology of Romanian Academy - Iasi branch, Romania

*Corresponding author email: bnechita@gmail.com

ABSTRACT

The study aimed to assess the technological potential of four indigenous *Saccharomyces cerevisiae* strains (*S. cer*. 4.1.11, *S. cer.* 4.3, *S. cer*. 4.6 and *S. cer*. 4.10), as possible sources for starter cultures. The experiments were carried out at micropilot level on the natural must of 'Fetească albă' cultivar. The evaluation of the yeasts was carried out according to the chemical parameters and volatile compounds analysed in the obtained wines compared to the wine obtained with a commercial starter culture (CSC). The values of the main physico-chemical parameters analyzed in the obtained wines were similar to those determined in the control wine. The average values of the volatile compounds with positive impact on the wine aroma ranged within the interval 177.46 - 217.81 mg/L, a higher value compared to the control wine, respectively 166.33 mg/L. The use of the indigenous strain *Torulospora delbrueckii* (*T.d* 10) in association with the strains *S. cer*. 4.1.11 or *S. cer*. 4.10 led to an increase of 12.56%, respectively 8.30% in glycerol concentrations, as well as an increase of 11.94% to 14.49% in the average concentration of volatile compounds. Harnessing the oenological potential of the yeasts tested in sequential fermentations proved dependent on the time allowed for the development of the strain *T.d* 10, namely 24 and 48 hours, as well as on the yeasts used. Thus, in the wines obtained by the association *T.d* 10/ *S. cer*. 4.10, in which the development of the culture *T.d* 10 was carried out for 48 hours, we noticed an increase of 12.52% and, respectively 32.95%, in the average of volatile compounds, compared to the monoculture wine for the same *S. cer*. 4.10 strain and to the control wine (CSC).

Keywords*:* yeast, killer factor, volatile compounds, sequential fermentation, starter cultures.

INTRODUCTION

Due to the increase in competition and market demand for quality wines, research in the oenological field has focused on the isolation and technological characterization of the potential of both *Saccharomyces* and non-*Saccharomyces* indigenous yeasts. Extensive study on the properties of non-*Saccharomyces* yeasts aimed their testing in co-culture in the

fermentation process to obtain the desired wines (Comitini *et al*., 2011; Loira *et al*., 2014; Taillandier *et al.*, 2014; Dasko *et al.*, 2015; Padilla *et al.*, 2017; Arslan *et al*., 2018; Benito *et al*., 2018; Benito *et al*., 2019; Mecca *et al*., 2020). The analysis of the oenological characteristics of *non-Saccharomyces* yeast strains proved that some species, *e.g*. *Candida pulcherrima, Kloeckera apiculata, Torulospora delbrueckii*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima, Hanseniaspora guillermandi,* have the genetic ability to produce a more varied range of enzymes compared to the *Saccharomyces cerevisiae* strains, enzymes that can influence the quality of wines (Benito *et al.*, 2019).
In Romania, the first studies in the field of isolation and characterization of yeasts were carried out starting in 1915. The activities of isolation, characterization and selection of yeasts from famous vineyards in Romania, led to the foundation of yeast collections in various research centers, representing currently valuable germplasm sources (Brînduşe *et al*., 2022). In order to recreate the conditions of natural fermentation, the recent research focused on the selection and characterization of the indigenous non-*Saccharomyces* strains isolated from the musts obtained in vineyards located in different geographical areas. The use of pure non-*Saccharomyces/Saccharomyces cerevisiae* strains in co-culture is a technological practice, which leads to diversifying wine styles and defining varietal characteristics and regional typicity. Tristezza *et al*., (2013), Benito *et al.* (2018), Benito *et al.* (2019) and Kontagiannatos *et al*. (2021) emphasize the need for continued isolation and selection of non-*Saccharomyces* and *Saccharomyces cerevisiae* strains to ensure that new combinations of starter cultures are available for use in future winemaking technologies. In this context, 30 *Saccharomyces cerevisiae* strains and one non-*Saccharomyces* strain were isolated from fermenting grape must obtained in Iasi vineyard area and characterized from the physiological, oenological and technological point of view (Nechita *et al.,* 2020). The selected indigenous strains were tested in fermentation processes with inoculation in monoculture, mixed and sequential cultures on sterilized must. Following the physico-chemical analysis of the obtained wines, only certain *Saccharomyces cerevisiae* and non-*Saccharomyces* strains complied with the criteria for obtaining starter cultures. The present study aimed the evaluation of the technological performance of selected indigenous yeast strains, at micropilot level, in mixed and sequential inoculation, in natural (non-sterile) must of the 'Fetească albă' cultivar. Also, the killer factor of the indigenous yeast was determined.

MATERIALS AND METHODS

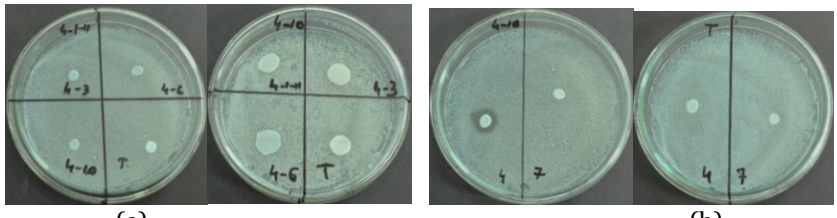
For conducting experimental studies were used four *S. cerevisiae* strains and one *T. delbrueckii* strain, selected from the Collection of microorganisms of the Research and Development Station for Viticulture and Winemaking Iasi (Romania), after testing in sterile must at laboratory level. The killer factor was assessed on YEPD-MB agar medium containing 3 mg methylene blue /100 mL medium, with pH 4.6. 10^5 CFU/mL suspensions of *Saccharomyces cerevisiae* and *Torulospora delbrueckii* strains were displayed on the surface of the agar medium, and the10^6 CFU/mL suspensions from each analyzed strain were inoculated on the Petri plates. The sensitivity of the tested strains to the killer factor was performed on the same YEPD-MB medium, in which the 10^5 CFU/mL suspensions of the tested strains were displayed on the plates and the 10^6 CFU/mL suspensions of the killer positive reference strains SP 39 (*S. cerevisiae* galactose; Sofralab, France) and Viniferm (*S. cerevisiae*; Agrovin, Spain), were inoculated in the center. The plates were incubated at 20°C for 48 hours. If no blue halos appear around the central yeast inoculum, it is assumed that they do not produce killer toxins. A blue halo around the inoculum of the reference strains, indicates sensitivity to the killer factor (Sangorrin *et al*., 2002). Must of the *Vitis vinifera* cultivar 'Fetească albă', used in the fermentation processes, was obtained according to the

industrial winemaking technology for white grapes, with a density of 1087 g/L, a sugar concentration of 206 g/L, a total acidity of 6.7 g/L $C_4H_4O_6$ and pH 3.46. In the experimental variant with 10^6 CFU/mL monoculture inoculation, four indigenous strains of *Saccharomyces cerevisiae* (*S. cer*) 4.1.11, 4.3, 4.6 and 4.10 were used, as well as a commercial starter culture *Saccharomyces cerevisiae* (CSC) used as a control (Zymaflore X5; Laffort, France). In the mixed culture, *Torulospora delbrueckii* (*T.d* 10) (10^5 CFU/mL) and each *Saccharomyces cerevisiae* strain (10^6 UFC /mL) were simultaneously inoculated, as a ratio of 1/10. In sequential fermentations, the above densities and ratio were preserved, with the mention that the *T.d* 10 strain was inoculated first, followed by the *Saccharomyces cerevisiae (S. cer.)* strains (after 24 or 48 hours). All fermentation processes were carried out at a temperature of 20±1°C. The wines obtained in the experimental variants were analyzed according to OIV methods (OIV, 2019). A Dujardin-Saleron DE2000 oenological distiller was used for ethanol (% vol.) and volatile acidity (g/L as acetic acid) analysis, while a pH-meter WTW INOLAB Level 1 was used for pH assay. The optical densities of the inoculums (OD 600 nm) were determined using an Analytik Jena Specord 200 plus UV-vis spectrophotometer. The aroma compounds of the wines were determined by GC-MS methods (OIV, 2019), on a gas-chromatograph with flame ionization detector GC-FID, Agilent 7890B. The concentrations of carbohydrates (glucose, fructose), acids (lactic, malic and acetic), polyols (2-3 butanediol and glycerol) and methyl alcohol were determined by the ^{1}H-NMR method (Bruker Avance III HD 600 spectrometer, operating 600.12 MHz for the ^{1}H nucleus; TopSpin 4.0.7 software). Data were reported as means of minimum three replicates, having specified the standard deviation (±).

RESULTS AND DISCUSSIONS

Determination of yeast killer factor

The selected indigenous strains *Saccharomyces cerevisiae* code 4.1.11, 4.3, 4.6 and 4.10 and the non-*Saccharomyces, Torulospora delbrueckii* code 10, were tested to reveal whether they produced killer toxins or were sensitive to this factor (Figure 1).



(a) (b)

Figure 1. Testing the presence of the killer factor (a) and the sensitivity to the killer toxin (b) of the yeast strains used for mixed and sequential fermentations **Note: 4.3, 4.10, 4.6, T. indicates the codes of the tested yeast strains; 4 and 7 indicates the codes for SP39 (Sofralab) and, respectively Viniferm (Agrovin) reference strains.*

According to the inoculation operations, between the strains, on the YEPD-MB agar medium, no blue halo was noticed around the centrally inoculated suspensions, proving that they do not produce killer toxins, as shown in Figure 1 (a). The sensitivity of the tested strains to killer toxins was determined on the same YEPD-MB medium; however, this time, after the inoculation of the suspensions of the investigated strains on the surface of the medium, the suspensions of the reference strains were inoculated in the center. In the latter case, a blue halo was noticed only in the case of the strain *Saccharomyces cerevisiae* 4.10 (Figure 1, b), showing that it is sensitive to the k_2 toxin from the reference culture code 4 (SP 39; Sofralab). The results obtained are consistent with the existing data in the literature, which

highlights the fact that some *Saccharomyces cerevisiae* strains are sensitive to the k_2 toxin, but not the non-*Saccharomyces* strains (Dabhole and Joishy, 2005).

Assessment of the fermentative potential of the S. cerevisiae indigenous strains in monoculture

The wines obtained in the fermentation processes in monoculture with the selected indigenous strains *S. cerevisiae* were analyzed by determining the physico-chemical parameters and the profile of some volatile compounds. The data obtained were interpreted in comparison to the values determined for the control wine (CSC) (Tables 1 and 2).

Table 1. Physico-chemical characteristics of wines obtained in monoculture

Parameter	CSC	*S. cer*. 4.1.11	*S. cer.* 4.3	*S. cer.* 4.6	*S. cer.* 4.10
Alcohol concentration, % vol	11.5 ± 0.08	12.0 ± 0.10	11.9 ± 0.09	11.7 ± 0.11	11.4 ± 0.06
Total acidity, g/L $C_6H_4O_4$	5.12 ± 0.10	5.04 ± 0.15	5.13 ± 0.09	5.17 ± 0.11	5.60 ± 0.10
Volatile acidity, g/L CH_3COOH	0.36 ± 0.04	0.30 ± 0.02	0.28 ± 0.04	0.28 ± 0.02	0.32 ± 0.01
pH	3.24 ± 0.01	3.23 ± 0.01	3.13 ± 0.02	3.18 ± 0.03	3.19 ± 0.02
Non-reducing extract, g/L	19.6 ± 0.4	19.4 ± 0.3	18.3 ± 0.2	18.3 ± 0.3	16.7 ± 0.2
Glucose, mg/L	538.2	730.8	514.8	315.0	351.0
Fructose, mg/L	nd	nd	1000.8	nd	462.8
Acetic acid, mg/L	300.6	295.2	210.6	219.0	277.8
Lactic acid, mg/L	2458.8	1845.0	3153.6	2220.3	2972.7
Glycerol, mg/L	12322.4	8844.8	13850.6	11814.6	12838.6
2,3 butanediol mg/L	616.5	448.0	495.9	497.7	530.1
Methanol, mg/L	32.64	30.08	27.84	19.52	23.68

**Values are mean ± SD, the bars indicate the standard deviations*

Table 2. Volatile compounds determined in white wines obtained from fermentation processes in monoculture with *Saccharomyces cerevisiae* indigenous yeast strains

Volatile compounds	CSC	*S. cer*. 4.1.11	*S. cer*. 4.3	*S. cer.* 4.6	*S. cer*. 4.10
Acetaldehyde, mg/L	10.15±0.06	5.29±0.03	7.85±0.05	3.71±0.10	4.69±0.04
Ethyl acetate, mg/L	39.55±0.10	36.98±0.12	27.54±0.09	27.87±0.05	28.83±0.03
1-Propanol, mg/L	14.96±0.05	14.00±0.02	18.12±0.04	13.84±0.06	13.65±0.04
2-Methyl-1-propanol, mg/L	32.27±0.07	27.64±0.14	31.75±0.02	28.85±0.12	30.09±0.03
Isoamyl acetate, mg/L	0.92±0.03	0.96±0.02	1.06±0.02	1.04±0.02	1.10±0.01
1-Butanol, mg/L	0.63±0.03	0.50±0.02	0.75±0.05	0.51±0.03	0.56±0.06
Ethyl lactate, mg/L	83.27±0.25	106.71±0.08	111.03±0.03	156.38±0.12	152.36±0.07

**Values are mean ± SD, the bars indicate the standard deviations*

Table 1 shows the chemical characteristics of wines obtained in monoculture. The results presented prove that all tested *S. cer.* strains lead to dry wines, similar to the control starter culture. Values close to those determined in the control wine were found in the case of alcohol concentration, total acidity, residual sugars and pH. Differences were noted in the case of volatile acidity, in the sense that in the wines obtained from indigenous *S. cer.* strains, the values were below the value of 0.36 g/L determined in the control wine. As regards the non-reducing extract, among the tested strains, *S. cer.* 4.1.11 achieved in wine the same value of the non-reducing extract as the control strain (CSC). The values obtained by the strains *S. cer.* 4.3. and *S. cer.* 4.6, were lower by 6.63%. Differences were also found in the case of glycerol and lactic acid concentrations. The values were higher compared to the CSC control wine by 4.02 and 11.03% in the case of glycerol in the wines obtained from strains 4.10 and 4.3, respectively by 22.10 % and 17.28% lactic acid in wines obtained with the same strains. Also, the methanol concentrations were lower compared to CSC (32.64 mg/L)

ranging between 19.52 mg/L and 30.08 mg/L. The assessment of the potential of the indigenous strains was also carried out according to the average concentrations of the volatile compounds with positive impact on the wine aroma (acetaldehyde, ethyl acetate, 2-methyl-1-propanol, isoamyl acetate, and ethyl lactate). The highest total average values were 217.0 mg/L and 218.81 mg/L in wines obtained from strains 4.6 and 4.10 (Table 2). In the case of wines obtained from strains 4.1.11 and 4.3, the average values were lower, respectively 177.46 mg/L and 179.14 mg/L, but they still exceeded the value obtained in the CSC control wine, respectively 166.33 mg/L. From the analysis of the data presented, it appears that the selected indigenous strains of *Saccharomyces cerevisiae* meet the criteria for use as possible sources for obtaining starter cultures.

Assessment of the fermentative potential of the indigenous strains in mixed cultures

The potential of indigenous strains *T.d 10* and *S. cer.* in mixed culture was assessed according to the values of the chemical parameters and the average of the volatile compounds with a positive impact on the wine aroma, compared to those determined in the wines obtained in the monoculture *S. cer.* and the starter culture CSC. The assessment of the different results obtained in the experimental variants can be explained by the interrelationships between the two strains associated in the fermentation processes. Thus, in the case of the increase in concentrations of aroma compounds, positive synergistic interrelationships are highlighted, passive interrelationships in the case of equal values, and negative interrelationships in the lower values, in comparison to those obtained in monoculture and CSC wines (Sadoudi *et al.*, 2013). Table 3 shows the chemical parameters of the wines obtained in mixed culture, in which the *Torulospora delbrueckii* (*T.d* 10) strain was associated with each selected *Saccharomyces cerevisiae (S. cer.)* strain. In this context, the fermentation processes were completed by obtaining dry wines, in which the parameters alcohol, total acidity, volatile acidity, and pH were comparable to those determined in wines obtained in monoculture with *S. cer.*, due to the simultaneous inoculation of both strains. However, a positive influence was found in wines obtained by associating *Td.* 10/ *S. cer.* 4.1.11 and *Td*. 10/ *S. cer*. 4.10, in which the glycerol concentrations increased by 12.56% and 8.30%, due to synergistic interrelations; this is a desired outcome, given the intensification of the sensation of vinosity, finesse and fullness of the wines.

Table 3. Physico-chemical characteristics of wines obtained in mixed culture

Parameters	*T.d* 10/ *S. cer.* 4.1.11	*T.d* 10/ *S. cer.* 4.3	*T.d* 10/ *S. cer.* 4.6	*T.d* 10/ *S. cer.* 4.10
Alcohol concentration, % vol	12.0 ± 0.11	11.8 ± 0.08	11.7 ± 0.09	11.4 ± 0.10
Total acidity, g/L $C_6H_4O_4$	5.60 ± 0.08	5.70 ± 0.11	5.07 ± 0.06	5.10 ± 0.10
Volatile acidity, g/L CH_3COOH	0.28 ± 0.09	0.28 ± 0.03	0.27 ± 0.05	0.28 ± 0.04
pH	3.21 ± 0.02	3.18 ± 0.03	3.22 ± 0.01	3.12 ± 0.02
Non-reducing extract, g/L	18.6 ± 0.4	19.0 ± 0.2	18.4 ± 0.3	16.3 ± 0.2
Glucose, mg/L	606.6	493.2	261.0	320.4
Fructose, mg/L	nd	nd	nd	1000.8
Acetic acid, mg/L	276.0	277.0	196.8	201.0
Lactic acid, mg/L	1921.5	2466.9	2007.0	3033.9
Glycerol, mg/L	10116.3	13850.6	10804.4	14001.4
2,3 butanediol mg/L	408.6	407.7	494.1	483.7
Methanol, mg/L	28.16	28.80	23.68	19.80

**Values are mean ± SD, the bars indicate the standard deviations*

Table 4 presents the profile of volatile compounds in the wines obtained from the mixed culture. The averages of volatile compounds with a positive impact on aroma were different, according to the association of *T.d* 10*/ S. cer*. strains, as follows: 201.54 mg/L (*T.d 10*/ S. *cer*.

4.1.11), 163.03 mg/L (*T.d* 10/ *S. cer*. 4.3), 190 .81 mg/L (*T.d* 10/ *S. cer*. 4.6) and 253.71 mg/L (*T.d* 10/ *S. cer.* 4.10). The average values of volatile compounds were higher by 11.94% and 14.49% in the wines obtained from the association of *T.d* 10/ *S. cer*. 4.1.11 and, respectively *T.d* 10/ *S. cer.* 4.10, compared to wines obtained in monoculture from the *S. cer.* 4.1.11 and *S. cer.* 4.10 strains. On the other hand, in the wines obtained by combining *T.d* 10/ *S. cer*. 4.3 and *T.d* 10/ *S. cer*. 4.6, the average values of volatile compounds decreased by 8.99% and 12.35%, compared to the values determined in the wines obtained in monoculture with *S. cer*. 4.3 and *S. cer*. 4.6, possibly due to negative interrelations; however, the values increased by 8.82% and 12.87% compared to the CSC control wine.

Table 4. Volatile compounds in white wines obtained from fermentation processes in mixed culture with indigenous strains of *Saccharomyces cerevisiae* (*S. cer*.) and *Torulospora delbrueckii* (*T.d* 10)

Volatile compounds	*T.d* 10/ *S. cer.* 4.1.11	*T.d* 10/ *S. cer*. 4.3	*T.d* 10/ *S. cer*. 4.6	*T.d* 10/ *S. cer*. 4.10
Acetaldehyde, mg/L	4.26±0.06	3.39±0.09	7.2±0.07	6.73±0.04
Ethyl acetate, mg/L	33.56±0.03	23.24±0.03	38.74±0.04	38.05±0.04
1-Propanol, mg/L	13.89±0.03	19.68±0.09	14.84±0.05	16.81±0.03
2-Methyl-1-propanol,mg/L	24.13±0.02	28.08±0.03	27.68±0.04	30.15±0.05
Isoamyl acetate, mg/L	0.99±0.01	1.03±0.01	1.11±0.01	0.9±0.01
1-Butanol, mg/L	0.52±0.05	0.73±0.05	0.60±0.00	0.70±0.08
Ethyl lactate, mg/L	139.32±0.74	108.42±0.88	116.01±0.80	178.08±0.03

**Values are mean ± SD, the bars indicate the standard deviations*

Assessment of the fermentative potential of the indigenous yeast strains in sequential cultures

The strategy to capitalize on the oenological potential of moderately alcoholic non-*Saccharomyces* yeasts, which generally produce most of the aroma compounds in the first days of the fermentation, has also been studied in sequential culture. From the analysis of the chemical parameters relevant differences were found in the case of volatile acidity, 0.26 g/L in the wines obtained from the *Td*. 10/ *S. cer*. 4.1.11 variant, with 24 hours inoculation, and *T.d* 10/*S. cer.* 4.10, with 48 hours inoculation, compared to the values of the volatile acidity determined in the wines obtained in monoculture (Table 5). The decrease in the volatile acidity values achieved in wines, through the association of the above strains, is attributed to the *T.d* 10 strain. During fermentation, this strain produces small concentrations of acetic acid that influence the volatile acidity (Bely *et al*., 2008; Benito *et al*., 2018).

Table 5. Physico-chemical characteristics of wines obtained in sequential culture

Parameters	*T.d* 10/*S. cer*. 4.1.11 (24 h)	*T.d* 10/*S. cer*. 4.3 (24 h)	*T.d* 10/*S. cer*. 4.6 (48 h)	*T.d* 10/*S. cer*. 4.10 (48 h)
Alcohol concentration, % vol	12.1 ± 0.06	12.1 ± 0.08	11.5 ± 0.12	12.1 ± 0.08
Total acidity, g/L $C_6H_4O_4$	5.05 ± 0.08	5.17 ± 0.12	5.85 ± 0.14	5.02 ± 0.04
Volatile acidity, g/L CH_3COOH	0.26 ± 0.02	0.29 ± 0.04	0.30 ± 0.04	0.26 ± 0.02
pH	3.32 ± 0.02	3.24 ± 0.04	3.16 ± 0.05	3.27 ± 0.01
Non-reducing extract, g/L	20.4 ± 0.3	19.6 ± 0.2	18.0 ± 0.2	19.6 ± 0.4
Glucose, mg/L	567.0	370.8	335.8	556.2
Fructose, mg/L	nd	nd	1000.8	nd
Acetic acid, mg/L	219.8	271.8	250.2	198.6
Lactic acid, mg/L	2164.5	2394.7	3276.9	2323.8
Glycerol, mg/L	10406.1	10442.0	15906.8	12520.2
2,3 butanediol mg/L	450.0	396.9	614.7	481.5
Methanol, mg/L	28.16	23.04	33.28	29.44

In the wines obtained by sequential fermentations, concentrations of glycerol determined varied depending on the association of *T.d* 10/*S. cer.* strains, but also on the time allowed to the singular evolution in the fermentation of the *T.d* 10 strain, aspect confirmed by the increased glycerol values, respectively 12520.2 mg/L and 15906.0 mg/L in the sequential variants with inoculation after 48 hours of *S. cer.* strains, compared to the values of 10406.0 mg/L and 10442 .0 mg/L, achieved in the case of inoculation of *S. cer*. after 24 hours. The volatile compounds analyzed in the wines obtained in the sequential variants were presented in Table 6. The influence of positive synergistic interrelationships was found in both sequential variants. In the wines obtained in the sequential variant *T.d* 10/*S. cer.* 4.1.11 and *T.d* 10/*S. cer*. 4.3, with inoculation at 24 hours and *T.d* 10/*S. cer*. 4.6 and *T.d* 10/*S. cer*. 4.10, with inoculation at 48 hours, the averages values of volatile compounds were, in order, 180.65 mg/L and 195.16 mg/L, respectively 211.93 mg/L and 249.09 mg/L. The increase in the mean value of the volatile compounds was determined mainly by the time allowed for the development of the *T.d* 10 strain. This aspect was evident in the case of *T.d* 10/*S. cer*. 4.1.11 and *T.d* 10/*S. cer.* 4.3 associations, in which the development and evolution of strain *T.d* 10, was carried out only for 24 hours; thus, the average values of volatile compounds increased by only 1.76 and 8.20% (Table 6).

Table 6. Volatile compounds determined in white wines obtained from sequential fermentations with indigenous strains *Saccharomyces cerevisiae* (*S. cer*.) and *Torulospora delbrueckii* (*T.d* 10)

Volatile compounds	*T.d* 10/ *S. cer*. 4.1.11 (24 h)	*T.d* 10/ *S. cer*. 4.3 (24 h)	*T.d* 10/ *S. cer*. 4.6 (48 h)	*T.d* 10/ *S. cer*. 4.10 (48 h)
Acetaldehyde, mg/L	4.22±0.08	5.02±0.08	5.25±0.03	4.66±0.04
Ethyl acetate, mg/L	31.26±0.03	32.06±0.13	27.69±0.05	23.35±0.14
1-Propanol, mg/L	15.37±0.04	20.73±0.06	14.73±0.04	15.53±0.06
2-Methyl-1-propanol, mg/L	22.98±0.07	32.97±0.02	32.24±0.02	27.18±0.03
Isoamyl acetate, mg/L	1.00±0.02	1.02±0.02	1.08±0.02	0.94±0.02
1-Butanol, mg/L	0.52±0.02	0.71±0.05	0.66±0.04	0.81±0.03
Ethyl lactate, mg/L	121.29±0.01	124.27±0.02	145.78±0.10	192.12±0.16

**Values are mean ± SD, the bars indicate the standard deviations*

In the case of the development of *T.d* 10 for 48 hours, in the wine obtained by the association of the *T.d* 10/*S. cer.* 4.10 strain, an increase of 12.52% and, respectively 32.95% in the average concentration of volatile compounds was determined, compared to the wines obtained in monoculture of *S. cer.* 4.10 strain and respectively to the CSC control wine.

CONCLUSIONS

The results of the present study indicated that the selected indigenous yeast strains possess appropriate oenological characteristics, as possible basic sources for obtaining new starter cultures for must fermentation. The wines obtained by monoculture inoculation of indigenous S*accharomyces cerevisiae* strains showed similar values of the main physico-chemical characteristics, compared to the control wine of commercial starter culture. Instead, the average values of the volatile compounds with positive impact on wine aroma were higher by up to 24%. The evaluation of the oenological potential of *Torulospora delbrueckii*/S*accharomyces cerevisiae* yeast association was carried out at micropilot level in non-sterile must. Although the same must inoculated with *T. delbrueckii* and *S. cerevisiae* strains was used in mixed and sequential cultures, the metabolic activity in the fermentation processes manifested differently, revealing both passive and synergistic, as well as negative interrelations. Thus, in the sequential cultures with inoculation of *S. cerevisiae* strains at 48 hours, the associations *T. delbrueckii* 10/*S. cerevisiae* 4.1.11 and *T. delbrueckii* 10/ *S.*

cerevisiae 4.10 were noted. Moreover, the positive synergistic interrelationships led to a decrease in volatile acidity and an increase in glycerol and aroma compound concentrations. Regarding the sequential fermentation, future studies should be directed towards obtaining new information on the technological potential of the selected indigenous yeasts, especially under the conditions of the use of non-sterile must, where the interrelationships between the pure non-S*accharomyces* and S*accharomyces* strains evolve in complex conditions.

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REFERENCES

1. Arslan E., Çelik Z.D. and Cabaroglu T. (2018). Effects of pure and mixed autochthonous *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on fermentation and volatile compounds of Narince wines. Foods 7(9): 147. doi: 10.3390/foods7090147.
2. Bely M., Stoeckle P., Masneuf-Pomarede I. and Dubourdieu D. (2008). Impact of mixed *Torulaspora delbrueckii*-*Saccharomyces cerevisiae* culture on high sugar fermentation. Int. J. Food Microbiol. 122: 312-320.
3. Benito Á., Calderón F. and Benito S. (2019). The influence of *non-Saccharomyces* species on wine fermentation quality parameters. Fermentation 5(3):54. https://doi.org/10.3390/fermentation5030054.
4. Benito S. (2018). The impact of *Torulaspora delbrueckii* yeast in winemaking. Appl. Microbiol. Biotechnol. 102(7): 3081-3094.
5. Brînduşe E., Nechita A., Ion M., Paşa R., Fîciu L. and Ciubucă A. (2022). Valorificarea biodiversităţii drojdiilor autohtone de vinificaţie în scopul obţinerii vinurilor cu tipicitate de areal viticol (Iaşi, Romania: PIM). pp. 19-27.
6. Comitini F., Gobbi M., Domizio P., Romani C., Lencioni L., Mannazzu I. and Ciani M. (2011). Selected non-*Saccharomyces wine yeasts* in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiol. 28: 873-882.
7. Dabhole M. and Joishy K. (2005). Production and effect of killer toxin by *Saccharomyces cerevisiae* and *Pichia kluyveri* on sensitive yeasts and fungal pathogens. Indian J. Biotechnol. 4: 290-292.
8. Dashko S., Zhou N., Tinta T., Sivilotti P., Lemut M.S., Trost K., Gamero A., Boekhou, T., Butinar L., Vrhovsek U. and Piskur J. (2015). Use of non-conventional yeast improves the wine aroma profile of Ribolla Gialla. J. Ind. Microbiol. Biotechnol. 42: 997-1010.
9. Kontogiannatos D., Troianou V., Dimopoulou M., Hatzopoulos P. and Kotseridis, Y. (2021). Oenological potential of autochthonous *Saccharomyces cerevisiae* yeast strains from the greek varieties of Agiorgitiko and Moschofilero. Beverages 7 (2): 27. Doi: 10.3390/beverages7020027.
10. Loira I., Vejarano R., Bañuelos M.A., Morata A., Tesfaye W., Uthurry C., Villa, A., Cintora I. and Suárez-Lepe J.A. (2014). Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality. LWT Food Sci. Technol. 59: 915-922.
11. Mecca D., Benito S., Beisert B., Brezina S., Fritsch S., Semmler H. and Rauhut D. (2020). Influence of nutrient supplementation on *Torulaspora delbrueckii* wine fermentation aroma. Fermentation 6 (1): 35. https://doi.org/10.3390/fermentation6010035.
12. Nechita A., Filimon V.R., Paşa, R., Damian D., Zaldea G., Filimon R. and Zaiţ M. (2020). Oenological characterization of some yeast strains isolated from the Iaşi vineyard Romania. Romanian J. Hort.1: 14.148.
13. OIV (2019). Compendium of international methods of wine and must analysis. Vol. I. International Organisation of Vine and Wine, Paris, France.
14. Padilla B., Zulian, L., Ferreres, À., Pastor, R., Esteve-Zarzoso, B., Beltran, G., and Mas, A. (2017). Sequential inoculation of native *non-Saccharomyces* and *Saccharomyces cerevisiae* strains for wine making. Front. Microbiol. 8: 1293. doi: 10.3389/fmicb.2017.01293.
15. Sadoudi M., Tourdot-Maréchal R., Rousseaux S., Steyer D., Gallardo-Chacón J.J., Ballester J., Vichie S., Guérin-Schneider R., Caixache J. and Alexandre H. (2012). Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-S*accharomyce*s and *Saccharomyces yeasts.* Food Microbiol. 32 (2): 243-253.
16. Sangorrín M., Zajonskovsky I., van Broock M. and Caballero A. (2002). The use of killer biotyping in an ecological survey of yeast in an old Patagonian winery. World J. Microbiol. Biotechnol. 18: 115-120.
17. Taillandier P., Lai Q.P., Julien-Ortiz A. and Brandam C. (2014). Interactions between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in wine fermentation: influence of inoculation and nitrogen content. World. J. Microbiol. Biotechnol. 30 (7): 1959-1967.