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EFFECT OF MIXED CULTURE WITH *TORULASPORA DELBRUECKII* AND *SACCHAROMYCES CEREVISIAE* ON PHYSICO-CHEMICAL AND SENSORY CHARACTERISTICS OF YOUNG WINES

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Abstract

The present study aimed to evaluate the effect of pure and mixed culture fermentations with autochtonous yeast strains Torulaspora delbrueckii and Saccharomyces cerevisiae on physico-chemical and sensory qualities of Fetească albă and Fetească neagră young wines, at pilot scale. Yeast strains were isolated during different stages of spontaneous alcoholic fermentation and selected for their potential role in winemaking: 26 strains, 17 Saccharomyces cerevisiae strains and 9 non-Saccharomyces strains, from genera Candida, Torulaspora and Debaryomyces, were evaluated in terms of extracellular enzymatic activity, fermentative and technological characteristics. From these, two Saccharomyces cerevisiae strains were selected for esterase activity, and two non-Saccharomyces strains, Torulaspora delbrueckii species were selected for ß-glucosidase and esterase activity. Different yeast strains influenced the physico-chemical characteristics of the wines. The sensory qualities of the young wines produced with mixed cultures were positively influenced.

***Key words**: mixed fermentation, wine yeasts, Torulaspora delbrueckii, Saccharomyces.*

INTRODUCTION

In the winemaking, commercial yeast strains of *Saccharomyces cerevisiae* are currently used in the form of active dry yeasts, which, if they are properly selected, ensure a fast fermentation with reduced risks.

These yeast strains have been selected from certain vine-growing areas of the world based on previously established criteria and there is currently a very wide offer of such products that are used to produce certain types of wines.

However, it was found that the wines produced with *S. cerevisiae* monocultures have shown a uniformity of the characters and a decrease in their aromatic complexity (Lambrechts et Pretorius, 2000; Rodriguez, 2010; Romano et al., 2003), while the natural fermentation of the musts leads to the production of wines of higher quality, with improved sensory properties.

Thus, in order to reproduce the conditions which characterized natural fermentations, numerous researches have been focused on the isolation and obtaining of pure culture of yeasts from non-*Saccharomyces* species.

Their screening according to oenological and technological properties highlighted the fact that not all strains from non-*Saccharomyces* species produce secondary metabolited with harmfull effects during fermentation (acetic acid, acetaldehyde, acetoin, ethyl acetate) and some of them produce and secrete several enzymes (esterases, glycosidases, lipases, b-glucosidases, proteases, cellulases, etc.) that could have a positive influence on the characteristics of the wine, mainly on the varietal aroma (Ciani et al., 2006; Gaensly et al., 2015; López et al., 2015; Strauss et al, 2001).

In recent years, new fermentation technologies based on the co-inoculation of non-*Saccharomyces* and *S. cerevisiae* yeast strains, or their sequential inoculation at different times were developed in order to improve the wines quality and complexity (Comitini et al., 2011; Contreras et al., 2014; González-Royo et al., 2015).

Torulospora delbrueckii strains have been frequently used in the last decade in the fermentation processes with sequential inoculation. Numerous studies put into evidence the ability of these strains to increase the aromatic complexity of wines (Agarbati et al., 2020;), to reduce the SO_2 content (Agarbati et al., 2020; Gonzalez-Royo et al., 2014), to increase the content in glycerol (Gonzalez-Royo et.al., 2014).

In Romania, until now, there has been a real interest and results in the isolation and oenological characterization only of yeast strains belonging to the genus *Saccharomyces*, very few works regarding the screening of non-*Saccharomyces* species, as well as the use of mixed cultures in the winemaking were reported (Brîndușe et al, 2020; Marian I. et al., .2022; Nechita, A. et al., 2020).

The present study aimed to evaluate the effect of mixed culture fermentations with autochtonous yeast strains *T. delbrueckii* and *S.cerevisiae* on physico-chemical and sensory qualities of Fetească albă and Fetească neagră young wines, at pilot scale.

MATERIALS AND METHODS

Twenty-six yeast strains belonging to *Saccharomyces*, *Candida* and *Debaryomyces* genus were screened for the production of extracellular b-glucosidase, esterase, pectinase and protease activity. From these, two *S.cerevisiae* strains, respectively 56 and 76, were selected for esterase activity, and two non-*Saccharomyces* strains*, Torulaspora rosei* species were selected for ß-glucosidase (strain 75) and esterase activity (strain 47) (Ion Marian et al., 2022). These strains were used in mixed culture fermentations using as raw material grapes from Fetească albă and Fetească neagră autochtonous varieties as fellow:

- *T. delbrueckii*, strain 75 + *S.cerevisiae*, strain 52 in case of Fetească albă variety;
- *T. delbrueckii,* strain 47 + *S. cerevisiae*, strain 76 for Fetească neagră variety.

The grapes were harvested at their physiological and technological maturity, when the must obtained from grapes of Fetească albă had a concentration in sugars of 225.6 g/L, a total acidity of 3.82 g/L tartaric acid, pH 3.9 and the must obtained from grapes of Fetească neagră showed a concentration in sugars of 235.6 g/L, a total acidity of 4.70 g/L tartaric acid pH 4.5.

Fermentations were carried out according to the following variants:

- monoculture with *S. cerevisiae* strain (10^6);
- co-inoculation of non-*Saccharomyces* strain (10^5) and a *S.cerevisiae* strain (10^6), simultaneous inoculation;
- sequential culture with inoculation of non-*Saccharomyces* strain (10^5) and a *S. cerevisiae* strain at an interval of 24 hours (10^6).

S. cerevisiae commercial strains (10^6) were inoculated as control.

Each experimental variant was represented by 10 kg of grapes. All fermentations were carried out in triplicate. The monitoring of the fermentations was carried out by daily determination of the following parameters: temperature (°C), density (g/L) and concentration in sugar of the must (g/L).

Density of the must was determined according to OIV method (OIV-MA-AS2-01B). The sugar concentration was determined by using a hand held digital refractometer and the results were expressed as an absolute value (OIV-MA-AS2-02 method). The completion of the fermentation process was established when the density value was 1000 g/L.

The young wines were analysed physico-chemically 6 months after grapes processing. The base parameters were determined according to OIV methods (Compendium of international methods of analysis, OIV 2022) are: alcoholic strength (OIV-MA-AS313-01 A), total acidity (OIV-MA-AS313-01), volatile acidity (OIV-MA-AS313-02), reducing sugar (OIV-MA-AS311-01A), total dry extract and unreducing extract (OIV-MA-AS2-03A).

The red wines were also analysed from phenolic profiles and color properties point of view.

The total phenolic content of the red wines was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The results were expressed in gallic acid equivalents, using gallic acid standard curve (0 - 0.1 mg mL^{-1}).

The anthocyanins determination was based on their transformation, under the action of bisulfite ion, into colourless derivatives (Ribéreau-Gayon and Stonestreet, 1965).

Condensed tannins were determined by the vanillin assay, with the absorbance measured at 500 nm (Swain and Hillis, 1959).

The determination of catechins (flavan 3-ols) is based on the reaction of the phloroglucinol ring with vanillin that produces a red colour with a maximum absorption at 500 nm (Pompei and Peri, 1971).

Phenolic acids were assessed according to Somers et al. (Somers, 1991) and is expressed as caffeic acid equivalents.

Chromatic parameters were determined according to the UV/Vis spectrophotometry method proposed by Glories (1984).

The sensory evaluation of wines was achieved by tasting organoleptic analysis, each sensory characteristics being appreciated by points at 1 to 5. Data processing was achieved by Dunnett t-tests, which treats one group as a control and compare all other groups against it, with a significance level of 0.05 or 0.01.

RESULTS AND DISCUSSIONS

The dynamic of the fermentation processes

For the Fetească albă variety, the fermentation process lasted for 9-14 days, with a must mass temperature of 18-21°C (Figure 1).

The two strains of *S. cerevisiae* used as monoculture treatments, represented by *S.cerevisiae* - commercial yeast, used as a control variant, and *S.cerevisiae* - strain 52, started the fermentation process in the first 24 hours. The fermentation lasted 11 days for the control variant and 9 days in the fermentation conditions with the strain 52.

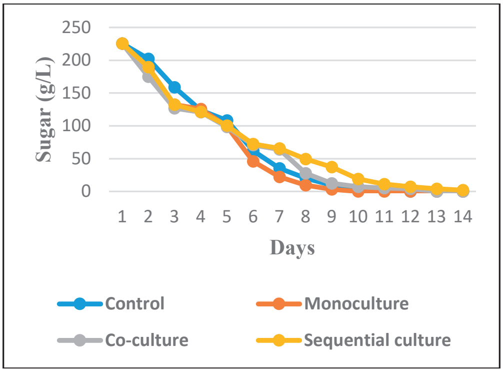

Figure 1. The sugar metabolisation curve during Fetească albă fermentation

The fermentation speed was around 20.16 g/L sugars/24 hours in the control and 24.04 g/L sugars/24 hours under fermentation conditions with strain 52. For the co-culture and sequential culture conditions, the fermentation had a similar behaviour in the first 6-7 days, after which, the fermentation speed decreased constantly until the end of the process, which lasted totally 12-14 days. Fermentation rate averaged 18.47 g/L sugars/24 h in the co-culture variant and 15.97 g/L sugars/24 h in the sequential culture variant.

Regarding the Fetească neagră variety, the fermentation was finished after around 10 - 12 days, the temperature of the must mass being around 17-21°C (Figure 2). The process lasted 11 days in the control and 10 days in the fermentation conditions with the *S. cerevisiae* - strain 76. The fermentation rate was on average 21.05 g/L sugars/24 hours in the control variant and 23.38 g/L sugars/24 hours under fermentation conditions with strain 76. Under co-culture and sequential culture conditions, the fermentation lasted 12 days with a speed of around 19.18 g/L sugars/24 h, respectively 19.40 g/L sugars/24 h.

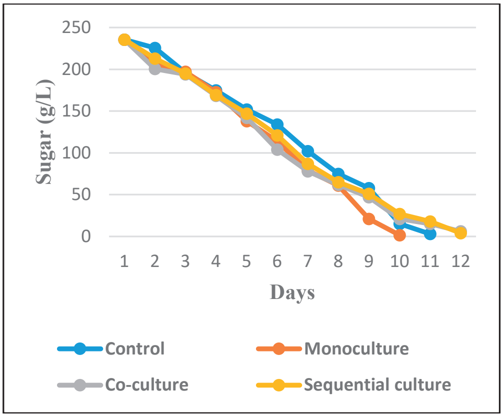

Figure 2. The sugar metabolisation curve during Fetească neagră fermentation

The physicochemical parameters of wines, depending on the different starter cultures used in fermentations are presented in Table 1.

Table 1. Physicochemical composition of wines

Grapevine variety	Parameters	Variants			
		Control	Monoculture	Co-culture	Sequential culture
Feteasca Alba	Alcohol content (% vol)	12.3±0.04	13±0.02**	12.3±0.03	12.5±0.06
	Sugar (g/L)	3.8±0.01	3.4±0.02	4±0.01**	2±0.03
	Total acidity (g/L tartaric ac.)	3.68±0.03	4.58±0.04**	5±0.02**	4.8±0.02**
	Volatile acidity (g/L CH_3COOH)	0.48±0.02	0.6±0.00	0.26±0.01**	0.58±0.02
	Total dry extract (g/L)	26.1±0.05	29.0±0.01**	24.8±0.01	24.8±0.04
	Unreduced extract (g/L)	21.29±0.03	24.9±0.01	20.8±0.00*	22.8±0.03
Feteasca Neagra	Alcohol content (% vol)	13.7±0.02	14±0.04	13.9±0.00	14±0.00
	Sugar (g/L)	3±0.03	1.62±0.02	6.06±0.04**	4±0.00**
	Total acidity (g/L tartaric ac.)	4.28±0.03	4±0.00	4.36±0.01	4.43±0.02**
	Volatile acidity (g/L CH_3COOH)	0.51±0.01	0.5±0.02	0.28±0.02**	0.46±0.04
	Total dry extract (g/L)	26.8±0.04	25.3±0.07	27.5±0.01**	26.3±0.05
	Unreduced extract (g/L)	23.8±0.01	23.7±0.03	19.44±0.01*	21.68±0.00

*The mean difference is significant at the P= 0.05 level;
**The mean difference is significant at the level P > 0.001 according to Dunnett t-tests.; [a] C.y. – commercial yeast

All fermentations with mixed inoculum of non-*Saccharomyces* and *Saccharomyces* (co-culture

and sequential culture) were completed with less than 4g/L residual sugars except Fetească neagră co-culture variant with a higher concentration of residual sugar, respectively 6.06 g/L, the wines obtained being semi dry.
The titratable acidity of white wine samples was higher in co-culture, sequential culture and monoculture variants compared with the control. For the red wine samples, all treatments produced similar titratable acidity values, which ranged between 4 g/L in monoculture variant and 4.43 g/L in the co-culture variant. The volatile acid content is very important for the wines health and is recommended to register a low value, best result being obtained after the co-culture treatment in both white and red wines.
The values of total dry extract were higher for Fetească alba wines fermented with authtonous *S. cerevisiae*, strain 52 and for Fetească neagră wines fermented in co-culture with *T.delbrueckii,* strain 47 and *S. cerevisiae*, strain 76.
The lowest values of non-reducing extract were registered for both types of wine in co-culture fermentation.
Concerning Fetească neagră wines, the achieved results indicated significant differences in the level of phenolic compounds between control and wines produced with *T.delbrueckii,* strain 47 + *S. cerevisiae*, strain 76 in different variants.
The influence of yeast strains combination on the anthocyanins profile was noted especially in case of co-culture variant, which significantly registered the highest value. In monoculture and sequential culture, values of anthocyanins closed to the control were obtained.
A higher content in phenolic acid, statistically assured compared to the control were observed in all fermentations (Table 2).
The most intense color was shown by Fetească neagră co-culture variant which registered also the highest content in anthocyanins. Lower intensity closed to the control was observed in monoculture variant.
The lowest tonality was observed in sequential culture. Similar values were recorded in the case of the other variants (Table 3).

Table 2. Polyphenolic compounds of Fetească neagră wines

Parameters	Variants			
	Control	Monoculture	Co-culture	Sequential culture
Catechins (mg/L)	0.21±0.1	0.23±0.00	0.21±0.00	0.21±0.03
Total polyphenols (mg/ GAE/L)	4603±2	4689±0**	4634±1**	4719±2**
Anthocyanins (mg/L)	345±1.73	345±3	392±2**	344±2
Tannins (g/L)	1.82±0.01	2.11±0.01**	1.9±0.03**	2.24±0**
Catechins (mg/L)	1.16±0	1.3±0.03**	1.18±0.02	1.39±0.02**
Phenolic acid (mg. caffeic acid/L)	422±1	468±0**	482±1**	484±0**

*The mean difference is significant at the P= 0.05 level.
**The mean difference is significant at the level P > 0.001 according to Dunnett t-tests.

Table 3. Chromatic properties of Fetească neagră wines

Parameters	Variants			
	Control	Monoculture	Co-culture	Sequential culture
Colour intensity	7.12±0.01	7.14±01	7.82±0.02	7.33±0.01
Hue	0.87±01	0.87±0.02	0.82±01	0.72±0.01
d 420% (% yellow pigments)	40.73±0.04	40.62±0.01	38.87±01	36.97±0.01
d 520 % (% red pigments)	46.35±0.03	46.50±0.01	47.31±0.02	51.16±02
d 620 % (% blue pigments)	12.92±0.02	12.89±01	13.81±0.01	11.87±0.04
dA%	42.12±0.02	42.47±01	44.32±0.01	52.27±0.04

Sensory analysis of wines

As expected in young wines, the fruity-flowery aroma have prevailed.
From the sensory point of view, among the Feteasca alba wines, the wine fermented with *T. delbrueckii*, strain 75 and *S. cerevisiae*, strain 52 applied in sequential culture stood out.
The wine was appreciated as being clear, straw-yellow in color, with great intensity. The flavors were classified in the normal category, in a complex mixture, the overall intensity of the flavors being rated as average. Floral (vine flowers, wildflowers), citrus (lemon, grapefruit) and honey (propolis) flavors predominate. The wine is dry, acidic, with a faint saltiness, and a little bitter. The alcoholic strength was assessed as normal. The light astringency given by a low tannic intensity makes these wines appreciated as balanced, correct, with medium aromatic persistence (Figure 3).

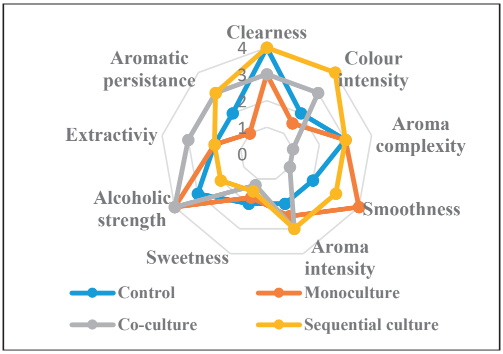

Figure 3. Sensory characteristics for Fetească albă wines

Among the Feteasca neagra wines, the wine obtained through co-culture (*T. delbrueckii*, strain 47 and *S. cerevisiae*, strain 76), stands out due to the fine flavor of black currants and pepper.

The wine was appreciated as being clear, ruby in color, with medium coloring intensity. The flavors are complex, delicate, with medium intensity. The wine is balanced, soft sweet, and with a weak salty sensation, without notes of bitterness. The alcoholic strength and extractivity were rated as normal. The wine presents a light astringency, round tannins with a normal intensity, and a correct balance. The aromatic persistence is long (Figure 4).

The enhance of aromatic intensity and complexity of Soave, Chardonnay and Vino Santo wines produced by multi-starter fermentation of *T. delbrueckii* strains and *Saccharomyces* yeasts in comparison with monoculture fermentation was also reported by Azzolini et al. (2015). Multi-starter fermentation greatly affected the content of several important volatile compounds, including 2-phenylethanol, isoamyl acetate, fatty acid esters, C_4-C_{10} fatty acids and vinylphenols which improved and enhanced wines' flavor.

The capacity of *T. delbrueckii* in sequential fermentations with *S. cerevisiae* to improve the aromatic complexity of wines by increasing their fruity flavour, while keeping spoilage attributes (volatile acidity, ethyl acetate and acetaldehyde) at suitable levels was reported by Loira et al. (2014).

According to Nechita et al. (2022) *T. delbrueckii/S. cerevisiae* yeast association at micropilot level led to a decrease in volatile acidity and an increase in glycerol and aroma compound concentrations.

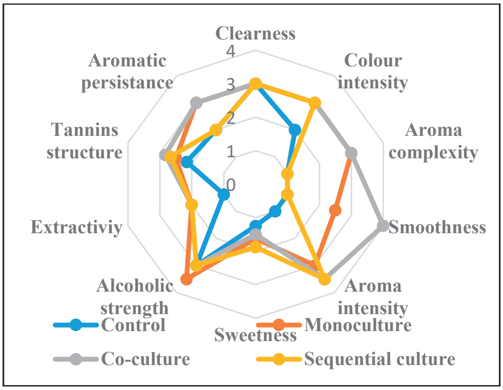

Figure 4. Sensory characteristics for Fetească neagră wines

CONCLUSIONS

The results presented in this study highlight the potential of autochthonous *Saccharomyces cerevisiae* strains in mixed culture with *Torulaspora delbrueckii* strains to enhance wines quality due to their impact on physico-chemical parameters and, especially, on the sensory properties of the wines. All fermentations with mixed inoculum of non-*Saccharomyces* and *Saccharomyces* (co-culture and sequential culture) were completed with less than 4 g/L residual sugars except Fetească neagră co-culture variant with a higher concentration of residual sugar, respectively 6.06 g/L, the wines obtained being semi dry. Significant differences between variants were recorded concerning some physico-chemical parameters and the polyphenolic compounds. Sensory analysis of wines pointed out the ability of *T. delbrueckii* in mixed culture with *S. cerevisiae* to improve the aromatic complexity of wines by increasing their fruity-flowery flavour. Further research is necessary to optimize the potential of this strains in wine industry.

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