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Assessment of the content in volatile substances in dry white and red wines obtained with different yeast strains from the "Trifeshti" wine center

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ABSTRACT

***Saccharomyces cerevisiae* is the predominant yeast species in spontaneous wine fermentations and thus it is the main responsible for the chemical and sensory properties of wines. The use of autochthonous yeast strains, besides assuring the maintenance of the typical sensory properties of the wines of any given region, can contribute to promoting or retaining the natural *S. cerevisiae* biodiversity. This study aims to determine specific enological characteristics of local yeasts and their potential in the production of volatile compounds and their influence on wine quality. The influence of several local yeasts strains on the chemical properties, the aromatic compounds, and finally, the sensory analysis of the white wine cv. 'Aligote' and the red wine cv. 'Cabernet Sauvignon' was analyzed obtained from the grapes production of 2018. The obtained results showed a positive influence of local yeasts on the chemical and sensory properties of dry red and white wines. They also contributed to the formation of a very complex flavor, being able to be used to produce "Trifeshti" wine with a unique and desirable aroma, with distinctive characteristics of the grape growing area.**

Keywords: *Saccharomyces cerevisiae,* local yeasts, aroma, volatile compounds, fermentation, sensory evaluation.

INTRODUCTION

One of the most important features of wines is their aroma, consisting of the content of a large number of volatile chemical components, with variable ratios. Wine aroma is a very complex concept since it is provided by a hundred different compounds with concentrations varying between 10−1 and 10−10 g/kg. Of course, the balance and interaction of all the present compounds (volatile or not) determine the wine's aromatic quality (López *et. al,* 2002; Perestrelo *et. al.,* 2019)
Wine is an alcoholic beverage made with the fermented juice of grapes. During the fermentation process, sugar transforms in ethyl alcohol due to a long series of biochemical reactions. Yeasts are the ones responsible for this process and the specific character of these reactions, is determined by a series of fermentative systems. Furthermore, the yeasts directly determine the course and direction of biochemical processes. From this point of view, the by-products, which play a major role in the wine flavor formation, are as important as the ethyl alcohol, i.e. the main fermentation product (Castino, 1988; Carpov *et.al.,* 1982; Rodopulo and Egorov, 1981; Rodopulo, 1987; Rodopulo, 1990). Wine flavor is due to volatile substances, perceptible by the human olfactory mucosa and various precursors, among

which the most important are glucosides, which in the process of preparing wine release volatile and odorous compounds, which participate in the formation of aroma (Gamero *et al.,* 2015; Carrau *et al.,* 2005; Carpov *et.al.,* 1982; Rodopulo and Egorov, 1981; Rodopulo, 1987; Rodopulo, 1990).

Under the influence of yeast, higher alcohols and volatile acids of the aliphatic chain are formed, which as a result of the action of esterase's form ethers composed of specific fruit aromas. The transition of amino acids to higher alcohols results not only in oxidative deamination, but also in the superannuation of amino acids with keto acids. Following the assimilation of amino acids by yeasts, a whole series of compounds are formed (Benavent, 1987).

MATERIALS AND METHODS

The research was carried out in the Laboratory of Biotechnology and Wine Microbiology at the Scientific-Practical Institute of Horticulture and Food Technologies (Republic of Moldova). The grapes were harvested at suitable technological maturity and processed according to the classical technology for white and red wine production in the conditions of micro-vinification. In the present research paper, local yeast strains (STr-1, S75Tr-2, S75Tr-4.4, ATr-2, ATr-2.3, MTr-4, M100Tr-1, M100Tr-4, C-S60Tr-2, C-S60Tr-4), isolated in the Trifeshti wine center were studied. The studies performed on the morphological, cultural, and physiological-biochemical properties, carried out with the use of Kudreavţeva identifier (Burian, 2003), established that the identified yeast strains belong to the *Saccharomyces cerevisiae* species. Active dry industrial yeast – Oenoferm freddo was used as a control for 'Aligote' white wine and Oenoferm Be-Red (Germany) – was a control for 'Cabernet Sauvignon' red wine.

Chemical analysis. The total sugars content (g/L) in musts was determined according to SM GOST 13192-73 (by the aerometric method). The total acidity of the wine was determined according to SM GOST 51621:2008 (by titration with bromthymol blue as an indicator). All determinations of the analyzed indicators were made in three repetitions. The gas chromatography technique was used to analyze wine aroma compounds.

Sensory analysis. The wines produced from grapes of the 2018 harvest season were subjected to sensory evaluation at the Scientific-Practical Institute of Horticulture and Food Technologies. The sensory profile of the analyzed aromas given by the use of different strains of local yeasts was determined by a paired sample test for both wines.

Statistical analyses. Differences in the physicochemical parameters between the musts and wines were assessed with a one-way analysis of variance (ANOVA) using the program GraphPad Prism 5.0 and an on-line calculator http://math.semestr.ru/.

RESULTS AND DISCUSSIONS

Table 1 Chemical composition of grapes from 'Aligote' and 'Cabernet-Sauvignon' cvs. (Harvest, 2018)

Grape cultivars	Sugar, g/L	Total acidity, $C_4H_6O_6$ g/L.	pH
'Aligote'	218,0±0,5	7,3±0,1	3,09±0,01
'Cabernet-Sauvignon'	255,0±0,5	5,9±0,1	3,22±0,01

Comparative analysis of the aromatic content of 'Aligote' dry white wine and 'Cabernet-Sauvignon' dry red wine (h.y. 2018) was performed by classical technology using different

yeast strains, which contributed to the identification of significant differences. The results obtained are shown in Tables 2 and 3.
The results illustrated in Tables 2 and 3 suggest that different strains of yeasts have an impact on the final content of volatile compounds in obtained white and red wines.

Table 2 Volatile substances content (mg/L) in dry white wines 'Aligote (h.y. 2018)

Compounds	Strain yeast					
	STr-1	S75Tr-2	S75Tr-4.4	ATr-2	ATr-2.3	Oenoferm freddo
Acetic aldehyde	15,4	13,8	10,5	12,7	11,3	13,5
Ethyl acetate	22,2	24,9	20,4	23,4	19,9	22,6
Methyl alcool, g/L	0,02	0,02	0,02	0,02	0,015	0,015
2-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
n-propanol	10,6	10,1	10,0	12,9	9,9	10,4
Isobutanol	22,4	22,5	20,7	31,7	20,7	23,0
n-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Isopentanol	155,6	151,1	145,5	161,1	141,9	148,0
Σ Higher alcohol	189,6	184,7	176,8	206,3	173,5	182,4

Esters are the most desirable group of compounds contributing fruity and floral aromas to the wine bouquet (Carrau *et. al*., 2008*). S. cerevisiae* synthesizes two major groups of esters during fermentation, namely the acetate esters of higher alcohols and the ethyl esters of medium-chain fatty acids. Such esters include ethyl acetate, isoamyl acetate, isobutyl acetate and phenylethyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate (Lambrechts and Pretorius, 2000)
In addition to the direct effect of acetaldehyde on the aromatic profile of the wine, it is equally important, if not more, its indirect effect due to its high reactivity with other compounds (Liu and Pilone2000; Styger *et al* 2011). Of special interest for the wine industry, it is acetaldehyde's binding activity with SO_2, the basic antimicrobial and antioxidant agent, forming a complex compound that offers limited protection to the produced wine (Styger *et al* 2011). Acetaldehyde also mediates condensation reactions of grape-derived anthocyanins with tannins into stable red wine pigments during winemaking (Eglinton *et al* 2004). As different strains of *S. cerevisiae* synthesize considerably different amounts of acetaldehyde, it is critical to choose a strain suitable depending on the type of wine produced (Klosowski *et. al,* 2017).
The major aldehyde synthesized by *S. cerevisiae* during wine fermentation is acetaldehyde, constituting over 90% of the total aldehyde content of wine (Styger *et. al,* 2011). Acetaldehyde is the last precursor in the anaerobic pathway before ethanol. Acetaldehyde, when present in low concentrations, confers a pleasant fruity aroma, but when in excess, it produces green and grassy off-flavors (Liu S-Q, and Pilone, 2000).
In 'Aligote' white wine, the concentration of acetic aldehyde varies in the range of values from 10.5 to 15.4 mg/L. Therefore, when using yeast strain S75Tr-4.4, the concentration of acetic aldehyde in wine is 10.5 mg / L (minimum value), while yeast strains S75Tr-2, and STr-1have a concentration of acetic aldehyde in a wine of 13.8 and 15, respectively, surpassing the control strain that registered only 13.5 mg/L. In 'Cabernet Sauvignon' red

wine, all 4 local yeast strains recorded higher acetaldehyde concentrations than the control, thus the local yeast strains recorded between 19.9 and 16.6 mg/L (MTr-4, M100 Tr-1respectively), while the control strain recorded a concentration of acetic aldehyde ~~e~~ of 16.1 mg/L.

Table 3 Volatile substances content (mg/L) in dry red wines 'Cabernet-Sauvignon' (h.y. 2018)

Compounds	Strain yeast					
	MTr-4	M100Tr-1	M100Tr-4	C-S60Tr-2	C-S60Tr-4	Oenoferm Be-Red
Acetic aldehyde	19,9	16,6	17,5	16,2	17,1	16,1
Ethyl acetate	23,7	21,2	25,8	22,2	27,7	21,5
Methyl alcool, g/L	0,02	0,01	0,02	0,01	0,01	0,01
2-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
n-propanol	9,6	10,0	10,1	10,2	10,8	11,3
Isobutanol	30,4	22,6	23,8	22,8	30,9	21,9
n-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Isopentanol	169,2	165,4	167,0	159,3	187,0	160,3
Σ Higher alcohol	210,0	199,0	193,3	193,3	229,7	194,4

The concentrations of acetaldehyde vary significantly between different types of wine, ranging approximately from 30–80 mg/L in white/red wines. In addition, this compound exerts a direct effect on the wine's aromatic profile and an indirect effect due to its high reactivity with other compounds.

The studied yeasts had a less significant influence on the n-butanol and 2-butanol in both white ('Aligote') and red wine ('Cabernet Sauvignon'), the determined values being lower than 0.5 mg/L.

The concentration of isobutanol in the wines obtained in the same way varies depending on the yeast strain used, and the range of variation in white wine was 20.7 mg/L in the case of S75Tr-4.4 strain to 31.7 recorded in ATr-2 yeast strain. The variation of n-propanol concentrations in the obtained wines is in the range of 9.6-11.3 mg/L. The amount of isopentanol in the wines obtained is about 60% of the sum of all higher alcohols. The highest concentration was detected in the wine obtained with C-S60Tr-4. Regarding the concentration of isopentanol in red wine ('Cabernet Sauvignon), it is noted that all 4 local strains of yeasts recorded values higher than the control (table 3). Thus, the highest concentration of isopentanol was recorded when using strain C-S60Tr-4 (30.9), followed by strains MTr-4 (30.1), M100Tr-4 (23.8 mg/L), compared to only 21.9 in the case of the control strain Oenoferm freddo. Another important component that is formed after the alcoholic fermentation process is ethyl acetate, which directly influences the organoleptic properties of the wines obtained.

The values of ethyl acetate in the case of white wine varied between 19.9 for yeast and 24.9 mg/L, and respectively 21.2 and 27.7 for red wines. Thus, in white wine, the lowest ethyl acetate concentration was 19.9 when using the ATr-2.3 yeast strain and the highest was 24.9 mg/L when using the S75Tr-2 yeast strain, while in red wine, the highest concentrations were recorded in the case of yeast. C-S60Tr-4 (27.7 mg/L), followed very closely by strain M100Tr-4 (25.8 mg/l), and the lowest concentration was found when using

the control yeast Oenoferm Be-Red (21. 5mg/L) and of the local yeast strain M100Tr-1 (21.2 mg/L), respectively.
According to the analysis of the volatile complex of the studied wines, it can be concluded that the methyl alcohol content is about 0.02 mg/L, which proves that the yeast strains do not significantly influence the concentration of methyl alcohol.

Sensory analysis.
The data of sensory evaluation of wines were processed in sensory profiles and represented in graphical form, figures 1 and 2. According to the obtained sensory profiles we can see that all produced wines have similar aromatic vectors. However, wines fermented with different local yeast strains isolated from the 'Trifeshti' wine center have a substantial effect on the aroma of wines, which is in accordance with the literature data had the most typical aroma, whereas the aroma of Oenoferm Freddo and Oenoferm Be-red wines was not so typical.
Our findings demonstrate that the more intense aroma of local yeasts wines was due to numerous factors, and we suppose that one of them could be a lower amount in total higher alcohols.

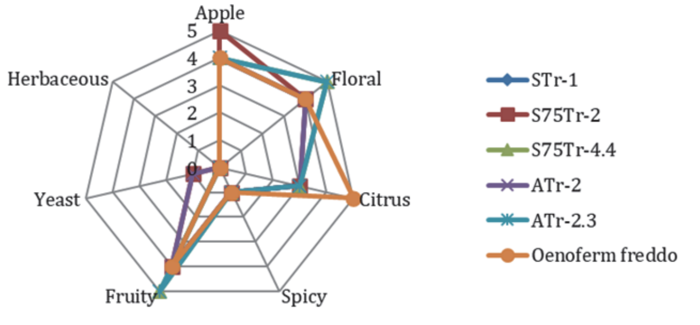

Fig 1. Sensory profiles of white wine 'Aligote' obtained with different yeasts strain.

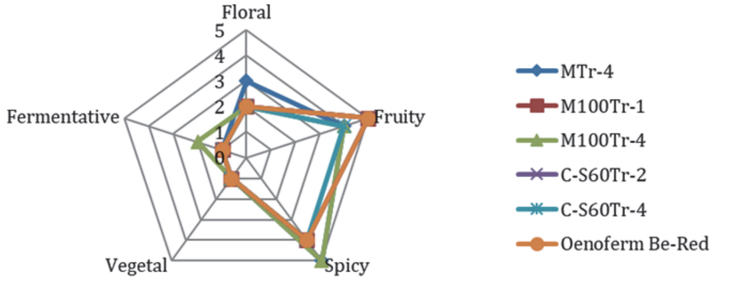

Fig 2. Sensory profiles of red wine 'Cabernet-Sauvignon' obtained with different yeasts strain.

CONCLUSIONS

The current study highlights the use of local yeast strains in winemaking and compared with commercial Oenoferm Freddo and Oenoferm Be-red, based on the obtained results demonstrate the enological potential of local yeast strains ATr-2.3, S75Tr-4.4, M100Tr-1 and C-S60Tr-2. These yeasts can enhance the production of wine with optimal content of alcohols, esters, and aldehydes that impact wine flavor. Our results show the potential use of yeast strains isolated from the 'Trifeshti' wine center (Republic of Moldova) for dry red and white wine production with distinctive characteristics of the area of grape cultivation.

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