The influence of new yeast strains from the indigenous flora of "Trifeshiti" Vineyard on the alcoholic fermentation process

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ABSTRACT

The use of selected yeasts for winemaking has clear advantages over traditional spontaneous fermentation. The selection of wine yeasts is usually carried out within the *Saccharomyces cerevisiae* species. Yeast strains produce different amounts of secondary compounds that impart specific characteristics to the wines. This suggests that it is necessary to isolate naturally occurring autochthons strains, which exhibit a metabolic profile that corresponds to each wine. The variability, the degree of adaptation as well as the widespread of yeasts in different biotypes enable the isolation of new yeast strains with properties that can influence the fermentative processes, for this reason, the activity of isolating and selecting yeasts strains from the vintage microbiota of Trifeshiti vineyard imposed as a necessity in the research and production activity. To be used in the current biotechnological practice, the *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36, and *Saccharomyces cerevisiae* CNMN-Y-37 yeast strains, isolated from the indigenous flora, were tested at the industrial level. The monitoring of the alcoholic fermentation involved the registration of the commencement time and the duration of the fermentation stages, as well as the dynamical evolution of the temperature, the sugar concentration, the alcohol content, and the total acidity. The new yeast strains were assessed as valuable biological material, recommendable in vine-growing practice, as they contribute to obtaining high quality wines that reflect the personality and potential of the cultivars specific to Trifeshiti vineyard.

Keywords: local, microbiota, physical-chemical parameters, industrial level.

INTRODUCTION

The transformation of grape must into wine is a complex process involving various microorganisms: yeasts, molds, and bacteria. The main step, alcoholic fermentation, is performed by yeasts. In natural fermentation, microflora comes from grape berries but also winery equipment and surroundings. Yeast biodiversity on grape berries is governed by various biotic and abiotic factors such as grape cultivar, climatic conditions, and viticultural practices (Pretorius, 2000; FLEET, 2008; Querol *et. al.*, 2018). Yeasts present on grapes are mainly from non-*Saccharomyces* genera (essentially *Hanseniaspora*, *Candida*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Cryptococcus* and *Rhodotorula* (Querol *et. al.*, 2018) while *Saccharomyces* genera are very rare. However, although non-*Saccharomyces* yeasts initiate fermentation and develop during the first hours, their population declines rapidly in favor of

Saccharomyces cerevisiae (*S. cerevisiae*), which becomes the dominant species until the end of alcoholic fermentation. The evolution of yeast populations during fermentation seems to be linked to several modifications that make the medium more selective. The establishment of nutrient depletion, anaerobic conditions, increased acidity, the production of sulfur dioxide, and increasing levels of ethanol (up to 15%v/v) results in a drop in yeast diversity (Fleet, 2008). This modification of the matrix environment allows the survival of *S. cerevisiae* because of its overall better resistance to stress compared to non-*Saccharomyces* species (Ciani *et al.*, 2010).

Producers have used wine starters for many decades to ensure proper fermentation initiation and the quality and reproducibility of wine. Indeed, starter yeasts allow efficient fermentation management that limits contaminations and avoids deviations due to interrupted or sluggish fermentations (Albergaria and Arneborg, 2016). These starter yeasts are selected for their specific metabolic properties: resistance to various stresses, fermentation capacity, or the presence of enzymatic activities (Binati *et al.*, 2019). The ability of *S. cerevisiae* to grow in a selective medium as described above, to carry out efficient and quick alcoholic fermentations, make this species a tool of choice as an oenological starter (Albergaria and Arneborg, 2016).

However, in recent years, non-*Saccharomyces* yeasts have been used for wine production since several yeast species have shown high oenological potential (Rossouw and Bauer 2017; Goold *et al.*, 2017). Indeed, yeasts like *Saccharomyces non-cerevisiae* (Alonso-del-Real, 2017; Alonso-del-Real, 2019), non-*Saccharomyces* (Bianti, 2019; Rossouw, 2017; Varela, 2016; Englezos, 2016; Benito, 2019; Binati *et al.*, 2019; Canonico *et al.*, 2019; Maturano *et al.*, 2018), and even natural hybrids (Bisson *et al.*, 2018; Bellon *et al.*, 2018; Kanter *et al.*, 2020; Origone *et al.*, 2018; Origone *et al.*, 2021) are of interest, because their different metabolisms compared to *S. cerevisiae* brings diversity to quantitative and qualitative composition of the final wine, for example, ethanol content, organic acids, aroma production, (Querol *et al.*, 2018; Comitini *et al.*, 2017; Rantsiou *et al.*, 2012).

Fermentative yeasts are used industrially in food biotechnologies in the production of wine, beer, and other products, having as main characteristic the capacity to produce the fermentation of simple carbohydrates in anaerobiosis, with the formation of ethyl alcohol and carbon dioxide (Querol *et al.*, 1992).

Yeasts responsible for the alcoholic fermentation in winemaking, as a rule, get in the grape must from the surface of the skin of vine grapes or through the direct administration of specific yeasts. The fermentation process can be initiated by the administration in grape must of the selected yeasts or the commercial active dry yeasts. The natural fermentation process takes place with wild strains from indigenous microflora from processed grapes (Querol *et al.*, 1992). One of the most significant technological advances in winemaking has been the control of the microbiological process by grape must inoculation using selected yeasts (Fleet and Heard, 1993). Today, the use of indigenous wine yeasts selected from each winegrowing centre is widely used (Rainieri and Pretorius, 2000). These local yeasts are supposed to be more competitive than commercial yeasts because they are better adapted to the ecological and technological features of their wine-growing area (Querol and Ramon, 1996; Lurton *et al.*, 1995).

MATERIALS AND METHODS

In order to optimize the alcoholic fermentation process by using some yeast strains selected from the indigenous flora, the *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36, and *Saccharomyces cerevisiae* CNMN-Y-37 yeast strains have been tested at an industrial level. The mash fermentation was

made in 20000 liters tanks and the following basic conditions have been provided so that the alcoholic fermentation should take place properly: the used mash was cleared, clarified, and sulfated in order to eliminate the spontaneous microbiota from the mash; the selected yeasts have been introduced in tanks in quantities that should guarantee from the beginning the optimal density of yeast cells/mL, necessary for the process of fermentation. When monitoring the fermentation process, there were registered the moment of starting and the duration (hours/days) of the fermentation stages, the tumultuous fermentation, the calm fermentation as well as in the dynamics of the main parameters: temperature ($t^{\circ}\text{C}$), sugar concentration (g/L), alcohol content (% vol.), volatile acidity (g/L CH_3COOH) and total acidity (g/L $\text{C}_4\text{H}_6\text{O}_6$). At the end of the process, the conditioned wines were analyzed from a physicochemical and organoleptic point of view.

The total sugars content (g/L) in musts was determined according to SM GOST 13192-73 (by the areometric method). Mass concentration of non-fermented sugars (g/L) was determined in wines according to SM GOST 13192-73 (by indirect titration method). The concentration of ethyl alcohol (% vol.) was determined by distillation, according to SM GOST 51653:2010. The volatile acidity of the wine was determined according to SM GOST 51654:2012 (by titration of the volatile acids separated from the wine by steam distillation and titration of the distillate). The total acidity of the wine was determined according to SM GOST 51621:2008 (by titration with bromthymol blue as an indicator). Determinations were made in triplicate.

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 on-line calculator; <http://math.semestr.ru/>

RESULTS AND DISCUSSION

The *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36 and *Saccharomyces cerevisiae* CNMN-Y-37 yeast strains, considered to be successful in the production of quality white and red wines have been verified on the musts obtained from the 'Chardonnay', 'Muscat Ottonel', 'Merlot' and 'Cabernet-Sauvignon' cultivars whose physical-chemical characteristics are presented in Table 1.

Table 1. The physical-chemical characteristics of the musts used for experiments

The musts used	Sugars, g/L	Total acidity, $\text{C}_4\text{H}_6\text{O}_6$ g/L.	pH
'Chardonnay'	215±1.0	7.2±0.1	3.15±0.01
'Muscat Ottonel'	220±1.0	6.4±0.1	3.21±0.01
'Merlot'	235±1.0	7.0±0.1	3.0±0.01
'Cabernet-Sauvignon'	230±1.0	7.3±0.1	3.18±0.01

We have to mention that for every must lot, a witness was provided for which we used as fermentation agent a commercial compound usually used in the technology of white and red wine production. The data obtained when monitoring the fermentation processes carried out with the three yeast strains are graphically presented in Figures 1, 2,3, and 4.

Figure 1. The dynamic of the physical-chemical parameters during the alcoholic fermentation process made by the *Saccharomyces cerevisiae* CNMN-Y-34 yeast strain in comparison with the control yeast

Figure 2. The dynamic of the physical-chemical parameters during the alcoholic fermentation process made by the *Saccharomyces cerevisiae* CNMN-Y-32 yeast strain in comparison with the control yeast.

Figure 3. The dynamic of the physical-chemical parameters during the alcoholic fermentation process made by the *Saccharomyces cerevisiae* CNMN-Y-36 yeast strain in comparison with the control yeast

Figure 4. The dynamic of the physical-chemical parameters during the alcoholic fermentation process made by the *Saccharomyces cerevisiae* CNMN-Y-37 yeast strain in comparison with the control yeast.

After testing the oenological value at an industrial level of the new selected yeast strains *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36 and *Saccharomyces cerevisiae* CNMN-Y-37, we established that they may be appreciated as biologic material useful for the wine-making practice in the production of quality dry white and red wines technology.

The data obtained when monitoring the fermentation processes point out the following aspects:

- the yeast strains *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36 and *Saccharomyces cerevisiae* CNMN-Y-37 fitted in the category of yeasts with a minimal degree of foaming in the first 24 hours from the beginning of the pre-fermentation stage (after this interval, the fermentation advances without foaming. From the perspective of this characteristic, the tested yeast strains are valuable because they offer the possibility to use the fermentation space in full capacity.
- the new selected yeast strains started the alcoholic fermentation after 24-28 hours from the introduction of the leaven in the must. In this stage, the musts grew turbid, and at the microscope, we can notice a great number of yeasts in an intense process of burgeoning, and the must temperature grew slowly with approximately 1°C. The relatively small duration of the pre-fermentation period is an advantage of the alcoholic fermentation process, always being preferred by the yeast strains that show this characteristic. In dynamic, the evolution of the alcoholic concentration curve emphasizes a more intense activity of sugar metabolism in the tanks considered as a witness, where a commercial compound was used as a fermentation agent.
- the tumultuous fermentation stage started after 48 hours and continued for 8-10 days, when the must temperature increased gradually, because of the increase of the metabolic activity concomitantly with the increase of the number of the yeast. In this stage, the temperature was verified in the fermentation devices twice a day, intervening to maintain it between 15 – 16°C for white wines and 28-29 °C for red wines. In the case of the four tested yeast strains, we noticed an average metabolism of sugars, which led to eventually obtaining some wines with special sensory characteristics;

- in the tanks with the newly selected yeast strains, the clarifying process started quickly at the end of the tumultuous stage, obtaining after 10 days relatively clear or lightly opalescent wines, and the yeast deposit formed after the first racking was compact, hardly removable.

At the end of fermentation, dry wines were obtained, with an alcoholic strength between 12.5-12.9% vol. alcohol for white wines and 13.5-13.8 % vol. alcohol for red wines (table 2,3), depending on the initial concentration of sugars from the substratum, which proves that the new tested yeast strains are alcoligene, being capable to metabolize almost completely the sugars from the fermentation medium.

As a result of determining the main structure characteristics, it follows that the wines made by the newly selected yeast strains from the indigenous flora of the Trifeshiti vine-growing region show the balanced concentration of the physicochemical characteristics (table 2,3).

Table 2. The main composition characteristics of the white wines were obtained by using new yeast strain testing in comparison with the control yeast

Physical parameters - chemical and organoleptic	'Chardonnay'		'Muscat Ottonel'	
	control yeast (IOC B-2000)	CNMN-Y-34	control yeast (IOC B-2000)	CNMN-Y-35
Alcohol, % vol.	12.6±0.1	12.5±0.1	12.9±0.1	12.9±0.1
Non-fermented sugars, g/L	2.1±0.2	2.0±0.2	4.0±0.2	3.7±0.2
Total acidity, g/L	6.7±0.1	6.8±0.1	5.9±0.1	5.8±0.1
Volatile acidity, g/L CH ₃ COOH	0.39±0.3	0.39±0.03	0.49±0.03	0.49±0.03
Organoleptic assessment, 0 - 10	8.1	8,1	8.2	8.2

Table 3. The main composition characteristics of the red wines obtained by using a new yeast strain testing in comparison with the control yeast

Physical parameters - chemical and organoleptic	'Merlot'		'Cabernet-Sauvignon'	
	control yeast (IOC R-9008)	CNMN-Y-36	control yeast (IOC R-9008)	CNMN-Y-37
Alcohol, % vol.	13.8±0.1	13.7±0.1	13.5±0.1	13.5±0.1
Non-fermented sugars, g/L	1.9±0.2	2.5±0.2	2.9±0.2	2.5±0.2
Total acidity, g/L	6.7±0.1	6.6±0.1	6.7±0.1	6.9±0.1
Volatile acidity, g/L CH ₃ COOH	0.49±0.03	0.45±0.03	0.56±0.03	0.53±0.03
Organoleptic assessment, 0 - 10	8.1	8.1	8.1	8.1

The tested yeast strains are considered valuable for obtaining quality dry white and red wines, these satisfy more conditions, which are: superior alcohol level, low volatile acidity, superior sugars-alcohol efficiency, etc.

The organoleptic appreciation of the analyzed wines emphasized their very good quality. All the wines presented a discreet, fine, specific flavor, being fruitfully well harmonized with the other components.

All these data offer a complete image of the oenological value of the new yeast strains, as well as their practical importance in the dry white and red wines production technology.

Taking into account the results, we recommend the carrying on of the research in order to go deeply into the aspects concerning the importance of the yeast strains origin, into defining the characteristics regarding the authenticity and specificity of the wine.

CONCLUSIONS

The verification at the industrial level, because of the fermentation and technological characteristics, allowed the appreciation of the newly selected yeast strains *Saccharomyces cerevisiae* CNMN-Y-34, *Saccharomyces cerevisiae* CNMN-Y-35, *Saccharomyces cerevisiae* CNMN-Y-36, and *Saccharomyces cerevisiae* CNMN-Y-37 as valuable biologic material, recommendable for the vine-growing practice.

Using the newly selected yeast strains in the alcoholic fermentation process at the industrial level has the following advantages: increased efficiency of the process by using at full capacity the fermentation spaces, a total transformation of sugars, rapid conditioning (clearing, separation from the deposit) and increasing the specificity degree of the Trifeshiti vine-growing region wines, contributing to their fame on the domestic and external market. The oenological value of the newly selected yeast strains allows their recommendation in the white and red dry wines production technology in Trifeshiti vine-growing region, these contributing to obtaining quality wines that reflect the personality and the potentiality of the cultivars specific to the region.

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