ISOLATION OF NEW TYPES OF YEASTS STRAINS FROM INDIGENOUS FLORA OF IAŞI VINEYARDS

NOI SUŞE DE LEVURI CU CARACTER ALCOOLIGEN RIDICAT IZOLATE DIN FLORA INDIGENĂ A PODGORIEI IAŞI

***NECHITA Ancuta*[1], *SAVIN* C.[1], *PAŞA Rodica*[1], *ZAMFIR C.I.*[2], *CODREANU Maria*[1]**
e-mail: ancuta.vasile@yahoo.com

Abstract. *The alcoholic fermentation of grapes must is highly desired when the yeasts are tolerant to high osmotic pressure due to high concentrated sugar and resistant to the toxic effects of the high alcohol concentration. In this context, the research led to the selections made from Iasi vineyard – Copou vine centre of new yeast strains with strong alcooligen character, Sacchoramyces genus. New strains of isolated and selected yeasts have the ability to achieve full alcoholic fermentation and has a good tolerance to high alcohol concentrations (15.0 to 16.0 vol % alcohol). They may also restart the alcoholic fermentation when the process was stopped at a concentration of 8.5 to 11.5% alcohol by volume and still have a residual sugar content of 60 - 100 g/L.*
Key words: *yeast, fermentation, tolerant*

Rezumat. *În procesul fermentaţiei alcoolice a mustului din struguri sunt foarte apreciate suşele de levuri tolerante la presiune osmotică ridicată, cauzată de concentraţi mari de zaharuri, şi rezistenţă la efectul toxic al concentraţiei mari de alcool. În acest context, cercetările întreprinse au dus la selecţia din plantaţiile centrului viticol Copou Iaşi a unor noi suşe de levuri cu puternic caracter alcooligen, aparţinând genului Sacchoramyces. Suşele noi de levuri izolate şi selectate au capacitatea de a realiza fermentaţia alcoolică integral şi posedă o bună toleranţă la alcool (15,0- 16,0 vol. % alcool). De asemenea, pot redeclanşa şi finaliza fermentaţiile alcoolice în cazul proceselor fermentative care s-au oprit la concentraţii de 8,5 – 11,5 vol. % alcool şi care au încă un conţinut în zaharuri rezidual de 60 – 100 g/L.*
Cuvinte cheie: *levuri, fermentaţie, toleranţă*

INTRODUCTION

In the alcoholic fermentation of grape musts are very popular yeast strains that are tolerant to osmotic pressure, caused by high concentrations of sugars and resistance to the toxic effects of high concentrations of alcohol. Through their use prevents stagnation of alcoholic fermentation, which can lead to the production of wines with a lower alcohol content and unfermented sugar residue. In recent years, stopping alcoholic fermentation is a phenomenon encountered in both large units and wine from small producers (Ekunsanmi and Odunfa S.A., 1990). For this reason wine research were discussed studies on the isolation of yeasts strains tolerant to high

[1] Reasearch and Development Station for Viticulture and Winemaking Iasi, Romania
[2] Oenological Research Center, Iasi Branch of the Romanian Academy, Iasi, Romania

concentrations of sugars and alcohol (Buescher W.A. et. Al., 2001; Oshoa, 2005; Tikka C. et.al., 2013).

In this paper we proposed the selection of yeast strains from Iasi vineyard – Copou vine, properties with high concentrations of alcohol tolerance, with the ability to restart, and to complete the alcoholic fermentation where it was stopped at a concentration of 8.5 to 11.5 % vol . alcohol and the residual sugar 60-100 g/L.

MATERIAL AND METHOD

New strains isolated in pure culture yeasts were preliminary tested in view of the technological (foaming, initiation and completion of alcoholic fermentation, clarity wine, yeast deposit type etc). Of the yeast strains tested were selected seven strains noted coded RF1, RF2 , RF3 , RF4 , RF5 , RF6 and RF7. The selected yeast strains, the next step was tested for tolerance to alcohol. Was used YPG culture medium (yeast extract, peptone, dextrose), after sterilization and cooling, alcohol was aseptically added in concentrations of 10%, 12%, 13%, 14% and 15%. Assessment of alcohol tolerance was performed by determining the dynamics (24, 48 and 72 hours), the optical density at 615 nm , and viability of yeast cells by staining smears with methylene blue. Yeast strains selected on the basis of tolerance to alcohol, the following experiment was verified using the sugar concentration must be 230 g/L and the wort was added to the rectified must in order to increase the sugar concentration 276 g/L. For each yeast strain to prepare a set of recipients with the capacity of 1000 mL were distributed in 750 mL of musts those inoculated with 4 x 10^6 cells/mL. Recipients were thermostated at 20°C. The wines were characterized physico - chemical OIV standards. The verification experiment refermentare capacity wines with sugar residue to use a wine with 11.5 % vol alcohol and 70 g/L sugars fermented. The amount of yeast inoculum was 8 x 10^6 cells/mL. The wines produced at the end of the experiment were characterized physico - chemical and organoleptic.

RESULTS AND DISCUSSIONS

With a view to the selection of new strains of yeast tolerant performance properties at high concentrations of alcohol have been studied 18 yeast strains have been isolated in pure culture. In the preliminary selection of all yeast strains isolated 11 yeast strains were eliminated by failure of technological characteristics: foaming, triggering delayed or stalled fermentation, producing quality wines with unsatisfactory organoleptic point of view. The selected yeast strains (RF1, RF2, RF3, RF4, RF5, RF6 and RF7) alcoholic fermentations were initiated by 18 to 20 hours, very little foam, and have completed the first 24 hours after 10 days the alcoholic fermentation, the wine to give dry, valued for organoleptic quality. Capacity development of yeast strains with alcohol concentrations of 10% vol., 12% vol., 13 % vol., 14 % vol., and 15 % vol. appreciated by determining the dynamics of the optical density at 615 nm of non inoculated culture medium. Increase in optical density indicates the ability of strains of yeast propagation under the conditions established in experiment (fig. 1 – 7).

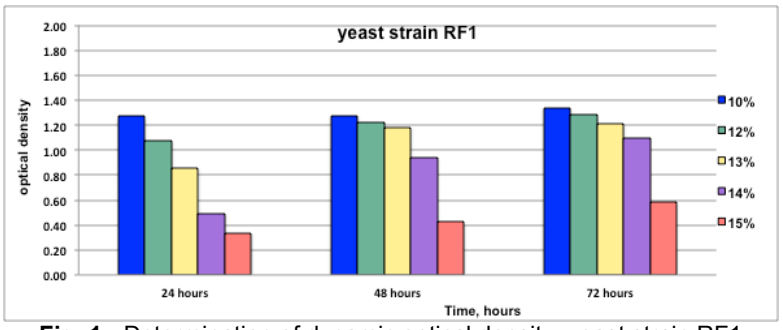

Fig. 1 - Determination of dynamic optical density, yeast strain RF1

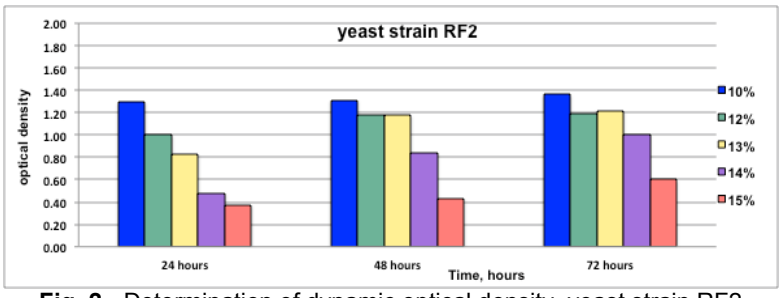

Fig. 2 - Determination of dynamic optical density, yeast strain RF2

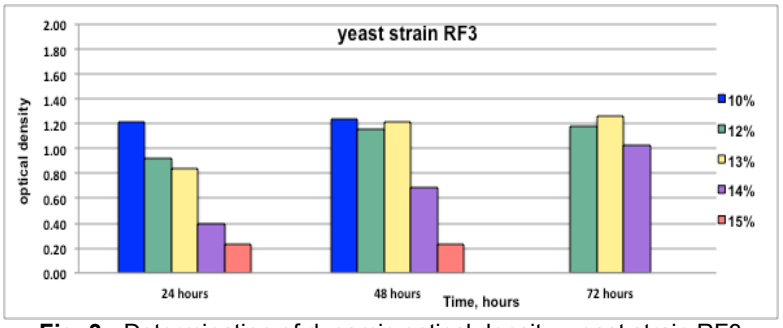

Fig. 3 - Determination of dynamic optical density, yeast strain RF3

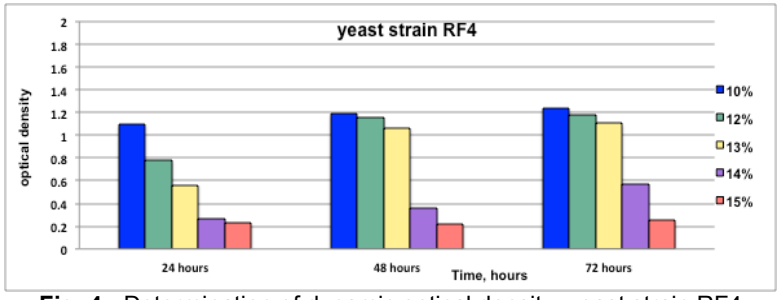

Fig. 4 - Determination of dynamic optical density, yeast strain RF4

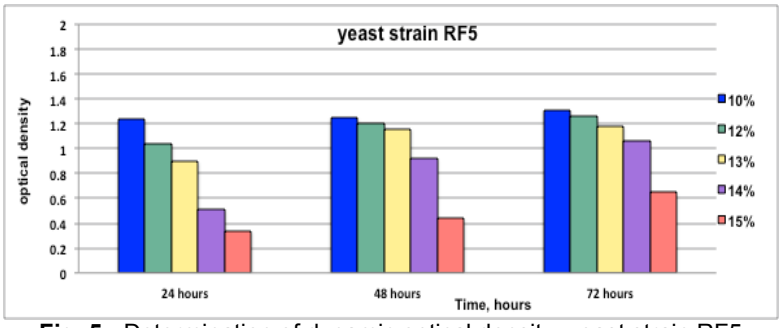

Fig. 5 - Determination of dynamic optical density, yeast strain RF5

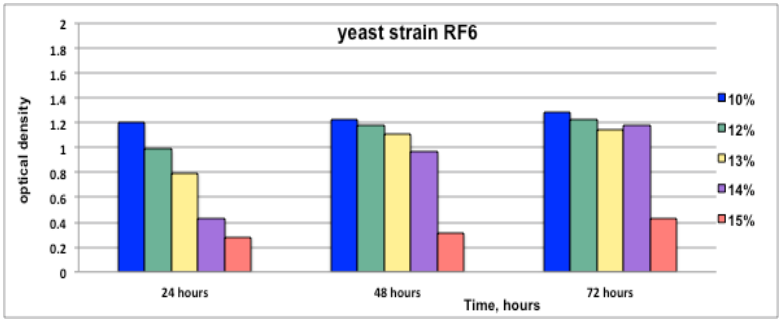

Fig. 6 - Determination of dynamic optical density, yeast strain RF6

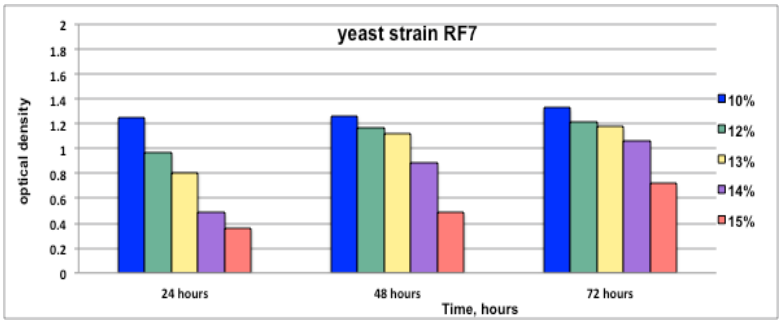

Fig. 7 - Determination of dynamic optical density, yeast strain RF7

After 24 hours of the onset of the experiment all strains tested reached the highest optical density at levels of 10% vol. alcohol. Along with increasing alcohol concentrations optical density values were becoming smaller. The results confirmed that the time lag in the concentration of alcohol 10% vol. is low and increases with increasing concentration.

In the following period of 48 hours the optical density increased to values close to those reached after 24 hours and yeast strains for the development of alcohol concentrations of 12-13 % vol. It was also an increase in the optical density of 42 % and 47 % alcohol concentrations of 14 % vol. Yeast strains for RF1, RF2, RF3 , RF5, RF6 and RF7 except strain RF4, whose optical density increased compared to the value at 24 hours only 26%.

Regarding the development of yeast strains for alcohol concentrations

of 15 % vol., optical density increased only by 10 % when RF6 strain, 20 % for RF1 and RF2 strains and 25 % for strain RF7. It will be appreciated that the alcohol concentration of 14-15 % vol. lag period of adaptation of yeast strains requires a longer time, namely 48 hours. Examination of methylene blue stained smears revealed that the number of viable cells as a percentage increase with increasing alcohol concentration.

Within 72 hours there was a progressive increase in the number of cells at concentrations of 14 % vol. alcohol, registering an increase of optical density by 13 % for yeast strain RF5, by 14% for yeast strain RF1, with 13 % for yeast strain RF2 and 17% pentru RF6 and RF7. Also, an increase in the optical density values found and for the development of yeast strains at a concentration that is 15 % alcohol and 27 % in the case of the yeast strains RF1, RF2 and RF6 and 30 % for yeast strains RF5 and RF7.

The data obtained by evaluating the dynamic optical density, it is clear that only five strains of yeast can be considered tolerant to alcohol concentrations of 15%. Yeast strains RF3 and RF4 are tolerant to high concentrations of alcohol, by means of the high number of viable cells after 24 hours, about 80 %.

Recovery capacity fermentation alcohol tolerant yeast strains was performed on must sugars concentrations of 230 g/L (table 1) and rectified concentrated grape must adjusted to the sugar concentration 276 g/L (tab. 2).

The amount of inoculum used was 4 x 10^6 cells/mL. Fermentations were conducted at 20°C. The wines were characterized physico - chemical and alcoholigenic results confirmed the ability of yeast strains tested in both alcoholic fermentation yielding dry wines with high alcoholic degrees depending on the sugar concentration musts used.

Table 1

Compositional characteristics of wines produced using yeast strains tolerant to alcohol the must with 230 g/L sugars

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	13,1	13,1	13,2	13,6	13,6
Total acidity, g/L $C_4H_6O_6$	4,2	4,2	4,1	4,3	4,3
Volatile acidity, g/L CH_3COOH	0,32	0,36	0,32	0,36	0,36
Reducer extract, g/L	21,6	21,0	21,8	21,06	21,6
Non-fermented sugars, g/L	-	-	-	-	-

Table 2

Compositional characteristics of wines produced using yeast strains tolerant to alcohol the must with 276 g/L sugars

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	15,9	15,7	15,9	15,8	16,0
Total acidity, g/L $C_4H_6O_6$	3,6	4,0	3,8	4,0	4,1
Volatile acidity, g/L CH_3COOH	0,48	0,51	0,48	0,36	0,45
Reducer extract, g/L	21,2	21,1	21,6	21,2	21,4
Non-fermented sugars, g/L	-	-	-	-	-

Ability alcohol tolerant strains in the refermented process was tested on a wine with an alcoholic strength of 11.5 % vol. and non fermented sugar residue of 70 g/L. Because during alcoholic fermentation which yielded this wine sold out a series of compounds essential for the development of yeasts (nitrogen, phosphorus), the wine used in the experiment was added 0.1 g diammonium phosphate and increased the amount of inoculum from the 8 x 10^6 cells / mL .

If the musts completion of the fermentation time was 10 days in this experiment , the length of the completion of fermentation was high, reaching 18 days. The wines were characterized physico - chemical (table 3), the data obtained showing that all strains tolerant to alcohol sugars are fermented entirely existing affording dry wine .

Table 3

Compositional characteristics refermented wine yeast strains using alcohol tolerance

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	14,9	15,7	15,9	15,8	16,0
Total acidity, g/L $C_4H_6O_6$	3,9	4,1	4,0	4,0	4,1
Volatile acidity, g/L CH_3COOH	0,48	0,51	0,48	0,36	0,45
Reducer extract, g/L	21,8	20,9	21,6	21,8	21,4
Non-fermented sugars, g/L	-	-	-	-	-

CONCLUSIONS

1. Following isolation of new activity alcooligene yeast strains were isolated in pure culture 18 strains from Iasi vineyard – Copou vine and the preliminary test selection based on technological characteristics were selected seven strains of yeast.

2. Yeast strains selected checked for tolerance to alcohol concentration in the range of 10 to 15 % shows that five are tolerant to high concentrations of alcohol (14-15 %) namely RF1, RF2, RF5, RF6 and RF7.

3. In the alcoholic fermentation restart of stagnated at 11.5 % vol. alcohol and 70 g/L sugars, alcohol - tolerant yeast strains have restart and completed fermentations leading to the production of dry wines.

REFERENCES

1. **Buescher W. A., Siler C. E., Morris J. R., Threlfall R. T., Main G. L., Cone G. C., 2001 -** *High Alcohol Wine Production from Grape Juice Concentrates*. Am. J. Enol. Vitic 2001 vol. 52 no. 4, pp. 345 - 351
2. **Osho A., 2005 -** *Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice*. African J Biotechnol. no. 4, pp. 660–662.
3. **Tikka C, Osuru HP, Atluri N, Raghavulu PC, Yellapu NK, Mannur IS, Prasad UV, Aluru S, K NV, Bhaskar M, 2013 -** *Isolation and characterization of ethanol tolerant yeast strains.* Bioinformation vol. 9(8), pp. 421-425
4. **Ekunsanmi T. J., Odunfa S.A., 1990 -** *Ethanol tolerance, sugar tolerance and invertase activities of some yeast strains isolated from steep water of fermenting cassava tubers*. Journal of Applied Bacteriology vol. 69, pp. 672–675.