COMPARATIVE STUDIES ON THE DYNAMICS OF FERMENTATION OF SELECTED WINE YEASTS

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ABSTRACT

Alcoholic fermentation is an anaerobic biochemical process of oxidation-reduction in which carbohydrates are metabolized by the action of yeast enzymes in major products (ethyl alcohol and carbon dioxide) and minor products (superior alcohols, aldehydes, acetic acid, glycerol, volatile acids and others). Typical agents of the alcoholic fermentation are from *Saccharomyces* genus, by fermentation resulting concentrations in ethylic alcohol higher that 8 alcoholic degrees. In this paper it was studied the dynamics of fermentation of 3 strains of *Saccharomyces ellipsoideus* wine yeast and were observed parameters such as the accumulation of alcohol, the release of CO_2, temperature, amount of oxygen released. It was found that alcoholic fermentation depends on medium factors but also on biotechnological qualities of yeasts selected for this purpose.

KAYWORD

Alcoholic fermentation - *Saccharomyces cerevisie var. ellipsoideus* – yeast - fermenter

INTRODUCTION

Saccharomyces cerevisie var. *ellipsoideus* yeast cells are intended to ferment the biomass in technological process, in order to obtain ethanol and CO_2. During these transformations, yeasts are subjects to oxidation process, to the stress derived from the development environment, and to the osmotic pressure which exists in dough starting from the simplest ones to the richest ones in sugar. Following this line, it is recommended to use viable methods in order to reduce the stress tolerance, the preservation period of fermentation capacity and preservation of the cells robustness in time (Banu, 2009).

Alcoholic fermentation is an anaerobic biochemical oxidation-reduction process where carbohydrates, under the action of the enzymes, are converted from yeast to main products (ethyl alcohol and CO_2) and secondary products (superior alcohols, aldehydes, acetic acid, glycerol, volatile acids, etc.). Typical agents of alcoholic fermentation belong to *Saccharomyces* types, which lead to concentrations greater than 8º of ethyl alcohol, using fermentation.

Saccharomyces yeasts were studied since a long time and some of their properties recommend them for industrial use. The most important properties of them are:
Alcoholigenic power - is the yeast's capacity of producing through fermentation a higher concentration of alcohol when sugar excess (*Saccharomyces cerevisiae* - *ellipsoideus* and *oviformis* cumulates 16-18º alcohol) exists in the environment. Some yeasts (*Kloeckera, Torulopsis)* have weak alcoholigenic power, and they are inhibited at 46º alcohol (Oprean, 2001).

Wine yeasts action in spontaneous and directed fermentation of grapes juice, having a well defined influence over the quality of the wine. Wine yeasts belong to *Saccharomyces*

type, and it had been established that this kind of yeast is the main agent of wine fermentation of the grapes juice. Picking of samples with superior ergonomics properties is based on the following properties: alcoholigenic power, osmo-tollerance and biomass fermentation capacity (Tița, 2001; Cotea, 1998).

MATERIALS AND METHODS

- Strain of *Saccharomyces ellipsoideus* DVFR, DVFR1, DVFR2
- Sartorius bioreactor with thermometer probe, CO_2, oxygen, biomass. Biomass sensor is optic and reading was made by comparing to a standard curve of wine yeast.

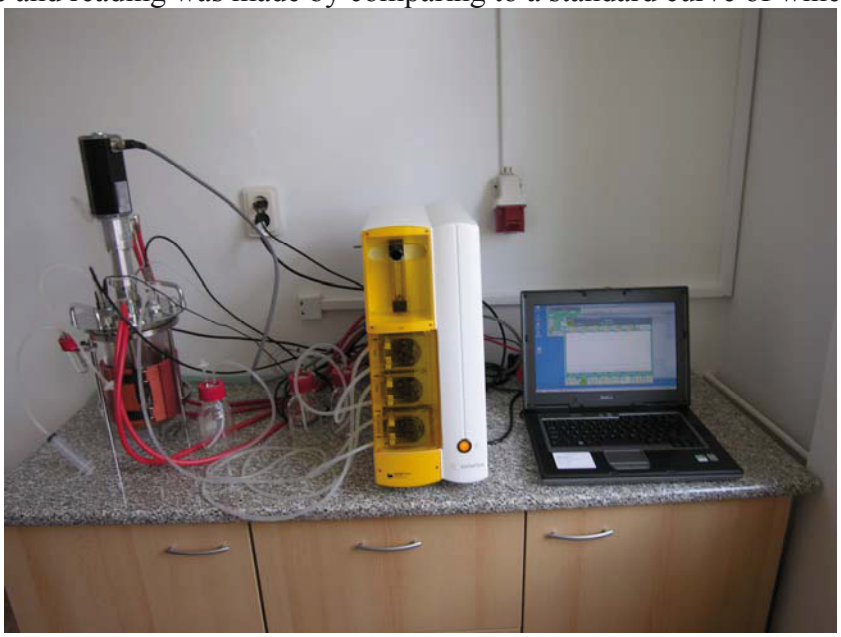

Fig.1.Fermenter BIOSTAT A plus SARTORIUS Bioreactor

Used methods were regarding the fermentation monitoring in the terms presented before and data transfer to the fermenter's database. The capacity of fermenter is 5 liters and fermentation process can be run in batch or feedback mode.

- Culture environment: malt containing: malt extract 15g/l, peptone 1g/l, maltose 12,5g/l, dextrin 2,5 g/l, phosphate dipotassic 1g/l, ammonium chloride 1g.l, pH 4,8 (Scharlau Chemie S.A., Spania).
- Fermentation time: 5 days – with monitoring of the CO_2 emission;
- Volatile acidity involves a part from fatty acids belonging to acetic series; they may exist as free acids or salts found in the tested biomass. The determination method consists in mixing the volatile acids from acetous biomass, using water vapors, with tartaric acid, and titration of distilled result using NaOH sollution, 0,1N of phenolphthalein (STAS 6182/ 2-86).
- Ethanol content: - alcoholic density was determined using the refractometer, method sensibility: 0,1% volume (STAS 6182/ 6-70).
- -pH. pH value was determined using the pH-meter, and the result considered was arithmetic average of five measurements for which the difference was not higher than 0,1 pH units. The pH meter was calibrated from 30 to 30 minutes, so that the measurements presented no susceptibility (STAS 6182/ 14-72).

- Total acidity was determined by titrating the sample with NaOH in presence of bromthymol blue, performing same measurements for different samples. Total acidity is expressed in g H_2SO_4 /l (STAS 6182/ 1-79).
- The glycerin from biomass is periodically oxidized with acid, and the resulted formaldehyde in presence of floroglucina forms a colored complex which may be identified using a photometric spectroscope (CECIL 1020 UV-VIS), the method's sensibility is 0,1 glycerin /l (STAS 6182/ 24-73).
- *The Potassium* was determined *using the* gravimetric method by precipitating the potassium with tetraphenil sodium borate in acid environment as tetraphenil potassium borate which was dried and weighed after isolation in a temperature controlled oven. The accuracy of measurement is 0,2% potassium/l (STAS 6182/ 30-74).
- *The Absorbance* was determined with CECIL 1020 UV-VIS photometric spectrometer, at 420nm

RESULTS AND DISCUSSIONS

From the graphics presented in Fig.2 it can be observed that the three types of wine yeasts *Saccharomyces ellipsoideus* DVFR,DVFR1, DVFR2, monitored at 22ºC, respectively 28ºC accumulates biomass and releases CO_2 during the entire cycle of fermentation, resulting some oscillations at the end of it, the most active stem being DVFR2. Although the three stems are derived from the same industrial source, its biological features provide superior properties, so it can be recommended for these specific technological procedures.

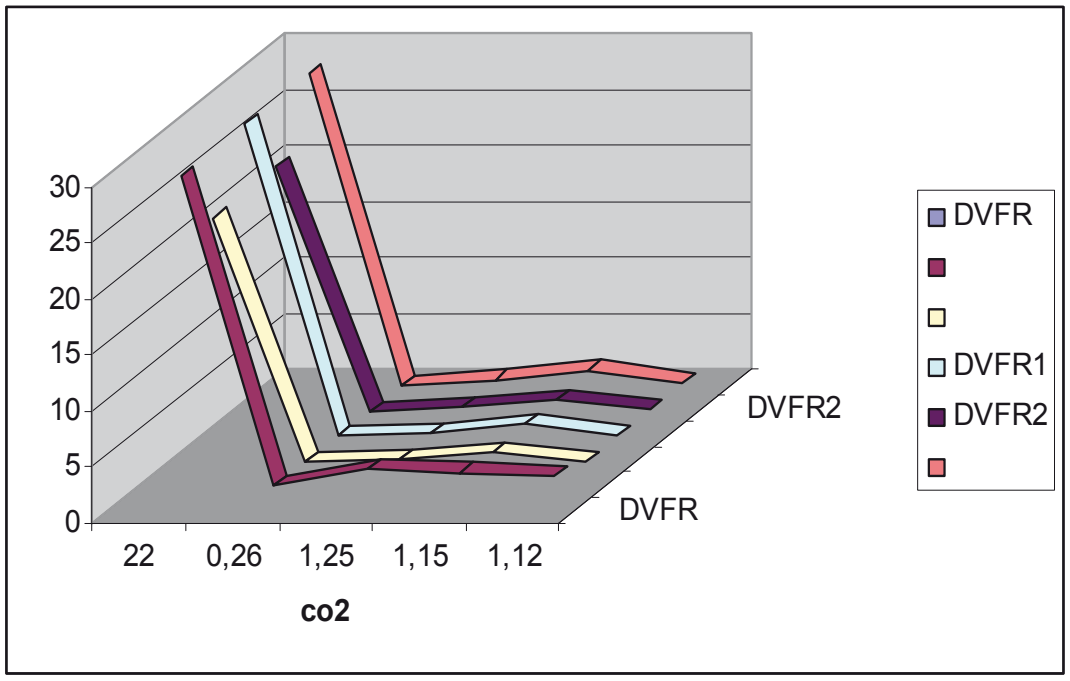

Fig.2. Fermentative evolution of DVFR, DVFR1, DVFR2 yeasts during five days

An analysis of information regarding the dynamics of the fermentation process for DVFR, DVFR1, DVFR2 may focus on the level that the three stems follow the same evolution function. So, let's consider the 28°C temperature.

The association of the two stems (X for DVFR and Y for DVFR1), related to their influence on the released amount of CO_2 , may be statistically expressed using covariance and correlation factor. If we name Dx_i the amount of CO_2 from cycle i for stem X and Dy_i the amount of CO_2 from cycle i for stem Y, where *i = {24;48;72;92}*, and M(Dx) , M(Dy) the averages of the released quantities of CO_2 on the four measurements, we can write:

M(Dx)=1,010

M(Dy)= 0.672

$$\operatorname{cov}(X,Y) = \frac{1}{4}\sum_{i=24}^{92}(Dx_i - M(Dx))(Dy_i - M(Dy)) = 0{,}116$$

Having a negative covariance (cov(X,Y) = 0,116 >0), we can say that the evolutions of the two amounts of CO_2 are oriented toward the same direction. The intensity of the connection can be determined using the correlation factor. First, has to be calculated the mean square deviation of CO_2 for both stems:

$$\sigma(X) = \sqrt{\frac{1}{4}\sum_{i=24}^{96}(Dx_i - M(Dx))^2} = 0.378$$

$$\sigma(Y) = \sqrt{\frac{1}{4}\sum_{i=24}^{96}(Dy_i - M(Dy))^2} = 0.712$$

Correlation factor will be: $\rho_{XY} = \frac{\operatorname{cov}(X,Y)}{\sigma(X)\cdot\sigma(Y)} = 0{,}530$ (the factor being usually specific for the interval (-1, 1)). This indicates an average association, so we can say that the process of fermentation of DVFR şi DVFR1 follow the same evolution pattern, in percentage of 49,2%, or that they are tending to evolve independently in percent of 50,8%.

The values of physio-chemical measurements are illustrated in Table 1, Figure 3. It can be observed that the DVFR2 strain presents better values than DVFR strain so we can say that it has superior biotechnological properties.

Tab.1. The values of physio-chemical measurements of vine yeast

No	Parameters	DVFR	DVFR1	DVFR2
1.	Reducing sugar (g/L)	1,24	1,12	1,28
2.	Volatile Acidity (g/L H_2SO_4)	0,42	0,31	0,12
3.	Ethanol content (%vol)	12,5	12,58	12,97
4.	PH	3,07	3,05	3,03
5.	Total Acidity (g/L H_2SO_4)	5,28	5,36	5,42
6.	Glycerol (g/l)	5,7	6,3	6,4
7.	Pothasium (g/L)	0,51	0,50	0,53
8.	Absorbance, 420 nm in 1cm	0,035	0,035	0,035

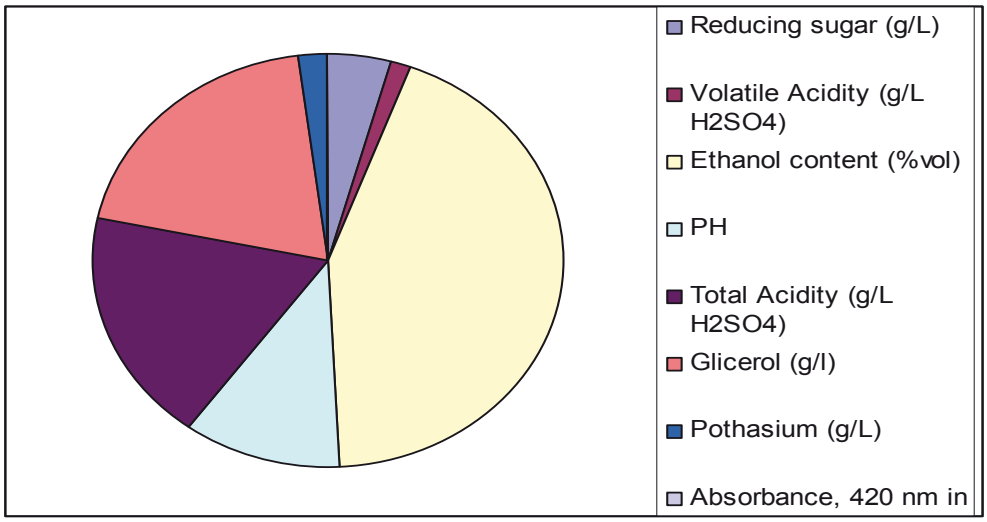

Fig. 3. The variation of phisico-chemical parameters that characterize the three strain of wine yeast DVFR, DVFR1, DVFR2

CONCLUSIONS

Following a fermentation cycle of stem wine yeast isolated from white grapes of Royal Feteasca, called DVFR,DVFR1,DVFR2 we can say:

- Wine yeast has a slower evolution, fermenting sugars from the environment at the highest value only after 72 hours, when stationary phase is installed.
- The temperature is direct proportional with sugar concentration; the fermentation speed is improving compared with the stems monitored at 22°C.
- We can see that the value of pH is influences the sugar fermentation being important in case of batch mode processing.
- Wine yeast *Saccharomyces ellipsoideus* DVFR, DVFR1, DVFR2 have similar properties but DVFR2 has the highest biotechnological value.
- The acidity is lowered in the case of DVFR2 and the level of glycerol is slightly increased; these will lead to a product with more smoothness and flavor.

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