IDENTIFICATION OF THE SACCHAROMYCES BAYANUS YEAST STRAINS BY DETERMINING THE VITAMINS CONTENT

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REZUMAT. Drojdiile utilizate într-un volum mare în industrie sunt: în industria fermentativă (alcool, vin, bere), produse de panificaţie şi în industria laptelui. Având o compoziţie chimică de valoare, acestea sunt o sursăde proteine în nutriţia umană, deoarece conţin aminoacizi şi un complex de vitamina B (Drăghici, et al. 2011). Unele elemente minerale (fosfor, sulf, fier, magneziu, etc.), factori de vitamine sau coenzime (vitamine B, acid paraaminobenzoic, co-enzima A, etc.) servesc drept activatori de reacţii enzimatice, care sunt specifice fermentaţiei alcoolice. În scopul obţinerii de vinuri de calitate, se vor folosi drojdii selecţionate, care au proprietăţi biotehnologice superioare (Oprean, et al. 2008). În lucrare s-au selectat trei soiuri de struguri, din care s-au izolat drojdiile Saccharomyces bayanus, iar identificarea lor a fost efectuată prin măsurarea conţinutului în vitamina B (B1, B2, B3, B5, B6), folosind metoda HPLC. Tulpinile de drojdie Saccharomyces bayanus au fost izolate din microbiota din strugurii autohtoni, din centrele de preparare a vinului Apold si Sebeş, centrul de prepararea al vinului Blaj-Tarnave si din centrul de preparare al vinului Jidvei-Tarnave. Soiurile de struguri folosiţi au fost: Iordana (SBIA), Riesling Italian (SBRI) şi Fetească Regală (SBFR). În scopul identificării acestor tulpini, s-au comparat cu proba de referinţă Saccharomyces bayanus EC 118 - din Spania (Gaspar, et al. 2011).

Cuvinte cheie: tulpini de drojdie, Saccharomyces bayanus, HPLC, vitamine

ABSTRACT. The yeasts are used in the industry on a high volume in the fermentative industry (alcohol, wine, beer), bakery and in the milk industry. Having a valuable chemical composition, they are a protein source in the human nutrition, containing aminoacids and B complex vitamins (Drăghici, et al 2011) .
Some mineral elements (phosphorus, sulfur, iron, magnesium, etc.), vitaminic factors or coenzymes (B vitamins, paraaminobenzoic acid, A co-enzyme, etc.) serve as activators of the enzymatic reactions which are specific to the alcoholic fermentation. In order to obtain quality wines, we use selected yeasts which have superior biotechnological properties (Oprean, et al 2008).
In this paper we selected three varieties of grapes, from which we isolated Saccharomyces bayanus yeasts, their identification was performed by measuring the content in B vitamins (B1, B2, B3, B5, B6) using the HPLC method. The Saccharomyces bayanus yeast strains were isolated from the microbiota of the native grapes from the Apold wine making centre – Sebes wineyard – Apold, Blaj wine making centre – Târnave wineyard, and the Jidvei wine making centre – Târnave wineyard. The grapes varieties we used were: Iordana (SBIA), Italian Riesling (SBRI) and Royal Feteasca (SBFR). In order to identify these strains, we compare them to the reference sample Saccharomyces bayanus EC118 - from Spain (Gaspar, et al 2011).

Keywords: yeast strains Saccharomyces bayanus, HPLC, vitamins

1. INTRODUCTION

The alcoholic fermentation process is an exoergic one and it is produced under the influence of the enzymes generated by the yeats. Mainly this combine itself with the reactive molecule called substrate; in this way intermediar compounds are formed so that it ends in a continuous cycle which can be repeated many times. The yeasts, through their viability guarantee the enzyme synthetisation, resulting a normal alcoholic fermentation process (*Oancea, 2011*).

Takin into account the high number of yeasts varieties which can be found in the grapes must, the problem of the isolation of the most active yeasts has arisen; these are called selected yeasts.

The *Saccharomyces bayanus* yeasts have as their composition, besides the proteins, also a significant amount of vitamins. The highly ranked liquids chromatography (HPLC) is an analytical method for determining the vitamins. Using the HPLC method, we determined the quantity of the vitamins: B1, B2, B3, B5, B6 of the SBIA, SBRI, SBFR and EC118 yeast strains.

The B1, B2, B3, B5 and B6 vitamins were identified and quantified using the HPLC method. HPLC is a simple and quick method used for their identification from different substrates, but which needs a series of adaptations due to the fact that the vitamins are not stable elements.

2. MATERIAL AND METHODS

Four yeast strains: *Saccharomyces bayanus* reference sample (EC118), *Saccharomyces bayanus* Iordana (SBIA), *Saccharomyces bayanus* Italian Riesling (SBRI), *Saccharomyces bayanus* Royal Feteasca (SBFR), from the collection of the Biotechnologies and Microbiology Research Centre of the SAIAPM Faculty.

Taking into account that the tiamin doesn't present retention to the standard C18 column, we modified the mobile phase by introducing a C18 AQ ProntoSil column, which was specially modified and also a mobile acid phase (oxalic acid).

The vitamins were extracted with extraction kits and then they were compared with the refference ones. The dillutions were performed using bi-distilled water, 20 minutes termostats; the dosage was according to the procedure. We compared the resulted values from the three strains (SBFR, SBRI, SBIA) with the reference sample EC118. The spectrographic detection was performed in the following way: for the vitamin B1 – λ: 246nm, B2 – λ: 267nm, B3 – λ: 260nm, B5 – λ: 240nm, B6 – λ: 290nm *(Redzepovic, 2002).*

The HPLC system we used was Knauer type with the following parameters: Eluent A-50mM H_3PO_4 with pH 2,5, Eluent B ACN, gradient: 0-2min 99%A, 2-8,5 min.30% A, 8,5-11 min.30% A, 11,02-15 min 99%A, pressure 77bar, inserter volume 10µl, temperature 40°C. Repetability ≤ 0,2% (*De Leenheer, 1985*).

3. RESULTS AND DISCUSSIONS

From tables 1, 2, 3, 4 and from the drawings in Figure 1, 2, and 3 which were resulted, we can observe that the isolated strains SBFR, SBRI, SBIA present identical characteristics in a percentage of 98% compared to the reference sample, EC118 strain. The variations may be considered as acceptable according to the agreed regulations.

The taxonomical framing of the studied yeasts, as well as their identification through the performed tests, lead to the development of their bio-diversity in the native natural environments.

Table 1. *Vitamin content for reference sample EC 118*

No.	Vitamins	T_r(min)	LOD (ng)	ESTD (mg)
1	Riboflavină (B2)	0,734	37,9	17,7
2	Acid pantotenic (B5)	1,368	15,4	8,2
3	Tiamină (B1)	2,233	39,5	14,8
4	Pyridoxină (B6)	2,987	15,9	7,9
5	Niacină (B3)	3,879	22,4	11,1

Table 2. *Vitamin content for strain SBFR*

No.	Vitamins	T_r(min)	LOD (ng)	ESTD (mg)
1	Riboflavină (B2)	0,821	35,7	17,9
2	Acid pantotenic (B5)	1,453	16,5	8,5
3	Tiamină (B1)	2,326	38,8	14,1
4	Pyridoxină (B6)	3,007	15,5	7,7
5	Niacină (B3)	4,264	21,9	11,0

Table 3. *Vitamin content for strain SBIA*

No.	Vitamins	T_r(min)	LOD (ng)	ESTD (mg)
1	Riboflavină (B2)	0,931	36,1	17,1
2	Acid pantotenic (B5)	1,368	15,9	8,4
3	Tiamină (B1)	2,313	39,1	14,7
4	Pyridoxină (B6)	3,002	15,3	8,0
5	Niacină (B3)	4,329	22,1	11,3

Table 4. *Vitamin content for strain SBRI*

No.	Vitamins	T_r(min)	LOD (ng)	ESTD (mg)
1	Riboflavină (B2)	0,734	37,4	17,7
2	Acid pantotenic (B5)	1,638	15,6	9,2
3	Tiamină (B1)	2,232	39,1	14,8
4	Pyridoxină (B6)	2,999	15,2	8,1
5	Niacină (B3)	4,221	22,6	14,2

1.Vitamin B2; 2.Vitamin B5; 3.Vitamin B1; 4.Vitamin B6; 5.Vitamin B3

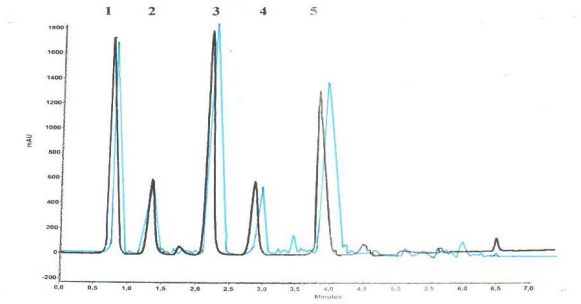

Fig.1. *The concentration of vitamins in the B complex (B1,B2,B3,B5,B6) in the SBFR yeast compared to the EC 118 reference sample*

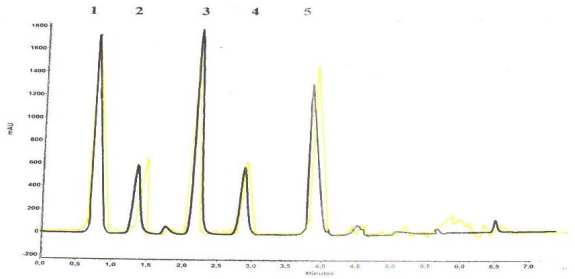

Fig.2. *The concentration of vitamins in the B complex (B1,B2,B3,B5,B6) in the SBRI yeast compared to the EC 118 reference sample*

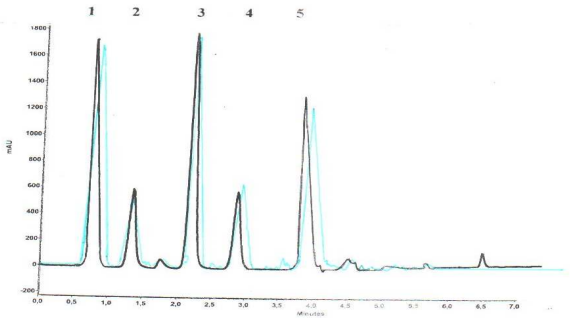

Fig.3. *The concentration of vitamins in the B complex (B1,B2,B3,B5,B6) in the SBIA yeast compared to the EC 118 reference sample*

The obtained results may be systematized for a statistical analisys regrarding the vitamins concentrations for the four yeast strains and the determination of the influence of the involved influences (table 4).

Table 5. *The determination of the influences of the involved factors regarding the vitamin concentrations for the yeast strains*

Vitamin \ Yeast	EC118	SBFR	SBIA	SBRI
Riboflavin (B2)	17.70	17.90	17.10	17.70
Pantotenic acid (B5)	8.20	8.50	8.40	9.20
Thiamine (B1)	14.80	14.10	14.70	14.80
Pyridoxin (B6)	7.90	7.70	8.00	8.10
Niacin (B3)	11.10	11.00	11.30	14.20

As the data presented in the above table presents constant values which were resulted at the end of each procedure, and not variable ones when measured sequencially, we cannot determine a mathematical model for the evolution of a characteristic depanding on the other. We can investigate two elements which present importance:

1) First, if the SBFR, SBIA and SBRI strains follow the reference sample EC118 pattern, vs. the concentration of vitamins;

2) Second, if the result from 1) is positive, the way the concentration of a vitamin influences the concentration of another.

In both cases we'll use the determination of the correlation coefficient, computed with the formula:

$$\rho_{XY} = \frac{\operatorname{cov}(X,Y)}{\sigma(X)\cdot\sigma(Y)}$$

where:

$$\operatorname{cov}(X,Y) = \frac{1}{4}\sum_{i=a}^{b}(x_i - M(x))(y_i - M(y))$$

is the covariance of the two characteristics, and

$$\sigma(X) = \sqrt{\frac{1}{4}\sum_{i=a}^{b}(x_i - M(x))^2} \quad \text{and}$$

$$\sigma(Y) = \sqrt{\frac{1}{4}\sum_{i=a}^{b}(y_i - M(y))^2}$$

are the square mean deviations, with x,y – discrete values of the X characteristics, respectively, Y, and M(x), M(y) – theyr means, i – the number of values which they take in the interval [a, b] (number of measurements).

1. In the first case, X is the vitamin concentrations for the referrence sample EC118, and Y is the concentrations for the other three yeast strains: SBIA, SBRI and SBFR (table 5).

Table 6. *The values of the coefficient of the yeast strains compared to the reference sample*

ρxy	Value	Description
EC118/SBFR	1.00	Maximum value; SBFR follows identically the pattern of the reference sample
EC118/SBIA	1.00	Maximum value; SBIA follows identically the pattern of the reference sample
EC118/SBRI	0.95	Very high value; the SBRI copies in a percentage og 95% the reference sample pattern

2. In the second case, X is the concentration of a vitamin, for all strains, and Y is the concentration of another vitamin, for all strains, again. The correlation coefficient is computed for all the combinations which can be obtained for each two of the vitamins (table 6).

Table 7. *The values of the correlation coefficient for two vitamin combinations*

ρxy	Value	Description
B2/B5	0.20	Low influence
B2/B1	-0.46	Medium influence, inversely
B2/B6	-0.51	Medium influence, inversely
B2/B3	0.11	Low influence
B5/B1	0.16	Low influence
B5/B6	0.55	Medium influence
B5/B3	0.95	Very high influence
B1/B6	0.87	High influence
B1/B3	0.44	Medium influence
B6/B3	0.74	High influence

4. CONCLUSIONS

The optimization of the fermentative process may be an important factor for the usage of selected cultures in the process of obtaining high quality wines.

From the statistical analisys we performed and the determination of the vitamins B1, B2, B3, B5, B6 in the studied strains, we can conclude that SBFR, SBRI and SBIA present indentical characteristics in a percentage of 98% compared to the reference sample EC118.

These results have as practical application, the usage of native strains, isolated from grapes from inland wineyards, so that we can preserve the specificity of the varieties and to lead to the decreasing of the similar imports.

It is recommended to select the valueable strains and to multiply them, so that we could have in our country selected yeasts for the wine, with native specificity.

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