

BIOTECHNOLOGICAL PROPERTIES OF FERMENTATIVE YEASTS OF THE GENUS *SACCHAROMYCES*

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ABSTRACT: Yeast strains studied are from collections of microorganisms of specialized centers, isolated and selected from spontaneous microflora and identified as belonging to the genus *Saccharomyces*. As a method of isolation and differentiation was used Koch method. In our study we made several analysis, such us: Determination of morphological characteristics of fermentation yeast, determination of physiological characteristics of yeast strains, fermentation capacity of the main sugars, ability of yeast to assimilate sugars, fermentation activity of yeasts by adding trehalose, using alcohol as the only carbon source, fermentation activity of yeast depending on temperature, fermentation activity of yeasts based on addition of vitamins. Physical characteristics of the cells depend on how and when of the division of nutritional substances held by cell. SCF 204, SCA 205, SCTS 206, SCHP309 and SET 102 have the dimensions between 4 and 7,58 μm and due to a smaller area, have a lower yield. Also, we can say that the addition of carbohydrate, respectively maltose content, promote positive capacity of the fermentation yeasts. Analysis of types of yeast studied, *Saccharomyces cerevisiae* shows the highest increase of fermentative capacity, and in this yeast sample with added 3% glycerol, glycerol register the maximum value recorded, respectively 19,459 g CO₂/g for dry substance and SCHDO 308. Following analyzes of selected yeast strains with temperature have revealed that favorable temperature fermentation activity is the 25° C, the most valuable being SCHP strain 309.

KEYWORDS: wine yeast, bakery yeast, brewer yeast, biotechnological properties

1. INTRODUCTION

Yeasts are, in terms of quality and economic the most important group of microorganisms that are used in production and marketing. Although technologies based on yeast continue to be used mainly in industries related to food and beverage products, there are various reasons for the initiation of new technologies.

In yeast technologies there is a remarkable past, but it is expected a future more impressive. To get a sorting that has certain characteristics that may be beneficial to exploited the knowledge required of characterization, classification and identification of yeasts. Only in this way can become – in the current scientific stage-a effective source of research with theoretical and practical applications. The theme of this paper is inscribed in the current study that concerns the biological and technological properties of yeasts used in production and technological processes that ensure high quality products, and how they can be controlled and conducted their metabolic activity.

Making industrial biotechnologies to obtain enzymatic preparations for extension of practical use have turned his attention of specialists focused on the identification of the microorganism capable of producing enzymes with catalytic properties as appropriate for the purpose for which it was obtained. With the help of enzymes produced by microorganisms it can speed up biochemical processes and improve production processes to improve food quality.

2. MATERIALS AND METHODS

Isolation and differentiation of fermentation yeast

Yeast strains studied are from collections of microorganisms of specialized centers, isolated and selected from spontaneous microflora and identified as belonging to the genus *Saccharomyces*. Were selected three strains of beer yeast (*Saccharomyces carlsbergensis*), of which the blank was acquired from the center of Freiburg (identified in this study with SCF 204), and two strains from the company Albacher

from Sebes (identified in this study with SCA 205) and Trei Stejari from Sibiu (identified in this study with SCTS 206).

Blank of *Saccharomyces cerevisiae* was purchased from the Biotechnology Research Center and Microbiological of Lucian Blaga University in Sibiu and was marked with

SCHCCBM 307. The other two strains come from Dr. Oetker (SCHDO 308) and from Pakmaya (SCHP 309).

Saccharomyces ellipsoideus blank comes from the Biotechnology Research Center and Microbiological of Lucian Blaga University in Sibiu and was noted with SEMCCBM 101, the other two strains I bought the wine center Jidvei (SEJ 103) and Târnavei (SET 102).

As a method of isolation and differentiation was used Koch method. This is based on the spread of microorganisms collected from natural environments in a nutrient medium and fixing cells detached from the solidification of the environment training by multiplication of colonies isolated from them.

Determination of morphological characteristics of fermentation yeast

To determine the morphological characteristics of yeast strains were made seeding techniques by loop on MMA medium. Yeast cells from an active young culture (24-48 hours) were seeded and incubated for 3 days at a temperature of 25 ° C.

It was also made with methylene blue staining of smears and yeast was identified at microscope using the object of immersion 90x.

Highlighting the capacity of training ascospores can be achieved by cultural methods or gypsum block method.

This character is of fundamental importance and allow to identify sporogenes or nonsporogenes yeast forms. The cultivation of yeast strains on Gorodkova medium and their incubation at 25 ° C for 3-5 days could be seen the presence

and number of ascospores and the shape and their position in asca.

Determination of physiological characteristics of yeast strains

Yeasts behave differently depending on the cellular osmotic pressure and osmotic pressure of the medium in which are the yeast cells. If the yeast cell is in a hypertonic medium (40% sucrose solution or 20% salt solution), yeast cells lose water, the cytoplasmic membrane is creasing and cell is in plasmolysis state. Then, the cell goes into a state of anaerobiosis and if this state is maintained, yeast cell dies. If the yeast cell is in hypotonic medium, it receives water, expands and moves into a state of turgidity when yeast cell splits.

Fermentation capacity of the main sugars

Is preparing a fluid medium from autolysate in which glucose is introduced whose fermentation will be tested, in concentration of 2%. The resulting environment is distributed into tubes with Durharm tubes and sterilize in autoclave at 120 ° C for 15 minutes. Of active yeast suspension (24-48 hours), take two drops and inoculate the medium which is incubated at 25 ° C. Observed daily for 10 days, if it releases carbon dioxide. The test is considered positive if the quantity of gas released is sufficient and if the bubble collected is to small than is testing with KOH to determine if CO₂ or air is released from solution.

Ability of yeast to assimilate sugars

To emphasize the ability to assimilate sugars yeast was used synthetic medium composition (g%): (NH₄) SO₄ - 0.5, KH₂PO₄ - 0.1, MgSO₄ 7H₂O - 0.05; agar - 2, add carbohydrates tested solution concentration of 2%. Sterilization is done in test tubes by autoclaving, at 120 ° C for 20 minutes. Basic medium with carbohydrates to be tested is poured into Petri dishes and allowed to solidify. The solidified medium are insemination with active loop of yeast cultures, the radial rays each culture and then incubated 3 days at 25 ° C. After 3 days incubation shows that the Petri dish, containing a carbohydrate, which develops only yeasts that assimilate carbohydrates.

To determine the replicative capacity of yeast using a multiplication medium and observed for 10 days the formation of biomass with biomass sensor. Daily total number of cells is determined by Thoma chamber.

Using alcohol as the only carbon source

In tubes with culture medium prepared with 3% ethanol, described in paragraph 1.1. is inoculated with a sterile loop to analyze the yeast suspension and incubated at 25 ° C for 3 days. The test is positive when the sediment is observed

(which involves cell growth), compared with the control sample without alcohol.

Fermentation activity of yeasts by adding trehalose

Trehalose for yeast is a backup energy source, and its accumulation occurs at every stage of multiplication. Lifespan of yeast cells depends on their content particularly trehalose, microorganisms showing stability in relation to external factors.

Yeast strains were selected:

Saccharomyces carlsbergensis: SCF 204 - blank, Saccharomyces carlsbergensis: SCA 205, Saccharomyces carlsbergensis: SCTS 206, Saccharomyces cerevisiae: SCHCCBM 307-blank test, Saccharomyces cerevisiae: SCHDO 308 in Saccharomyces cerevisiae: SCHP 309, Saccharomyces ellipsoideus: SEMCCBM 101 - blank test , Saccharomyces ellipsoideus: SEJ 103, Saccharomyces ellipsoideus: SET 102. Composition of media used in alcoholic fermentation is: Must the malt in g / l (MM): malt extract - 15.0, peptone - 1.0, maltose - 12.5, Dextrins - 2.5, potassium phosphate - 1.0, ammonium chloride - 1.0, pH - 4.8

Fermentation activity of yeast depending on temperature

To determine the fermentation ability of nine strains of yeast culture medium was used as the malt wort have been introduced 2 ml of inoculum, samples were fermented for six days at 22 ° C and 25 ° C. The amount of CO₂ released was measured periodically, aiming at development of pre-fermentation conditions.

Fermentation activity of yeasts based on addition of vitamins

To determine the fermentation ability of nine strains of yeast in the presence of exogenous vitamin B1 and B6 was used as culture medium MM malt mash, malt mash enriched with vitamin B1 (MM1) and malt mash enriched with vitamin B6 (MM2) which were introduced 2 ml of inoculum, samples were fermented for six days at 22 ° C. The amount of CO₂ released was measured periodically, aiming at development of pre-fermentation conditions.

3. RESULTS AND DISCUSSIONS

Results of determination of morphological characteristics of yeast strains

As a result of the analyses carried out yeasts Saccharomyces carlsbergensis formed colonies with a diameter between 2-4 mm on the MMA. Examined under the microscope the cells exhibited a spherical or oval shape, being singular or arranged in pairs, as can be seen in the figure below.

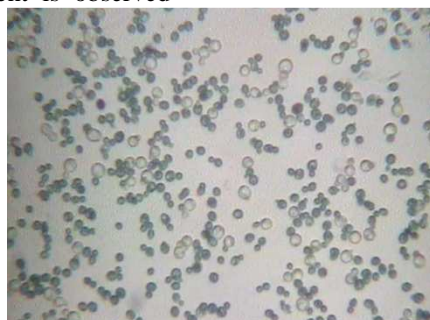


Figure 1. Saccharomyces carlsbergensis

Yeasts *Saccharomyces cerevisiae* observed under the microscope (native prepared) have an ellipsoid shape, white-gray. *S. cerevisiae* cells fall into three groups: the first group sizes are placed cells with dimensions 4,5 – 10,5 x 7-21 μ m, those in the second group have size 2,5 – 7 x 4,5 – 11 – 18,5 μ m, and the last group, covers cells with the smallest dimensions, 3,5-8 x 5 (11.5) – 17,5 μ m.

Yeasts *Saccharomyces ellipsoideus* takes the form of spherical cells, design, cylindrical, elongated, arranged in isolation or in pairs and occasionally form chains and agglomerates. The dimensions of the new strains of yeast have been identified and analysed centrally. Physical characteristics of the cells depend on how and when of the division of nutritional substances held by cell. SCF 204, SCA 205, SCTS 206, SCHP309 and SET 102 have the dimensions between 4 and 7,58 μ m and due to a smaller area, have a lower yield.

Results of determining the physiological characteristics of yeast strains

Results of determination of the main sugar fermentation capacity

The new strains of yeast have been seeded in culture medium of liquid malt mash at a temperature of 22 ° C for 3 days. Technological properties of yeasts have been studied from the point of fermentative test, in additional contact with maltose content of 5%, 10% and 15%. It can be seen that the fermentation yeasts varies depending on the amount of maltose content added, but also during fermentation. It can be seen that the greater amount of CO₂ that was recorded after the addition the content of 15% by adding maltose after a period of 72 hours. High values indicate that after 72 hours with the addition of 15% maltose content, yeasts increase their shelf life by superior, with a fermentative capacity of 45-55% higher compared with the blank.

Compared with the blank, yeast strains with the addition of 5% maltose content registered a higher fermentative activity, as the yeast *Saccharomyces ellipsoideus* versus the other two types of yeast.

Thus conclude that the addition of carbohydrate, respectively maltose content, promote positive capacity of the fermentation yeasts.

Results on the yeast's ability to assimilate sugars

It is known that yeasts are facultative anaerobic. Under conditions of aerobiose sugars are assimilated to carbon dioxide and water, and thus a large amount of energy required for rapid growth and multiplication. The pH established and recorded was 4.5 – 6.5. The data obtained shows that the addition of sugar inhibits easy fermentative activity due to the quantity of sugar in excess which cannot be fully assimilated by yeast. Sugar content as a percentage of 5% constitutes an added moderated which results in differences of about 20-30% between the work of the blank and the fermentative of the sample.

Unlike *Saccharomyces ellipsoideus* and *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae* as a result of the addition of sugar and maintained at a temperature of 27 ° C recorded higher values, demonstrating a high fermentation capacity.

Results on alcohol use as the only source of carbon in the activity of yeast strains

The ability of yeasts to ferment in environments with high osmotic pressure depends on the correlation between total product glycerolul and retained by yeasts. To highlight the effect of fermentative yeasts on preservation activity has added glycerol in a proportion of 1%, 2% and 3%. Glycerol added the biggest concentration after 3 days produced a significant growing to the fermentative capacity of the yeasts. This is due to partial or total period of the lag that occurs in the production of CO₂, needed the biosynthesis of glycerol. Analysis of types of yeast studied, *Saccharomyces cerevisiae* shows the highest increase of fermentative capacity, and in this yeast sample with added 3% glycerol, glycerol register the maximum value recorded, respectively 19,459 g CO₂/g for dry substance and SCHDO 308.

Results on the fermentation activity of yeasts by adding trehalose

Biotechnological properties of yeasts have been investigated from the viewpoint of fermentative capacity of nine strains of yeast in contact with exogenous trehaloses added in content of 5 and 10%.

It was used as a culture medium in which malt must have been introduced by 2 ml of inoculum, samples are fermented for six days at 22° C. The quantity of CO₂ was measured periodically, while pursuing the development of fermentation in default conditions.

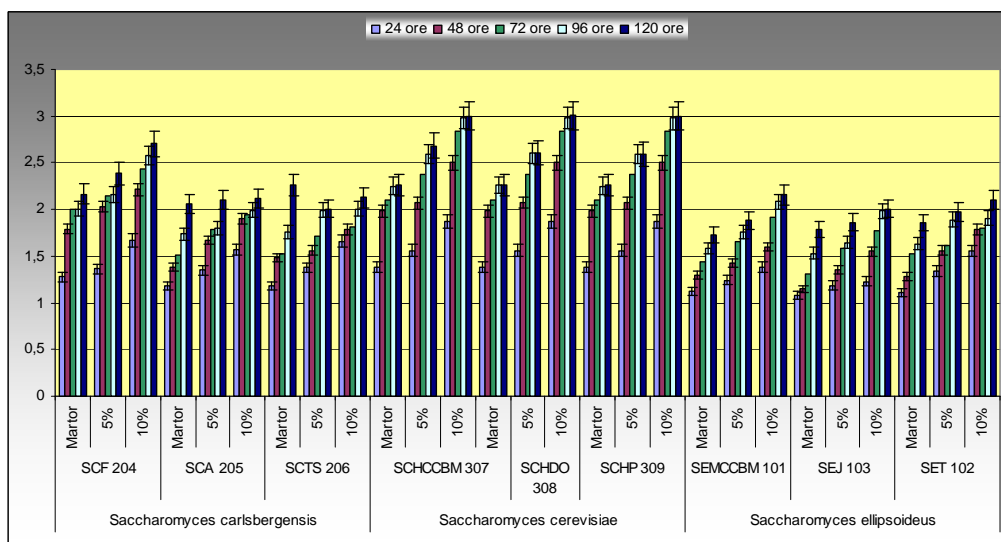


Figure 2. Evolution of *Saccharomyces* yeast strains activity with exogenous addition of 5% and 10% trehalose

Results on yeast fermentation activity with temperature.

Following analyzes of selected yeast strains with temperature have revealed that favorable temperature fermentation

activity is the 25 ° C, the most valuable being SCHP strain 309, as can be shown in the figure below:

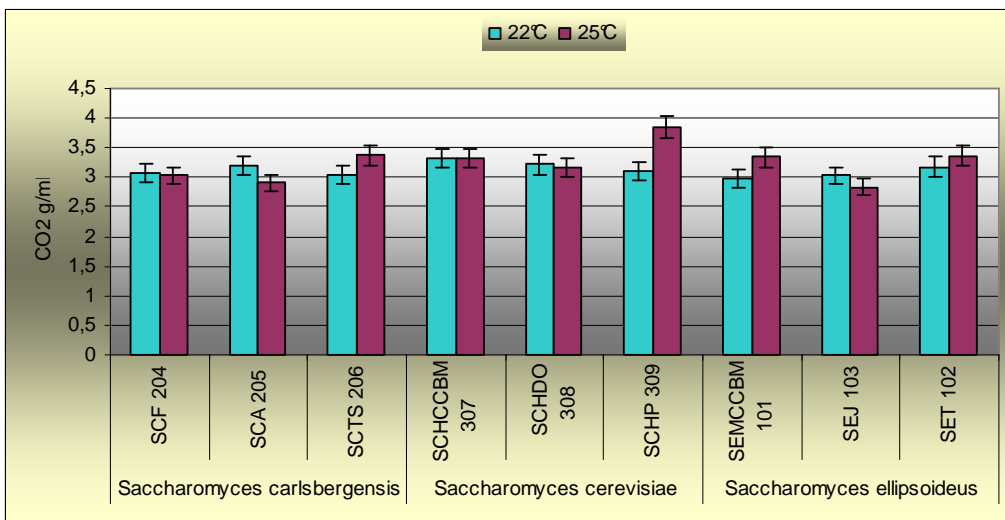


Figure 3. Evolution of the fermentative activity of yeast strains to temperature 22 ° C and 25 ° C.

Results on yeast fermentation activity depending on the addition of vitamins

Fermentation activity of yeast was analyzed according to the addition of vitamins in malt wort medium. As can be seen in Figure 4, concentration

and type of vitamins influences the fermentative activity of yeasts, such addition of vitamin B1 for the most efficient yeast strain was SCHDO 308, with a total release of CO₂ from 3.426 g/100 ml . SET-102 strain of the highest fermentative activity in culture medium enriched with vitamin B6

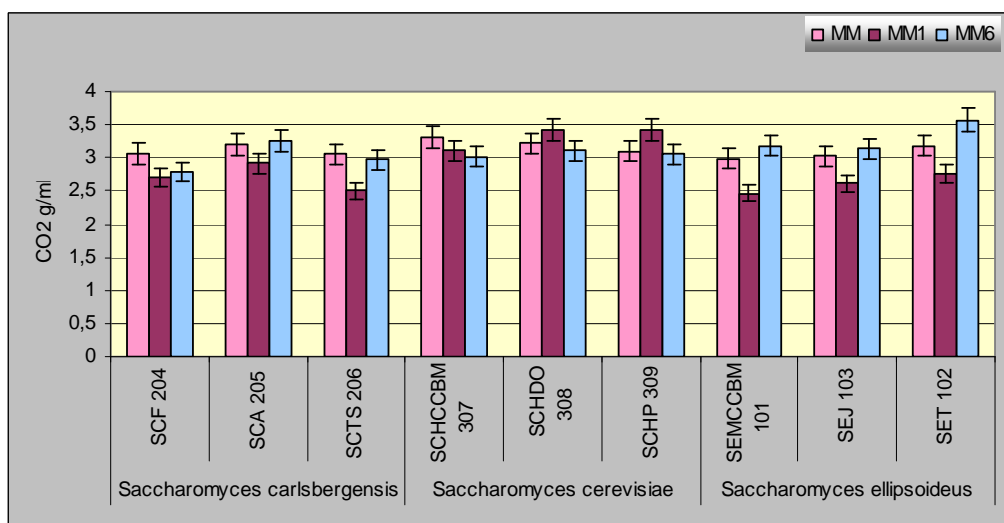


Figure 4. Dynamics fermentative activity of yeast strains in MM, MM1 and MM6

4. CONCLUSION

Saccharomyces cerevisiae yeast were observed microscopically ellipsoidal shape, white-gray, and *Saccharomyces ellipsoideus* yeast takes the form of spherical, ellipsoidal, cylindrical, elongated cells, arranged singly or in pairs and occasionally form chains and clusters.

Physical characteristics of cells depend on the mode and time division held cell nutrient was found that strains.

It was found that the strains SCF 204, SCA 205, SCTS 206, SCHP309 and SET 102 have the dimensions between 4 and 7,58 μm and due to a smaller area, have a lower productivity.

The addition of vitamins in malt wort medium, lead to a sharp dynamic fermentative process of yeast strains.

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