# PRACTICAL APPLICATIONS OF YEAST STRAINS WITH SUPERIOR BIOTECHNOLOGICAL PROPERTIES

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**ABSTRACT:** From collections of microorganisms of specialized centers yeast strains were studied, isolated and selected from spontaneous microflora and identified as belonging to the genus *Saccharomyces*. In our study we made several analysis, such us: Determination of biochemical composition of yeast strains- determination of moisture and dry, determination of ash, determination of lipids by the gravimetric method, the concentration of yeast cells by direct methods. The average dry yeast within the three types of such: for *Saccharomyces carlsbergensis* determined by SU average is between 32-35%, for *Saccharomyces cerevisiae* is between 34-36%, and *Saccharomyces ellipsoideus* SU is between 31-33%. The analysis performed shows that the average percentage of ash for yeast samples taken is between: *Saccharomyces carlsbergensis* ash 5-11%, for *Saccharomyces cerevisiae* is 8-9 %, the average value determined for Saccharomyces ellipsoideus ash is 13%. The percentage of crude protein for yeast strain taken was: for *Saccharomyces carlsbergensis* 50-54%, for *Saccharomyces cerevisiae* 40-50%, for *Saccharomyces ellipsoideus* 60%. There is a higher amount of lipids in the case of beer and wine yeasts, compared with yeast dough. Assessment of the level of activity of microbial populations is required in biotechnological research and determining the microbial load is an important indicator to watch. *Saccharomyces ellipsoideus* yeast strain, unlike other types of yeast, have the greatest number of cells / ml of sample analyzed, respectively 37 cells / ml sample (SET 102).
**KEYWORDS:** wine yeast, bakery yeast, brewer yeast, biotechnological properties.

## 1. INTRODUCTION

The group generically called "wine yeast" includes species of the genus *Saccharomyces,* which can be active in the grape, characterized by high concentration in sugars and acid pH, fermented yeast capable of producing higher concentrations of 10%.vol. alcohol and which are adapted to doses of sulfur dioxide added to the must to control the fermentative process. It was established that the main agent of alcoholic fermentation of grape must is *Saccharomyces cerevisiae* Hansen, variety *ellipsoideus* (Hansen) Dekker, after the nomenclature established by Lodder and Kreger -VANRIJ, 1952 ( Anghel et al., 1991).

The first bottom fermentation beer yeast were isolated in pure culture of the Carlsberg Laboratory (Denmark) by E.C. Hansen (1908), culture considered a distinct species, *Saccharomyces carlsbergensis*. From taxonomic point of view this species is identical with *Saccharomyces uvarum* (wine yeast) and the new name was accepted for yeast with lower fermentation, a period of decades. As the use of new investigative techniques, it was established that the differences between *Saccharomyces uvarum, Saccharomyces carlsbergensis* and the yeast called original *Saccharomyces cerevisiae* classic are insignificant, that now, taxonomiții include all three in one species, *Saccharomyces cerevisiae* and for respect of tradition and convenience, bottom fermentation yeast (for beer of type "lager", "Pilsen") return to the name of *Saccharomyces carlsbergensis*, and for beer of type "ale", top fermentation *Saccharomyces cerevisiae*. To the fermentation of dough is using yeast belonging alcooligene of the genus *Saccharomyces* (Meyer) Rees and the species *Saccharomyces cerevisiae*. (Oprean, 2002, Oprean et al,2009a.) In terms of quality, yeasts may have different capacities to adapt to the environmental conditions offered in bakery technology. Yeast cells found in cake or dry yeast are surrounded by air and to maintain vital functions they assimilate by breathing the intracellular compounds reserve (glycogen and trehalose) through enzymes, components of respiratory chain to end products ($CO_2$ and $H_2O$). While the cells are suspended in water, leaven, dough, occurs anaerobic condition which requires "connection and switch" in the breathing process of fermentation (Tița et al, colab).

## 2. MATERIALS AND METHODS

Determination of biochemical composition of yeast strains

Determination of moisture and dry. (Oprean, 2002)

Determination of water content of a product can be achieved by direct methods, determining the water content itself, or by indirect methods, determining the product dry. Water content in the latter case is determined by the difference between material weighed before drying and dry weight.

Determination of ash.

Ash is determined from the dry yeast to constant weight by calcination at a temperature of 800°C. The calculation is based on the difference between the weight of the capsule before and after calcination, reporting is performed at 100 g yeast.

Determination of lipids by the gravimetric method (Soxhlet method).

Lipids of different foods are solubilised and extracted with organic solvents (ethyl ether, petroleum ether, etc..) After removal of solvent, the residue obtained weighed. Leaching and lipid extraction is performed using Soxhlet apparatus.

Counting methods and techniques of yeast.

Yeast cells and mold spores may include, by direct microscopic examination, with citometrelor (Thoma counting chambers). For counting place a drop of cell suspension for analysis on the central platform in the right area delineated. Suspension is placed over a slide which is supported by two side platforms and thus between cytometer slide and create a liquid film height equal to the drum of the central platform (0.1 mm).

Studied in a preparation microscope objective magnification x 40, when the microscopic field can be viewed a group of 16

elementary squares, which are cells whose surface is more than half of 4x4 squares within which elementary. Cell are counts from several microscopic fields and calculated the average number of cells on a basic square. Number of cells present in a cc of suspension to be analyzed are determined by the formula:

$N = n \cdot 4 \cdot 10^6 \cdot k$

where: n-average number of cells on a square elementary; k-factor of dilution;

Practical applications of yeast strains with superior biotechnological properties Yeast *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus a*re able to drive the alcoholic fermentation so by the biotechnology dominant properties were selected the strains: SCF 204-marked B1, SCTS 206-marked B3, SCHCCBM 307- marked P1, SCHP 309- marked P3 , SEMCCBM 101- marked V1 and SET 102 – marked V3 noted to obtain high quality wine products.

Biomass (in malt wort and incubated five days at 22°C) of the six strains was centrifuged at 4500 r / min. The supernatant was separated from the biomass, which was washed with distilled water. Biomass of the six strains of yeast were subjected autolysed for 24 hours in the thermostat at 50°C (autolysis is a process in which cells produce their own lytic enzymes, is a slow process that takes place in a period of order of days, which is an inconvenience. Yeast autolysis, a process carried out on an industrial scale to obtain autolysed yeast, requires 12 to 24 h at 45-50°C), plasmolysis in 20% NaCl for 24 h (in dissolving chemical are salts, sugars, acetic esters, compounds that induce plasmoliza, extracting intracellular material, generally without distorting it).(Oprean, 2003, 2005) Yeast autolysated and plasmolysed have undergone: - the alcoholic fermentation, to determine the ability of fermentation of the six strains of yeast, was used as culture medium malt wort MM, in which were introduced 2 g of biomass for six days at 22°C – on the accumulation of biomass (BĂNĂDUC et.al, 2011, 2012).

## 3. RESULTS AND DISCUSSIONS

Results of determining the biochemical characteristics of yeast strains.

Results of determination of moisture and dry yeast strains.

From the figure below shows that the average moisture within the three types of yeast as follows: - for *Saccharomyces carlsbergensis* water percentage is between 65-68% - for *Saccharomyces cerevisiae* water percentage is between 64-66% - for *Saccharomyces ellipsoideus* water percentage is between 67-69%.(Pietro et al., 2006),

Mean dry matter, as Figure 1, within the three types of such yeast: *Saccharomyces carlsbergensis* average value determined for dry matter is between 32-35% for *Saccharomyces cerevisiae* determined average dry matter is between 34-36 % average value determined for *Saccharomyces ellipsoideus* dry matter is between 31-33%.

***Results of determination of ash and crude protein of yeast strains.***

Ash is determined from the dry yeast to constant weight by calcination at a temperature of 800°C. The calculation is based on the difference between the weight of the capsule before and after calcination, reporting is performed at 100 g yeast. Of the total amount of crude protein, only a certain percentage is the "crude protein" (natural). If the nine yeast strains analyzed crude protein contains between 60 and 90% crude protein, which shows high biological value of pure protein. The average percentage of crude protein for this strain of yeast taken range from: the average value determined for Saccharomyces carlsbergensis crude protein is between 50-54%; for Saccharomyces cerevisiae determined average crude protein is between 40-50%; for Saccharomyces ellipsoideus average value determined for the crude protein is 60%.(OLOSUTEAN et al,2011, Oprean et al, 2009 b.)

***Results of determination of lipid strains of yeast***

Lipids or practice what is known as "crude fat" is on average 2-5% of dry matter, but in some yeasts (Endomyces vernalis, Torulopsis lipofera), lipid amount can reach 80% of dry matter. Extraction using ethanol / chloroform, we obtained the following results: Analyzing Figure 10 shows a larger amount of fat in the case of beer and wine yeasts, compared with yeast dough.(

***Results of assessing the concentration of yeast cells by direct methods (Thoma counting chamber)***

To highlight yeast alive to be used staining method of the preparation with 0.1% methylene blue solution. By this method the dead cells will stain blue, and the living will remain uncolored or weakly colored. The ratio of live and dead cells is an indicator of quality yeast culture: the quality is good if the percentage does not exceed 5%. The following figure are results from analysis of Saccharomyces yeast suspension

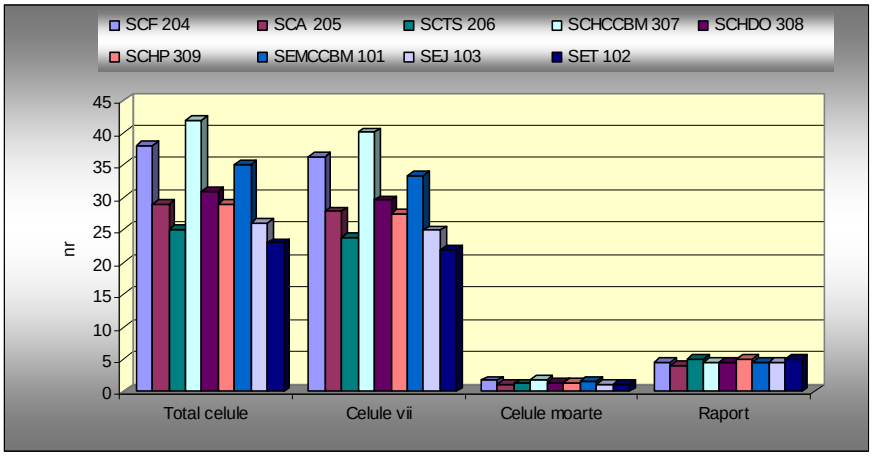


**Figure 1.** Total yeast cells during fermentation of beer, bread and wine

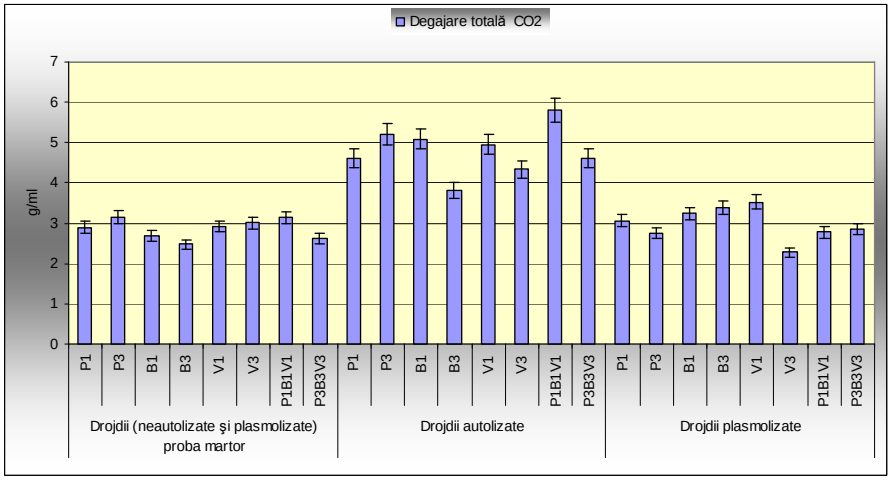


**Figure 2.** Evolution of the fermentative activity of yeast autolysates and plasmolizate by the blind

In this procedure counts were performed in three stages of development of yeast, namely: the first day of incubation at 22 ° C, the third day of incubation and end process on the sixth day.

Yeast *Saccharomyces carlsbergensis, Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus* are able to drive so that the properties of alcoholic fermentation biotechnology dominant strains were selected: SCF 204-marked B1, B3 SCTS 206-marked, marked SCHCCBM 307-P1, P3 SCHP 309-noted SEMCCBM 101-102-SET marked V1 and V3 noted to obtain high quality wine products.(Oprean, 2005, 2006).

As shown in Figure 3, showed the yeast autolysates increased fermentative activity, of which the most valuable evidence is P1B1V1, a direct proportion of the three selected yeast strains

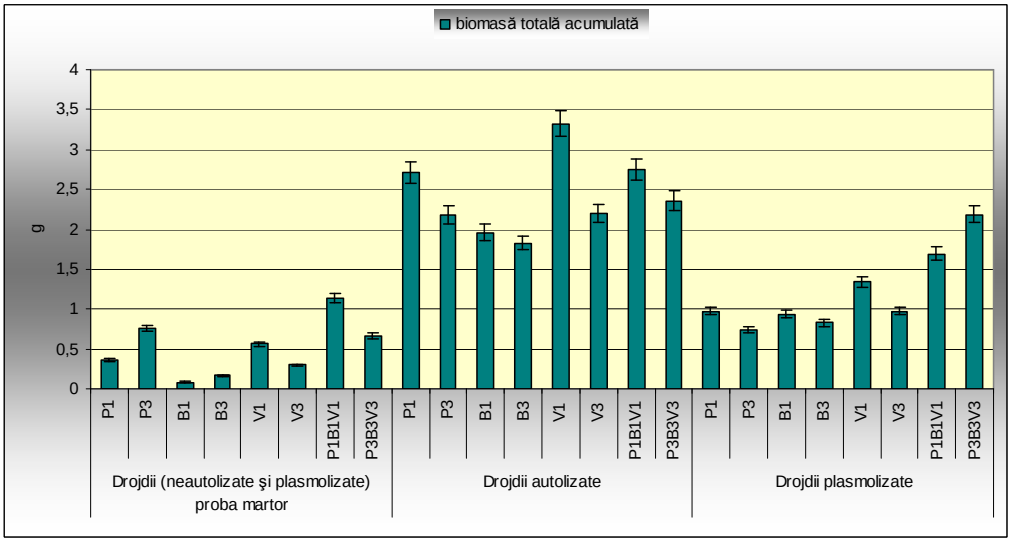


**Figure 3.** The amount of biomass accumulated during the fermentation of yeast and plasmolizate autolysates by the blind

Biomass (in malt wort and incubated five days at 22°C),of the six strains was centrifuged at 4500 r/min. The supernatant was separated from the biomass, which was washed with distilled water. Biomass of the six strains of yeast were autolysed for 24 hours in the thermostat at 50°C. The amount of accumulated biomass was recorded by yeast autolysated, V1 is the most valuable strain (Pop et.al, 1999, 2002).

## 4. CONCLUSIONS

Following the determinations made, the average dry yeast within the three types of such: for *Saccharomyces carlsbergensis* determined by SU average is between 32-35%, for *Saccharomyces cerevisiae* due to SU average is between 34-36%, and the average value determined by *Saccharomyces ellipsoideus* SU is between 31-33%.

The analysis performed shows that the average percentage of ash for yeast samples taken is between: the average value determined *Saccharomyces carlsbergensis* ash is between 5-11%, for *Saccharomyces cerevisiae* due to ash average is between 8-9%, the average value determined for *Saccharomyces ellipsoideus* ash is 13%.(Tița et al, 2010).

The percentage of crude protein for yeast strain taken so determined as follows: for *Saccharomyces carlsbergensis* determined average crude protein is between 50-54%, for *Saccharomyces cerevisiae* determined average crude protein is between 40-50%, the average value determined for *Saccharomyces ellipsoideus* crude protein is 60%. (Oprean, et al, 2010; Tănăsescu,C. 2009).

The analysis of the three types of yeast *Saccharomyce* there is a higher amount of lipids in the case of beer and wine yeasts, compared with yeast dough (Tofan et al, 2002).

Assessment of the level of activity of microbial populations is required in biotechnological research and determining the microbial load is an important indicator to watch.

*Saccharomyces ellipsoideus* yeast strain, unlike other types of yeast, have the greatest number of cells / ml of sample analyzed, respectively 37 cells / ml sample (SET 102) (Oprean et.al, 2010,2011).

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