OPTIMIZATION OF INVERTASE PRODUCTION BY YEAST STRAINS FROM THE GENUS *SACCHAROMYCES*

— research paper —

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Abstract: This paper investigates the influence of vitamins and zinc acetate on the synthesis of the enzyme invertase by nine yeast strains belonging to the genus *Saccharomyces*, namely species *Sacch. carlsbergensis (*beer yeast), *Sacch. cerevisiae* (bread yeast) and *Sacch. ellipsoideus* (wine yeast). From each yeast species, one strain was provided by collections of specialized centers and other two strains were isolated and selected from industrial microbiota belonging to the genus *Saccharomyces*. Invertase activity of yeast strains of different origins was determined by using the Schoorl chemical method. Three solutions for the improvement of the cultivation broth were tested: the addition of a vitamins complex (1 ml /l), the addition of 5 mg/l zinc acetate and the addition of both vitamins complex and zinc acetate. The results obtained in this study show that the invertase activity of the nine strains of yeast *Saccharomyces* studied increase with the supplementation of the nutritive substrate with each of the solutions tested. The highest invertase activity is obtained on a culture medium enriched with vitamins complex combined with $(CH_3COO)_2Zn$, followed by vitamins complex; the addition of zinc acetate doesn't influences greatly the enzyme production. No big differences in the enzyme synthesis are observed for yeasts from the same specie. As invertase producer, the yeast SCHCCBM 307 (from the Biotechnology and Microbiology Research Center at Lucian Blaga University in Sibiu) was the best on the control substrate (malt wort) and on the substrate enriched with both vitamins and acetate and the yeast SEJ 103 (from the Jidvei Center) was the best on media enriched only with vitamins.

Key words*:* Yeast, vitamins, malt wort, $(CH_3COO)_2Zn$

INTRODUCTION

As a result of their metabolic activity, yeasts of the genus *Saccharomyces*

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form several categories of products which include structural constituents of cell addition, extra and intracellular enzymes (Zarnea et al., 1984). Enzymes are formed during growth of microorganisms (trophophase), as a result of oxidative metabolism and aerobic fermentative. Trophophase corresponds to the period of rapid multiplication and accumulation of biomass in growing cell culture media rich in nutrients and adequate physical conditions (Bahrim, 1999).

Invertase is produced by various strains of microorganisms, but of these, yeasts, especially *Saccharomyces cerevisiae* and *Saccharomyces carlbergensis* have become the most widely used to obtain enzyme preparations (Bahrim, 1999). Even within the same culture of yeast invertase exists in several forms. For example, intracellular invertase has a weight of 135 000 Daltons, while extracellular invertase has a weight of 270 000 Daltons (National Research Developement Corporation, 2003).

Very important for the potential of yeast to produce invertase is the composition of culture medium and the physical growth conditions. Regarding the substrate composition, it was found that invertase formation by cells is greatly stimulated by the presence of sucrose (Bisson et al., 1998).

This research investigates the action of some enzymes and of zinc acetate on the production of enzyme invertase by three types of yeasts belonging to the *Saccharomyces* genus, namely *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus* obtained from specialised laboratories and strains isolated from industrial sources. The chemical method Schoorl was used to evaluate the influence of the selected compounds on the potential of yeasts strains to produce invertase.

MATERIALS AND METHODS

Materials

Three yeast strains were chosen: *Saccharomyces carlsbergensis (*beer yeast), *Saccharomyces cerevisiae* (bread yeast) and *Saccharomyces ellipsoideus* (wine yeast). From each yeast type, two kinds of strains were analysed: strains aquired from the collections of specialized centers (serving as blind samples) and strains isolated and selected from the spontaneous microbiota identified in the lab as belonging to the genus *Saccharomyces*.

The blind sample *Sacch. carlsbergensis,* was purchased from the Freiburg Center (identified in this study as SCF 204), the other two strains were delivered by the companies Albacher Sebes (abreviated in this study as ACS

205) and Trei Stejari Sibiu (noted in this study as SCTS 206).
The blind sample *Sacch. cerevisiae* was purchased from the Biotechnology and Microbiology Research Center at Lucian Blaga University in Sibiu and was noted by SCHCCBM 307. The other two strains come from Dr. Oetker (SCHDO 308) and from Pakmaya (SCHP 309).
The blind sample *Sacch. ellipsoideus* came from the Biotechnology and Microbiology Research Center at the University Lucian Blaga of Sibiu and was noted SEMCCBM 101. The other two wild strains of *Sacch. ellipsoideus* were purchased from the Jidvei Center (SEJ 103) and Târnavei (SET 102).
Yeasts were cultivated on malt wort, which is the natural source of nutrients, especially because 90-92% of dry matter is carbohydrates (Oprean, 2003).

Experimental design

In order to analyse the influence of different compounds on the production of invertase and the invertase activity of yeast, four tests were made:
1. No other additions (control sample);
2. Addition of a vitamins complex in the malt wort, with the concentration of 1 ml/l. Based on the requirements for growth, vitamins tested were: thiamine (5 mg / 5 ml), riboflavin (2 mg / 5 ml), pyridoxine (2 mg / 5 ml), nicotinamide (20 mg / 5 ml) and D panthenol (3 mg / 5 ml).
3. Addition to malt wort of zinc acetate $(CH_3COO)_2Zn$, 5 mg/l;
4. Addition in malt wort of both the vitamins complex and $(CH_3COO)_2Zn$ in the same concentrations as they were added in the previous experiments: 1 ml/l, respectively 5 mg/l.

Extraction of the enzyme from yeasts

Because the intracellular invertase is an enzyme that does not diffuses across the membrane, the enzyme was obtained by extraction from the compressed yeast. For this, 10 g of compressed yeast were well mixed with sand for 10-15 minutes to destroy the cell walls; 30 ml distilled water and 0.5 ml toluene (as antiseptic) were added. The mixture was maintained for 2 hours at 37°C for autolysis of cells which were not destroyed by mixing. The sample was mixed again, bringed to 100 ml with distilled water and then centrifuged at 4000 rpm for 20 minutes. The solution obtained contains active invertase and can be kept refrigerated for 3 - 4 days.

Determination of invertase activity

The Schoorl method was used to determine the reducing carbohydrates formed as a result of invertase action on sucrose of known concentration (Mironescu and Mironescu, 2000).
Reactives used were: sucrose 2% solution buffered to pH 4.5 with acetic acid; sodium acetate solution 0.2 m; carbohydrates reagents, toluene.
Two samples were considered for each analysis: a sample with 1 ml solution extracted from yeast (as described before) and a sample with 1 ml solution extracted from yeast which was boiled for enzyme inactivation. In both balloons 10 ml 2% sucrose solution were added, mixed and then maintained for 30 minutes at 37° for enzymatic reactions. After 30 minutes, 9 ml water was added in each ballon in order to reach the final volume of 20 ml and mixed well. The reducing sugars formed after hydrolysis were determined with the Schoorl method, by using 5 ml from each sample. The amount of 0.1 N sodium thiosulphate used in titration of the samples was determined and from the special Schoorl Table the amount of inverted sugar (day, in mg) corresponding to sucrose hydrolysed in 5 ml sample taken into the analysis was determmined.
Invertase activity was expressed as μmoles of sucrose hydrolysed in a minute by 1 ml enzyme preparation or 1 g yeast, in the working conditions gave:

$$\text{Invertase activity} = \frac{0{,}95 \cdot z.i}{342 \cdot 10^{-6} \cdot c \cdot t} \cdot d \text{ , } \mu \text{ mol / g yeast}$$

where:
c - the amount of enzyme extract taken in analysis, ml (c = 1 ml);
I.S. - the amount of invert sugar produced by the enzyme, g;
d - dilution (d = 40);
t - the maintaing time, minutes (t=30).
0.95 - factor to transform the invert sugar expressed as sucrose;
$342 \cdot 10^{-6}$ - 1 μmole sucrose, g.

RESULTS AND DISCUSSIONS

The results obtained at the analysis of the invertase activity of yeast strains of beer, brewer and wine according to the culture medium are shown in Figure 1. In the case of yeasts of the genus *Saccharomyces*, the potential to synthesise invertase is dependent on the composition of culture medium.
The gradual increase in invertase activity of the nine yeast strains reveals that the enrichment of culture medium has a strong influence, with positive results on the invertase synthesis by all studied microorganisms.

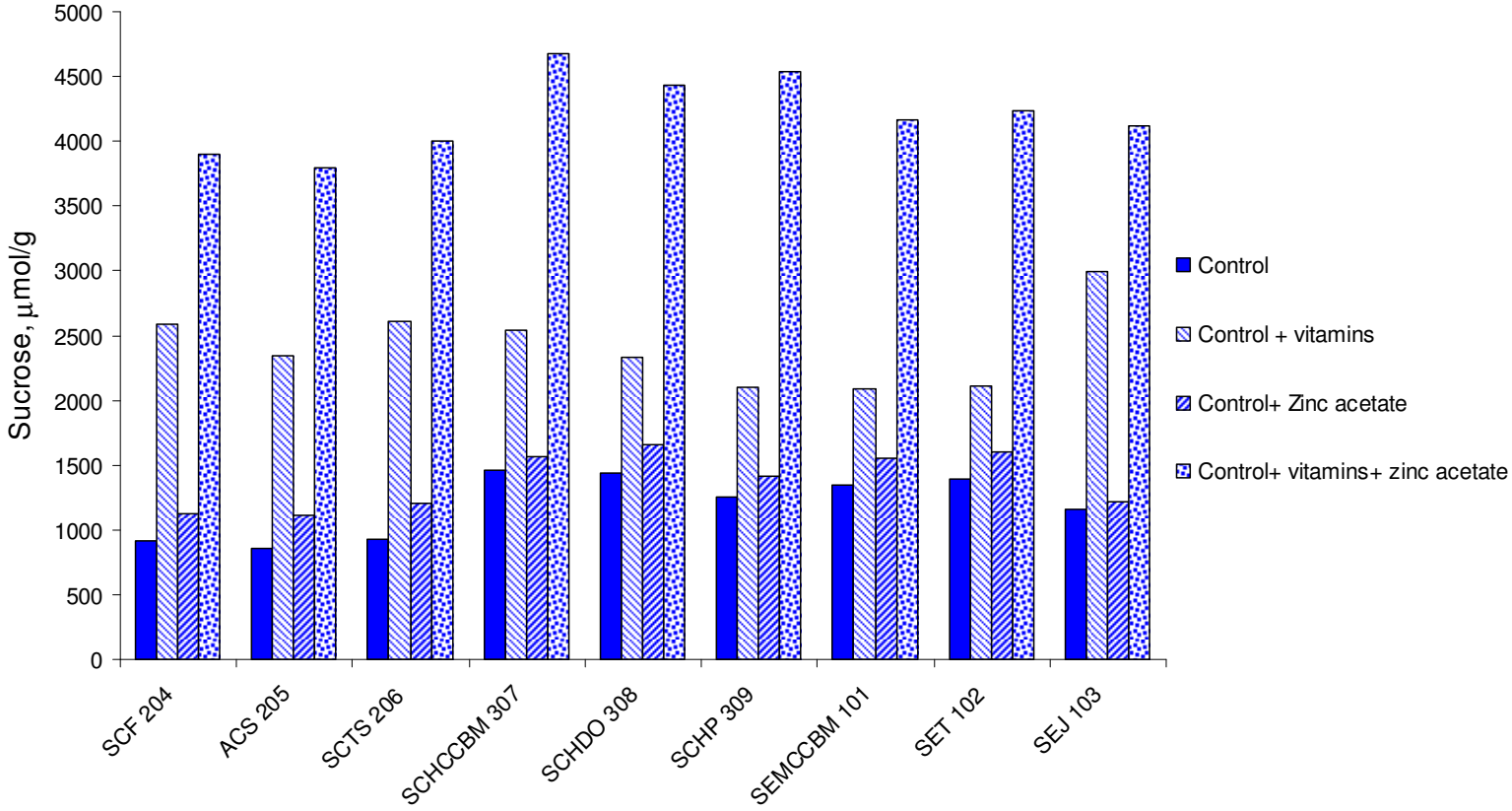

Figure 1. Invertase activity of yeast strains *Saccharomyces* analysed in media with vitamins complex, with $(CH_3COO)_2Zn$ and with combined vitamin complex and zinc acetate in comparison with the control sample

As shown in Figure 1, the best results are obtained when the yeast strain grows on medium enriched with vitamins complex and zinc acetate, followed by those grown on medium enriched with vitamins.
The addition of only zinc acetate doesn't influence very much the production of invertase by the studied yeasts.
A comparison between the nine strains of yeast regarding the influence of vitamin complex + $(CH_3COO)_2Zn$ shows that the most powerful strain is SCHCCBM 307 (4678 μmol / g yeast) with the largest increase (1.23 times) than SCA 205 strain showed the lowest value of all nine strains (3789 μmol / g yeast), as shown in Figure 1.
In conditions of complex addition of vitamins and zinc acetate in the malt wort, the invertase activity of yeast strains is increased 4.26 times for SCF 204 compared to blank, from 4.42 times to 4.31 times in the case of FAS 205 and 4.31 times for SCTS 206. Yeast strain selected from this criterion as the better invertase producer is SCA 205, which is *Sacch. carlsbergensis* provided from Albacher company, Sebeş.
If SCHCCBM strain 307 has an increase of 3.21 times higher than the control sample, SCHDO 308 has an increase of 3.07 times and SCHP 309 strain increased by 3.60 times compared with the blind sample. The strongest growth invertase activity is recorded for SCHP 309, a strain of *Sacch. cerevisiae* from Pakmaya company.
In the case of wine yeasts, the invertase activity of strain SEMCCBM 101 increases by 3.09 times higher than the control sample, for SET 102 times 3.04 and 3.55 for SEJ 103 times (Figure 1). The largest increase is recorded by strain SEJ 103 (3.55 times the control sample), a strain isolated from valuable features wine center Jidvei.
The invertase activity described by the yeast strains of beer, bread and wine grown on malt wort medium enriched with vitamins complex, with zinc acetate and vitamins complex + $(CH3COO)_2Zn$ shows the maximum increased:

- for the brewers' yeast strains, the strain SCA 205 isolated from Albacher company from Sebeş grew by 4.42 times more than the blind sample;
- for the baking yeast strains, the strongest growth of invertase activity was recorded for the strain SCHP 309, isolated from Pakmaya company, with an increase of 3.07 times more than the control sample;

- ❖ the strain SEJ 103 increased by 3.55 times more than the control sample, a strain with valuable features isolated from the wine center Jidvei.

Analysing the results obtained, the average amount of invertase (μmol / g yeast) recorded by all nine strains of yeast is 4205 μmol / g yeast.

CONCLUSIONS

The methods chosen in this research for enriching the cultivation substrate with different growth factors have been positive, the yeast invertase activity increased significantly for all samples, compared with the blind samples.

Between the nine yeast strains analysed, the best invertase-producing strain was SCHCCBM 307 (4678 μmol sucrose consumed/g yeast) with an increase of 1.23 times more than the SCA 205, strain which recorded the lowest value of all nine strains (3789 μmol / g yeast).

Mean values of invertase produced are depending on the composition of culture medium:

- for the malt wort medium the average value was 1194 μmoli sucrose consumed/g yeast;
- for the malt wort medium with vitamins complex the average value was 2411 μmoli sucrose consumed /g yeast;
- for the malt wort medium with zinc acetate the average value was 1384 μmoli sucrose consumed /g yeast;
- for the malt wort medium with vitamins complex and with zinc acetate, the average value was 4205 μmoli sucrose consumed/g yeast, the best result.

The amount of invertase obtained by growing yeast strains on the malt wort medium with vitamins complex + $(CH_3COO)_2Zn$ is higher than the control sample or the other media enriched with only vitamins complex or zinc acetate.

REFERENCES

1. Bahrim G., Microbiologie tehnicã, Editura Evrika, Brăila, 1999
2. Bisson L., Coonsd, Kruckeberg, A., Lewis, D.A., Yeast sugar transporters, Crit. Rev. Biochem. Mol. Biol., 1998, 28, 259-308.
3. Mironescu V., Mironescu M., Obţinerea şi asigurarea calităţii produselor zaharoase, Editura Universităţii "Lucian Blaga" Sibiu, 2000
4. Oprean L., Microbiologia şi controlul calităţii microbiologice a alimentelor, Ed. Univ. „Lucian Blaga", Sibiu, ISBN 973-651-608-3, 2003, p. 226.

5. Zarnea G., et al., Bioingineria preparatelor enzimatice microbiene, Editura Tehnică, Bucureşti, 1983.