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# THE STATISTIC EXPERIMENTAL SHAPING OF INFLUENCE OF MEDIUM FACTORS UPON THE QUANTITY OF INVERTASE PRODUCED BY *SACCHAROMYCES* YEASTS

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## Abstract

*As a result of the microbian metabolism complexity and of the special sensibility of living cells depending on the medium culture conditions, but, also due to the variable quality of nutritive medium compounds, the elaboration of the biotechnological processes became a necessity. Therefore the statistical shaping is very useful tool in research activity or for conceiving and leading the fermentative processes.*

***Keywords**: culture medium, yeast, wort extract, efficiency, wheat germs, statistical, dispersion*

## Introduction

The microorganism's activity and behavior can be affected by medium conditions such as *p*H variations or different concentration of nutrients. The conceiving of a mathematical model presumes identification and quantification of biological and physical processes exerting a special influence upon microorganisms' evolution in a certain context. For this purpose it is necessary to know and check the biochemical parameters that influence the growth of Saccharomyces yeast (Anghel, 1991; Bahrim, 1999).

The statistic processing has in view to establish some correlation, between quantity of yeasts invertase and simultaneous action of the two quantitative factors chosen: the quantity of wheat germs and the quantity of wort extract. For this purpose a series of culture medium were conceived, trough concentration variations of two nutritive sources chosen as studying factors like independent variables.

In order to establish the best correlation the factorial experiments were carried out and the results were performed trough the regress equation.

## Experimentals

As a source of yeast was used baker yeast (*Saccharomyces cerevisiae)* from ROMPAK S.A. with 32.5% dry matter, and 46.54% protein content (N · 6.25). As essential medium for the yeast′s growing it was used industrial medium adapted to the laboratory conditions: 40cm$^3$ wort, 0.08g $(NH_4)_2HPO_4$, 0.08g $KH_2PO_4$, 0.02g $Mg(NO_3)_2$ and 0.02g $KNO_3$ (Segal, 2000).

Starting to this medium a series of culture medium were conceived trough concentration variations of the two nutritive sources chosen (wheat germs and wort extract). The growth of the yeast was studied in the same conditions of time, temperature, *p*H and stirring.

The general work scheme was: the baker yeast sizing at $10^6$ cells / cm$^3$ was suspended in 40cm$^3$ nutritive medium, in aseptic conditions. The tests were maintain on mechanical agitator (230 rot/min), at 30°C and pH = 4.5 for 24 hours. Then the tests were centrifuged 25 minutes at 4000 rot/min for obtaining the biomasses. The obtained biomasses were studied following the determination of humidity and dry substance.

In order to appreciate the degree of increasing of commercial strain of yeast in the work conditions we determinate growth by global evaluation of biomasses forming with determination of optical density at $\lambda$ = 600 nm, initial and at the end of cultivation.

One unit of invertase activity represents the number of inverted sugar micromoles released by hydrolytic action of one cm$^3$ crude enzyme preparation (or 1 g d.m.), during one minute in the following conditions: 20% sucrose as substrate, 0.02 M acetate buffer pH = 4.6, at 45°C.

## Results and Discussions

This study supposed a regression and correlation analysis. The regression is studying the type of dependence between variables, and correlation measures the degree of this dependence on (Pop, 2004).

It had in view the following characteristics:

A = quantity of invertase
$x_1$ = quantity of wheat germs
$x_2$ = quantity of wort extract

First, it was studied linear simply dependence, like:

$$A = A(x1); \quad A = A(x2);. \qquad [1]$$

The data selection had in view is shown in table 1.

**Table 1.** The variation of invertase quantity depending on the two variable take separately

| Nr.crt. | $x_1 = x_2$ | A(x1) | A(x2) |
|---|---|---|---|
| 1. | 0.05 | 2754 | 2695 |
| 2. | 0.10 | 3814 | 3064 |
| 3. | 0.15 | 4516 | 3772 |
| 4. | 0.20 | 6234 | 4093 |
| 5. | 0.25 | 6235 | 4100 |
| 6. | 0.30 | 6242 | 4105 |
| 7. | 0.35 | 6247 | 4109 |

In a rectangular axes system, the pair of experimental dates ($x_i$, $y_i$) represents a crowd of points, which can approximate a line, named regression line of Y characteristic in relation with independent variable X.

The linear dependence between A and $x_1$ or $x_2$ can be generic restore by an equation like this:

$$y = a + b \cdot x \qquad [2]$$

Where the regression coefficients *a* and *b*, are calculated with *the smallest square points method of Gauss.*

By analog method we can define a regression line of X characteristic in relation with independent variable Y. The equation is:

$$x = \alpha + \beta \cdot y \qquad [3]$$

The coefficient of correlation, independent on the measurement units of characteristics is a quantitative expression of a linear dependence.

The formula for this is founded in specialty literature:

$$r_{xz} = \frac{N\sum_{i=1}^{N} X_I Y_I - \sum_{i=1}^{N} X_I \cdot \sum_{i=11}^{N} Y_i}{\left[N\sum_{i=1}^{N} X_i^2 - \left(\sum_{i=1}^{N} X_i\right)^2\right]\left[N\sum_{i=1}^{N} Y_i^2 - \left(\sum_{i=1}^{N} Y_i\right)^2\right]}, \qquad [4]$$

The results of regression and correlation analysis are shown in table 2.

**Table 2.** The results of simple linear regression and correlation analysis

| $y = a + b \cdot x$ | The coefficient a | The coefficient b | Coefficient of correlation |
|---|---|---|---|
| $A = A(x1)$ | 12181±2557 | 2712±571 | 0.9052 |
| $A = A(x2)$ | 4751±1177 | 2755±263 | 0.8748 |

The conclusion is between the variables compared exists a certain linear dependence because the correlation coefficients had values over 0.85. Those dates can replace, in good measure, the effectuation of a great number of experiments.

The next step was to evaluate statistic the linear multiple dependences, like:

$$A = A(x1, x2) \qquad [5]$$

This study it was necessary for evaluate the influence of simultaneous action of both factors. The results are shown in table 3.

Linear multiple dependence between A and ($x_1$ and $x_2$) can be related by equation like:

$$y = a + b \cdot x1 + c \cdot x2, \qquad [6],$$

were the coefficients of regression a, b and c, are calculated with *the smallest square points method of Gauss.*

The results of regression and correlation analysis are shown in table 4.

**Table 3.** Bidimensional dependences

| Nr.crt. | x1 | x2 | A=A(x1,x2) |
|---|---|---|---|
| 1. | 0.05 | 0.05 | 19070 |
| 2. | 0.10 | 0.10 | 23729 |
| 3. | 0.10 | 0.10 | 24275 |
| 4. | 0.10 | 0.10 | 26488 |
| 5. | 0.15 | 0.15 | 29836 |
| 6. | 0.15 | 0.15 | 27979 |
| 7. | 0.20 | 0.20 | 29287 |
| 8. | 0.20 | 0.20 | 23845 |
| 9. | 0.20 | 0.20 | 32519 |
| 10. | 0.20 | 0.20 | 37930 |
| 11. | 0.25 | 0.25 | 37925 |
| 12. | 0.30 | 0.30 | 37936 |

**Table 4**. The values obtained for the coefficients of liner multiple regression

| $y = a + bx_1 + cx_2$ | Coefficient a | Coefficient b | Coefficient c | Coefficient of multiple correlation |
|---|---|---|---|---|
| $A = A(x1, x2)$ | 1.5248 | 5.3722 | 3.1789 | 0.8986 |

Through the evaluation of quantity of yeasts invertase it was established for the polynomial equation obtained a respond surfaces variation. In order to establish the optimal composition of culture medium it was made a spatial interpolation obtaining the special dependence from figure 1.

The respond surface present in figure 1 permitted to establish the optimal concentration of nutrients for which we obtain maximal values of invertase activity of yeasts biomass biosynthesis.

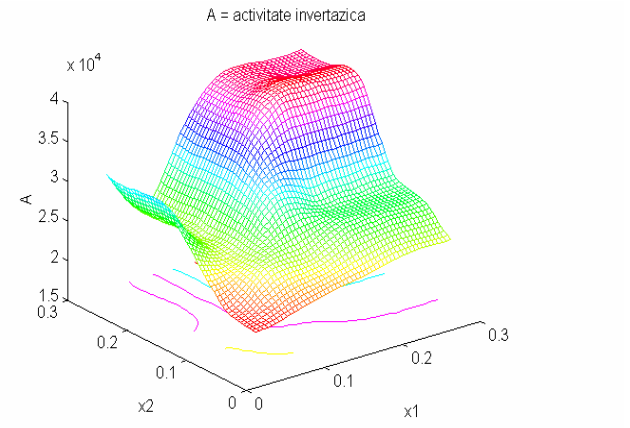


**Fig. 1.** The evolution of correlation between wheat germs concentration and wort extract in medium composition and yeasts invertase activity

## Conclusions

After the statistical study were selected the factors which have an essentially influence upon the invertase activity. Results that two independent variables in the fermentative medium are essential for carbon and nitrogen, minerals, growing factors contributions and the fact that they have a favorable influence upon qualitative and quantitative point of view on the increasing the biomass quantity of baker yeast *Saccharomyces cerevisiae.* The results of statistical shaping are shown that the optimal concentration for the increase of quantity yeasts biomass are 4.75 g/cm$^3$ wheat germs and 3.75 g/cm$^3$ wort extract.

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