STUDIES ABOUT THE GLUCOOXIDASE ACTIVITY DURING WINE FERMENTATION

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Abstract
It was studied the glucose oxidase activity during wine fermentation using UV-VIS spectrophotometer kinetic method. It was shown that the glucose oxidase activity decreased strongly after ten days of fermentation.
Key words: glucose oxidase, wine, fermentation

INTRODUCTION

Glucose oxidase (β-^{D}-glucose: oxygen 1-oxidoreductase; EC 1.1.2.3.4) catalyzes the oxidation of β-D-glucose to gluconic acid, by utilizing molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide.

Glucose oxidase is widely used for the determination of free glucose in body fluids (diagnostics), in vegetal raw material, and in the food industry. It also has many applications in biotechnologies, typically enzyme assays for biochemistry including biosensors in nanotechnologies. It is often extracted from *Aspergillus niger*.

GOx is a dimeric protein, the 3D structure of which has been elucidated. The active site where glucose binds is in a deep pocket. The enzyme, like many proteins that act outside of cells, is covered with carbohydrate chains (figure 1).

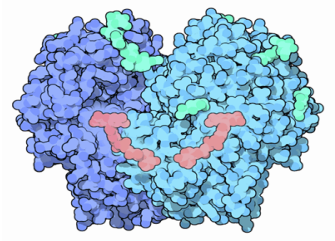

Fig. 1. Glucose oxidase [1]

MATERIALS AND METHODS

The glucose oxidase enzyme is coupled with a second peroxidase enzyme system - o-dianisidine according to the reaction:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{GOx} \text{D-Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{o-Dianisidine reduced} \xrightarrow{HRP} \text{o-Dianisidine oxidated} + H_2O$$

The hydrogen peroxide oxidizes o-dianisidine in presence of peroxidase to form a colored product, which is a colorimetric test at 460 nm.

Reagents:

1. Glucose solution, 200 mg / ml
2. Acetic acid buffer solution -sodium acetate 0.1 M, pH 5.5
3. o- Dianisidine solution1%
4. Horse radish peroxidase 60 U / ml prepared in buffer 2
5. Glucose oxidase 1-4 mg / ml isolated from Aspergillus niger
6. Must in different days of fermentation

In two spectrophotometer cuvettes are introduced the reagent as presented in *Table 1*.

Table 1. Work mode

Reagents	Sample	Standard
O-dianisidine 1% (ml)	2,6	2,6
Glucose (ml)	0,2	-
HRP (ml)	0,1	0,1
GOx (ml)	0,1	-
DW (ml)	-	0,2

The optical density of the sample with enzyme is determined at 540 nm and compared to a blank at time zero and in 10 seconds intervals for 10 minutes.

Results calculation

The change in optical density at 460 nm is plotted versus time on the initial linear portion of the curve.

The specific enzyme activity is expressed in units /mg of protein and take into account the dilution factor applied.

$$\frac{D.O._{\cdot sample} \times 5 \times 0{,}0182}{D.O._{\cdot standard}}$$

The glucose oxidase activity on glucose is represented in figure 2.

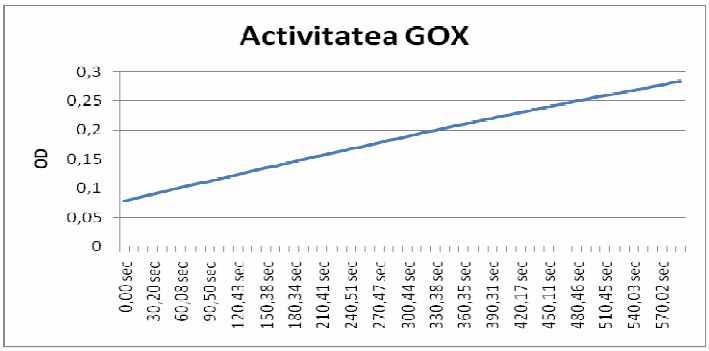

Fig.2 The glucose oxidase activity on glucose

RESULTS AND DISCUSSIONS

It was studied the glucooxidase activity in the first ten days of must fermentation.

Fermented mash samples were made at Bay Zoltan Institute for Biotechnology, Szeged and then were analyzed spectrophotometrically at the University of Oradea (Spectrophotometer Specord 210 Plus UV-VIS Analytic Jena and the software Win ASPECT PLUS).

The grape samples were physically crushed to make must by a fruit press (Green Star GS-1000). 3 l red must were placed to two fermenters, which were incubated at 21±2°C. In these fermenters we could continuously monitoring the pH, temperature and dissolved oxygen.

Samples of must were not sterilized before fermentation. For the determination of glucooxidase activity were taken samples at the starting point and for the first six days of fermentation.

The experiments were made as presented in *Table 2*.

Table 2. The determination of glucose oxidase activity in wine fermentation

Reagents	Sample	Standard
O-dianisidine 1% (ml)	2,6	2,6
Must * (ml)	0,2	-
HRP (ml)	0,1	0,1
GOx (ml)	0,1	-
DW (ml)	-	0,2

*Must in different days of fermentation

The experimental results are presented in figure 3.

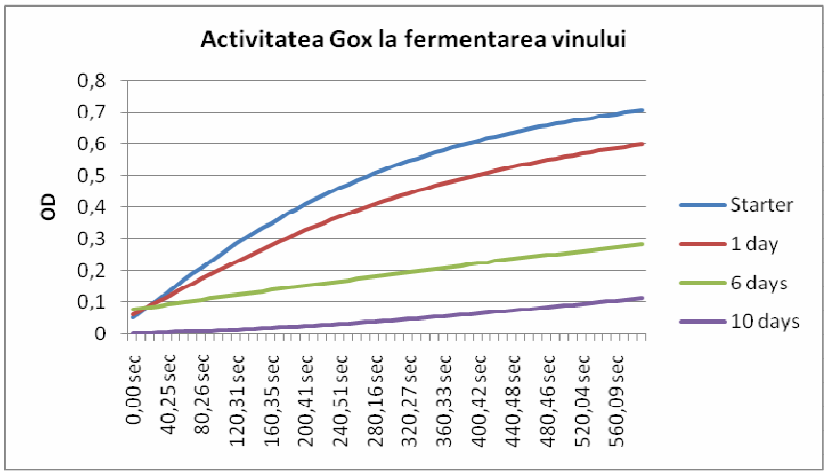

Fig.3 Glucooxidase activity in ten days of wine fermentation

CONCLUSIONS

Glucooxidase activity is related with the glucose content in must fermentation. In the first days of fermentation the Gox activity is higher but after ten days of fermentation it strongly decreased.

Aknowledgements

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