INFLUENCE OF NUTRITIVE SUBSTRATE ON ACTIVITY OF SOME HYDROLASES FROM FUNGUR *RHIZOPUS STOLONIFER*

INFLUENȚA SUBSTRATULUI NUTRITIV ASUPRA ACTIVITĂȚII UNOR HIDROLAZE LA CIUPERCA *RHIZOPUS STOLONIFER*

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Abstract*. Amylases are some of the most important industrial enzymes that have a wide range of applications. Although they come from different sources and have different patterns of action, in industry are mainly produced by microorganisms. Enzymes from microbial sources meet industrial demands due to their higher efficiency and thermostability. The purpose of this study is to investigate the influence of nutrient substrate represented by grinded cereal caryopses on α amylase, β amylase and γ amylase activity from saprophytic fungus Rhizopus stolonifer. Enzymatic determinations were made using culture liquid of fungus, at 3 time intervals: 3, 6 and 9 days. After data interpretation it was observed that the activity of the three enzymes was influenced by the nature and concentration of nutritive substrate and by fungal culture age. Thus, the highest values were obtained for glucoamylase in barley medium variants at concentration of 30 g/l in the first time period.*
Key words*: Rhizopus stolonifer, amylases, cereal caryopses*

Rezumat*. Amilazele sunt unele dintre cele mai importante enzime industriale, ce au o gamă largă de aplicații. Deși acestea provin din diverse surse și prezintă modele diferite de acțiune, în industrie sunt produse în principal din microorganisme. Enzimele din surse microbiene îndeplinesc cerințele industriale datorită randamentului lor superior și a termostabilității. Scopul acestui studiu constă în investigarea influenței substratului nutritiv reprezentat de cariopse de cereale măcinate asupra activității α amilazei, β amilazei și γ amilazei la ciuperca saprofită Rhizopus stolonifer. Determinările enzimatice au fost realizate, utilizând lichidul de cultură al ciupercii, la 3 intervale de timp: 3, 6 și 9 zile. În urma interpretării datelor s-a observat că activitatea celor trei enzime a fost influențată atât de natura și concentrația substratului nutritiv cât și de vârsta culturii. Astfel, cele mai ridicate valori au fost obținute în cazul glucoamilzei la variantele de mediu cu orz la o concentrație de 30 g/l în primul interval de timp.*
Cuvinte cheie*: Rhizopus stolonifer, amilaze, cariopse de cereale*

INTRODUCTION

Amylases are some of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. These enzymes

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represent approximately 30% of global enzymes production (Sivaramakrishnan et al., 2006). Although they come from different sources (plant, animals, microorganisms) and have different patterns of actions (Hagenimana et al., 1992), in industry are produced mainly from microorganisms. Enzymes from microbial sources meet industrial requirements due to their higher efficiency and thermostability. Due to the increasing demand for these enzymes in various fields of human activity, there is interest in the development of enzymes with superior proprieties such as raw starch degrading amylases suitable for industrial applications and their production techniques with low cost (Burhan et al., 2003).

Extent of involvement of biological catalyst in various industry fields, agriculture, environmental protection, medical and environmental diagnosis, development of medicinal remedies based on enzymes, and/or their activators, the obtain and use of renewable sources of energy and biofuels motivates the importance and actuality of investigation that are related to the selection of new active producers of enzymes and development of modern processes for increase/stabilize their biosynthetic capacity (Ciloci et al., 2011).

Many *Rhizopus* species are capable of producing α amylases and glucoamylases, and are important in the industrial production of glucoamylase and production of various alcoholic beverages (Mertens and Skory, 2007; Soccol et al., 1994; Higgins, 1995).

Various chemical and physical factors are affecting growth and amylase production, such as temperature, pH, incubation period, moisture, agitation, but above all the cultivation medium composition (carbon and nitrogen sources). Interactions of these parameters can have a significant influence on the enzyme production (Sivaramakrishnan et al., 2006; Grata et al., 2008). To meet the growing demands of the industries is required amylase production with low cost. Synthetic media are very expensive and unprofitable, so they must be replaced with agricultural byproducts more economical and available which are considered to be good growth substrates for amylases producing microorganism (Kunamneni et al., 2005; Saxena and Singh, 2011). The objective of this study was to monitor the influence of nutritive substrate represented by grinded cereal caryopses from three species: wheat, corn and barley on α, β and γ amylases activity produced by fungus *Rhizopus stolonifer*.

MATERIAL AND METHOD

Microorganism used for conducting experiments - *Rhizopus stolonifer* was isolated from germinated cereal caryopses. For this purpose were used three species of cereals: wheat, corn and barley. Wheat and corn caryopses came from the storage place of the Enterprise of Cereal Products from Chișinău, Republic of Moldova and the barley caryopses were taken from a private household in Grebleşti village, from Străşeni aria, Republic of Moldova. The fungus was inoculated in the form of 8 mm in diameter discs on liquid Leonian medium (K_2HPO_4 1.25 g, $MgSO_4$. $7H_2O$ 0.625 g, peptone 1 g, glucose 20 g, distilled water 1000 ml) (Constantinescu, 1974), from whose composition carbon source – glucose was replaced with different amounts of grinded cereal caryopses, resulting three medium variants: V1 with 10 g/l, V2 with 20 g/l and V3 with 30 g/l, plus a control version where medium composition remained

unchanged. Liquid, stationary cultures of *Rhizopus stolonifer* were incubated under the dark conditions at 28 C. Enzymatic determinations were made at three time intervals: 3, 6, and 9 days using fungus culture liquid. The determination of α and β amylases was performed using the Noelting-Brenfeld method (Artenie VI., et al., 2008), and the γ amylase measurement was made using dinitrosalicylic reagent method (Cojocaru, 2009). Enzyme activity was reported to the amount of total soluble protein determinate by Bradford method (Artenie et al., 2008). Experiments were performed in triplicate and for graphical representation of the data averages were calculated.

RESULTS AND DISCUSSIONS

The use of carbohydrates as carbon source is a common practice in the microbial fermentation process. Composition and concentration of the medium plays an important role in the growth and productions of extracellular α amylases in fungi and bacteria. The rate at which carbon sources are metabolized can often influence the production of biomass or production of primary or secondary metabolites from microorganisms (Zhou and Jiang, 1991; Laoide and McConel, 1989; Srivastava and Baruah, 1986).

The influence of nutritive substrate on the α amylase activity determined at three time intervals in culture liquid of *Rhizopus stolonifer* is illustrated in figure 1. In the first time interval α amylase values are quite high, the highest values being observed in medium variants with barley caryopses and the lowest in those with corn. Maximum value was recorded in variant V3 from barley (1.2115 UA/mg protein). There is no definite correlation between nutritive substrate concentration and enzyme activity, except for wheat samples where α amylase activity increases with cereal caryopses amount from culture liquid. In the next time interval there is a decrease in enzyme activity in all experimental variants, regardless of nutritive substrate used, except variant V3 from corn, where α amylase activity intensifies (0.5965 UA/mg protein). In the last time interval a slight increase in enzyme activity takes place, maximum value being observed at V3 variant form barley (0.6682 UA/mg protein).

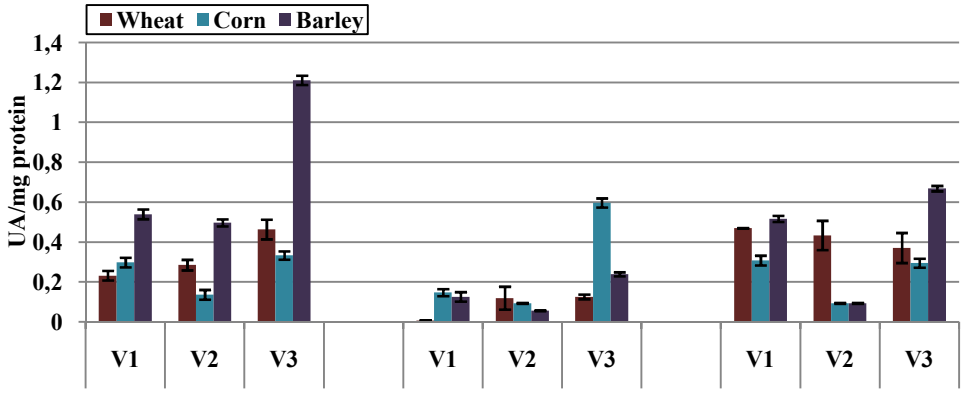

Fig. 1 - Influence of nutritive substrate on the α amylase activity in fungus *Rhizopus stolonifer*

As shown in figure 2, β amylase activity recorded significantly lower values compared with α amylase activity, and in many of the variants could not be detected. In the first time interval was possible to measure enzyme activity only in 3 experimental variants (V2 wheat – 0.0591 UA/mg protein, V2 corn – 0.2109 UA/mg protein, V3 corn – 0.221 UA/mg protein) and only in 4 in the next 2 time intervals (at 6 days: V1 wheat – 0.1986 UA/mg protein, V1 barley – 0,0863 UA/mg protein,V2 corn – 0.2325 UA/mg protein, V2 barley – 0.0231 UA/mg protein; at 9 days: V1 corn – 0.0725 UA/mg protein, V2 corn – 0.1025 UA/mg protein, V2 barley – 0.1864 UA/mg protein, V3 corn – 0.0891 UA/mg protein). Maximum value was observed after 6 days of incubation at a 20 g/l concentration of grinded corn caryopses.

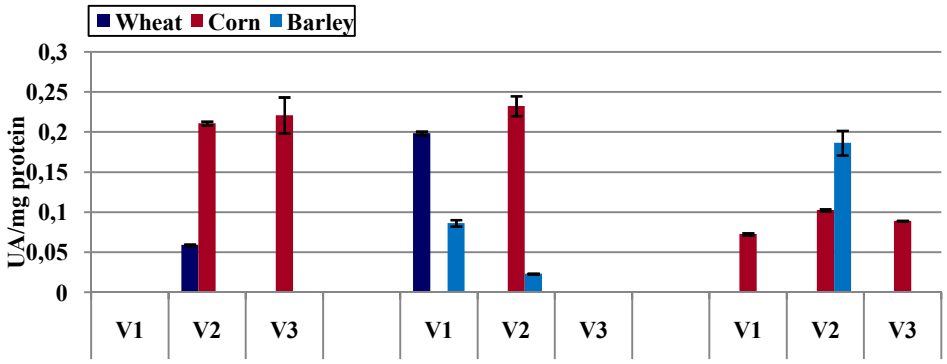

Fig. 2 - Influence of nutritive substrate on β amylase activity in fungus *Rhizopus stolonifer*

γ-Amylase activity recorded in the first time interval the highest values for all medium variants regardless of the nature of nutritive substrate used (fig. 3). Maximum activity was recorded in variant V3 barley (27.553 U/ml/mg protein), and the minimum in variant V1 wheat (9.089 U/ml/mg protein). There is no obvious correlation between concentration of cereal caryopses and the enzyme activity in most experimental variants, except medium variants with barley, where glucoamylase activity increases with nutritive substrate concentration. Although the higher is amount of grinded caryopses, the higher is starch concentration, the amylase production may not follow this pattern and maximum amylase activity, maximum biomass and protein concentration can be obtained at different concentrations of nutritive substrate. We could obtain the maximum biomass amount at a higher concentration of nutritive substrate, but not a maximum amylase activity (Ayogu and Amadi, 2010). In the next time interval enzyme activity decreases in most work variants, except variant V3 (26.012 U/ml/mg protein) from corn, where a slight increase in enzyme activity takes place. Lowest values are also recorded in medium variants with wheat caryopses. In the last time interval enzyme activity decreases more. The highest value was recorded in variant V1 from corn (9.111 U/ml/mg protein) and the lowest in variant V1 from barley (0.5242 U/ml/mg protein).

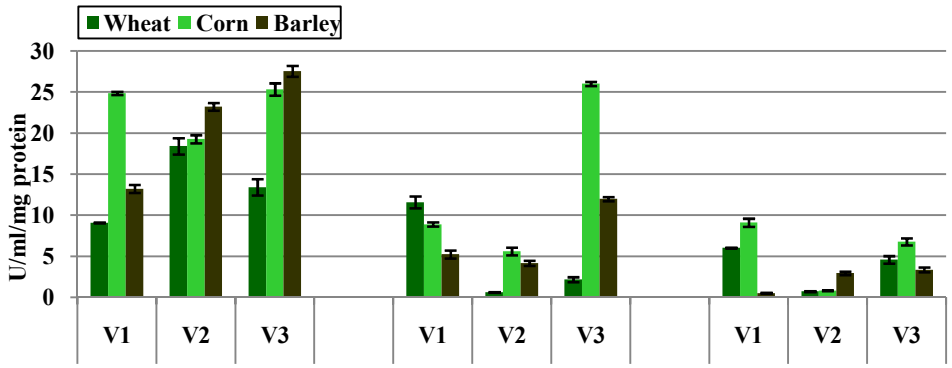

Fig. 3 - Influence of nutritive substrate on γ amylase activity in fungus *Rhizopus stolonifer*

CONCLUSIONS

Most favorable nutrient substrates for amylase synthesis are represented by grinded barley and corn caryopses, as the highest values of the three studied enzymes activity were observed in medium variants that contained barley and corn caryopses, and the maximum value of enzymatic activity was recorded for glucoamylase at a 30 g/l concentration after three days of incubation. Also fungal culture age had a strong influence on amylase synthesis, thus the highest activity was observed after three days of incubation.

Fungus *Rhizopus stolonifer* is not a good β amylase producer, the activity of this enzyme recording low values throughout the experiment, and in many variants enzyme activity could not be detected.

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