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Effect Of Medium Composition On Enzymatic Protein Hydrolysis In Heterogeneous Systems

Zakirova Muyassar[1*] , Zairova Khulkar[1] , Artikova Rano[2]

[1]Tashkent Pharmaceutical Institute, Aybek str., 45, 100015 Tashkent, Uzbekistan

[2]Tashkent State Agrarian University, University str., 2, 100140 Tashkent province, Uzbekistan

Abstract: At present, the use of enzyme preparations is an integral part of most industrial and agricultural processes.

In the process of fermentation of multi-component raw materials, enzymes undergo denaturation under the influence of various factors, such as thermal inactivation, which depends not only on temperature, but also on the presence of substances of different nature, formed after hydrothermal treatment of the raw materials.

In complex substrate mixtures, the introduction of alcohols into the medium can regulate the adsorption and stable state of neutral proteinases. This allows to remove the negative effect of insoluble particles of reaction mixture.

Keywords: enzyme, fermentation, thermal inactivation, grain, sugar must, alcohol, denaturation, melanoidines, oximethylphurphural, yeast, adsorption, proteinase.

Introduction

At present, in the field of biotechnology, there is a growing trend of transition to resource-saving technologies, taking into account the specifics of the production industry.

The main production factors negatively affecting the yield and quality indicators of the product in alcohol production are high temperature conditions at hydrothermal treatment of grain, poor quality of sugar must, extreme technological parameters of fermentation for yeast (relative to nitrogen nutrition), etc.

In the process of fermentation of multi-component raw materials, enzymes undergo denaturation under the influence of various factors, such as thermal inactivation, which depends not only on temperature, but also on the presence of substances of different nature, formed after hydrothermal treatment of the raw materials. For example, many compounds (melanoidines, oxymethylphurphurol, etc.) can also cause inactivation of enzymes under conditions where thermal inactivation does not occur or occurs at a low rate, in addition, these processes are accelerated with temperature rise.

As our experiments have shown, during thermal treatment of the raw materials due to the process of denaturation of accompanying protein substances and melanoid formation an even more heterogeneous complex substrate mixture is obtained.

In such complex substrate mixtures, a certain part of enzymes, due to the nonspecific interaction with insoluble particles in the medium, have been bound.

This leads to the fact that in alcohol production used enzymes (including proteinases) in the fermentation process lose their activity due to interaction with various components of the processed raw materials, moreover, this process is accelerated with increasing temperature.

Therefore, during the development of effective ways to use enzymes, we have made an attempt to recognize the mechanisms of inactivation of a particular type of enzymes, in particular proteolytic enzymes of microbial origin, in various conditions.

Materials and methods

Materials We used proteolytic enzyme from bacteria microorganisms – Bacillus amyloliquefaciens (Neutraza, "Novozymes", Denmark).

Water-soluble, salt-soluble (10% NaCI), alcohol-soluble (80% ethanol) and alkaline soluble (0.2% NaOH) proteins isolated in the respective solutions from high grade wheat flour were used as protein substrates. Insoluble particles of crushed wheat grain washed with distilled water before and after hydro-thermal (125-128°C) processing were used as solid phase.

Products of hydrolysis of proteins of wheat flour

We poured 1 ml of 10% suspension of wheat flour (or flour made from rice chaff) in 0.1 M universal buffer with the respective pH (for neutral proteinase pH 7.0) in the test tube and then added 1 ml of enzyme solution with a concentration of 0.2 units·ml-1 of activity. The mixture was stirred and kept for some time (10, 30, 60, 90 and 120 min) in a thermostat at 37°C and then 2 ml TCA (trichloroacetic acid) was added to the sample to stop the enzymatic reaction. Next, the settled solution was filtered through a paper filter, 1 ml of filtrate was taken out and 5 ml 0.5 M solution of sodium carbonate was added. While stirring, 1 ml of working solution of Folin was added. Solutions that were settled a little became blue in color. Their intensity was determined by a photoelectric colorimeter (at wave length of 660 nm) and compared with the control sample in the cuvette with layer thickness of 10 mm.

The content of the hydrolysis products R, [μmol·ml-1], was determined by the formula:

$$R = \frac{D \cdot 4}{1{,}72} \qquad (1)$$

Analysis of the activity and thermal stability of proteinase Proteinase activity was determined by a modified method of Anson [1] using proteins extracted from wheat flour as a substrate. Thermo stability of the enzyme was studied in incubation medium containing 0.27-0.3 units·ml-1 activity (in some cases in the medium there were also present solid particles of wheat flour before and after the hydro-thermal treatment, which had been washed with distilled water) and heated to 50°C 0.1 M universal buffer at the desirable pH of the medium. The enzyme activity was measured at 37°C in 0.1 M buffer containing 0.027-0.03 units·ml-1 activity and 1% solution of albumin.

Results and discussion

Influence of insoluble structural components of grain on proteinase activity

First of all, attention was drawn to the fact that works on the study of the action of proteinases on water-soluble proteins in the presence of the solid phase, usually studied the effect on these substrates of

adsorbed enzymes, which showed that the adsorption of the enzyme may be accompanied by a decrease in catalytic activity, accompanied by a simultaneous increase in the resistance of enzymes to the effects of elevated temperature and storage. However, this pattern is not common, as there are known cases when adsorption leads to destabilization, although it happens quite rarely.

In order to study the above facts, at the beginning we tested water-rinsed wheat flour insoluble particles as well as insoluble grain bard particles as an insoluble phase.

Fig. 1. presents an illustration of the data obtained on the influence of solid phases on the catalytic properties of protolithic enzymes using neutraze. The presented data show that due to adsorption immobilization on the surface of solids the activity of enzymes decreases. In the case of durum particles obtained from wheat flour, this decrease is insignificant, and in the case of bard solids testing, some decrease in neutraze activity was observed.

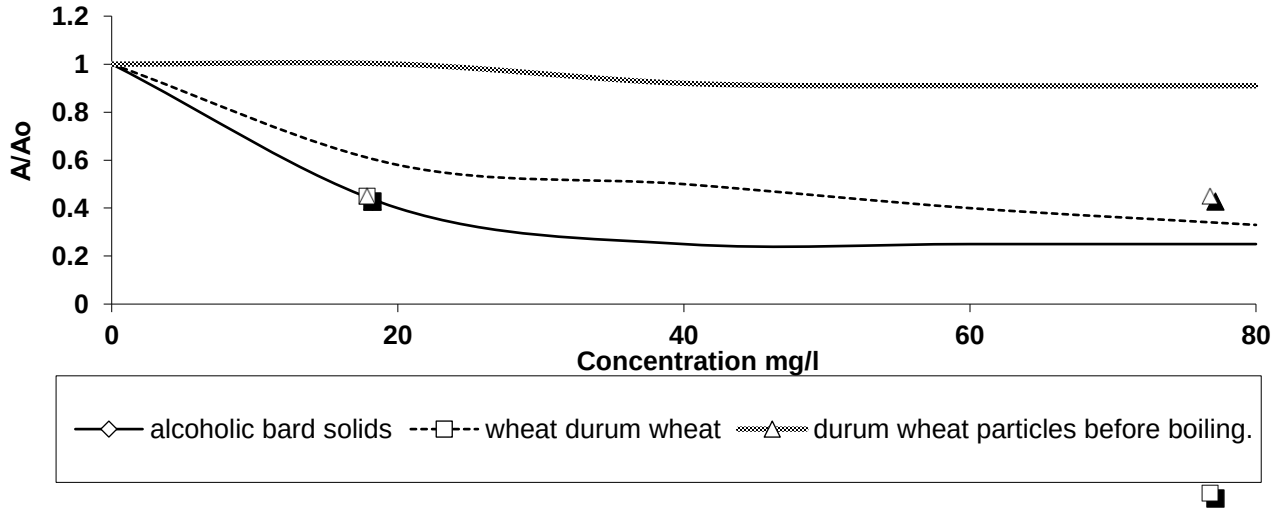

Fig. 1. Influence of concentration (mg/ml) of suspended solids on activity of B. amyloliquefaciensneutraze.

It should be noted that in the liquid phase of the boiled mass the products of melanoid formation and caramelization are accumulated, which inhibit the enzymatic activity of proteinases.

Fig. 2. shows data on the influence of bard filtrate on the activity of neutral proteinase, which shows that in the presence of filtrate bard activity of neutral proteinase decreases. In the case of water extraction of wheat flour there is no decrease in the activity of the enzyme.

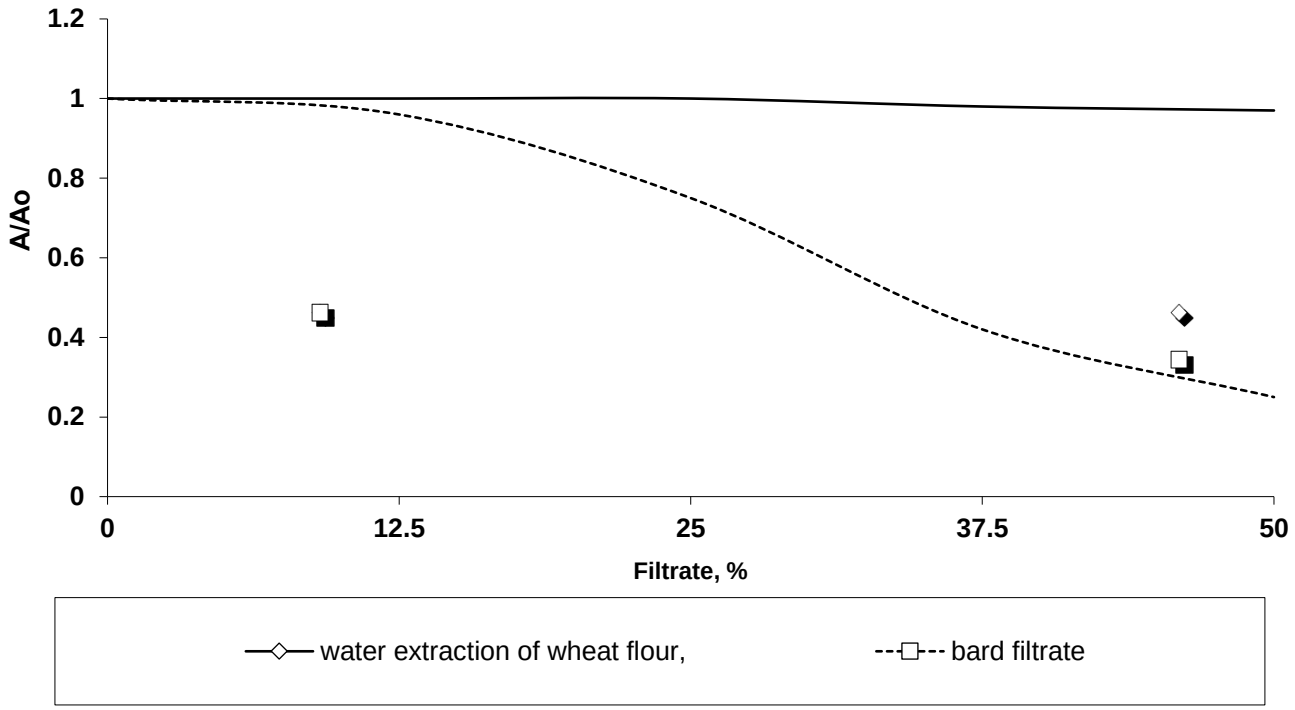

Fig.2. Effect of water extraction of wheat flour and bard leachate on neutral proteinase activity.

It was also found that bard solids also affect the stability of the neutral proteinase. In Fig. 3. represents the kinetics of neutral proteinase inactivation in the presence of solids. From the presented data we can see that at 50 0C in 0,1 M universal buffer pH 7,0 the time of proteinase semi inactivation in the absence of solid particles is 45 min, and in the presence of solid particles derived from the grain bard 25 min.

Similar data were obtained for wheat germ proteinase trials.

Thus, in complex substrate mixtures, when the enzymatic reaction takes place at the interface, where heterogeneity is determined by the multicomponent system, a certain part of enzymes in the medium are in an adsorbed state on the surface of the insoluble substrate phase. At the same time on certain surfaces (in our experiments the surface of insoluble particles obtained from the grain bard) there is not only a decrease in catalytic activity, but also observed the destabilization of enzymes.

To clarify the above assumption, we studied the effect of various desorbing agents on the stability of enzymes in systems with insoluble phases and the results of the study were discussed in the next section of the dissertation.

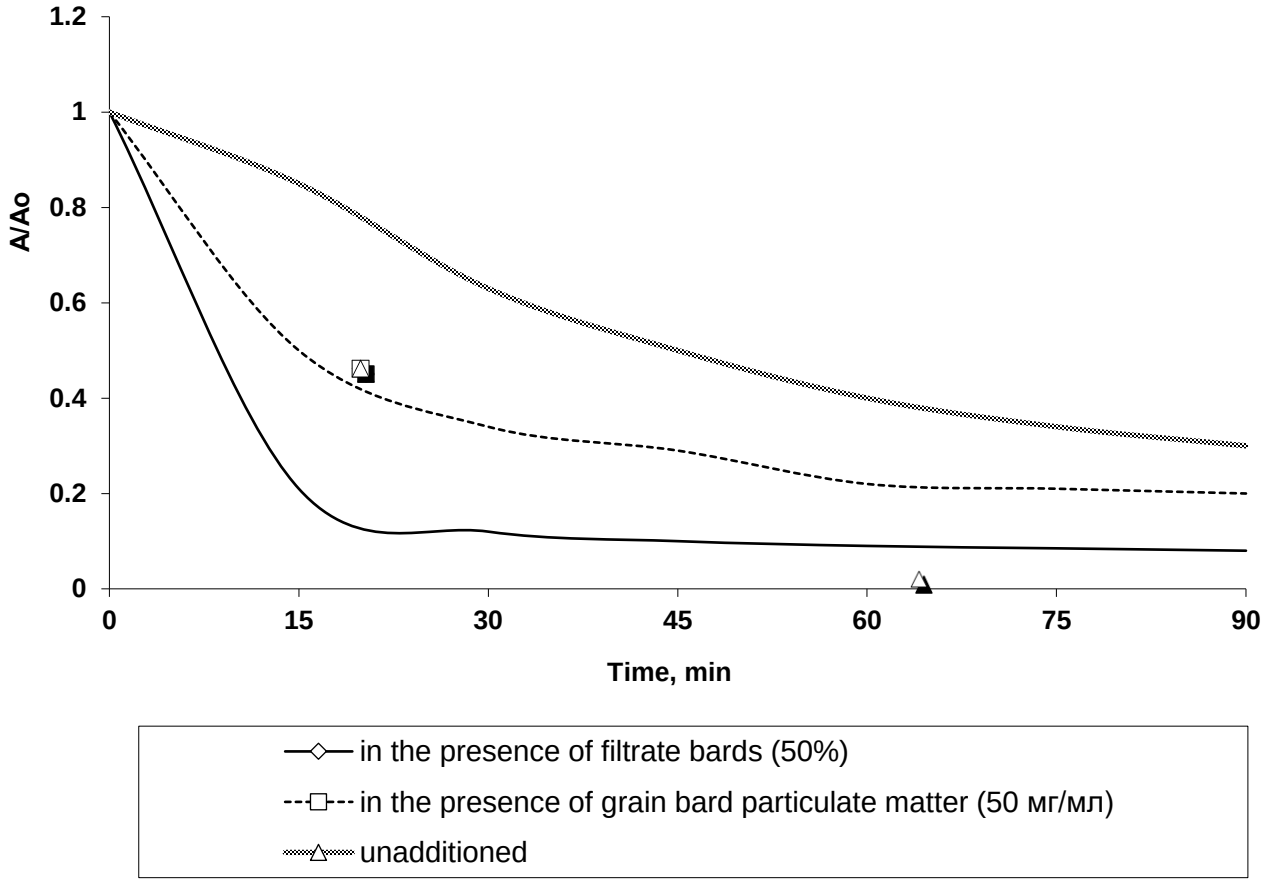

Fig. 3. Stability of neutral proteins under different conditions

Effect of desorbing agents on proteolytic enzyme activity in heterogeneous systems

In complex substrate mixtures, the adsorption state of enzymes can be controlled by adding desorbing agents. Such agents may include alcohols, high concentrations of salts, increased content of surfactants, etc. The adsorption state of the enzyme can be controlled by adding desorbing agents.

It can be expected that addition of ethanol to the medium could lead to desorption of the enzyme on the one hand, and on the other hand, if the enzyme is firmly adsorbed, to change the conformational stable state of adsorbed enzymes. As a result, this should necessarily affect the catalytic properties of the enzymes.

The data on studying the effect of ethanol on the activity and stability of neutral proteinase are shown in Figure 1. 4 of which show that specific activity of neutral proteinase decreases in the presence of ethanol. The decrease in catalytic activity of neutral proteinase in aqueous-alcohol solutions is also accompanied by a decrease in their resistance to high temperatures. Thus, for example, at 50 0C in 0.1 M universal pH 7.0 buffer the time of neutral proteinase semi inactivation in the absence of ethanol was 45 min, whereas in the presence of 15% of ethanol it was 12 min in total. (fig. 4.)

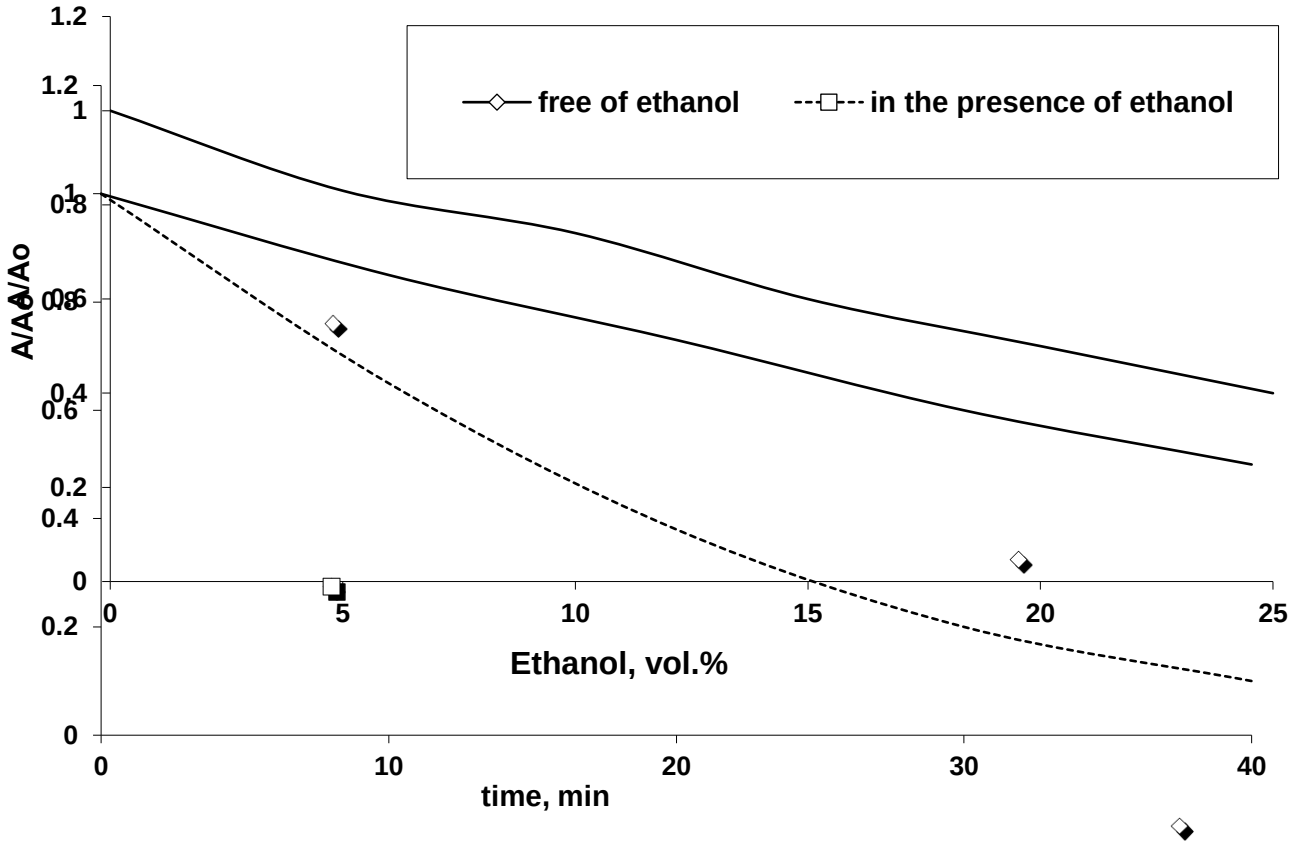

Fig.4. Ethanol influence on activity (a) and stability (b) of neutral proteinase. Stability was studied at 15% of ethanol volume.

Conclusions

In the case when insoluble particles of grain bard were used as a solid phase, addition of ethanol led to desorption of neutral proteinase which was accompanied, as it should be expected from previous experiments, by reduction of destabilizing effect of solid phase.

Analysis of the obtained results presented in Fig.4 presents that complete release from destabilizing effects of the solid phase in the presence of ethanol occurs with insoluble particles obtained from wheat flour.

Thus, in complex substrate mixtures the adsorption and stable state of neutral proteinases can be regulated by introduction of alcohols into the medium. This removes the negative effect of insoluble particles in the reaction mixture.

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