

## Enzymatic Hydrolysis of Various Proteins of Wheat in Heterogeneous Conditions

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**Abstract:** Enzymatic hydrolysis of different proteins isolated from wheat flour by neutral proteinase (neutraza "Novozymes") was studied. It was shown, that hydrolysis of alkaline proteins was 10-11 times higher as compared with albumin from wheat, 3-4 times higher than alcohol soluble proteins and 2-2.5 times higher than globulins. It was found that, hydrothermal treatment of wheat flour decreased the rate of protein hydrolysis. The rate of hydrolysis of native alkaline soluble proteins was 4-6 times higher than denaturated proteins. The rate of hydrolysis of denaturated water-soluble proteins is 3-5 times higher as compared with native protein (albumin). It was shown that product of thermal degradation of raw materials also influence on the rate of protein hydrolysis.

**Keywords:** Proteins, Wheat flour, Isolate, Hydrolysis, Proteinase, Heterogeneous system.

### Introduction

In heterogeneous systems where the reaction occurs at the border between the phases or in complex substrate mixtures where heterogeneity is determined by the multi-component systems containing soluble and insoluble compounds some part of the enzymes or substrates in the medium are in a bound state in the surface [4, 5, 7].

Moreover, not only the soluble components, but also the insoluble components of the processed raw material can interact with certain functional groups of proteins while playing the role of the solid phase and thus making the enzyme conformational unstable by screening the enzyme molecules against the attack of the substrate [2]. Such components most often are natural inhibitors which are products formed during the preliminary, often thermal, processing of raw materials, organic acids, high concentrations of salts, high content of surface-active substances, etc. For example, in the preliminary preparation of the raw material for fermentation, because of the need to sterilize the medium and in order to soften and dissolve the substrates the raw material is heat-treated [3, 6]. At the same time, while sterilizing, softening and destructing the cell walls of the plant material, denaturation of proteins, formation of melanoidins and oxymethylfurfural decomposition of hexoses, etc occur. Change of the state of the substrate phase also occurs, which additionally affects the catalytic properties of the enzymes used.

To a certain extent, this may affect the operational activity and stability of the enzymes. The aim of this work is to study the influence of various factors on the activity and stability of proteolytic enzymes in the processing of starch-containing raw materials.

## Materials and methods

### Materials

We used proteolytic enzyme from bacteria microorganisms – *Bacillus amyloliquefaciens* (Neutraza, “Novozymes”, Denmark).

Water-soluble, salt-soluble (10% NaCl), 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<sup>-1</sup> 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  $R$ , [ $\mu\text{mol}\cdot\text{ml}^{-1}$ ], was determined by the formula:

$$R = \frac{D \cdot 4}{1.72} \quad (1)$$

### 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<sup>-1</sup> 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<sup>-1</sup> activity and 1% solution of albumin.

## Results and discussion

### Enzymatic hydrolysis of various proteins of wheat

Fig. 1 shows the kinetics of hydrolysis of various protein fractions of wheat flour with a neutral proteinase from *Bac. amyloliquefaciens*.

From the data presented it is evident that alkaline soluble proteins have the highest rate of hydrolysis, whereas water-soluble and alcohol-soluble proteins are difficult to hydrolyze by neutral proteinase. The initial rate of hydrolysis of the alkaline soluble proteins was 10-11 times higher as compared with albumin, 3-4 times higher than alcohol soluble proteins and 2.0-2.5 times higher than salt-soluble proteins. The reactions occurred in aquatic

environment, although the protein was in insoluble state. Enzymatic hydrolysis of alkaline proteins with neutral proteinase proceeded more intensively than with albumin.

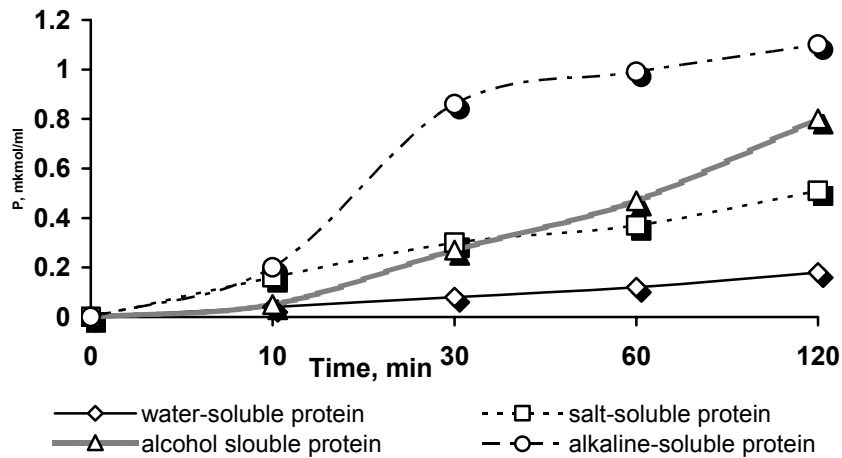


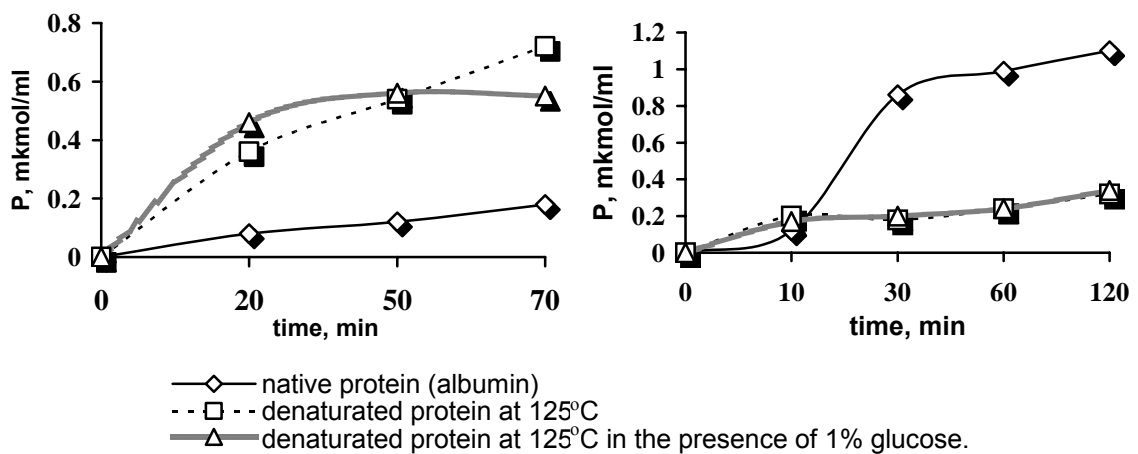
Fig. 1 Kinetics of hydrolysis of various fractions of proteins of wheat

The same trend and regularities of the hydrolysis process were observed in the hydrolysis of the samples of wheat flour selectively released from the various accompanying proteins.

### Effect of hydro-thermal treatment of the hydrolysis of proteins

When plant material is heat-treated the enzymatic hydrolysis of proteins changes as a result of the ongoing process of their denaturation.

In hydro-thermal treatment of raw materials the proteins actively undergo thermal denaturation. It should be noted that denatured proteins undergo enzymatic hydrolysis in a different manner. Fig. 2b shows the kinetics of hydrolysis of water-soluble proteins of wheat subjected to heat treatment which shows that the type of dependence of hydrolysis of different proteins is different. It turned out that the preliminary heat treatment has a positive effect on the enzymatic hydrolysis of water-soluble proteins. The rate of hydrolysis of denatured water-soluble proteins is 3-5 times higher compared to native protein (albumin).



a) alkaline soluble proteins

b) water-soluble proteins

Fig. 2 Influence of preliminary heat treatment of the substrate on the enzymatic hydrolysis of wheat proteins

On the other hand, it was found that heat treatment has an adverse effect on the alkaline soluble, salt-soluble and alcohol-soluble proteins.

From the data presented in Fig. 2a it is evident that the rate of enzymatic hydrolysis of denatured alkaline soluble proteins is lower than that of native protein. Similar results were obtained in the case of hydrolysis of other salt-soluble and alcohol-soluble proteins.

The first stage of the technological process of grain conversion to alcohol, is a hydro-thermal processing of raw materials. In this case proteins undergo thermal denaturation. Denatured proteins are differently exposed to enzymatic hydrolysis. On the Fig. 3 was demonstrated hydrolysis protein-containing substrates derived from distilleries. The data presented that the denatured proteins of wheat flour after the hydro-thermal treatment (138-140°C) are not good substrates for the enzyme action. Hydrolytic products were 3-4 times fewer than native proteins.

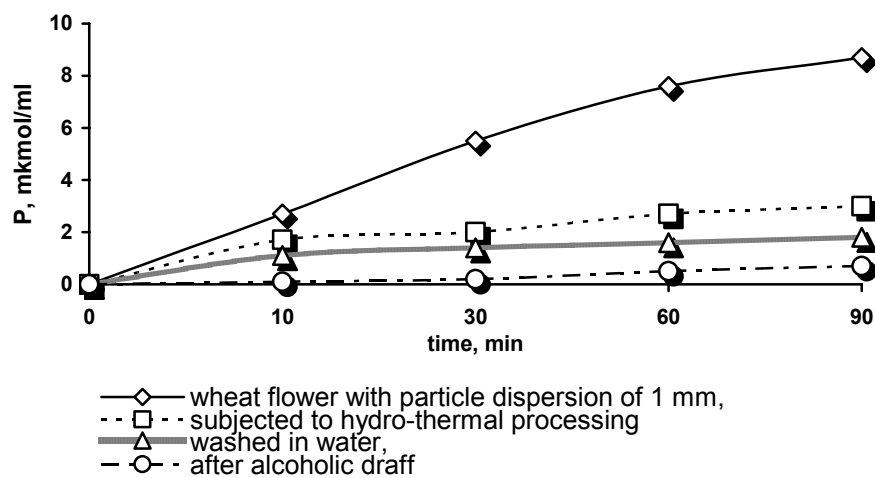


Fig. 3 Kinetic curves of hydrolysis of protein amyloid material

It should be noted that in the liquid phase (suspension) of sodden mass melanoidins and caramelization products accumulate inhibiting the enzymatic activity of proteinase. Evidence of these assumptions can be seen from the data obtained by the hydrolysis of proteins of wheat flour in the presence of filtrate of sodden mass. Fig. 4 presents data on the effect of filtrate of sugarized mash on the kinetics of hydrolysis of wheat proteins, which shows that in the presence of sugarized mash hydrolysis of native proteins of wheat is substantially reduced, hence the rate of hydrolysis decreases by 3-3.5 times.

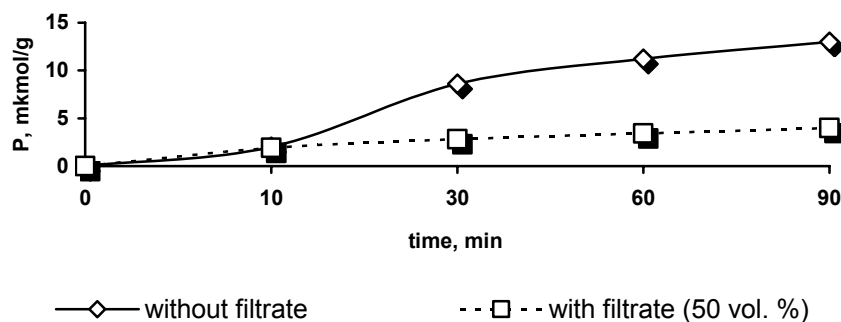


Fig. 4 Effect of filtrate of sugarized mash on the kinetics of hydrolysis of proteins of wheat flour (premium variety of flour)

Conditions: 10% suspension of flour in a 0.05 M phosphate buffer with pH 7.0, concentration of neutral proteinase 0.05 ml·ml<sup>-1</sup>, temperature – 45°C.

Thus, the preliminary hydro-thermal processing of protein substrates leads to a significant decrease in their hydrolysis. As this kind of exposure can occur in conditions of hydro-thermal treatment of sodden starch material, this explains the low degree of hydrolysis of proteins observed in the technological processes of the production of alcohol.

### *Influence of insoluble structural components of the grain on the activity of proteinase*

Fig. 5 provides an illustration of data obtained for the influence of solid phases on the catalytic properties of proteolytic enzymes using neutrasa. From the data presented it becomes obvious that due to adsorption immobilization on the surface of the solid particles the activity of the enzymes decreases. For the solid particulate matter derived from wheat flour this decrease is slight, and in the case of tested particles from the draff there was a slight decrease in the activity of neutralization.

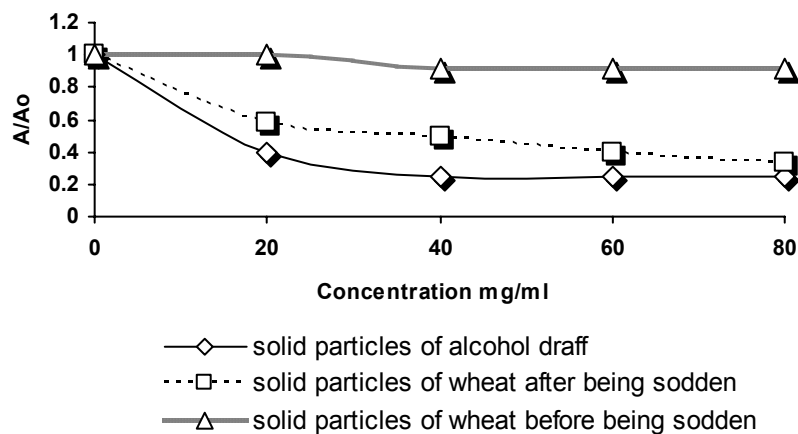


Fig. 5 Effect of concentration (mg·ml<sup>-1</sup>) of solid particles of the mixture on the activity of neutralization *Bac. amyloliquefaciens*

## Conclusions

In complex substrate mixtures when the enzymatic reaction occurs at the border of phases where heterogeneity is determined by the multiple components of the system, as well as due to hydro-thermal processing of raw materials under extreme conditions, a decrease in hydrolysis of substrates is observed. Moreover, on certain surfaces (in our experiments, the surface of insoluble particles derived from draff) not only a decrease in catalytic activity is observed, but also destabilization of the enzymes.

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