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ENZYMATIC HYDROLYSIS KINETIC ANALYSIS OF THE VARIOUS SOURCE PROTEINS

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Abstract: The chemical composition marble goby and sunflower meal is presented. The experimental results of enzymatic hydrolysis velocity in dependence of the protein concentration in substrate systems are set. Michaelis constants values for industrial proteolytic enzymes Corolase ® L10 and Corolase ® L7089 are calculated. The application experimental - theoretical kinetic analysis for protease / proteins congeniality determine in complex dispersion substrate systems expediency was confirmed. It is determined that microbial enzyme drag Corolase ® L7089 has a higher congeniality to proteins of all the tested substrates than plant enzyme drag Corolase ® L10.

Keywords: Michaelis constant, congeniality Corolase ® L10 and Corolase ® L7089.

INTRODUCTION

Currently, many countries are having a growing food and feed animal protein shortage.

One of the ways to alleviate this situation, specially with feeding proteins hydrolyzate production. This products are highly digestible food with a high content of free amino acids and various molecular weights peptides.

Protein hydrolysates are widely used for amino acid formulas enrichment and balancing for various cereals, flour, minced products, pet and farm

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animals, birds, fish farming, as well as for various soups, sauces, salads, fish, meat and vegetable dishes.
The raw material for hydrolysates production can different food waste and shallow fish, as well as various high - protein vegetable meals (Rumyantseva 2007) (Rumyantseva and Dunchenko 2007).
In the production of protein hydrolysates can be used chemical (acid and alkaline) and enzymatic hydrolysis methods.
Despite its affectivity the acidic and alkaline proteins hydrolysis are not widely used as it accompanied by destruction and isomerisation of some amino acids due to high temperature, extreme pH values, and corrosive chemicals large quantities.
In the case of enzymatic hydrolysis the amino acids are not change, but it's formed a complex protein degradation products mixture. Their molecular weight ratio depends upon the enzyme properties, the type of raw materials and the process conditions. The negative effects of enzymatic hydrolysis - is the dangerous of microbiological process expansion.
The microbial spoilage risk can be reduce with various chemical preservation drugs, or hydrolysis process intensifying by high active enzyme drag application. Such enzymes drags should have maximum congeniality to raw material proteins and have a property to keep its activity due to potential inhibition by reaction products.
Quantitative assessment the enzyme congeniality to the substrate was reviewed by fundamental enzymatic processes kinetics works.
This property of the enzyme - substrate systems is described in the Michaelis - Menten equation in the form of the Michaelis constant (Km). This constant value - is the substrate concentration at which the rate of the process is half the maximum. In accordance to this approach, for low Km values corresponds high enzymatic congeniality to the substrate and the higher catalytic process efficiency .
The classical attitude to enzymatic reactions kinetics examines the enzyme - substrate system in a condition of solution. In real technological processes, this system is a multi-component complex mixture of substrate and industrial enzyme drag, partially dissolved and partially suspended. Usually industrial enzyme drag contains several individual enzymes. Such substrate systems include minced tissue suspension are typical in hydrolysate processing from vegetables and fish raw and have not tested before by kinetic analysis. Obviously, experimental and theoretical ki-

netic analysis of such systems contains a scientific novelty and practical interest.

Thus, the aim of the present work was to assess the congeniality degree of the most common industrial photolytic enzyme drag to fish and plants proteins. In accordance with the main aim, the following tasks were examined in the research:

- to determine the chemical composition substrates, taken to research;
- at the basis of the experimental data about substrates chemical composition to create enzyme - substrate model system;
- to The experimental dependence of the enzymatic hydrolysis and to calculate the values of Michaelis constant for the considered enzyme - substrate systems;
- to get experimental functional relationship between enzymatic hydrolysis velocity substrate concentration and to calculate the Michaelis constant values for tested enzyme - substrate systems;

MATERIALS AND METHODS

For substrate – enzyme system formation were used the following kinds of raw materials - minced Azov goby (Gobius melanostomus) fillet and sunflower meal.

For research was used the photolytic enzyme drag Corolase® L10 and Corolase® L7089 by Enzymes GmbH (Germany) with a declared activity of 850 UHb/g.

The raw maternal chemical composition were rated by the moisture, ash, fat content, total nitrogen (TN) and short protein fragments nitrogen (NPN) amount.

Fermentoliz process velocity was rated by the amount of hydrolysed protein at the incubation time, which was determined by short protein fragments nitrogen amount and was calculated under equation:

$$V = \frac{6{,}25 \cdot (NPN - NPN_0)}{\tau},$$

where NPN - short protein fragments nitrogen amount after incubation, mkg/100g; NPN_0 - - short protein fragments nitrogen amount of raw material, mkg/100g; τ - incubation duration, min.

The moisture, ash, fat content, total nitrogen (TN) and short protein fragments nitrogen (NPN) amount was determinates by standard meth-

ods. Determination of short fragments nitrogen was performed under the Kjeldahl method after high molecular weight proteins precipitation with threechloroacetic acid.
The Kjeldahl nitrogen auto analyzer VELP Scientifica was used for different nitrogen forms determination.

RESULTS AND DISCUSSION

The raw material chemical composition study results are represented in Table 1.

Table 1. The chemical composition of raw material for enzyme - substrate systems formation.

The research object	Nitrogen substances', mg/100 g			Moisture, %	Fat content, %	Ash, %
	Total nitrogen, (TN)	Short protein fragments nitrogen (NPN_0)	Protein nitrogen (TN-NPN_0)			
Minced Azov goby fillet	2750	160	2590	74,8	3,4	4,6
Sunflower meal	6336	85	6251	9,8	1,05	3,4

The obtained data about raw material chemical composition, allowed to form water dispersed enzyme - substrate system with a range of protein concentrations 0 ... 60 mg/100 g and the concentration of enzyme drug Corolase ® L10 and Corolase ® L7089 10 mg/100g.
The hydrolysis process was carried out at a temperature of 50ºC at 120 minutes duration with constant stirring.
Experimental dependences of enzymatic hydrolysis velocity from substrate protein concentration at the base of fish minced muscle are shown at figure 1. Approximated equations are: $V = -0{,}0001\ [S]^3 - 0{,}0011\ [S]^2 + 1{,}3067\ [S]$ for enzyme drag Corolase ® L10 and $V = -4\ 10^{-6}\ [S]^3 - 0{,}0176\ [S]^2 + 1{,}8979\ [S]$ for Corolase ® L7089. The decision of these equations for V=0,5 Vmax, allowed to obtain Michaelis constant with the following values:
Corolase® L10 - $K_m = 18{,}9$;

Corolase® L7089 - $K_m = 15{,}4$.

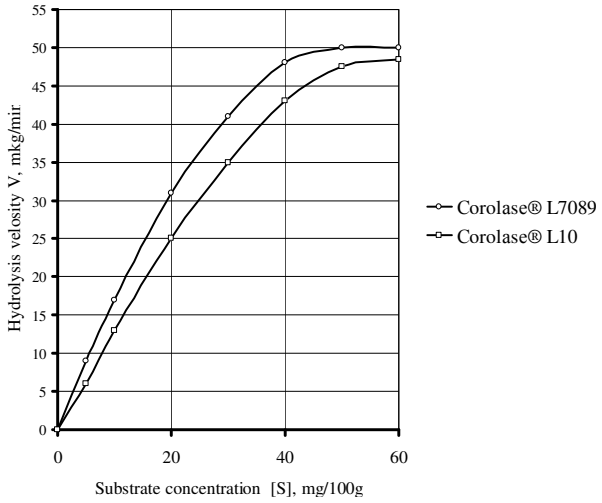

Figure 1. Enzymatic hydrolysis velocity dependences
from fish minced substrate protein concentration

A similar study for the enzyme - substrate systems based on sunflower meal (Fig. 2). Allowed to obtain approximate equation: V = -0,0003 [S] ^{3} +0,00112 [S] ^{2} +1,0546 [S] for Corolase ® L7089 and V = -0,0003 [S] ^{3} +0, 0176 [S] ^{2} +0,5957 [S] for Corolase ® L10.

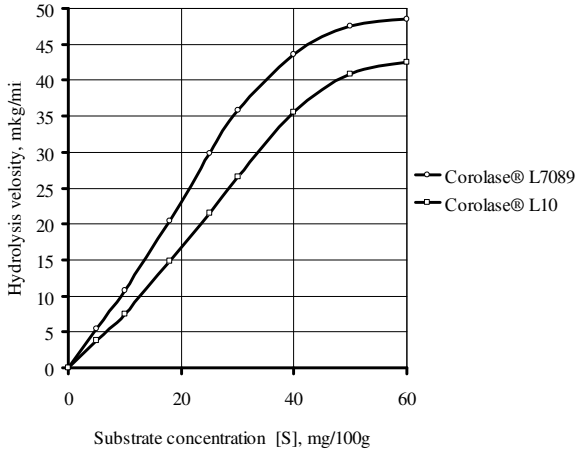

Figure 2. Enzymatic hydrolysis velocity dependences
from sunflower meal substrate protein concentration

In this case, the Michaelis constant value is 23.69 for enzyme drag Corolase ® L10 and 18.21 for Corolase ® L7089.
From a comparative analysis the obtained Michaelis constant values, follows that the enzyme drag Corolase ® L7089 has a higher congeniality for the protein substrate of all tested systems.
These results can be explained by protein composition special features the possible presence of protease inhibitors in the sunflower meal.

CONCLUSIONS

1. It is confirmed expediency of experimental - theoretical kinetic analysis application for protease / proteins congeniality determine in complex dispersion substrate systems.
2. Experimentally determined that Azov goby proteins are able for more intensive enzymatic hydrolysis by Corolase ® L10 and Corolase ® L7089 than sunflower meal.
3. It was revealed that microbial enzyme drag Corolase ® L7089 has a higher congeniality to proteins of all the tested substrates than plant enzyme drag Corolase ® L10.

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