**УНИВЕРСИТЕТ ПО ХРАНИТЕЛНИ ТЕХНОЛОГИИ -
ПЛОВДИВ**

**UNIVERSITY OF FOOD TECHNOLOGIES -
PLOVDIV**

**SCIENTIFIC WORKS
Volume LX
Plovdiv, October 18-19, 2013**

НАУЧНА КОНФЕРЕНЦИЯ С МЕЖДУНАРОДНО УЧАСТИЕ

"ХРАНИТЕЛНА НАУКА, ТЕХНИКА И ТЕХНОЛОГИИ 2013"

'FOOD SCIENCE, ENGINEERING AND TECHNOLOGIES 2013'

НАУЧНИ ТРУДОВЕ

Том LX

Пловдив, 18- 19 октомври 2013

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НАУЧНИ ТРУДОВЕ

SCIENTIFIC WORKS

ТОМ LX, св. 1

VOLUME LX

2013

ISSN 1314-7102

Докладите в сборника са рецензирани и одобрени от Редакционната колегия.

Академично издателство на УХТ - Пловдив
UFT Academic Publishing House, Plovdiv
2000

НАУЧНИ ТРУДОВЕ
ТОМ LX
"ХРАНИТЕЛНА НАУКА, ТЕХНИКА И ТЕХНОЛОГИИ – 2013"
18-19 октомври 2013, Пловдив

SCIENTIFIC WORKS
VOLUME LX
„FOOD SCIENCE, ENGINEERING AND TECHNOLOGIES – 2013"
18-19 October 2013, Plovdiv

FISH PROTEINS ENZYMATIC HYDROLYSIS KINETIC'S ANALYSIS

Bandurenko Galina[1], Vinnov Aleksey[2]
1-National University of Food Technologies, Kyiv, Ukraine,
2-National university of life and environmental sciences, Kyiv, Ukraine
e-mail: Aleks2174@yandex.ru

Abstract
The enzymatic marble goby protein hydrolysis process experimental kinetic diagrams analysis is represented. The results can be used to assess the protease inhibition degree by reaction products.
Keywords: *Michaelis constant, affinity, Corolase® L10, competitive inhibition.*

АНАЛИЗ КИНЕТИКИ ПРОЦЕССА ФЕРМЕНТАТИВНОГО ГИДРОЛИЗА БЕЛКОВ РЫБЫ

Бандуренко Г. М.[1], Виннов А. С.[2]
1-Национальный университет пищевых технологий, г. Киев, Украина,
2-Национальный университет биоресурсов и природопользования, г. Киев, Украина
e-mail: Aleks2174@yandex.ru

Аннотация
Представлен анализ экспериментальных кинетические кривых ферментативного гидролиза белков бычка. Полученные результаты могут быть использованы, для оценки степень ингибирования протеазы продуктами реакции.
Ключевые слова: *Константа Михаэлиса, сродство, Королаза® L10, конкурентное ингибирование.*

Introduction.

Protein digested (protein hidrolizates) food and feed products characterized with better nitrogen assimilation level than other protein products, due to short peptides and free amino acids high content. Technology of these products is based at multi - component protein plant or animal origin raw materials hydrolysis by proteases catalyses. In industry, for protein hidrolizates production, proteolytic plant origin enzyme drug Corolase® L10 and microbial origin Protoryzin, Corolase® L7089 are mostly prevalent. Industrial enzymatic hydrolysis processes are complicated by complex colloid state and substrate composition. It is obvious, that during polycomponent dispersed protein substrates enzymatic hydrolysis individual enzymes inhibition by hydrolysis products is possible [1]. Qualitative and quantitative assessment of this process can be implemented by theoretical and experimental kinetic analysis by the classic Michaelis - Menten equation (1):

$$V = \frac{V_m [S]}{[S] + K_m} \qquad (1)$$

This equation combines the initial stage process velocity (V), maximal reaction velocity (V_m) and substrate concentration [S], over the Michaelis constant (K_m). This constant is strictly individual for each enzymatic process and characterizes the enzyme/substrate affinity degree. The Michaelis constant value decreasing demonstrates their higher affinity [2].

The Michaelis - Menten equation is based on the classical kinetic scheme of the enzymatic reaction: E +S ↔ ES → E +S.

In practical multicomponent enzyme - substrate system, differential Michaelis - Menten equations form more accurately accepts the enzymatic process peculiarities:

$$-\frac{\mathrm{d}\sum[S]}{\mathrm{d}\tau} = \frac{V\sum[S]}{K_m' + \sum[S]} \qquad (2)$$

The K'_m constant by physical essence is similar to the Michaelis constant, but takes into account the complex dissolved and suspended protein substrates structure, enzymatic processes multistage and diversity flow [3].

This equation integration with initial conditions $\Sigma[S] = \Sigma[S]_o$, $\tau = 0$ leads to the expression, which determines the reaction products concentration in the process duration dependence (2):

$$\sum[P] = V'_m \tau - K'_m \ln \frac{\sum[S]_o}{\sum[S]_o - \sum[P]}, \quad (3)$$

where Σ[P]-the total amount of the reaction products, τ-process duration, K'_m - the apparent Michaelis constant, V'_m - apparent maximum speed of the process, $\Sigma[S]_o$ – initial protein - substrates amount.

This expression is in integrated form. It determines the reaction evolutional time (kinetic curve), for which the Michaelis – Menten equation is performed for a long period after the enzymatic reaction beginning.

For process analysis in the practical purposes, the integral Michaelis – Menten equation should be transformed to the linear form. The Woker - Schmidt method is most useful for this purposes. In these coordinates, the Michaelis – Menten equation integral form of the equation takes the form (4):

$$\frac{\sum P}{\tau} = V'_m - K'_m \frac{1}{\tau} \ln \frac{\sum[S]_o}{\sum[S]_o - \sum[P]} \quad (4)$$

At the multicomponent protein substrates enzymatic hydrolysis, particular interest attaches to the enzyme competitive inhibition by reaction products.

In this case the reaction scheme is following:

$$E_i + S_i \leftrightarrow \Sigma ES \rightarrow E_i + P_i$$
$$E_1 + P_1 \leftrightarrow E_1 + P'_1$$
$$\ldots\ldots\ldots\ldots\ldots\ldots$$
$$E_i + P_i \leftrightarrow \Sigma E_i P_i \rightarrow E_i + P_i^j \quad (5)$$

Under these conditions, linearized integral Michaelis - Menten equation, acquires the following interpretation (6):

$$\frac{\sum P}{\tau} = \frac{V'_m}{1 - \frac{K'_m}{K_i}} - \frac{K'_m \left(1 + \frac{\sum[S]_o}{K_p}\right)}{\tau \left(1 - \frac{K'_m}{K_i}\right)} \ln \frac{\sum[S]_o}{\sum[S]_o - \sum[P]}$$

Constant K_i, characterizes the enzyme reversible binding process by reaction's products in competitive inhibition process. One or another individual enzyme E_i, extrication is possible as a result EP complexes natural decay and as a result of the new catalysis cycle.

In this case, a new comprehensive kinetic constant K_{eff} appears

$$K_{eff} = K'_m \left(1 + \frac{\sum[S]_o}{K_i}\right) \Big/ \left(1 - \frac{K'_m}{K_i}\right) \quad (7)$$

From expression, describes the K_{eff} value analysis, follows that due Ki value change for some kinetic curves amount with $[S]_o$ different value, the slope of their linearized form in the Woker - Schmidt coordinates also will be changed [4].

Decreasing K_i value, and therefore the decreasing values inclination angle tangent, will show the more high products reaction affinity to the enzyme compared with the original substrate.

At least, in the case when the $K_i << K'_m$ inclination angle tangent can take negative values, which probably corresponds to the stage of the almost full competitive substitution of the initial substrate by intermediate reaction products.

This assumption is probably realistic in case of prolonged proteolysis in composite protein systems and presented theoretical approach to the kinetic analysis can be used to predict the process trend, but requires experimental verification.

Thus, this work tangent is to estimate the Michaelis - Menten integral form equation with Woker - Schmidt linearization possibility for kinetic analysis of the proteolysis in composite multicomponent substrate system.

To accomplish this aim it is necessary to solve the following tasks:

- to study the protein-raw materials chemical composition accepted for accepted for further investigations;
- to form enzyme - substrate system with a different $[S]_o$ value on the basis of raw materials chemical composition obtained experimental data;
- to receive the adopted proteins model systems enzymatic hydrolysis experimental kinetic curves;
- to calculate and to estimate the experimental kinetic curves, linearized in the Woker - Schmidt coordinates inclination angle tangent values changes.

Material and methods

In the research, as protein raw materials for substrate system formation was used Azov sea round goby (Gobius melanostomus) fillet. Hydrolysis reactions were performed under vegetable origin proteolytic enzyme drug Corolase® L10 produced by Enzymes GmbH (Germany).

Raw fish material chemical composition was estimated by content of ash, moisture, general and non-protein nitrogen, lipids extracted by ethyl ether.

Enzymatic process was assessed by the accumulation of non-protein nitrogenous substances.

Determination of total nitrogen, moisture, lipids was carried out by standard methods. Determination of non-protein nitrogen (NPN)) by Kjeldahl method (automatic analyzer VELP Scientifica) after high-molecular proteins coagulation by trichloroacetic acid with a final concentration - 5%.

Results and Discussion

Raw materials chemical composition study results is presented in table 1.

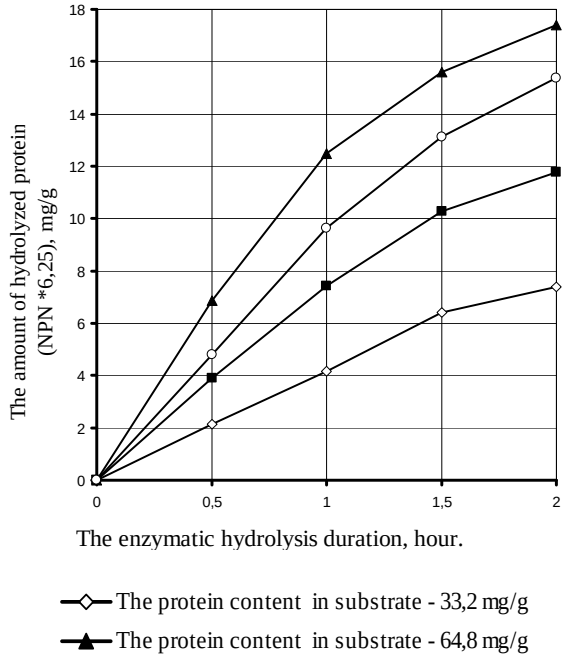
Table 1- Raw materials chemical composition for enzyme-substrate systems formation

Nitrogenous substances, mg/100 g			Moisture, %	Lipids, %	Ash, %
Total nitrogen, (TN)	Nonprotein nitroger, (NPN)	Protein nitrogen, (TN-NPN)			
2750	160	2590	74,8	3,4	4,6

The obtained data allowed to get in the form of water dispersants enzyme - substrate systems from minced Azov sea goby fillet with a range of the protein substrates concentrations 30 - 60 mg/g and the concentration of the enzyme drug Corolase® L10 – 0,1 mg/g.

Hydrolysis reactions were performed at the temperature 50ºC for 120 minutes at constant paddling with frequency 3 min^{-1}.

The experimental kinetic curves is presented on the fig 1.



The protein content in substrate - 33,2 mg/g
The protein content in substrate - 64,8 mg/g

Fig.1 The enzymatic hydrolysis kinetic curves

Obtained experimental curves can be reliably approximated by cube polynoms. Their differentiation determine that in the accepted time interval, the enzymatic reaction has damping process character by NPN amount.

Woker - Schmidt coordinates kinetic curves linearization results with the subsequent approximation by the linear function, are presented in table 2.

Table 2.- The Woker - Schmidt coordinates linearised kinetic curves approximating equations

The protein substances concentration in substrate, mg/g	Approximation equation	tgφ *
64,80	0,0137x+0,0393	0,0137
54,27	0,0149x+0,0529	0,0149
43,74	0,0163x+0,0631	0,0163
33,20	0,0206x+0,0497	0,0206

$$*\,\mathrm{tg}\varphi = \mathrm{K'_m}\left(1+\frac{\sum[S]_o}{\mathrm{K_i}}\right)\Big/\left(1-\frac{\mathrm{K'_m}}{\mathrm{K_i}}\right) \qquad (8)$$

The presented results are displaying that the kinetic curves linearized in the Woker - Schmidt coordinates inclination angle tangent value decreases due to initial substrate concentration incrementing. This indicates the Corolase® L10 drug individual enzymes competitive inhibition existence by reaction products.

Obtained results can be used to predict the required hydrolysis depth.

Inactivation of the enzyme at a certain stage will allow to receive hydrolyzate with definite molecular mass peptides.

The obtained results also can be used to select the substrate system characteristics, which provides enzyme minimal inhibition.

Conclusions:

1. From experimentally-theoretical result analysis was determined that the Woker - Schmidt coordinates can be applied for Corolase® L10 drug individual enzymes reversible inhibition identification.

2. The obtained results can be set as the basis for theoretical prediction of the various protein substrates hydrolysis depth in feed and food fermented products processing.

3. Established the depth controlling possibility of real industrial substrates enzymatic hydrolysis by enzyme drug entered amount.

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SCIENTIFIC CONFERENCE WITH INTERNATIONAL PARTICIPATION
‘FOOD SCIENCE, ENGINEERING AND TECHNOLOGIES 2013’

НАУЧНИ ТРУДОВЕ

Том LX

TRAVAUX SCIENTIFIQUES

Volume LX

Съставител: **доц. д-р инж. Пантелей Денев**

Предпечатна подготовка: **Център за компютърни технологии и комуникации при УХТ, Пловдив**

Формат: **70/100/16**
Тираж: **250**
ISSN 1314-7102

Академично издателство на УХТ - Пловдив
UFT Academic Publishing House, Plovdiv
2000

Изданието е одобрено от Издателския съвет на УХТ