ТЕХНОЛОГИЯ ПЕРЕРАБОТКИ ГИДРОБИОНТОВ

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APPLICATION OF FOSTER – NUEMANN METHOD FOR ESTIMATION OF EFFICIENCY OF PROTEIN ENZYMATIC HYDROLYSIS OF BLACK SEA ATHERINA

Abstract. The estimation of efficiency of technological modes of protein enzymatic hydrolysis of the black sea atherina by Foster – Nuemann method is made. By the linearization of experimental curves the apparent Michaelis's constant for substrates with different moisture based on the black sea atherina muscle tissue was obtained. From the experimental Michaelis's constant values comparison, it follows that in a system with high humidity Protosubtilin G3x enzymes affinity to substrate proteins is higher than in natural humidity systems. The obtained value of Michaelis constant for the theoretical process allows to assert that the enzymatic hydrolysis of the considered enzyme-substrate systems with the addition of 50 % of water does not meet any damping.

Key words: fish protein hydrolysates, objective estimation of enzymatic hydrolysis damping level, black sea atherina, Foster – Nuemann method, Michaelis – Menten integral equation.

Introduction

Fish protein hydrolysates production and their application in feed, food and specialty products allows to solve the problem of raw materials complex processing and to improve fish processing enterprises economic efficiency [1].

Enzyme-substrate system on raw fish basis are viscous mixtures, which consists of dissolved, dispersed and emulsified protein and fat components. Proteins hydrolysis in such multiple substrates associated with a complex inhibitory factors, among which the most probable diffusion damping, individual enzymes competitive inhibition by reaction products, specific substrates depletion, etc.

This process is extremely tricky for formalization and modeling and therefore estimation of efficiency of protein substances enzymatic hydrolysis inhibition in such complex systems is an important practical and theoretical problem. This problem decision allows to select the most effective proteolyses process modes [2].

An objective assessment of enzymatic hydrolysis damping can be obtained from the analysis of experimental kinetic dependences presented in linearized Michaelis – Menten integral equation by Foster – Nuemann method [3].

All considered experimental enzyme-substrate modeling systems must have equal protein / enzyme proportion and different mass fraction of added water. With the help of such enzyme-substrate systems, the proteolysis diffusion and competitive damping integrated assessment can be obtained.

Integral form of the Michaelis – Menten equation has the form:

$$\sum[P] = V't - K'_m \ln \frac{\sum[S_0]}{\sum[S_0] - \sum[P]}, \quad (1)$$

where $\sum[P]$ – total amount of reaction products; t – process duration; K'_m – apparent Michaelis constant; V' – apparent maximum process velocity; $\sum[S]_0$ – initial total amount of proteins in the substrate.

The apparent Michaelis constant and the apparent maximum process velocity in the integral equation is similar to classical Michaelis – Menten equation corresponding figures, but it takes into account a set of complex processes, including damping occurring in multicomponent enzyme-substrate systems.

The less K'_m value, the higher substrates congeniality, available in the system, to a set of individual enzymes, i. e. the less influence of different damping factors on hydrolysis intensity.

The numerical value of the apparent Michaelis constants can be derived from equation (1) by its conversion it's converting to the linear form in Walker – Schmidt coordinates:

$$\frac{\sum P}{t} = V'_m - K'_m \frac{1}{t} \ln \frac{\sum [S]_0}{\sum [S]_0 - \sum [P]}.$$

The apparent Michaelis constant K'_m is numerically equal to these direct line tangents of inclination angle, and it will characterize the process of enzymatic hydrolysis at the suitable for linearization process intervals of duration.

According to the Foster – Nieman method at the process starting point when $t \to 0$, enzymatic hydrolysis damping is minimal or completely absent, under enzyme evently distribution at substrate entire volume requament.

At this time point, all the enzyme molecules will be saturated with substrate.

Auxiliary direct lines drawn from the Walker – Schmidt coordinate zero point will cross linearized experimental graphics of the Michaelis – Menten integral equation for enzyme-substrate systems with substrate mass fraction $\sum [S]_{0i}$ at the points corresponding to the enzymatic hydrolysis process at initial moment.

The tangents of inclination angle of these theoretical direct lines (tg α) will be equal to:

$$\text{tg } \alpha = \frac{\sum [P]/t}{\frac{1}{t} \ln \frac{\sum [S]_0}{\sum [S]_0 - \sum [P]}}. \quad (2)$$

Under the requirement $t \to 0$, the numerator and denominator of equation (2) tends to zero, which leads to indetermination type 0/0.

By L'Hopital's rule application:

$$\lim_{t \to 0} = \frac{\sum [P]}{t} = \frac{d \sum [P]}{dt}, \quad (3)$$

$$\lim_{t \to 0} = \frac{\text{In}(\sum [S]_0 / \sum [S])}{t} = \frac{1}{\Sigma [S]_0} = -\frac{d \sum [S]}{dt}. \quad (4)$$

Having divided the expression (3) by the expression (4), tg α = $\sum [S]_0$.

Thus, direct lines drawn from the initial point (requirement $t \to 0$) will have tangents of inclination angle numerically equal to the relevant initial protein concentration in substrate systems.

Theoretical lines drawn through the intersection points will describe the relationship, which will characterize the enzymatic hydrolysis process in the coordinates Walker – Schmidt at the initial moment, when damping is minimized or absent. Comparison of the theoretical values of Michaelis constant with the experimental ones allows obtaining enzymatic hydrolysis damping level for investigsted protein substrates.

Submitted assumptions of hydrolysis damping level determining hase theoretical format and require experimental verification.

In this connection, the aim was to estimate proteins enzymatic hydrolysis damping in viscous substrate systems with different hydronic module. Such enzyme-substrate systems investigations will permit to estimate the aggregated diffusion and enzymatic hydrolysis reaction products damping.

In order to achieve assigned aim in the present work, the following tasks were considered:

– to clarify the chemical composition of raw fish accepted for studies and to form necessary enzyme-substrate systems;

– to obtain experimental kinetic curves of model system's protein enzymatic hydrolysis;

– to determine the values of the apparent Michaelis constants for experimental K'_m and theoretical K''_m relationship;

– from obtained apparent Michaelis constants values comparison to estimate damping effects of investigated raw materials protein enzymatic hydrolysis process.

Materials and methods

In the experimental part of the work, black sea atherina (Atherina mochon pontica) and microbiological origin enzymatic drag – protosubtilin G3x were used as the main raw materials.

To reduce the impact of autolytic processes raw fish were decapitated and the internal organs were removed.

At substrate systems formation prepared raw fish was minced by grinder with 3 mm grating hole diameter.

In the experiment we used the viscous fish substrates with natural moisture content and substrates with 25 and 50 % distilled water addition.

Obtained samples were stirred, warmed up to 50 °C and further milled in a blender at 1000 rpm for 1 minute with simultaneous dry enzyme drag addition in amount of 10 mg/g of protein.

At the same time, to suppress putrefactive processes, 0.5 % sodium metabisulfite ($Na_2S_2O_5$) was entered into the system.

The obtained enzyme-substrate system were guided for incubation at 50 °C. Hydrolysis was carried out in a water oven during 360 minutes at natural pH.

To obtain the data, necessary for calculations and complete raw materials and created substrates characterization the moisture, total, non-protein nitrogen, fat extracted with ethyl ether mass fraction and ash were determined.

Determination of these parameters was performed by the standard and conventional methods.

Enzymatic process flow was assessed by the accumulation of non-protein nitrogen compounds (NPN) in the hydrolyzate after high molecular proteins precipitation with trichloroacetic acid and filtration. NPN amount was determined by the Kjeldahl method (analyzer VELP Scientifica).

Results and discussion

The chemical composition of raw material and the formed protein substrates are listed in Tabl. 1.

From the presented data it can be assumed that the protein substrates enzymatic hydrolysis will mostly followed by diffusion damping effect. Competitive inhibition by reaction products at the first process stage will probably be insignificant due to the low amount of nonprotein nitrogen in the substrate (approximately 8 % of the total content).

Table 1

Chemical composition of experimental substrates based on the black sea atherina muscle tissue

Parameter	The substrate with the natural moisture	Substrate with hydronic module 0.5	Substrate with hydronic module 0.5
Moisture, %	78.60	82.86	85.73
Total nitrogen (TN), mg/100 g	2570.80	2060.50	1713.8
Non-protein nitrogen (NPN), mg/100 g	205.60	158.40	137.03
Protein nitrogen (PN = TN – NPN), mg/100 g	2365.20	1892.10	1576.75
Fat content, %	2.81	2.25	1.87
Ash, %	2.53	2.02	1.69

Experimental dependences, which describe the dynamics of accumulation of protein hydrolysis products in enzyme-substrate systems with natural moisture and hydronic module 0.25 and 0.5 are shown in Fig. 1.

To assess the extent of diffusion process damping, it is necessary to linearize the experimental dependence in Walker – Schmidt coordinates with construction of auxiliary lines for the process characterization at the initial process point $t \rightarrow 0$ for each substrate type.

Tangent of inclination angle of the theoretical line drawn through the intersection point of the linearized experimental and auxiliary lines will be numerically equal to the apparent Michaelis constant K''_m in any damping absence.

Specified graphic transformation realization is shown in Fig. 2.

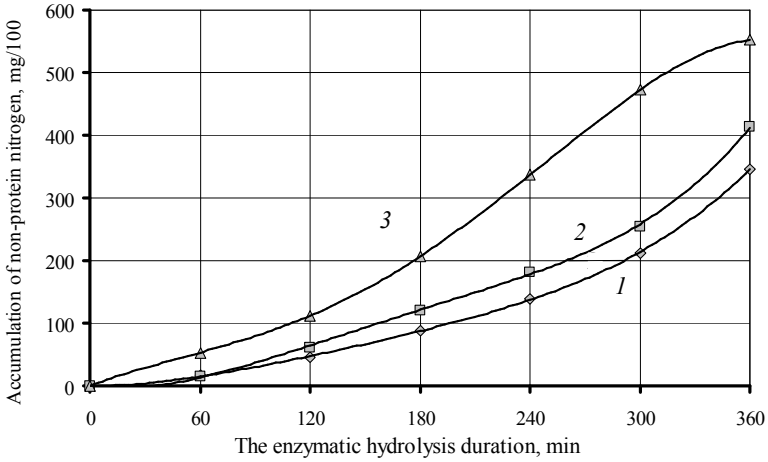

Fig. 1. Protein enzymatic hydrolysis products accumulation dynamics of enzyme-substrate systems with different moisture on the atherina muscle tissue basis:
1 – enzyme-substrate system with hydronic module – 0;
2 – enzyme-substrate system with hydronic module – 0.25;
3 – enzyme-substrate system with hydronic module – 0.5

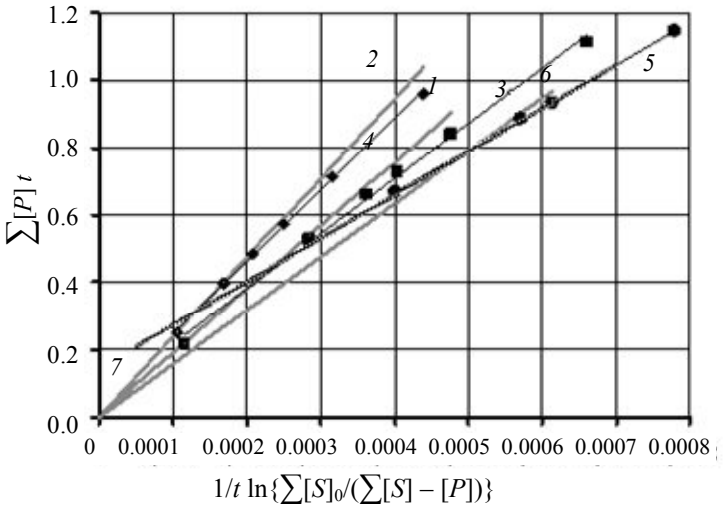

Fig. 2. Experimental dependences, auxiliary and theoretical lines in Walker – Schmidt coordinates: *1* – linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0;
2 – auxiliary line for the enzyme-substrate system with hydronic module equal to 0;
3 – linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0,25; *4* – auxiliary line for the enzyme-substrate system with hydronic module equal to 0.25; *5* – linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0.5;
6 – auxiliary line for the enzyme-substrate system with hydronic module equal to 0.5;
7 – theoretical direct line which characterize the substrate's protein hydrolysis process in Walker – Schmidt coordinates without any damping

Quantitative characteristics of the linearized experimental, theoretical and auxiliary lines, coordinates of intersection points are shown in Tabl. 2.

The obtained values of the apparent Michaelis constant for the experimental and theoretical dependences describing the enzymatic hydrolysis of high molecular proteins in the investigated substrates, as the process with considerable high hydronic module value influence.

For the systems with natural moisture, various damping factors reduce the enzymes and protein substrates congeniality by 1.65, and for the systems with hydronic module by 0.25–1.27 times.

Increasing the hydronic module value to 0.5 creates practically ideal enzyme-substrate system based on the black sea atherina and enzyme drag protosubtilin G3x. In this case, the ratio of the theoretical and experimental apparent Michaelis constant values is close to one (0.98).

Table 2

Linearized experimental, theoretical and auxiliary lines coefficients

Lines description	The equation which describes obtained direct lines in Walker – Schmidt coordinates	Apparent Michaelis constant value
Linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0	$0.0352-\frac{2126.9}{t}\ln\frac{\sum[S]_0}{\sum[S]_0-\sum[P]}$	2126.90
Linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0.25	$0.0537-\frac{1636.4}{t}\ln\frac{\sum[S]_0}{\sum[S]_0-\sum[P]}$	1636.40
Linearized in Walker – Schmidt coordinates experimental dependence for enzyme-substrate system with hydronic module equal to 0.5	$0.1606-\frac{1264.2}{t}\ln\frac{\sum[S]_0}{\sum[S]_0-\sum[P]}$	1264.2
Auxiliary line drawn from the coordinates origin for the enzyme-substrate system with hydronic module equal to 0	$\frac{2365.2}{\tau}\ln\frac{\sum[S]_o}{\sum[S]_0-\sum[P]}$	$\mathrm{tg}\,\alpha_1=\sum[S]_{01}{}^*$
Auxiliary line drawn from the coordinates origin for the enzyme-substrate system with hydronic module equal to 0.25	$\frac{1892.1}{\tau}\ln\frac{\sum[S]_o}{\sum[S]_0-\sum[P]}$	$\mathrm{tg}\,\alpha_2=\sum[S]_{02}{}^*$
Auxiliary line drawn from the coordinates origin for the enzyme-substrate system with hydronic module equal to 0.5	$\frac{1576.7}{\tau}\ln\frac{\sum[S]_o}{\sum[S]_0-\sum[P]}$	$\mathrm{tg}\,\alpha_2=\sum[S]_{03}{}^*$
Theoretical direct line, which characterizes the process Of substrate protein hydrolysis in Walker – Schmidt coordinates without any damping	$0.1432-\frac{1292.4}{\tau}\ln\frac{\sum[S]_o}{\sum[S]_0-\sum[P]}$	1292.4
Intersection points coordinates for linearized experimental and auxiliary lines: for enzyme-substrate system with hydronic module equal to 0; for enzyme-substrate system with hydronic module equal to 0.25; for enzyme-substrate system withhydronic module equal to 0.5	(0.000148; 0.349) (0.000210; 0.397) (0.000514; 0.810)	

$^*\sum[S]_{01}$ – high molecular proteins concentration in the enzyme-substrate system with hydronic module equal to 0;

$^*\sum[S]_{02}$ – high molecular proteins concentration in the enzyme-substrate system with hydronic module equal to 0.25;

$^*\sum[S]_{03}$ – high molecular proteins concentration in the enzyme-substrate system with hydronic module equal to 0.5.

From the foregoing results, the necessity of further research to improve understanding of hydronic module value increasing in enzyme-substrate systems becomes obvious.

If the observed beneficial effect is mainly connected to overcoming diffusion damping of protein enzymatic hydrolysis by viscosity reducing in the water-diluted substrates, the similar effect can be also observed as a result of effective viscosity reduction by agitation.

Conclusion

1. It was determined that accepted in studies the substrate systems, based on the black sea atherina (Atherina mochon pontica) muscle tissues contain a small amount of low molecular protein com-

pounds, that allows to consider the effects of the enzymatic process of competitive damping at the initial stages insignificant.

2. From the character of the received experimental curves of accumulation of protein enzymatic hydrolysis products, it follows that the intensive process damping takes place at the initial hydrolysis stages. The diffusion damping nature is testified by the increasing of the velocity process, which is accompanied by substrate viscosity decreasing.

3. From the experimental curves linearization, the values of the apparent Michaelis constants for the substrates with different moisture based on black sea atherina muscle tissue were defined. From the comparison of their values, it follows that in a system with high humidity protosubtilin G3x enzymes complex has higher congeniality to ubstrate's proteins than in the systems with natural water content.

4. The obtained value of the apparent Michaelis constant for the theoretical process states that enzymatic hydrolysis in the discussed enzyme-substrate systems with 50 % water addition does not occur with any damping.

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ПРИМЕНЕНИЕ МЕТОДА ФОСТЕРА – НЕЙМАНА ДЛЯ ОЦЕНКИ ЭФФЕКТИВНОСТИ ФЕРМЕНТАТИВНОГО ГИДРОЛИЗА БЕЛКОВЫХ ВЕЩЕСТВ ЧЕРНОМОРСКОЙ АТЕРИНЫ

Проведена оценка эффективности технологических режимов ферментативного гидролиза белковых веществ черноморской атерины с помощью метода Фостера – Неймана. В результате линеаризации полученных экспериментальных кривых установлены значения кажущихся констант Михаэлиса для субстратов различной влажности на основе мышечной ткани черноморской атерины. Из сравнения их значений следует, что в системе с повышенной влажностью сродство комплекса ферментов ферментного препарата протосубтилин Г3х

к белкам субстрата выше, чем в системах естественной влажности. Полученное значение кажущейся константы Михаэлиса для теоретического процесса позволяет утверждать, что ферментативный гидролиз рассмотренных фермент-субстратных систем с добавлением 50 % воды не встречается с каким-либо торможением.

Ключевые слова: рыбные белковые гидролизаты, объективная оценка степени торможения процесса ферментативного гидролиза, черноморская атерина, метод Фостера – Неймана, интегральное уравнение Михаэлиса – Ментен.

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