Orthogonal central composite planning application for Azov sardelle proteins enzymatic hydrolysis process investigation

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Анотація. *Уточнено дані з хімічного складу тюльки азовської. Розглянуто можливість застосування ортогонального центрального композиційного планування при дослідженні процесу ферментативної деградації білкових речовин сировини ферментним препаратом Протосубтилін Г3х. Отримано параметри рівнянь регресії другого порядку для опису експериментальних поверхонь відгуку для індикаторних показників процесу. Визначено оптимальні умови ферментолізу.*
Ключові слова: *ферментативний гідроліз, тюлька азовська, рівняння регресії, ферментний препарат, білковий гідролізат.*

Abstract*. Azov sardelle chemical composition data was clarified. The possibility of orthogonal central compositional planning applying for raw proteins enzymatic degradation process by enzyme drag Protosubtylin G3x was discussed. The adequate second order regression equations parameters to describe the experimental response surfaces for process indicators were obtained. The optimal conditions for enzymatic hydrolysis are determined.*
Key words*: enzymatic hydrolysis, Azov sardelle, regression equation, enzyme drag, protein hydrolyzate.*

Introduction

Significant food and feed animal protein shortages dedicates the necessity of development and improving various resource-saving technological processes. One of the promising directions of such processes development is the enzymatic protein hydrolysates production from row underutilized fish species and fish processing by-products.

Generally, protein hydrolysates find their application as the components of food and special products, various animal's fodder blends [1].

The main element of fish protein hydrolyzate technology development is the raw materials proteins enzymatic hydrolysis conditions investigations. At this research stage should be obtained polynomial adequate mathematical model, which describes both individual and complex process conditions (factors) interactions impact at the raw materials protein hydrolyses product accumulation (response function).

The necessary model for experiment laboriousness reducing can be obtained under plural factorial experiment mathematical planning methods.

Due to the proteolysis processes nature, can be confidently assert that the response function description by the polynom of first order would be insufficient, and the regression equation required degree problem, remains open. Obvious that the high polynomial degree leads to a significant enhance labor input, excessive reagents consumption.

The most appropriate for enzymatic hydrolysis process study is the orthogonal central composite designs application, as in contrast to the rototabele planning, this method guaranteed allows to get star points coordinates accessibled to research range.

In this context, the main aim of this work was to determine necessary and sufficient regression equation degree, obtained by the implementation of orthogonal central composite design application in investigation of shallow Azov-Black Sea fish proteins enzymatic degradation process.

Table 1

Research orthogonal central composite design for Azov sardelle proteins enzymatic hydrolysis process.

Experimental point number		Experimental design in coded form			Experimental design in true form		
		the hydrolysis duration, min.	the enzyme drag dose,% *	hydronic module,% *	the hydrolysis duration, min.	the enzyme drag dose,% *	hydronic module,% *
1	the plan core	-1	-1	-1	60	0,1	10
2		1	-1	-1	360	0,1	10
3		-1	1	-1	60	0,5	10
4		1	1	-1	360	0,5	10
5		-1	-1	1	60	0,1	50
6		1	-1	1	360	0,1	50
7		-1	1	1	60	0,5	50
8		1	1	1	360	0,5	50
9	star points	-1,287	0	0	17	0,3	30
10		1,287	0	0	403	0,3	30
11		0	-1,287	0	210	0	30
12		0	1,287	0	210	0,56	30
13		0	0	1,287	210	0,3	4
14		0	0	-1,287	210	0,3	56
15	plan center	0	0	0	210	0,3	30
16		0	0	0	210	0,3	30

** to raw fish mass*

To achieve this goal in the work the following tasks were discuss:

- to clarify consumption raw fish chemical composition;
- to obtain the experimental points value for enzymatic process orthogonal central composite design;
- to verify the experimental equation regression adequacy and, in necessity, to carry out further studies by higher order experimental design;
- to obtain fish raw proteins enzymatic hydrolysis process optimal parameters with received regression equation application as an objective function.

Materials and methods

In the experimental part of the work, as the raw material was used a massive, underutilized shallow fish - Azov sardelle (Clupeonella cultriventris). Enzymatic hydrolysis was carried out by commercial microbial proteolytic enzyme drag - Protosubtilin G3x.

To characterize created enzyme - substrate systems, in the raw fish, the moisture and crude protein (TNx6,25) content, fat extracted by ethyl ether and ash amount were determined

The progress of enzymatic hydrolysis process was assessed by free tyrosine accumulation, which amount was determined by the colorimetric method with Folin-Ciocalteu color reaction.

The free tyrosine amount determination was performed twice for each experimental point: after the sedimentation of non hydrolyzed proteins by boiling (Tir_f) and after the sedimentation of thermo stable proteins by trichloroacetic acid (Tir_{tcl}).

The total tyrosine amount (Tir) determination was performed after raw fish samples complete alkaline hydrolysis.

In order to form approachable for implementation orthogonal central composite plan (table 1) were taken experiment parameters of three-factor design which shown in table 2. All studies were performed at the native raw fish pH and hydrolysis process temperature - 54 °C.

Presented experimental design allows to get the regression equation not higher than second order. In the case if the obtained regression equations, has insufficient adequacy to experimental data, it was stipulated plan expansion to obtain a higher degree regression.

Results and discussion

The Azov sardelle chemical compo-

Table 2

The central three factor orthogonal composite second order design parameters

Variation levels	The coded parameters		
	the hydrolysis duration, min.	the enzyme drag dose,% *	hydronic module,% *
Overhead	360	0,5	50
Inferior	60	0,1	10
Basic	210	0,3	30
Variation step	150	0,2	20
Star shoulder	193	0,26	26

** to raw fish mass*

Table 3

Azov sardelle chemical composition

The indicator	Values of indicators
Moisture, %	60,6
Crude protein, % (TN x 6,25)	14,60
Fat content, %	23,2
Ash, %	1,60
Tir, mg/100 g	1306,27
Tir_f, mg/100 g	141,05
Tir_{tcl}, mg/100 g	130,00

sition resulting studies data (table 3) characterize this kind of fish as high-fat protein material.

This row fish proteins enzymatic hydrolysis allow to obtain a product with a sufficiently high nitrogen compounds content. Fat, released by hydrolysis, can be relatively easily separated by gravity methods.

The actual experimental conditions for Azov sardelle proteins enzymatic hydrolysis and the resulting values of its implementation for response functions are shown in table 4.

The resulting response surface, characterized free tyrosine accumulation in the enzyme - substrate systems based on the Azov sardelle are presented in figures 1 and 2.

The responsible surfaces for free tyrosine accumulation after high-temperature precipitation of nonhydrolyzed proteins is an inclined concave plane with a weak bend in process duration interval of 100-200 min, in enzyme drag dose dependence.

The responsible surfaces character suggests that the process intensity at the initial stage, (before bend zone) is lower than at final stage.

Response surface describing the free tyrosine accumulation after nonhydrolyzed proteins precipitation by trichloroacetic acid solution is an inclined convex plane.

Generally, this dependence has an ongoing character to the whole examined enzyme dose range.

Adequacy assessing of the mathematical model for Azov sardelle proteins enzymatic hydrolysis by enzyme drag Protosubtilin G3x showed that the experimental response surface described by the second order regression equations (table 5) with an accuracy not less than 95,6%.

Thus, it could be argued that second order orthogonal central composite designs application sufficient for protein enzymatic degradation investigation in the considered enzyme-substrate systems.

The hydrolysis process optimization was executed with the application of obtained regression equations as the objective function. Optimization allowed to obtain following the most appropriate process parameters (table 6).

The proteins enzymatic hydrolysis process optimum parameters comparative analysis shows that for maximum accumulation of low molecular weight protein fragments, non precipitable by trichloroacetic acid solution, the enzyme-substrate system must formed with a high dose of enzyme drug (0,55%) and hydronic module equal to 56%.

The maximum accumulation of free tyrosine with considering of thermostable protein fragments can also be obtained at high enzyme drag doses, but at low (4%) hydronic module values.

Conclusion

1. It was found that Azov sardelle (Clupeonella delicatula), accepted in research, contains 14,60% of proteins, 23,2% fat and 60,6% moister.

Revealed protein sufficient high content provides a basis to recommend this type of raw material for enzymatic protein hydrolysates production.

Table 4

Experiment plan actual parameters and response functions indicators values

Experimental point number	Experiment plan actual parameters in true form			Response function experimental values	
	the hydrolysis duration, min.	the enzyme drag dose,% *	hydronic module,% *	Tir_f, mg/100g row fish	Tir_{tcl}, mg/100g row fish
1	60	0,1	10	261,22	255,75
2	360	0,1	10	541,74	482,10
3	60	0,5	10	364,90	348,56
4	360	0,5	10	636,08	602,25
5	60	0,1	50	271,18	240,00
6	360	0,11	50	444,76	498,75
7	60	0,5	50	387,60	373,26
8	360	0,49	50	659,81	692,16
9	17	0,28	30	270,35	277,88
10	403	0,31	30	642,94	646,73
11	210	0	30	180,52	169,00
12	210	0,56	30	577,32	594,75
13	210	0,3	4	460,94	434,57
14	210	0,3	56	507,95	469,32
15	210	0,3	30	510,68	451,44
16	210	0,3	30	522,99	472,14

* to raw fish mass

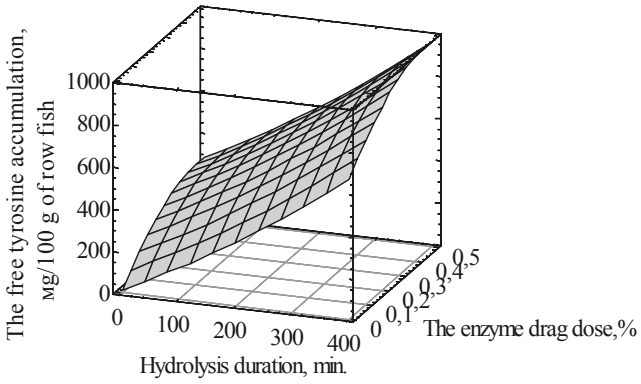

Figure 1. The free tyrosine accumulation in Azov sardelle proteins hydrolysis process after the precipitation of nonhydrolyzed proteins by boiling (hydronic module 30%)

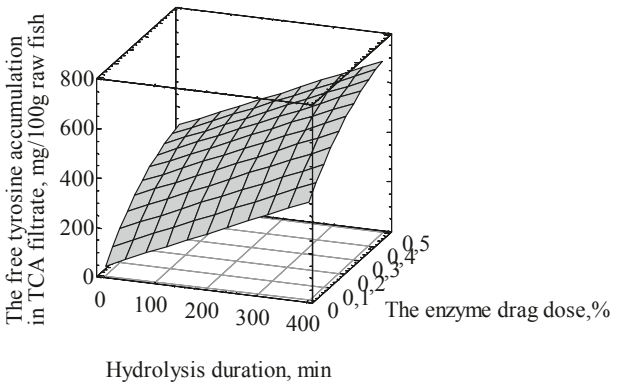

Figure 1. The free tyrosine accumulation in Azov sardelle proteins hydrolysis process after the precipitation of nonhydrolyzed proteins by trichloroacetic acid (hydronic module 30%)

Table 5

Regression equations describing free tyrosine accumulation in Azov sardelle proteins enzymatic hydrolysis process by Protosubtilin G3x

Process indicators	The regression equation
Tir_f	38,61 + 1,41 τ + 1148,58 C - 1,37 H - 0,00098 $τ^2$ +0,107088 τ C - 0,005 τ H - 1397,0 C^2 + 4,54707 C H + 0,018 H^2
Tir_{tcl}	222,82 + 1,46 τ + 679,82 C + 5,58 H - 1,14 C τ - 0,014 τ H - 574,96 C^2 + 4,24 C H - 0,055 H^2

where: τ - hydrolysis duration, min; C – added to the system enzyme drag amount, % to the raw fish mass; H – hydronic module, % to the raw fish mass.

Table 6

The Azov sardelle proteins enzymatic hydrolysis optimal parameters by enzyme drag Protosubtilin G3

Process parameters	Process indicators	
	Tir_f	Tir_{tcl}
The hydrolysis duration, min	374	380
The enzyme drag dose,%	0,42	0,55
Hydronic module,%	4,0	56,0

2. Application for experiment planning the second order orthogonal central composite designs allows to receive regression equation adequately describes the experimental response surface for considerated indicators.

3. The Azov sardelle proteins enzymatic hydrolysis by enzyme drag Protosubtilin G3x process parameters optimization showed that the maximum protein low molecular fragments accumulation is observed after 380 minutes in the system with hydronic module - 56% and enzyme drag dose – 0,55%.

For maximum accumulation of thermostable protein fragments is optimal enzymatic hydrolysis during 374 min in the system with hydronic module - 4% and enzyme drag dose – 0,42%.

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