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HYDROLYSATE OF OVALBUMIN: PRODUCTION AND EVALUATION OF FUNCTIONAL PROPERTIES OF PEPTIDES

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Abstract

Chicken eggs proteins and their derivatives, like protein hydrolysates, peptides and amino acids, possess high nutritional value and provide a wide range of biological activity. They serve as sources for development of functional ingredients that draw the attention of specialists in the food production and biomedical industries, as well as the livestock feed industry. Enzymatic hydrolysis of proteins is a popular process for obtaining bioactive peptides with multifunctional properties. The purpose of this study is to obtain a hydrolysate of ovalbumin with a high degree of hydrolysis and to determine its functional and technological parameters. The research presents a two-stage scheme of ovalbumin hydrolysis with the help of pepsin and trypsin which provide high degree of hydrolysis (82–83%). The fractional composition of the hydrolysate is determined. The fractional composition is represented by three main fractions (high, medium and low molecular weight). The summarized antioxidant activity (SAA) of the hydrolysate is considered within the dynamics of the hydrolysis process. The highest SAA value was noted after 2 hours, and it amounted to 170.23 mg/l; at the end of hydrolysis the SAA value was equal to 114.31 mg/l. When analyzing the SAA, it was found that the main contribution to the summarized antioxidant activity of the ovalbumin hydrolysate is made by peptides of the medium molecular fraction. The microfiltration process, used in the research, made it possible to separate high-molecular compounds, which led to an increase in the SAA of the hydrolysate to 189.9 mg/l. The main functional and technological parameters of the hydrolysate are determined in this research. The comprehensive study of the biological activity and functional characteristics of egg protein hydrolysates and their peptides provides a theoretical basis for expanding the range of functional ingredients obtained from food proteins and for replenishing the range of functional foods.

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Introduction

Proteins are an important macronutrient that provides catalytic, structural, regulatory, receptor, energy, transport, protective and respiratory functions in the human body. In addition, some dietary proteins may provide a positive effect on body functions and/or human health with the help of bioactive peptides release. Recently the close attention of scientists around the world has been attracted to the production and analysis of bioactive peptides, necessary for production of functional ingredients [1]. As a rule, bioactive peptides are oligopeptides that are included in the sequence of a protein molecule and that can be released in result of enzymatic hydrolysis, microbial fermentation or digestion in the gastrointestinal tract. Peptides with a low molecular weight are easily digested by the body, are available for nutrition of people with various disorders and diseases of the digestive system, and do not cause allergic reactions [2,3].

Studies have shown that protein hydrolysates (peptides) feature a wide range of biological activities. They possess immunomodulatory, anticancer, antihypertensive, antioxidant, anti-inflammatory, antilipidemic, osteoprotective, antimicrobial, and other useful properties [2]. In addition to biological activity, food-derived protein hydrolysates (peptides) have different physicochemical properties, including good solubility, ability of lipid binding, foaming and emulsification, etc., depending on their composition, sequence and length, which makes them quite demandable for their inclusion into the composition of food products [4].

For the recent years the demand for bioactive protein hydrolysates, obtained from natural sources in the food industry, has dramatically increased. Those researches are valuable due to opportunity to use the bioactive peptides as food additives (to increase the biological and nutritional

value of the product; the development of functional foods). In addition, consumers are increasingly aware that proper nutrition (i.e. not only balanced, but also functional) is directly related to their physical well-being and contributes to the prevention of various diseases [5,6].

Among various food sources of animal origin, eggs, in particular chicken eggs, are considered as inexpensive food products, but at the same time rich in nutrients, with a well-balanced composition of essential amino acids [7]. Eggs contain many biologically active compounds with antimicrobial, immunomodulatory, antioxidant, anticarcinogenic, hypotensive, and other properties [8].

In addition, chicken eggs are one of the most popular foods that are consumed all over the world. An egg contains an average of 13% proteins, which consist of several functionally active proteins: ovalbumin (about 54%), ovotransferrin (12–13%), lysozyme (3.4–3.5%), ovomucoid, ovomucin (1.5–3.5%), ovoglobulins (2%). Egg protein is considered a vital source for obtaining bioactive protein hydrolysates and peptides with multifunctional properties and potential application in healthy nutrition [9,10].

Since Fujita et al. [11] in 1995 identified the first bioactive peptide derived from egg protein (ovokynin — Phe-Arg-Ala-Asp-His-Pro-Phe-Leu), the interest to research and development of new bioactive peptides from this source has been rising vigorously. Hydrolysates and peptides have been obtained from various protein-rich egg structures, like egg white, egg yolk and eggshell [11].

In the review article, written by Moreno-Fernández et al. [11], the researcher presents data obtained from numerous researches devoted to the production and evaluation of the activity of enzymatic hydrolysates and peptides, prepared from egg white. These peptides have a hypoallergenic effect, and also exhibited antioxidant, antihypertensive, anti-inflammatory, hypocholesterolemic, and antidiabetic activities.

In the work of Venkatachalam et al. [12] reported data on enzymatic hydrolysis and production of bioactive hydrolysates/peptides from egg protein using various proteases (alcalase, ficin, protamex, and neutralase). The authors studied their influence on the parameters like the efficiency of hydrolysis, foaming and free radicals inactivation ability.

Although the isolated egg protein fractions (ovalbumin, lysozyme, ovotransferin) have been used in researches as a substrate for enzymatic hydrolysis and the production of bioactive peptides, more often the hydrolysis process is carried out using whole egg white using enzymes such as alcalase, flavorzyme, pepsin, bromelain, trypsin, α-chymotrypsin, papain, etc. [13].

It has been noted that peptides obtained from egg protein as a result of in vitro digestion "imitation" are resistant to in vivo digestive enzymes, which is a great advantage when they are delivered orally (as part of food products) [11].

Thus, the interest in technologies for the enzymatic modification of food proteins, including egg protein, remains extremely high.

The article by Zhamsaranova et al. [14] describes the production of an enzymatic hydrolysate of soy protein, while gel filtration was used to chromatograph it, and the summarized antioxidant activity of the obtained three main fractions (high, medium, and low molecular weight) was determined. The research of the authors B. A. Bolkhonova et al. is devoted to the selection of operable parameters for obtaining peptides of an egg white [15].

The purpose of this research is to obtain a hydrolysate of ovalbumin with a high degree of hydrolysis and to determine its functional and technological parameters.

Objects and methods

To obtain a hydrolysate, ovalbumin (Igreca SAS, France) and proteolytic enzymes pepsin (Reakhim, Russia), trypsin (Spofa, Czech Republic) were used as an object of study.

At the first stage, one-stage hydrolysis of ovalbumin was run separately with pepsin and trypsin. A 1% protein solution was prepared. Pepsin-aided hydrolysis was run in 0.1 M HCl (pH = 1.6) at a temperature of 39 °C for 1 to 5 hours after the introduction of the enzyme. Trypsin-aided hydrolysis was run in 0.1 M NaHCO3 (pH = 7.8) also at 39 °C for 1 to 5 hours after the introduction of the enzyme. For both enzymes the researchers used the different enzyme-substrate ratios: 1:30, 1:20, and 1:10.

At the second stage of research, a two-stage process of protein hydrolysis was used. The first stage of hydrolysis of ovalbumin (pepsin-aided hydrolysis) was run in 0.1M HCl (pH = 1.6) for 5 hours. During the second stage of hydrolysis (trypsin-aided hydrolysis for 3 hours), the pH of the reaction mixture was changed to 7.8 by adding 10% NaOH.

The content of total nitrogen was determined with the help of Nessler's reagent according to GPM.1.7.2.0027.151, the content of amine nitrogen in non-hydrolyzed raw materials and the resulting hydrolysates was determined by the method of formal titration (Sorens method) according to GPM.1.2.3.0022.152.

The degree of hydrolysis (DoH) of the protein was calculated by the following formula:

$$DoH = \left(\frac{N_{AA} - N_{AAo}}{N_{OA} - N_{AAo}}\right) \times 100\%, \quad (1)$$

where

N_{OA} is the content of total nitrogen,%;

N_{AAo} is the content of amine nitrogen in non-hydrolyzed raw material,%;

N_{AA} is the content of amine nitrogen in the hydrolysate after hydrolysis for a certain period of time, %.

[1] GPM.1.7.2.0027.15 "Determination of total nitrogen with Nessler's reagent in immuno-biological medications" Retrieved from https://pharmacopoeia.ru/wp-content/uploads/2016/09/GPM.1.7.2.0027.15-Opredelenie-obshhego-azota-s-reaktivom-Nesslera-v-immunobiologicheskih-lekarstvennyh-preparatah.pdf Accessed January 24, 2023. (In Russian)

[2] GPM.1.2.3.0022.15 "Determination of amine nitrogen by formol and iodometric titration methods" Retrieved from https://pharmacopoeia.ru/wp-content/uploads/2016/10/GPM.1.2.3.0022.15-Opredelenie-aminnogo-azota-metodami-formolnogo-i-jodometricheskogo-titrovaniya.pdf Accessed January 24, 2023. (In Russian)

The parameters of enzymatic hydrolysis were optimized with Mathcad 15 software. In order to find the maximum value of the response function and the corresponding values of the factors, a standard procedure was run to find the maximum of the function of two variables in a limited domain of definition. To obtain the value of the desirability function from the values of the response function, the Harrington formula was used [16].

The obtained hydrolysate was separated into peptide fractions by gel filtration on the G-55 molselect [14]. The hydrolysate was fractionated on a 1×25 cm column filled with molselect, while the gel height in the column was equal to 20 cm. The amount of exposed sample was 1 cm^3. Distilled water was used as the eluent. After applying the hydrolysate to the column, the hudrolysate was eluted in a stream of water at a rate of 35 cm^3/h. Sampling was carried out at rate of 1 cm^3. The absorbance of the eluate was determined at a wavelength of 210 nm on a Cary 300 spectrophotometer (Varian Optical Spectroscopy Instruments, Australia). Each selected fraction was additionally analyzed to determine the content of peptides by the method of Warburg and Christian [17], after that the percent content of each fraction was calculated in relation to the total number of components.

The macromolecular compounds in the hydrolysate composition were separated out by microfiltration through commercial filters Sartorius Minisart™ NML Syringe Filters, Sterile, with a pore diameter of 0.2 μm.

The summarized antioxidant activity (SAA) of the peptides was assessed by the amperometric method on the "Tsvet-Yauza-01-AA" antioxidant activity analyzer (Khimavtomatika, Russia) according to GOST R54037–2010[3]. The concept of the amperometric method is to measure the electric current that occurs during the oxidation of the tested substance (or mixture of substances) on the surface of the live electrode at a certain potential, and to compare the obtained signal with the signal of the standard (quercetin) under the same measurement conditions. The parameter is characterized as the summarized content of water-soluble antioxidants in the analyzed sample (mg/dm^3 or mg/l).

Solubility was determined according to GPM.1.2.1.0005.15[4], the viscosity of the solution — with a liquid capillary viscometer (glass capillary viscometer PVZh-2, Ekokhim, Russia) according to GPM.1.2.1.0015.15[5], foaming and foam stability were determined according to GOST 23409.26–78[6].

pH was determined by the potentiometric method using ph-009 (measurement range from 0 to 14, error ±0.1 pH, Kelilong, China) according to GOST 32892–2014[7], density was determined by the areometric method (AON-1 hydrometer, Khimlaborpribor, Russia) according to GOST R54758–2011[8].

The mass fraction of moisture and the mass fraction of solids were determined according to GOST 54607.4–2015[9].

Appearance, color, smell and taste were determined according to GOST R ISO 22935–2–2011[10].

The experimental data were processed by calculation of mean values (M), with standard error of the mean (m), and parametric evaluation criterion (Student's t-test). The results were considered significant when the threshold of differences significance was reached ($p \leq 0.05$)

Results and discussion

Ovalbumin was enzymatically converted with the proteolytic enzymes pepsin and trypsin, which are available on the Russian market and are relatively inexpensive. In addition, the application of these proteases imitates (simulates) the natural process of protein hydrolysis in the human body, which makes it possible to use the resulting hydrolysates orally (as part of food products) [18].

As noted above, at the first stage of the research, the conditions for one-stage enzymatic hydrolysis of ovalbumin were arranged separately by pepsin and by trypsin. During the study, after 1, 3 and 5 hours of hydrolysis, aliquots of the hydrolysate were sampled and analyzed to determine content of amine nitrogen. The content of total nitrogen and amine nitrogen was preliminarily determined in a sample of non-hydrolyzed raw materials. The degree of protein hydrolysis (SH) was calculated according to formula 1.

The results of a one-step process of ovalbumin hydrolysis, assessed by its degree (DoH) by the enzymes used (pepsin and trypsin), are presented below, respectively, in Figures 1 and 2.

From the data presented above in the Figure 1, it follows that in all variants of ovalbumin pepsin-aided hydrolysis, a significant increase in hydrolysis degree was observed. The highest efficiency was noted after 5 hours in the second variant of hydrolysis (ESR ratio 1:20) and DoH was 70.4 ± 0.21% ($p \leq 0.05$).

[3] GOST R54037–2010 "Food stuffs. Determination of water-soluble antioxidants content by amperometric method in vegetables, fruits, products of their processing, alcoholic and soft drinks" Retrieved from https://docs.cntd.ru/document/1200084226 Accessed January 24, 2023. (In Russian)

[4] GPM.1.2.1.0005.15 "Solubility" Retrieved from https://pharmacopoeia.ru/wp-content/uploads/2016/10/OFS.1.2.1.0005.15-Rastvorimost.pdf Accessed January 24, 2023. (In Russian)

[5] GPM.1.2.1.0015.15 "Viscosity" Retrieved from https://pharmacopoeia.ru/wp-content/uploads/2016/10/OFS.1.2.1.0015.15-Vyazkost.pdf Accessed January 24, 2023. (In Russian)

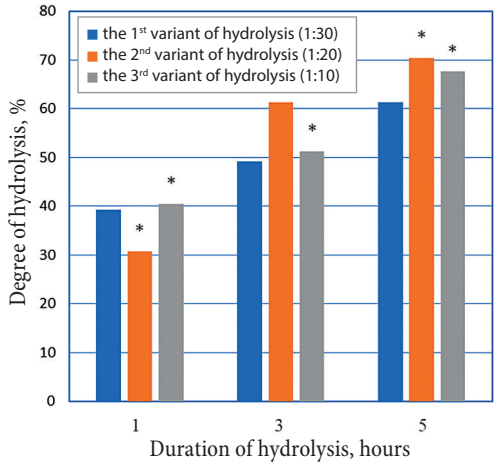
[6] GOST 23409.26–78 "Fluid self-hardening sand mixtures. Method for determination of surfactants solutions foaming ability and stability of foam" Retrieved from https://docs.cntd.ru/document/1200025337 Accessed January 24, 2023. (In Russian)

[7] GOST 32892–2014 "Milk and dairy products. Method of pH determination" Retrieved from https://docs.cntd.ru/document/1200114186 Accessed January 24, 2023. (In Russian)

[8] GOST R54758–2011 "Milk and milk products. Methods for determination of density" Retrieved from https://docs.cntd.ru/document/1200089992 Accessed January 24, 2023. (In Russian)

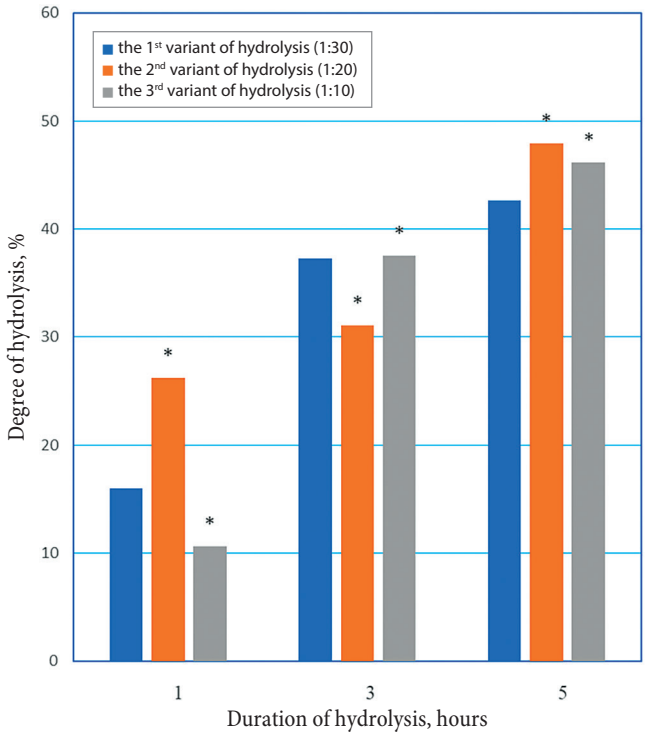
[9] GOST 54607.4–2015 "Public catering services. Methods of laboratory quality control of products of public catering. Part 4. Methods for determination of moisture and dry substances" Retrieved from https://docs.cntd.ru/document/1200127216 Accessed January 24, 2023. (In Russian)

[10] GOST R ISO 22935–2–2011" Milk and milk products. Sensory analysis. Part 2. Recommended methods for sensory evaluation" Retrieved from https://docs.cntd.ru/document/1200085798 Accessed January 24, 2023. (In Russian)



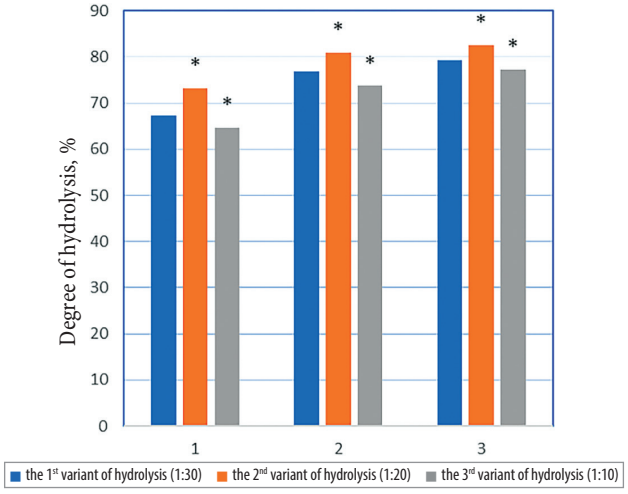
* significant differences between hydrolysis variants ($p \leq 0.05$).

Figure 1. Dynamics of the degree of ovalbumin hydrolysis by pepsin



* significant differences between hydrolysis variants ($p \leq 0.05$).

Figure 2. Dynamics of the degree of ovalbumin hydrolysis by trypsin



* significant differences between hydrolysis variants ($p \leq 0.05$).

Figure 3. Dynamics of the degree of ovalbumin hydrolysis by trypsin in a two-stage process

As can be seen from the Figure 2, in all variants of hydrolysis of ovalbumin with trypsin, an increase in its degree was also observed. The highest efficiency was noted for the second hydrolysis variant after 5 hours (ESR1:20) and DoH amounted to $47.9 \pm 0.49\%$ ($p \leq 0.05$).

At the second stage of research, in order to obtain a higher degree of protein splitting, a two-stage hydrolysis process was used, in which the enzyme productions pepsin and trypsin were introduced sequentially. The variant with the simultaneous introduction of enzymes was not considered, because the conditions for their action (pH values) are different. In a two-stage process, hydrolysis was at first run with pepsin for 5 hours at ESR1:20 (Figure 1, variant 2), after that trypsin was added (at ESR equal to 1:30, 1:20 and 1:10) and hydrolysis continued for another 3 hours. The obtained data on the degree of hydrolysis (DoH) of ovalbumin with trypsin in a two-stage process are shown below in the Figure 3.

From the data presented in the Figure 3, it follows that the two-stage hydrolysis process (first stage was run with pepsin at ESR equal to 1:20 for 5 hours, and then with trypsin at ESR equal to 1:20 for 3 hours) contributed to an increase in degree of hydrolysis up to the maximum value — $82.56 \pm 0.66\%$ ($p \leq 0.05$).

This hydrolysate can be characterized as a "deep" hydrolysate, i.e. it features a high degree of protein splitting. It can be used to produce the functional hypoallergenic food products [19].

Based on the obtained data, factorial experiments using mathematical methods were run to optimize the enzymatic hydrolysis of ovalbumin.

Main parameters for optimization:

- Duration of hydrolysis
- Enzyme-substrate ratio (ESR)

Results of a two-factor experiment

Based on the planned levels (Table 1 and Table 2), a matrix of a two-factor experiment was drawn up, according to which 9 experiments were run. When compiling the structure of the matrix, it was taken into consideration that during all experiments, each level of any factor occurs once along with each level of any other factors.

Table 1. Levels of the analyzed factors

Factor	Level		
	1	2	3
X1, Duration, hours	1	3	5
X2, Enzyme-substrate ratio	1:10	1:20	1:30

Table 2. The levels of the analyzed factors in a two-stage process: at first — pepsin-aided hydrolysis for 5 hours (at ESR1:20), and then with trypsin for 3 hours

Factor	Level		
	1	2	3
X1, Duration of trypsin hydrolysis, hours	1	2	3
X2, Enzyme-substrate ratio	1:10	1:20	1:30

Results of the regression analysis of the response function

To obtain the response function, multivariate non-linear regression was performed with the help of the second-order polynomial in *Statistica 10* software, and the equations 2–4 were obtained.

Response function that characterizes the degree of pepsin-aided hydrolysis of ovalbumin is as follows:

$$Y(x_1, x_2) = 0.259x_1^2 - 331.167x_2^2 + 5.317x_1 + \\ + 685.713x_2 - 350.96 \quad (2)$$

Response function that characterizes the degree of trypsin-aided hydrolysis of ovalbumin is as follows:

$$Y(x_1, x_2) = 0.355x_1^2 - 301.457x_2^2 + 4.122x_1 + \\ + 671.575x_2 - 389.572 \quad (3)$$

Response function that characterizes the degree of two-stage hydrolysis of ovalbumin by pepsin and trypsin:

$$Y(x_1, x_2) = -2.468x_1^2 - 713.1x_2^2 + 34.541x_1 + \\ + 1011.57x_2 - 987.56 \quad (4)$$

An assessment of the quality of regression equations is presented below in the Table 3.

Table 3. Evaluation of the regression levels quality

Quality parameters of the regression equation	Parameters of pepsin-aided hydrolysis	Parameters of trypsin-aided hydrolysis	Parameters of two-stage hydrolysis
Multiple Correlation Index	R = 0.981	R = 0.973	R = 0.9922
Determination coefficient	$R^2 = 0.986$	$R^2 = 0.944$	$R^2 = 0.985$
Fisher's criterion	F = 37.72 p < 0.002	F = 31.56 p < 0.002	F = 63.46 p < 0.001

From the parameters given in the Table 3, it follows that the obtained regression equations feature high accuracy and statistical reliability.

Results of solving the extremal problem

In order to find the maximum value of the response function and the corresponding factors values, the standard procedure for determination of the maximum of two variables function in a limited domain of definition was run. When solving the extremal problem, the following solution was obtained (Table 4).

Table 4. Solution of extremal problems

Enzyme	The value of factors at the maximum point	The value of the response function at the maximum point
Pepsin	$X_1 = 7$ ч, $X_2 = 1{:}22$	Y = 77.631%
Trypsin	$X_1 = 7$ ч, $X_2 = 1{:}30$	Y = 79.92%
Pepsin (5 hours, ESR1:20) + trypsin	$X_1 = 3$ ч, $X_2 = 1{:}19$	Y = 88.275%

The influence of technological parameters on the degree of ovalbumin hydrolysis by enzyme preparations can be assessed by contours of "desirability" depending on the values of these factors (duration of the process and ESR) (Figures 4–6).

In our case, the higher the value of the desirability function, the higher the degree of hydrolysis (the value of Y response function at the maximum point, Table 4). At the same time, the range of values of the desirability function, equal to 0.63–0.37, is considered an acceptable and sufficient level [16].

Thus, in the course of processing of the experimental data, the factors contributing to the intensification of the enzymatic conversion of ovalbumin and the optimal parameters of the process were identified. For a one-stage process of pepsin-aided hydrolysis, the duration was 7 hours, enzyme-substrate ratio was equal to 1:22. For a one-stage process of trypsin-aided hydrolysis, the duration of hydrolysis was also equal to 7 hours, while enzyme-substrate ratio was equal to 1:30.

For a two-stage hydrolysis process, first pepsin-aided hydrolysis lasted for 5 hours at an enzyme-substrate ratio of 1:20, then with trypsin it lasted for 3 hours at an enzyme-substrate ratio of — 1:19.

As noted earlier, peptides of protein hydrolysates possess a whole range of biological properties, including antioxidant ones [2]. The study showed that peptides manifest their antioxidant properties in several ways — that is inhibition of free radicals, chelation of metal ions of variable valence, inactivation of reactive oxygen intermediates or reduction of hydroperoxides [18].

During the study, the dynamics of changes in the summarized antioxidant activity (SAA) during the hydrolysis of ovalbumin was determined. Sampling was carried out every hour for 8 hours long. The SAA of the non-hydro-

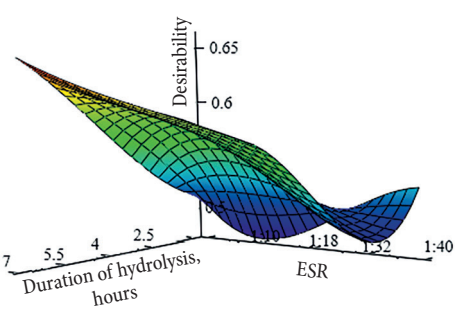


Figure 4. Contour of desirability of degree of pepsin-aided hydrolysis of ovalbumin

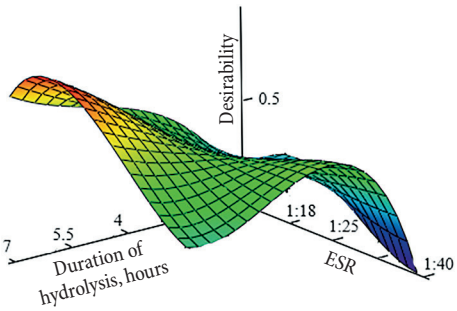


Figure 5. Contour of desirability of degree of trypsin-aided hydrolysis of ovalbumin

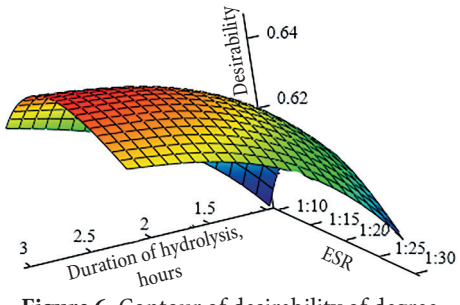


Figure 6. Contour of desirability of degree of two-stage hydrolysis of ovalbumin (at first with pepsin, then with trypsin)

lyzed protein was preliminarily determined. The data obtained is presented below in the Figure 7.

From the obtained results, presented above in the Figure 7, it follows that the dynamics of changes in SAA depended on the duration of the hydrolysis process and showed a wavy pattern. The maximum SAA value was noted after 2 hours of hydrolysis and amounted to 170.23 mg/l, which was almost 10 times higher than SAA before the beginning of hydrolysis (0 hours) ($p \leq 0.05$). Then, the SAA index decreased to 59.03 mg/l by the 5th hour, and then increased again almost 2 times ($p \leq 0.05$) an hour later, remaining almost at the same level until the end of the hydrolysis process. At the end of hydrolysis, the SAA value was equal to 114.32 mg/L. The obtained results prove that peptides with high antioxidant activity were formed during the ovalbumin hydrolysis.

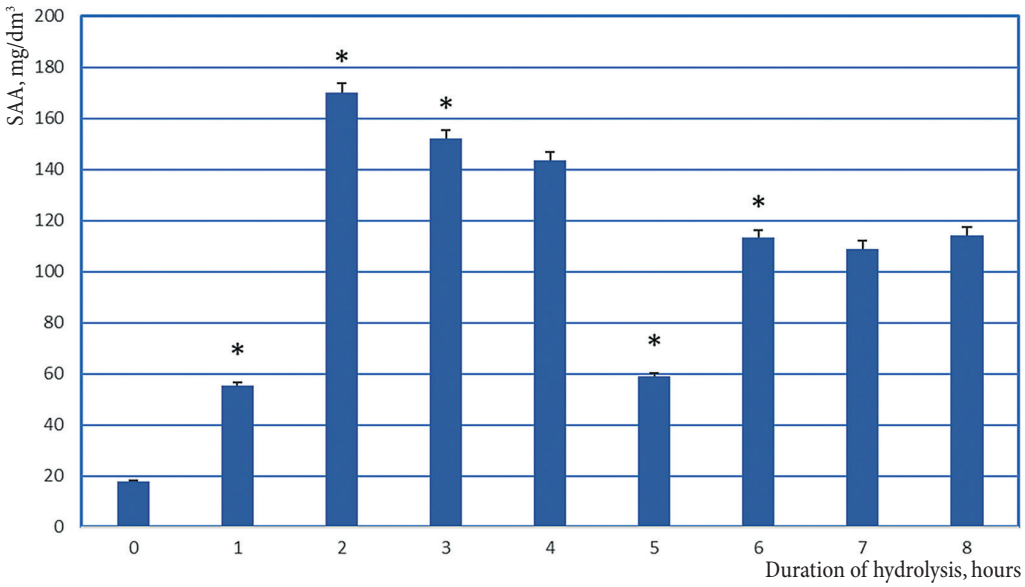
According to the literature, ovalbumin makes up 54% of proteins of an egg white and most of the identified antioxidant peptides are derived from it. Thus, ovalbumin is a rich source of antioxidant peptides that can be released under the action of gastrointestinal proteases [20, 21].

As follows from the literature data, most biologically active antioxidant peptides derived from dietary proteins consist of 2 to 20 amino acid residues, although some longer peptides have also been described [22].

Therefore, further in the research the fractional composition of the hydrolysate obtained after 8-hours two-staged hydrolysis process was studied with the help of gel chromatography method and the antioxidant activity of the obtained fractions was found.

Fractionation data are presented below in the Figure 8, from which it follows that as a result of egg white gel chromatography, the sample was divided into three main fractions (conditionally to the fraction of high molecular weight (I), medium molecular weight (II) and low molecular weight (III)):

1) Fraction I (11–20 vials) — eluate volume amounted to 10 ml;
2) Fraction II (21–33 vials) — eluate volume amounted to 13 ml;
3) Fraction III (34–80 vials) — eluate volume amounted to 47 ml.



* significantly significant differences in SAA subject to hours of hydrolysis ($p \leq 0.05$).

Figure 7. Dynamics of changes in the summarized antioxidant activity of egg hydrolysate peptides

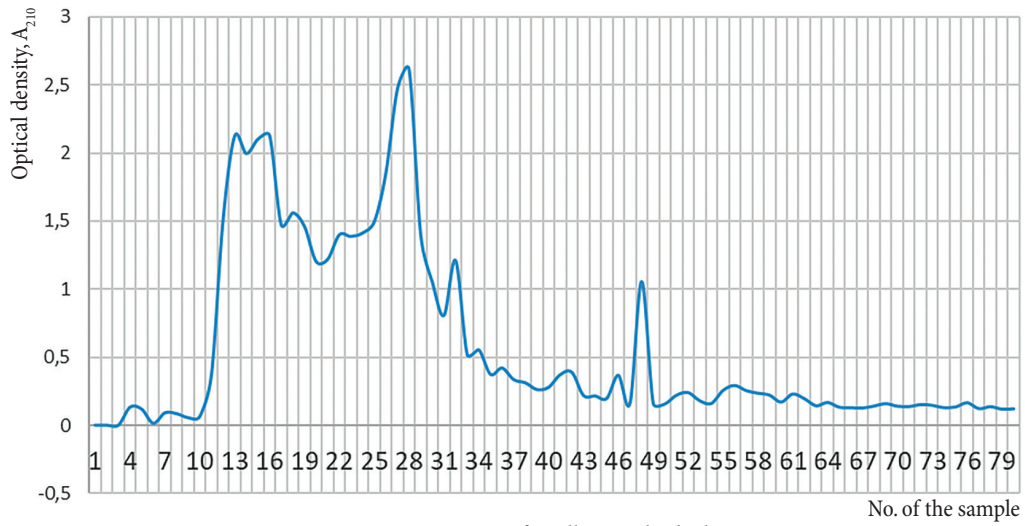


Figure 8. Fractionation of ovalbumin hydrolysate

The protein concentration in the hydrolysate was equal to 7.54 mg/mL. The protein concentration in the fractions was also 0.27 mg/mL in fraction I, 0.3 mg/mL in fraction II, and 0.02 mg/mL in fraction III, respectively. Thus, the proportion of proteins in fraction I was equal to 2.7 mg (35.8% protein), fraction II — 3.9 mg (51.7% protein), and fraction III — 0.94 mg (12.5% protein).

The summarized antioxidant activity of the three fractions at the same protein concentrations was comparatively assessed. The results of the assessment are presented in the Table 5 below.

Table 5. Results of antioxidant activity of peptide fractions

No. of the sample	No. of the fraction	Concentration of protein in the fraction, mg/ml	Summarized antioxidant activity, mg/l
1	I	0.27	11.2 ± 0.21
2	II	0.27	12.28 ± 0.23
3	II	0.3	13.54 ± 0.25*1
4	I	0.02	0.9 ± 0.02*1,2,3
5	II	0.02	4.9 ± 0.10*1,2,3,4
6	III	0.02	3.0 ± 0.06*1,2,3,4,5

*1,2,3,4,5 — significantly significant differences in reference to, respectively, the 1,2,3,4,5 sample ($p \leq 0.05$)

As it follows from the data in the Table 5, at equal protein concentrations the fraction II (medium molecular weight) (the samples 2, 3, and 5) featured the highest SAA. Thus, we can conclude that the main contribution to the antioxidant activity of ovalbumin hydrolysate is made by peptides of the average molecular weight fraction.

To separate macromolecular compounds from the hydrolysate obtained in result of high degree hydrolysis of protein (82.56%) and SAA equal to 114.32 mg/l, the hydrolysate was microfiltrated. This process also made it possible to increase the SAA to 189.9 mg/l.

Literature data testify that the identification and definition of peptides responsible for antioxidant activity is of great theoretical and practical interest. The authors Benedé et al. [23] showed that pepsin-aided 3-hours proteolysis of egg white at pH 2 and at temperature of 37 °C with an ESR ratio of 1:100 made it possible to obtain a peptide fraction with a molecular weight below 3 kDa, which featured an antioxidant activity 3 times greater than that of the native egg white. Four ovalbumin peptides were found whose antioxidant activity was caused by the presence of tyrosine at the N-terminus.

In addition to amino acid sequence, the molecular weight of peptides can also influence their antioxidant activity. Akbarian et al. [18] showed that the maximum antioxidant activity of hydrolyzed corn gluten protein was observed within the range from 0.5 to 1.5 kDa. In addition, it was found that the higher the degree of hydrolysis, the lower the antioxidant activity of the peptides. This process is associated with their further splitting to free amino acids with significantly lower antioxidant activity.

In further studies, the functional and technological parameters of the obtained egg hydrolysate were determined in comparison with ovalbumin solution. Summary data are presented below in the Table 6.

Table 6. Functional and technological parameters of ovalbumin hydrolysate

№	Parameter	Ovalbumin solution	Hydrolyzed ovalbumin
1	Appearance	Homogeneous liquid	Homogeneous liquid
2	Color	Creamy white	Milk transparent
3	Flavor	With a specific flavor	Tasteless
4	Smell	Weak specific odor	Odorless
5	Dry matter content,%	2.0 ± 0.1	2.0 ± 0.1
6	Moisture contents,%	98.0 ± 1.9	98.0 ± 1.9
7	Density, g/ml	0.999 ± 0.001	0.999 ± 0.001
8	pH	6.8 ± 007	7.8 ± 0,1
9	Protein content, mg/ml	3.85 ± 0.04	3.85 ± 0.04
10	Degree of hydrolysis,%	—	82.56 ± 0.66*
11	Solubility,%	100.0	100.0
12	Viscosity, mP	4.47 ± 0.04	4.08 ± 0.04*
13	Foaming ability,%	50.0 ± 0.50	100.0 ± 0.10*
14	Foam stability, min.	130.0 ± 2.6	65. 0 ± 1.3*
15	Summarized antioxidant activity, mg/l	17.78 ± 0.52	189.9 ± 3.79*

* reliably significant differences in the hydrolysate compared to the parameters of ovalbumin solution ($p \leq 0.05$)

As follows from the data in the Table 6, the hydrolysate of ovalbumin compared with its solution shows a row of differences. First of all, it features a high degree of protein hydrolysis (the 10th parameter) and SAA, which is 10.7 times higher than this parameter of the solution (the 15th parameter). The hydrolysate has a lower viscosity (by 8.7% less compared to the ovalbumin solution), 2 times higher foaming ability and 2 times lower foam stability; odorless and tasteless. These parameters will be taken into consideration when using the hydrolysate as an ingredient in a functional food products.

The work of Chinese researchers Chen et al. (2012–2018) showed that the functional properties of egg powder can be modified by enzymatic conversion. They have developed a whole series of egg protein powders (gel-like, foamy, emulsion, instant powder, etc.). A number of works by these authors are devoted to the production of egg protein hydrolysates and the study of peptides. Various enzymes were used to obtain such hydrolysates as papain [24,25], trypsin [26], pepsin [27], and other proteases [28]. The authors found that the functional properties of hydrolysates and peptides are directly affected by the type of enzyme, its concentration, pH, and duration of hydrolysis time. The obtained hydrolysates have high biological activity (in particular, antioxidant)

and improved functional characteristics (emulsification, foaming ability, solubility, etc.). From these hydrolysates, antioxidant peptides were obtained, purified and identified. Two purified peptides were synthesized [28]. In addition, the authors studied the influence of ultrafiltration [26] and types of drying (lyophilization, spray drying) [25] on the functional and technological parameters of hydrolysates.

Conclusions

Thus, a hydrolysate with a high degree of hydrolysis (82.56%) was obtained by a two-stage process of enzymatic conversion of ovalbumin using mathematical methods for planning an experiment. The optimal parameters of this process were determined: first, pepsin-aided hydrolysis lasted for 5 hours at an ESR of 1:20, and then trypsin-aided hydrolysis took over for 3 hours at an ESR of 1:19.

The SAA of the hydrolysate was determined, and the function of this parameter on the duration of the hydrolysis process was determined. This dependence had wavy pattern. The highest SAA value was recorded after 2 hours of hydrolysis and amounted to 170.23 mg/l. After 8 hours of hydrolysis, the SAA value was equal to 114.31 mg/l.

Fractionation of the ovalbumin hydrolysate allowed its separating into 3 main fractions (of high, medium and low molecular weight respectively). The peptides of the medium molecular fraction of hydrolysate showed the highest SAA values.

The microfiltration process made it possible to partially remove macromolecular compounds from the hydrolysate, which led to an increase of SAA value up to 189.9 mg/l.

The main functional and technological parameters of ovalbumin hydrolysate were determined.

Ovalbumin hydrolysate can be recommended as a component of functional hypoallergenic and antioxidant food products.

This way, the comprehensive study of the biological activity and functional characteristics of egg protein hydrolysates and their peptides provides a considerable theoretical basis for expanding the range of functional food ingredients derived from food proteins and thus expanding the assortment of functional food products.

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