Identifying Bioactive Peptides from Poultry By-Products

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Abstract.
Bioactive peptides derived from food proteins are becoming increasingly popular ingredients due to their beneficial effect on the immune system and other functional properties. We aimed to develop a technology for obtaining peptides from poultry by-products and identify their bioactivity.

Pepsin was the main reagent for the *in vitro* enzymatic hydrolysis. Specialized equipment and methods were used to determine the key indicators. The molecular weight and bioactivity of the resulting peptides were calculated by using the Peptide Mass Calculator and PeptideRanker online resources.

First, we developed a flow chart for obtaining bioactive peptides and produced hydrolysates from poultry by-products. The hydrolysates had identical physicochemical parameters, with no significant differences. The molecular weight distribution revealed that most protein fractions were represented by peptides with a molecular weight below 20 kDa. Then, we evaluated the bioactivity of the peptides. The hydrolysate obtained using pepsin with an activity of 30 units per 100 g of material showed higher bioactivity in the FD peptides (0.922094). The hydrolysate obtained using pepsin with an activity of 45 units per 100 g of material had greater bioactive properties in the CYG peptides (0.947378).

Based on the results, we designed a flow chart for obtaining hydrolysates from poultry by-products and evaluated the bioactive properties of the peptides obtained. For further work, these properties should be confirmed by *in vitro* experiments to determine the reliability of our data and identify specific bioactive properties of the peptides.

Keywords. Peptides, hydrolysis, hydrolysates, waste-free technologies, *in vitro*, bioactive properties

Получение и идентификация биоактивных пептидов из вторичных сырьевых ресурсов переработки птицы

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Аннотация.
Биоактивные пептиды, полученные из пищевых белков, становятся все более популярными на рынке пищевых ингредиентов. Они способствуют укреплению иммунного статуса организма, а также обладают другими функциональными свойствами. Цель исследования состояла в разработке технологии получения пептидов из вторичных сырьевых ресурсов переработки птицы и идентификации их биоактивности.

В качестве основного реагента для проведения исследования использовался фермент пепсин. Ферментативный гидролиз проводили *in vitro*. Для определения основных показателей применяли специальное оборудование и методики. Молекулярную массу и биоактивность полученных пептидов рассчитывали с помощью онлайн-ресурсов Peptide Mass Calculator и PeptideRanker.

На первом этапе исследования была разработана принципиальная схема производства биоактивных пептидов. Были получены гидролизаты из вторичных сырьевых ресурсов переработки птицы. По физико-химическим показателям сухие гидролизаты были идентичны друг другу, значимых различий не выявлено. Из результатов анализа молекулярно-массового распределения выявлено, что основные фракции представлены пептидами с молекулярной массой ниже 20 кДа. В гидролизате образца № 1, полученного с применением пепсина активностью 30 ед. на 100 г сырья, большей биоактивностью обладают пептиды FD. Их биоактивные свойства равны 0,922094 ед. Три пептидные последовательности гидролизата образца № 2, полученного с применением пепсина активностью 45 ед. на 100 г сырья, обладают биоактивными свойствами. Большей биоактивностью обладают пептиды CYG (0,947378 ед.).

Была разработана принципиальная схема получения гидролизатов из вторичных сырьевых ресурсов переработки птицы. Проведена оценка биоактивных свойств полученных пептидов. Для дальнейшей работы биоактивные свойства следует подтверждать экспериментальными исследованиями *in vitro*, которые помогут определить достоверность полученных данных и конкретные биоактивные свойства изучаемых пептидов.

Ключевые слова. Пептиды, гидролиз, гидролизаты, безотходные технологии, *in vitro*, биоактивные свойства

Introduction

Meat is a source of complete protein in the human diet. Meat proteins have a high nutritional value and therefore contribute to the normal physiological status. They are converted into various forms during cooking, processing, and digestion. For example, bioactive peptides obtained from meat proteinsthrough an enzymatic reaction can help maintain the immune status of the human body. Not only muscle meat is a good source of protein and bioactive peptides, but offal is as well. Therefore, meat by-products have increasingly been studied and used to produce functional ingredients and bioactive peptides [1–6].

Consumers are becoming increasingly aware of the immune-boosting properties of bioactive peptides derived from dietary proteins. Bioactive peptides are small fragments of dietary proteins, mostly consisting of 2–20 amino acid residues. They can be ligands and therefore have many targets in the human body, such as the immune, cardiovascular, digestive, and endocrine systems [7–11].

Hydrolysis is the main process of isolating peptides. Many studies confirm that the proteins of meat by-products are hydrolyzed by proteolytic enzymes under controlled conditions, enabling bioactive peptides to form. The main proteolytic enzymes that can be used to hydrolyze proteins are pepsin, trypsin, chymotrypsin, corolase, papain, as well as enzymes of microbial origin, such as *Neutrase* from *Bacillus amyloliquefaciens* and *Alcalase* from *Bacillus licheniformis* [12–15].

The efficacy and safety of bioactive peptides must be proven by clinical studies on living organisms before they can be approved for use by regulatory and supervisory authorities [16–18].

Some food-derived peptides have antioxidant, immunomodulatory, antihypertensive, anticancerous, anti-inflammatory, antimicrobial, hypocholesterolemic, intestine-modulatory, antidiabetic, opioid, and metal-chelating properties [19–23]. Their biological activity mainly depends on their amino acid composition, sequence, length, and charge [24, 25].

Currently, hundreds of peptides with different biological action have been identified and isolated from various food sources, including milk, whey, eggs, fish, rice, soybeans, peanuts, chickpeas, corn, and algae [24].

However, only a few of them are marketed as functional nutraceutical products. For example, bioactive peptides derived from milk and fish are more commonly used as food ingredients than peptides from other food sources. Table 1 presents some bioactive peptides obtained from various protein sources and lists their properties [26].

Antioxidant peptides usually contain hydrophobic amino acids and residues of histidine, phenylalanine, tryptophan, or tyrosine.

Thus, the food industry needs to develop technologies for isolating bioactive peptides from by-products, including meat by-products.

We aimed to develop a technology for obtaining peptides from poultry by-products and study the bioactivity of some peptide sequences.

To achieve this aim, we set to:
– select enzymes and develop a scheme for hydrolyzing a homogeneous mass of chicken skin;
– develop two alternative schemes for producing bioactive peptides through the hydrolysis of poultry by-products;
– conduct an electron microscopy of the obtained hydrolysates;
– determine the main physicochemical parameters of the hydrolysates; and
– perform a comparative analysis of the peptides.

Study objects and methods

We studied broiler chicken skin in a homogeneous state. First, we selected proteolytic enzymes and hydrolysis conditions that could increase the bioactivity and yield of hydrolysates. In particular, we chose a

Table 1. Some bioactive peptides and their properties

Таблица 1. Некоторые биоактивные пептиды и их свойства

Protein source	Peptide sequence	Bioactive properties
Cow's milk whey lactoferrin	*Glu-Asn-Leu-Pro-Glu-Lys-Ala-Asp-Arg-Asp-Gln-Tyr-Glu-Leu*	Osteoblast-proliferating
Beans (*Phaseolus vulgari*)	*Gly-Leu-Thr-Ser-Lys*, *Leu-Ser-Gly-Asn-Lys*, *Gly-Glu-Gly-Ser-Gly-Ala*, *Met-Pro-Ala-Cys-Gly-Ser-Ser*, and *Met-Thr-Glu-Glu-Tyr*	Anticancerous
Soy	*Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro-Gly-Arg*	Immunomodulatory
Chicken egg lysozyme	*Val-Ala-Trp-Arg-Asn-Arg-Cys-Lys-Gly-Thr-Asp*, *Trp-Arg-Asn-Arg-Cys-Lys-Gly-Thr-Asp*, *Ala-Trp-Ile-Arg-Gly-Cys-Arg-Leu*, *Trp-Ile-Arg-Gly-Cys-Arg-Leu*, and *Ile-Arg-Gly-Cys-Arg-Leu*	Antioxidant
Egg white	*Ala-Glu-Glu-Arg-Tyr-Pro*, *Asp-Glu-Asp-Thr-Gln-Ala-Met-Pro*, *Pro-Val-Asp-Glu-Asn-Asp-Glu-Gly*, *Gln-Pro-Ser-Ser-Val-Asp-Ser-Gln-Thr-Ala-Met*, and *Glu-Glu-Arg-Tyr-Pro*	Antioxidant
Casein	*Tyr-Gln-Lys-Phe-Pro-Gln-Tyr-Leu-Gln-Tyr*	Antihypertensive
Egg yolk	*Ile-Arg-Trp* and *Ile-Gln-Trp*	Antidiabetic
Tuna	*Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro*	Antihypertensive
Freshwater clam (*Corbicula fluminea*)	*Val-Lys-Pro* and *Val-Lys-Lys*	Hypocholesterolemic
Soy	*Tyr-Val-Val-Asn-Pro-Asp-Asn-Asp-Glu-Asn* and *Tyr-Val-Val-Asn-Pro-Asp-Asn-Asn-Glu-Asn*	Hypocholesterolemic

Table 2. Characteristics of the enzyme used in the study

Таблица 2. Характеристика фермента, используемого в исследовании

Indicator	Description
Composition	Pepsin based on *Rhyzomucor miehei* (CAS: 9001-92-7)
Origin	Microbial
Assumed splitting	$Phe^1 + Val$, $Gln^4 + His$, $Glu^{13} + Ala$, $Ala^{14} + Leu$, $Leu^{15} + Tyr$, $Tyr^{16} + Leu$, $Gly^{23} + Phe$, $Phe^{24} + Phe$, and Phe^{25}
Form, color	White powder
Activity, units per 1 g	At least 300 000
Activation temperature, °C	30 ± 2
Producer	Meito Sangyo Co., Ltd., Japan
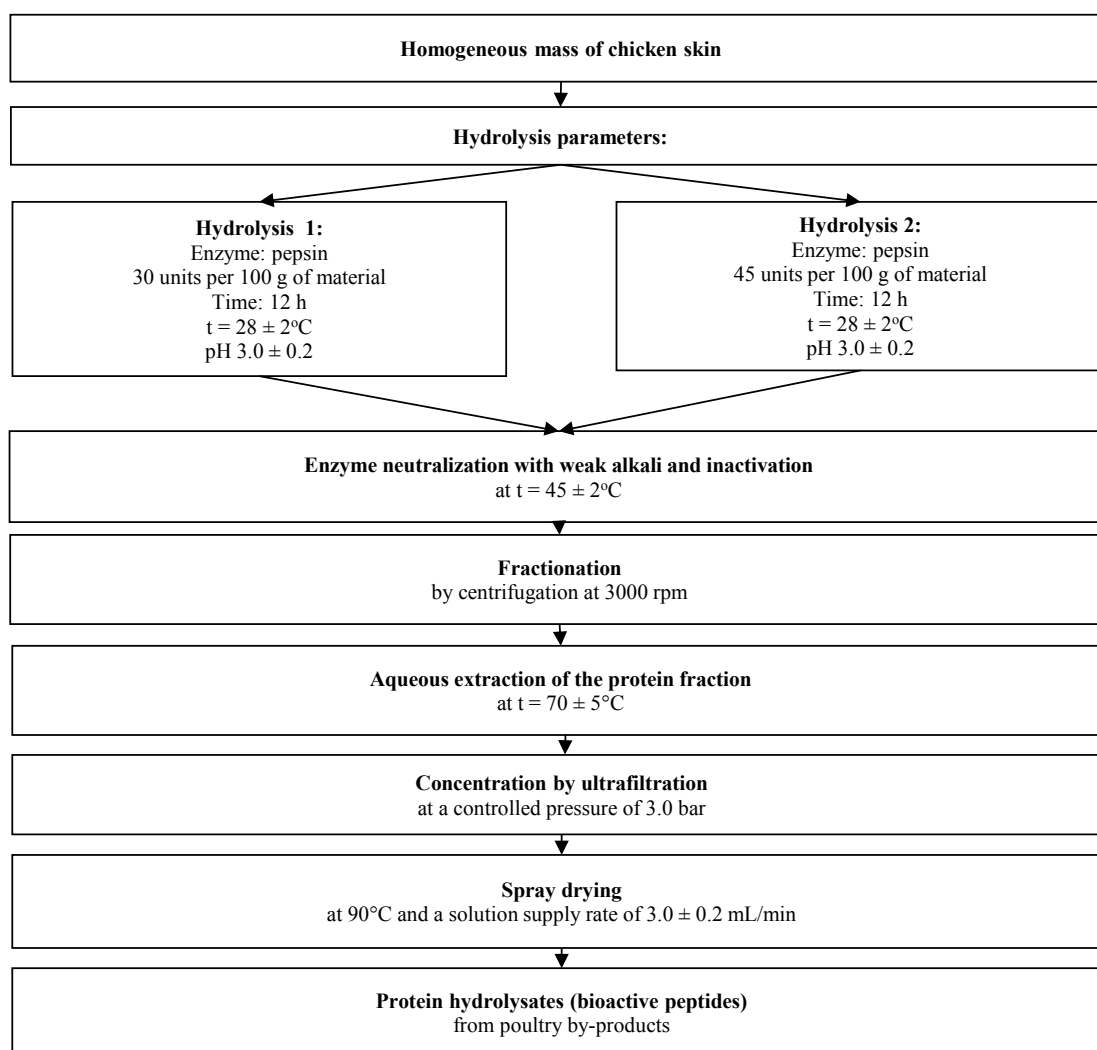
Figure 1. Flow chart for producing bioactive peptides from poultry by-products using hydrolysis (two variants)

Рисунок 1. Схема производства биоактивных пептидов с применением гидролиза (в двух вариациях) из вторичных сырьевых ресурсов переработки птицы

pepsin produced by Meito Sangyo Co. (Japan) for our experiments as a potentially more suitable enzyme for obtaining peptides from poultry by-products (Table 2).

As can be seen in Table 2, the enzyme has high proteolytic properties and is activated at temperatures that are favorable for protein structures.
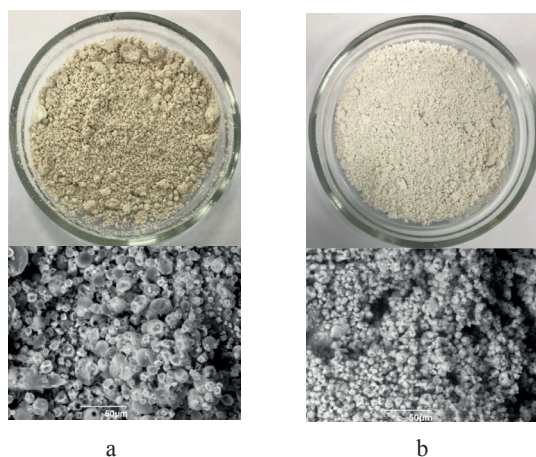
Figure 2. Protein hydrolysates from poultry by-products:
a – Sample 1 based on pepsin with an activity of 30 units
per 100 g of material; b – Sample 2 based on pepsin with
an activity of 45 units per 100 g of material

Рисунок 2. Белковые гидролизаты из вторичных сырьевых
ресурсов переработки птицы: a – образец № 1, полученный
с применением пепсина активностью 30 ед. на 100 г сырья;
b – образец № 2, полученный с применением пепсина
активностью 45 ед. на 100 г сырья

Table 3. Physicochemical parameters of the hydrolysates

Таблица 3. Основные физико-химические показатели
гидролизатов

Parameters, %	Sample 1	Sample 2
Protein	90.7 ± 0.1	91.4 ± 0.2
Fat	0.60 ± 0.03	0.40 ± 0.06
Moisture	8.1 ± 0.1	7.8 ± 0.2
Ash	0.60 ± 0.04	0.40 ± 0.03

Enzymatic hydrolysis was carried out *in vitro*. MM-5 laboratory magnetic stirrers were used to ensure a uniform treatment of the materials with an enzyme solution throughout the experiment (100 rpm, $28 \pm 2°C$). The acidity was maintained with 1M hydrochloric acid. The hydrolysates were dried in a B-290 Mini Spray Dryer (Buchi, Sweden) at $100 \pm 2°C$ and at a solution supply rate of 3.5–4.0 mL/min. The dried hydrolysates were microphotographed with a JEOL JED-2300 electronic microscope (Japan). The mass fraction of protein was determined on a RapidN Elementar nitrogen (protein) analyzer. This analyzer uses the Dumas method that involves combusting the samples and registering total nitrogen on a thermal conductivity detector. Molecular weight distribution was performed by polyacrylamide gel electrophoresis in the presence of an anionic detergent (sodium dodecyl sulfate). The amino acid sequence of the peptides was determined by matrix-activated laser desorption/ionization on a MALDI Biotyper (Bruker). The molecular weight was calculated by using the Peptide Mass Calculator. The bioactivity of the peptides was assessed *in silico* using the PeptideRanker online server that ranks peptides by the predicted probability of their bioactivity. The structure of the peptides was modeled by using the PepDraw online tool.

The experiments were performed at the Department of Animal Origin Food Technology and the Scientific and Educational Center of the Research and Innovation Department, Kemerovo State University.

Results and discussion

The flow chart for producing bioactive peptides from poultry by-products is presented in Fig. 1. It includes two sets of parameters for enzymatic hydrolysis of a homogeneous mass of chicken skin.

Chicken skin was homogenized with a laboratory homogenizer. The homogeneous mass was hydrolyzed with pepsin in two variations during 12 h at $28 \pm 2°C$. The enzyme was then thermally inactivated at $45 \pm 2°C$ and neutralized with a weak alkali to pH 7.0 ± 2.0. Next, the hydrolysate was fractionated into protein and fat parts using a CM 6 M Multi centrifuge at 3000 rpm. The protein part was then subjected to thermal water extraction in order to dissolve the protein fractions at $70 \pm 5°C$. The resulting solution was filtered using a MFU-R-45-300 laboratory ultrafiltration unit (Russia) at a controlled pressure of 3.0 bar and a difference of 0.2–0.5 kgf/cm^2 in the discharge and return headers. The protein part of the solution went into the retentate, while the water and minerals went into the permeate. Further, protein hydrolysate samples were obtained by spray drying at $90 \pm 2°C$ and a solution supply rate of 3.0 ± 0.2 mL/min. The samples were then microphotographed with an electronic microscope (magnified 500 times), as can be seen in Fig. 2.

First, we evaluated the color and particle size of the hydrolysates. As we can see in Fig. 2, the samples differed in color, with sample 1 having a darker creamy color and sample 2 having a whitish color. Also, the hydrolysates differed in particle size, although they were dried under the same conditions. In particular, sample 2 had a more finely dispersed structure, which can be explained by a deeper hydrolysis of this sample.

Next, we determined the main physicochemical parameters of the hydrolysates for further studies (Table 3).

As can be seen in Table 3, the samples had no significant differences in physicochemical parameters. Since the hydrolysates had a high protein content (over 90%), they can be classified as a high-protein product.

Next, we analyzed the distribution of protein fractions by polyacrylamide gel electrophoresis in the

Table 4. Molecular weight distribution of the hydrolysates

Таблица 4. Молекулярно-массовое распределение образцов гидролизатов

Molecular weight, kDa	Distribution of peptide fractions by molecular weight, %	
	Sample 1	Sample 2
200	1.063760	2.194861
150	4.905115	2.328694
100	2.186618	4.007342
85	14.60372	0.948302
60	6.369427	3.001682
50	4.813185	0.776231
40	3.224112	1.697767
30	0.965264	2.011318
25	1.267319	5.766289
20	4.392935	11.56317
Less than 20	56.20855	65.70435

Table 5. Amino acid sequence of the hydrolysates

Таблица 5. Аминокислотная последовательность исследуемых гидролизатов

Parameter	Sample 1	Sample 2
Amino acid sequence in a one-letter code*	PILG/PILV/ILA/ILG/ILV/ ILT/PSV/GVS/HGL/HVI/SVP/SV/FD/NW	ILKH/PSPV/PSVP/GHG/SPV/SVP/AYG/ CYG
Peptide chain length	41	27
Isoelectric point (pI)	6.05	8.84

* A – alanine; C – cysteine; D – aspartic acid; E – glutamic acid; F – phenylalanine; G – glycine; H – histidine; I – isoleucine; K – lysine; L – leucine; M – methionine; N – asparagine; P – proline; Q – glutamine; R – arginine; S – serine; T – threonine; V – valine; W – tryptophan; Y – tyrosine.

* A – аланин; C – цистеин; D – аспарагиновая кислота; E – глутаминовая кислота; F – фенилаланин; G – глицин; H – гистидин; I – изолейцин; K – лизин; L – лейцин; M – метионин; N – аспарагин; P – пролин; Q – глутамин; R – аргинин; S – серин; T – треонин; V – валин; W – триптофан; Y – тирозин.

presence of an anionic detergent, sodium dodecyl sulfate. Table 4 shows the molecular weight distribution of the hydrolysates.

As can be seen in Table 4, most peptide fractions had a molecular weight below 20 kDa, especially those in sample 2. Thus, we can assume that the hydrolysates contained peptides with bioactive properties, especially sample 2.

Then, we determined the amino acid sequence of the hydrolysates under study (Table 5).

According to Table 5, sample 1 had a chain of 41 peptides, while sample 2 was represented by 27 peptides. We can also see that the first and the second variants of hydrolysis, which used pepsin with lower and higher activity, respectively (Fig. 1), split the protein into 14 and 8 fragments of amino acids (peptide sequences), respectively.

Next, we used online resources to determine the molecular weight and bioactivity of individual peptide sequences, as well as modelled their structure (Tables 6 and 7).

The PeptideRanker service has a threshold value of 0.5 for peptides' bioactive properties, i.e. any pep-tide with an estimated value above 0.5 is ranked as bioactive. However, literature shows that using a higher threshold, particularly 0.8, reduces the number of false positive results. Therefore, we assessed the bioactive properties of peptides based on a threshold bioactivity value of 0.8 and the maximum value of 1.

According to Table 6, the FD peptides (38–39 in the sequence) and the NW peptides (40–41 in the sequence) had greater bioactivity values of 0.922094 and 0.934148, respectively. Their structure shows aromatic rings which are mainly represented by phenylalanine and tryptophan.

As can be seen in Table 7, sample 2 had three peptide sequences with high bioactive properties. They were the CYG peptides (25–27 in the sequence), the GHG peptides (13–15), and the AYG peptides (22–24), with bioactivity values of 0.947378, 0.839383, and 0.815664, respectively. Structurally, these bioactive peptides had aromatic rings represented by the aromatic α-amino acid of tyrosine.

We found no correlation between the bioactivity values of the peptides and their molecular weight.

Table 6. Individual peptide sequences for hydrolysate Sample 1

Таблица 6. Характеристика отдельных пептидных последовательностей гидролизата образца № 1

The number of peptide in the chain	Peptide sequence in a one-letter code*	Molecular weight, g/mol	Bioactivity	Peptide structure
1–4	PILG	398	0.492674	
5–8	PILV	440	0.291507	
9–11	ILA	315	0.237443	
12–14	ILG	301	0.480965	
15–17	ILV	343	0.123381	
18–20	ILT	345	0.154222	
21–23	PSV	301	0.166459	
24–26	GVS	261	0.109533	
27–29	HGL	325	0.446783	
30–32	HVI	367	0.0887486	
33–35	SVP	301	0.194373	
36–37	SV	204	0.0523218	
38–39	FD	280	0.922094	
40–41	NW	318	0.934148	

* A – alanine; C – cysteine; D – aspartic acid; E – glutamic acid; F – phenylalanine; G – glycine; H – histidine; I – isoleucine; K – lysine; L – leucine; M – methionine; N – asparagine; P – proline; Q – glutamine; R – arginine; S – serine; T – threonine; V – valine; W – tryptophan; Y – tyrosine.

* A – аланин; C – цистеин; D – аспарагиновая кислота; E – глутаминовая кислота; F – фенилаланин; G – глицин; H – гистидин; I – изолейцин; K – лизин; L – лейцин; M – метионин; N – аспарагин; P – пролин; Q – глутамин; R – аргинин; S – серин; T – треонин; V – валин; W – триптофан; Y – тирозин.

Table 7. Individual peptide sequences for hydrolysate sample 2

Таблица 7. Характеристика отдельных пептидных последовательностей гидролизата опытного образца № 2

The number of peptide in the chain	Peptide sequence in a one-letter code*	Molecular weight, g/mol	Bioactivity	Peptide structure
1–4	ILKH	509	0.162711	
5–8	PSPV	398	0.390668	
9–12	PSVP	398	0.327229	
13–15	GHG	269	0.839383	
16–18	SPV	301	0.222562	
19–21	SVP	301	0.194373	
22–24	AYG	309	0.815664	
25–27	CYG	341	0.947378	

* A – alanine; C – cysteine; D – aspartic acid; E – glutamic acid; F – phenylalanine; G – glycine; H – histidine; I – isoleucine; K – lysine; L – leucine; M – methionine; N – asparagine; P – proline; Q – glutamine; R – arginine; S – serine; T – threonine; V – valine; W – tryptophan; Y – tyrosine.

* A – аланин; C – цистеин; D – аспарагиновая кислота; E – глутаминовая кислота; F – фенилаланин; G – глицин; H – гистидин; I – изолейцин; K – лизин; L – лейцин; M – метионин; N – аспарагин; P – пролин; Q – глутамин; R – аргинин; S – серин; T – треонин; V – валин; W – триптофан; Y – тирозин.

We should note that the detection of more bioactive peptides by the *in silico* method should be confirmed by further *in vitro* experiments with various modifications of peptides. Based on literature, we can assume that some peptide sequences obtained in our study may have antimicrobial and antioxidant activity due to the presence of proline and leucine that have such properties [26]. Further *in vitro* experiments can determine the reliability of our results and identify specific bioactive properties of the studied peptides.

Conclusion
Based on the research results, we designed a flow chart for obtaining hydrolysates from poultry by-products. Then, we evaluated the bioactive properties of the peptides obtained. The hydrolysate sample obtained with a lower pepsin activity (30 units per 100 g of material) had greater bioactivity values in the FD and NW peptides (0.922094 and 0.934148, respectively). The sample obtained with a higher pepsin activity (45 units per 100 g of material) had higher bioactivity in the CYG, GHG, and AYG peptides (0.947378 units, 0.839383, and 0.815664, respectively). However, these data obtained *in silico* need to be confirmed by *in vitro* experiments for further work. Such experiments will determine their reliability and identify specific bioactive properties of the peptides.

Contribution

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Patshina MV, Voroshilin RA, Osintsev AM. Global biomaterials market: Potential opportunities for raw materials of animal origin. Food Processing: Techniques and Technology. 2021;51(2):270–289. (In Russ.). https://doi.org/10.21603/2074-9414-2021-2-270-289

2. Arihara K, Zhou L, Ohata M. Bioactive properties of Maillard reaction products generated from food protein-derived peptides. Advances in Food and Nutrition Research. 2017;81:161–185. https://doi.org/10.1016/bs.afnr.2016.11.005

3. Chalamaiaha M, Keskin Ulug S, Hong H, Wu J. Regulatory requirements of bioactive peptides (protein hydrolysates) from food proteins. Journal of Functional Foods. 2019;58:123–129. https://doi.org/10.1016/j.jff.2019.04.050

4. Bhat ZF, Kumar S, Bhat HF. Bioactive peptides of animal origin: A review. Journal of Food Science and Technology. 2015;52(9):5377–5392. https://doi.org/10.1007/s13197-015-1731-5

5. Bazhenova BA, Zhamsaranova SD, Zabalueva YuYu, Gerasimov AV, Zambulaeva ND. Effects of lingonberry extract on the antioxidant capacity of meat paste. Foods and Raw Materials. 2020;8(2):250–258. https://doi.org/10.21603/2308-4057-2020-2-250-258

6. Kaur K, Chhikara N, Sharma P, Garg MK, Panghal A. Coconut meal: Nutraceutical importance and food industry application. Foods and Raw Materials. 2019;7(2):419–427. https://doi.org/10.21603/2308-4057-2019-2-419-427

7. Chernukha IM, Mashentseva NG, Afanasev DA, Vostrikova NL. Biologically active peptides of meat and meat product proteins: A review. Part 1. General information about biologically active peptides of meat and meat products. Theory and Practice of Meat Processing. 2019;4(4):12–16. https://doi.org/10.21323/2414-438X-2019-4-4-12-16

8. Chernukha IM, Mashentseva NG, Afanasev DA, Vostrikova NL. Biologically active peptides of meat and meat product proteins: A review. Part 2. Functionality of meat bioactive peptides. Theory and Practice of Meat Processing. 2020;5(2):12–19. https://doi.org/10.21323/2414-438X-2020-5-2-12-19

9. Chalamaiah M, Yu W, Wu J. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. Food Chemistry. 2018;245:205–222. https://doi.org/10.1016/j.foodchem.2017.10.087

10. Milentyeva IS, Davydenko NI, Rasshchepkin AN. Casein proteolysis in bioactive peptide production: Optimal operating parameters. Food Processing: Techniques and Technology. 2020;50(4):726–735. (In Russ.). https://doi.org/10.21603/2074-9414-2020-4-726-735

11. Ryazantseva KA, Agarkova EYu, Fedotova OB. Continuous hydrolysis of milk proteins in membrane reactors of various configurations. Foods and Raw Materials. 2021;9(2):271–281. https://doi.org/10.21603/2308-4057-2021-2-271-281

12. Fu Y, Zhang Y, Soladoye OP, Aluko RE. Maillard reaction products derived from food protein-derived peptides: Insights into flavor and bioactivity. Critical Reviews in Food Science and Nutrition. 2020;60:3429–3442. https://doi.org/10.1080/10408398.2019.1691500

13. Rizzello CG, Tagliazucchi D, Babini E, Sefora Rutella G, Taneyo Saa DL, Gianotti A. Bioactive peptides from vegetable food matrices: Research trends and novel biotechnologies for synthesis and recovery. Journal of Functional Foods. 2016;27:549–569. https://doi.org/10.1016/j.jff.2016.09.023

14. Voroshilin RA. Technology of enzymatic-acid hydrolysis of bone raw material in production of gelatine. Theory and Practice of Meat Processing. 2021;6(3):279–284. https://doi.org/10.21323/2414-438X-2021-6-3-279-284

15. Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food Chemistry. 2012;135(4):3020–3038. https://doi.org/10.1016/j.foodchem.2012.06.100

16. Grishin DV, Podobed OV, Gladilina YuA, Pokrovskaya MV, Aleksandrova SS, Pokrovsky VS, *et al.* Bioactive proteins and peptides: Current state and new trends of practical application in the food industry and feed production. Problems of Nutrition. 2017;86(3):19–31. (In Russ.). https://doi.org/10.24411/0042-8833-2017-00041

17. Skata R. Bioactive peptides and probiotics for functional meat products. Meat Technology. 2017;170(2):40–43. (In Russ.). [Ската Р. Биоактивные пептиды и пробиотики для функциональных мясных продуктов // Мясные технологии. 2017. Т. 170. № 2. С. 40–43.].

18. Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Laganà A. Recent trends in the analysis of bioactive peptides in milk and dairy products. Analytical and Bioanalytical Chemistry. 2016;408(11):2677–2685. https://doi.org/10.1007/s00216-016-9303-8

19. Dinika I, Utama GL. Cheese whey as potential resources for antimicrobial edible film and active packaging production. Foods and Raw Materials. 2019;7(2):229–239. https://doi.org/10.21603/2308-4057-2019-2-229-239

20. Daliri EB-M, Oh DH, Lee BH. Bioactive peptides. Foods. 2017;6(5). https://doi.org/10.3390/foods6050032

21. Lafarga T, Hayes M. Bioactive protein hydrolysates in the functional food ingredient industry: Overcoming current challenges. Food Reviews International. 2017;33(3):217–246. https://doi.org/10.1080/87559129.2016.1175013

22. Vilcacundo R, Martínez-Villaluenga,C, Hernández-Ledesma B. Release of dipeptidyl peptidase IV, *α*-amylase and *α*-glucosidase inhibitory peptides from quinoa (*Chenopodium quinoa* Willd.) during *in vitro* simulated gastrointestinal digestion. Journal of Functional Foods. 2017;35:531–539. https://doi.org/10.1016/j.jff.2017.06.024

23. Babich O, Dyshlyuk L, Noskova S, Sukhikh S, Prosekov A, Ivanova S, *et al. In vivo* study of the potential of the carbohydrate-mineral complex from pine nut shells as an ingredient of functional food products. Bioactive Carbohydrates and Dietary Fibre. 2019;18. https://doi.org/10.1016/j.bcdf.2019.100185

24. Kehinde BA, Sharma P. Recently isolated antidiabetic hydrolysates and peptides from multiple food sources: A review. Critical Reviews in Food Science and Nutrition. 2020;60(2):322–340. https://doi.org/10.1080/10408398.2018.1528206

25. Ashaolu TJ. Antioxidative peptides derived from plants for human nutrition: Their production, mechanisms and applications. European Food Research and Technology. 2020;246(5):853–865. https://doi.org/10.1007/s00217-020-03479-y

26. Lavelli V, Proserpio C, Gallotti F, Laureati M, Pagliarini E. Circular reuse of bio-resources: The role of *Pleurotus* spp. in the development of functional foods. Food and Function. 2018;9(3):1353–1372. https://doi.org/10.1039/C7FO01747B