Determination of the functional properties of protein hydrolysates by the *in silico* method

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Abstract. Bioactive peptides are of increasing interest to scientists. The development of peptidomics and bioinformatics contributes to a deeper study of peptides obtained by enzymatic hydrolysis of raw materials. In this work, the *in silico* method was used to study the properties and biological value of peptides identified in protein hydrolysates obtained by microbial fermentation of the broiler chicken gizzards in whey with the addition of bifidobacteria and propionic acid bacteria. The activity of the peptides was determined using the BIOPEP database. Potential toxicity and such properties as hydrophobicity, hydropathicity, hydrophilicity, molecular weight of each peptide were predicted through the tool ToxinPred. The potential activity of the peptides was evaluated using the PeptideRanker database. The conducted studies made it possible to identify bioactive peptides TR, SY, VW, PPP, SW in hydrolysates, which have various physiological effects. Several peptides with high potential biological activity have also been identified. Due to the fact that not all peptides obtained during the fermentation of raw materials have been studied, *in silico* methods allow us to assess the feasibility of isolating certain peptides from hydrolysates.

1 Introduction

The market for specialized food products is constantly expanding. Scientists are finding new sources of functional ingredients that affect the physiological functions of the body. One of such promising functional ingredients is food protein hydrolysates.

Protein hydrolysates are commonly used in specialized food products due to the fact that hydrolyzed proteins are more easily absorbed by the human body, and in addition, they contain bioactive peptides with a variety of physiological properties: antitumor, antioxidant, antihypertensive, antithrombotic, antidiabetic, antimicrobial, immunomodulatory, and others [1, 2].

Peptides have numerous advantages over small molecules: high biological activity, high specificity, and high penetration. However, peptides can also have negative properties such as toxicity, immunogenicity, and instability [3].

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To predict both positive and negative properties of peptides, the *in silico* method is used as the fastest and time saving method [4]. This method selects suitable enzymes for hydrolysis in order to obtain maximum bioactivity, reveals the relationship between the structure and function of the [5] peptide. Many scientists have confirmed the convenience and reliability of virtual screening as a means of predicting the activity of peptides [5-7].

The most widely used open access databases are BIOPEP [8], ERP-Moscow [9], ToxinPred [10], PeptideRanker [11], and PEPBANK [12]. They contain data on the origin of the protein, on the main chemical and structural characteristics, the biological activity of peptides, toxicity and allergenic properties. As noted in [13], the development of peptidomics and bioinformatics contributes to a better understanding of biologically active food ingredients that can be used in the future for the development of new functional and specialized products.

The aim of this research was to investigate the properties of peptides obtained by microbial fermentation of the broiler chicken gizzards in whey in the presence of bifidobacteria and propionic acid bacteria using the *in silico* method.

2 Materials and Methods

2.1 Preparation of Protein Hydrolyzates

Protein hydrolyzate was obtained by microbial fermentation of the broiler chicken gizzards in whey with the addition of concentrates of bifidobacteria and propionic acid bacteria. The control sample was obtained by fermentation without the addition of bacteria to the whey. The technology for obtaining a protein hydrolyzate is shown in Fig. 1 and described in detail in an earlier research [14].

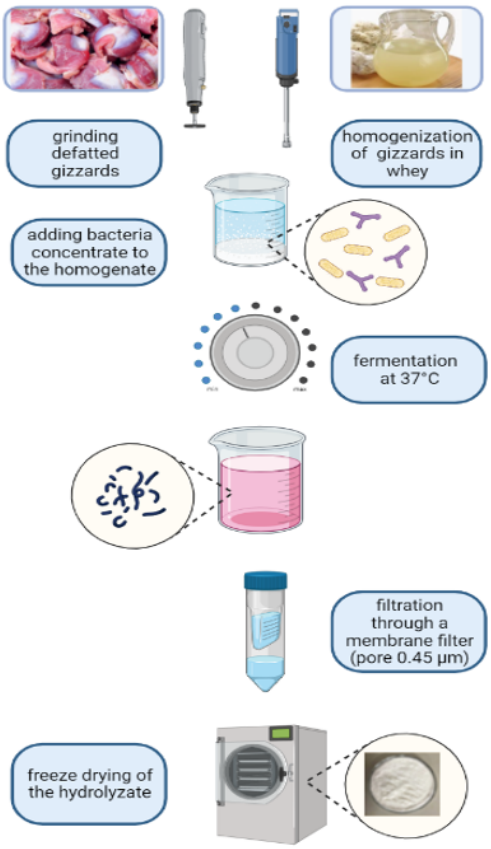

Fig. 1. Technological scheme for obtaining protein hydrolyzate (Created with BioRender.com)

2.2 In Silico Analysis of the Hydrolysates Properties

The aqueous and alcoholic extracts of the hydrolysates were examined for peptide content using UPLC-ESI-Q-TOF-MS analysis on the UHPLC 1290 Infinity system with time-of-flight mass spectrometric detector Agilent 6545XT AdvanceBio LC/QTOF (Agilent Technologies, USA).

The activity of the peptides identified in the hydrolysates was determined using the BIOPEP database. Potential toxicity and such properties as hydrophobicity, hydropathicity, hydrophilicity, molecular weight of each synthesized peptide were predicted through the tool ToxinPred. The potential activity of the peptides was evaluated using the PeptideRanker database.

3 Results and Discussion

The results of peptide identification (Tab. 1) showed the presence in the hydrolysates of peptides with different physiological activity, established by the BIOPEP database. Tab. 1 also provides information on potential toxicity and such properties as hydrophobicity,

hydropathicity, hydrophilicity, and molecular weight of each synthesized peptide obtained from the ToxinPred database.

Table 1. Bioactive peptides in protein hydrolysates

Sequence	Activity (based on BIOPEP)	Properties of the peptide (based on ToxinPred)				
		toxicity	hydrophobicity	hydropathicity	hydrophilicity	molecular weight
TR	dipeptidyl peptidase IV inhibitor	Non-Toxin	-0.97	-2.60	1.30	275.32
SY	ACE inhibitor	Non-Toxin	-0.12	-1.05	-1.00	268.28
	dipeptidyl peptidase IV inhibitor					
VW	ACE inhibitor	Non-Toxin	0.46	1.65	-2.45	303.38
	antioxidative					
	dipeptidyl peptidase IV inhibitor					
	alpha-glucosidase inhibitor					
PPP	ACE inhibitor	Non-Toxin	-0.07	-1.60	0.00	309.39
SW	dipeptidyl peptidase IV inhibitor	Non-Toxin	0.05	-0.85	-1.55	291.32

Peptides with high potential biological activity were identified using the PeptideRanker database, among them there are also peptides with potential antioxidant activity - PHHSSASCCLW, PPHM, HGVCWIY (Fig.2).

As can be seen in Fig. 2, peptides with potential biological activity PHHSSASCCLW and GGPPPPPPHPG are defined as toxic in the database ToxinPred. These peptides were tested in the BIOPEP database to confirm the determined information and, according to the information in this database, these peptides are not toxic.

Du et al. (2022) used the *in silico* method and the BIOPEP database to evaluate the properties of goat milk whey protein hydrolysate [6]. Li et al. (2020) used the PEPstr Server to predict the structure of synthesized duck breast peptides, and also assess their potential toxicity and physico-chemical properties using the ToxinPred tool [7]. Other studies of protein hydrolysates by the *in silico* method are also known to assess their structure and properties [15]. Various tools and programs are used for this purpose [16].

Query Peptides

Peptide ID	Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
	KEPPPGM	-0.41	Non-Toxin	-0.22	-1.53	0.67	0.00	754.99
	HGVCWIY	-0.84	Non-Toxin	0.21	0.77	-1.50	0.50	877.13
	PGTHPLLVF	-0.79	Non-Toxin	0.18	0.79	-0.94	0.50	980.31
	SGAPM	-0.81	Non-Toxin	0.07	0.18	-0.30	0.00	461.59
	PAVVSCLPGPL	-0.85	Non-Toxin	0.19	1.30	-0.71	0.00	1052.45
	PPPGV	-0.34	Non-Toxin	0.10	-0.20	-0.30	0.00	465.61
	HGSPGHGWVL	-1.04	Non-Toxin	0.08	-0.29	-0.74	1.00	1046.31
	GRGHIWGQM	-0.83	Non-Toxin	-0.11	-0.77	-0.42	1.50	1041.34
	PHHSSASCCLW	1.39	Toxin	-0.04	-0.06	-0.71	1.00	1227.52
	ICIMAPIAF	-0.74	Non-Toxin	0.39	2.52	-1.24	0.00	978.40
	VGICIYCL	-0.42	Non-Toxin	0.35	2.54	-1.40	0.00	883.25
	VICFFSVW	-0.71	Non-Toxin	0.40	2.41	-1.74	0.00	1000.33
	GLGGAWAF	-1.08	Non-Toxin	0.31	1.01	-1.09	0.00	777.99
	KVPPPRPPL	-0.50	Non-Toxin	-0.24	-0.93	0.30	2.00	1000.37
	GSAPCPG	-0.56	Non-Toxin	0.03	-0.07	-0.17	0.00	587.73
	PGGPGPAM	-0.68	Non-Toxin	0.10	-0.29	-0.23	0.00	682.90
	IHPF	-0.77	Non-Toxin	0.22	0.62	-1.20	0.50	512.66
	PPHM	-0.70	Non-Toxin	-0.07	-1.12	-0.45	0.50	480.63
	PCSIF	-0.84	Non-Toxin	0.21	1.48	-1.00	0.00	565.74
	GCTF	-0.89	Non-Toxin	0.16	1.05	-0.97	0.00	426.53
	QPPQPALAGLVF	-1.32	Non-Toxin	0.11	0.50	-0.68	0.00	1237.64
	VAPWIMM	-0.39	Non-Toxin	0.33	1.69	-1.40	0.00	847.20
	PFGAFCNVW	-0.79	Non-Toxin	0.21	0.86	-1.24	0.00	1040.32
	IVCWLPAF	-0.67	Non-Toxin	0.38	2.14	-1.56	0.00	948.30
	GGPPPPPPHPG	0.30	Toxin	-0.04	-1.42	-0.05	0.50	1006.28
	PPPHPFPVALL	-0.66	Non-Toxin	0.16	0.47	-0.78	0.50	1184.60
	GFPFPGIHW	-0.74	Non-Toxin	0.23	0.22	-1.19	0.50	1057.35

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Fig. 2. Results of analysis of peptides with potential biological activity *in silico*

4 Conclusion

The results of the research of protein hydrolysates by the *in silico* method showed the possibility of obtaining data on peptides synthesized in the course of enzymatic hydrolysis in the substrate in a short time. There are several tools and databases for assessing the properties of peptides, the most widely used are the BIOPEP database, ToxinPred, and the PeptideRanker. Due to the fact that not all peptides obtained during the fermentation of raw materials have been studied, *in silico* methods allow us to evaluate the prospects for the isolation of certain peptides from hydrolysates.

Acknowledgments

This research was funded by Russian Science Foundation № 23-26-00153, https://rscf.ru/project/23-26-00153/

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