MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Analysis of the structure and efficacy of soybean meal antimicrobial peptides**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
BEBT-21
Wu Yufan
Scientific supervisor
Olena Okhmat, Ph.D.,
Assoc. Prof.
Reviewer
Iryna Voloshyna, Ph.D.,
Assoc. Prof.

Kyiv 2025

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty: Chemical and Biopharmaceutical Technologies
Department: Biotechnology, Leather and Fur
First (Bachelor's) level of higher education
Specialty: 162 Biotechnology and Bioengineering
Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Wu Yufan

1. Thesis topic **Analysis of the structure and efficacy of soybean meal antimicrobial peptides**

Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat

approved by the order of KNUTD “05” March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)		
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)		Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)		

I am familiar with the task:

Student _____ Wu Yufan

Scientific supervisor _____ Olena OKHMAT

Abstract

Wu Yufan. Analysis of the structure and efficacy of soybean meal antimicrobial peptides. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

In this thesis, soybean meal protein was used to explore its potential in the development of natural antimicrobial peptides. With the increasing problem of antibiotic resistance, the development of safe, efficient and low toxic new antibacterial agents has become a hot research direction. As a by-product of soybean processing, soybean meal is rich in protein, which is a potential source for the preparation of antimicrobial peptides and provides high-quality raw materials for the preparation of bioactive peptides.

In this study, the amino acid sequence of β-conglycinin was obtained from the NCBI database, and the peptide fragments were obtained by BIOPEP to simulate the digestion process of pepsin, proteinase K and papain. Then toxicity analysis was performed with ToxinPred to screen out non-toxic peptides. The CAMPR3 and APD3 databases were used to predict the antibacterial and functional properties of the peptides, and the peptides with antibacterial potential such as SCPAASRPGCHKNTCGL, PVWR and APNNMR were screened. Finally, molecular docking experiments using AlphaFold3 showed that the above peptides had a strong binding affinity with the target proteins in *E. coli* (pTM value >0.88), which verified the antibacterial activity of the peptides.

The results showed that there were indeed peptides with good antibacterial activity in soybean meal, and these peptides exhibited low toxicity, high binding affinity and strong predictive antibacterial properties. This study provided a theoretical basis for high-value utilization of soybean meal resources, and also provided a reference for the development and structure optimization of new natural antimicrobial peptides. In the future, its practical efficacy can be further verified by

in vitro experiments, and its application prospects in food preservation, animal feed, medicine and other fields can be explored.

Key words: Antimicrobial peptides, β-conglycinin, Soy meal, Bioinformatics analysis

TABLE OF CONTENT

Introduction

With the aggravation of antibiotic resistance, the development of safe, efficient and low-toxicity novel antimicrobial agents has become the focus of global attention. Antimicrobial peptides, as a class of naturally occurring small molecule peptides, are regarded as ideal alternatives to traditional antibiotics due to their broad-spectrum antimicrobial activity, low drug resistance and environmental friendliness.

Soybean meal, as a by-product of soybean oil and fat processing, has a protein content of more than 50%, making it a high-quality resource for the development of bioactive peptides. It contains β-accompanied by soybean globulin and other components with potential antimicrobial activity. This study takes soybean meal protein as the research object and systematically analyzes the structure and function of its antimicrobial peptides by means of bioinformatics, aiming at tapping the potential of high-value utilization of soybean meal and providing theoretical support for the development of new natural antimicrobial agents.

This thesis is divided into four chapters: Chapter 1 reviews the current status of antimicrobial peptide research and the preparation method of antimicrobial peptide from soybean meal; Chapter 2 describes in detail the experimental materials, bioinformatics tools and technical routes; Chapter 3 demonstrates the results of structural analysis of β-accompanied by soybean globulin, active peptide screening and molecular docking results; and Chapter 4 concludes the results of the research.

Through systematic research, this thesis provides a scientific basis for the deep development of soybean meal resources and the molecular design of antimicrobial peptides, and also lays the foundation for subsequent experimental validation and industrial application.

The research topic of study is the structure and efficacy analysis of antimicrobial peptides of β-accompanied soybean globulin origin from soybean meal.

The research object of the study is to screen low-toxicity and high-efficiency antimicrobial peptides from soybean meal and to promote their application in food and medicine.

The research target of the study is β-accompanied soybean globulin

The research method of the study are bioinformatics analysis.

CHAPTER 1
LITERATURE REVIEW

1.1 Meaning of Antimicrobial Peptides

With the development of global economy and the improvement of human living standards, global food safety issues as well as physical health are of great concern. Antimicrobial peptides, as a kind of natural small molecule alkaline peptide widely found in nature, have antipathogenic microbial activity. Compared with antibiotics, antimicrobial peptides are characterized by a short existence time in the water body[1], less toxicity, low drug resistance, and a wide range of inhibitory effects on fungi, bacteria and viruses [2]. Therefore, antimicrobial peptides have great potential as an alternative to antibiotics, and also have broad application prospects in the fields of medicine and breeding [3].

1.2 Research Progress of Antimicrobial Peptides from Different Sources

Among them, the integration of information of different sources of antimicrobial peptides is shown in Tab.1.1.

Table 1.1 – Integration of antimicrobial peptide information from different sources

Name	Source	Sequences	Hydrolysis enzyme	Enzymatic conditions	Framework	Literatures
PLN-1	*Lactobacillus plantarum*	TELAKKLLVK	Trypsin, pepsin, papain and proteinase K	Incubate at 37℃ for 5h	alpha-helix	4
VK13	*plasma* (medicine)	AAGGVKKPKKAAAA KKSPKKKPKKKPAAA	Pepsin, trypsin, proteinase K	Incubate at 37℃ for 5h	alpha-helix	5
HNP1	*human neutrophil*	ACYCRIPACIAGER RYGTCIYQGRLWAF CC	Trypsin, pepsin, lysozyme	37°C, pH 7.0-8.0	alpha-helix	6

Name	Source	Sequences	Hydrolysis enzyme	Enzymatic conditions	Framework	Literatures
AtR472	safrantha (Triticum aestivum)	LCVSYSVRPSCKFV GRSNWKRNTCLEWC DQLFLFCFLIGGEM ANS	Trypsin, enteric protease, papain	37°C, pH 7.5-8.5	alpha-helix or β-folding	7
P9	plant microbiota	LIRCSRTCLQYKTS RFMRW	Trypsin, Papain, Protein K	37°C, pH weak base	alpha-helix	8
Chiono-dracine	ice fish	FFGHLYRGITSVVK HVHGLLSG	Trypsin, Papain, Protein K	37°C, pH weak base	alpha-helix	9
C2	coconut milk	MGQARGAVSSVAGR AQGM	Trypsin, proteinase K, papain	37°C, pH weak base	alpha-helix	10
Ps-AFP1	pea (Pisum sativum)	RQLKSSRRGALVCV RLKLCSAILSRGLS CGMFSCNARR	Trypsin, pepsin, proteinase K	37°C, neutral or slightly alkaline	alpha-helix	11
VM-3Y	insects	FLYIIGKLLSGLL	Trypsin, pepsin, proteinase K	37°C, neutral or slightly alkaline	alpha-helix	12

1.3 Domestic and International Research Status of Soybean Antimicrobial Peptide

1.3.1 Domestic Research Status of Soybean Antimicrobial Peptide

Listeria monocytogenes is a foodborne pathogen that seriously jeopardizes human health, Xie Boyang[13] et al. found that soybean globulin antimicrobial polypeptide can interact electrostatically with the surface of Listeria monocytogenes cell membranes to damage the cell membrane, disrupt the respiratory metabolism of Listeria monocytogenes by decreasing the activity of ATPases, and also interfere with Listeria monocytogenes DNA replication and expression, leading to Listeria monocytogenes death, thus treating foodborne viral infections. In addition to the treatment of foodborne pathogenic bacterial infections,

soybean meal can also be used for soybean active peptide extraction, soybean peptide functional beverage preparation, etc. Hu Yalan[14] et al. used Aspergillus oryzae as the strain and soybean meal as the raw material, and the liquid fermentation method will be used for the preparation of functional beverages with the soybean peptide obtained from the isolation. In food preservation, mold contamination is the main cause of spoilage of fresh fruits and vegetables, pasta products and baked goods, and the alkaline peptides in soybean globulin can counteract mold contamination. Hao Man[15]et al. found that the antimicrobial peptide of soy protein has very good inhibitory effect on Aspergillus niger, and it has significant preservative effect in milk and bread, which can inhibit the growth of bacteria in cow's milk, inhibit the decomposition of proteins and the rancidity of cow's milk, maintain the freshness of cow's milk, and prolong the preservation period of cow's milk, and has the advantage of serving as a natural preservative. Sun Xiu-Xiu[16] et al. in the use of soybean basic polypeptide (GBP) in the treatment of Escherichia coli, it was found that it can expose the hydrophobic end of the cell surface, so that the leakage of alkaline phosphatase increases, destroying the permeability of the inner and outer membranes of *E. coli* cells and increasing the sensitivity of GBP to rifampicin and erythromycin, which leads to the immediate entry of o-nitrophenyl β-D-galactopyranoside into the cells and the reaction with intracellular β-galactosidase to produce a yellow o-nitrophenyl β-D-galactoside. reacted to produce yellow o-nitrophenol, which inhibited the growth of *E. coli*.

AMPs are expected to become a new type of green antibiotics under the background of "alternative antibiotics". However, natural AMPs have problems such as low antibacterial activity , high toxicity and poor stability, which limit their application in livestock and poultry production. Song Xueying[17] et al. proposed a bioinformatics-based approach to modify existing AMPs, as well as the use of bioinformatics molecular design and prediction tools to modify AMPs, which provides a theoretical basis for obtaining new AMPs with high activity and stability. Antimicrobial peptides are mainly modified by peptide chain length, net charge number, hydrophilicity and hydrophobicity, and stability to ligand protease

binding as a way to improve the activity of AMPs. Based on natural antimicrobial peptide sequences, novel antimicrobial peptides can be obtained by amino acid substitutions, hybridization between different peptides, and removal of inactive amino acid residues to shorten the peptide chain length in order to improve the activity and safety of peptides. At present, Zhang Weimin[18] et al. studied the design of antimicrobial peptides using natural antimicrobial peptides as templates, and the results showed that genetically engineered antimicrobial peptides, compared with natural antimicrobial peptides, have a strong effect on tumor cells and no killing effect on normal cells, which provides a new option for the treatment of tumor diseases. In the field of bioinformatics, Janssen[19] et al. designed a cascade of computational models for sequence derivation and living language embedding based on the identification of AMPs and their functional types, called AMPLATZER. AMPLATZER aims to distinguish between AMPs and non AMPs and predict their functional types. The method achieved high improvement in both AMP recognition and AMP function prediction compared to existing methods.AMPLATZER showed better performance on the independent dataset, with F1 scores (1.45%-6.13%), MCC (2.92%-12.86%), and AUC (5.13%-8.56%) as well as AP (9.20%- 21.07%) all improved. Lower bias was achieved on the D2 dataset by 10-fold cross-validation, with an R improvement of 18.82%-19.46%. In order to improve the efficiency of AMP use, Hao-Ran Deng[20] et al. carried out a peptide-based nanostructuring approach, where nanotechnology improves the effectiveness and safety of AMPs, significantly increases the duration of antimicrobial action, and enhances the elasticity of AMPs by encapsulating them within nanoparticles, thus preventing enzymatic hydrolysis or degradation. Nanotechnology is emerging as a very promising medical application, and with the increase in antibiotic-resistant bacteria, silver nanoparticles offer a promising alternative to combat bacterial infections[21] , Silver nanoparticles are small particles made of pure silver that have the ability to inhibit bacterial growth and reduce infection rates. By absorbing water, silver nanoparticles release ions that interact

with the bacterial cell wall, preventing the bacteria from multiplying and spreading.

1.3.2 Foreign Research Status of Soybean Antimicrobial Peptide

Freitas C S[22] et al. separated aqueous extracts of soybean meal, a by-product of soybean oil refining, by gel filtration chromatography to obtain two fractions, in both fractions most of the peptides were found to be concentrated in the α-subunit of β-accompanied by the α-subunit of soybean globulin or in both regions of the α-main subunit, the peptide pools from both fractions inhibited the growth of both Gram-positive and Gram-negative foodborne pathogens and were not toxic to murine bone marrow or fibroblasts without toxicity, but inhibited human glioblastoma proliferation. And the three-dimensional structure of the β-accompanied soybean globulin structural domain containing the best AMP candidate was determined by molecular modeling to have an α-helical conformation. In the interest of food safety, Roy S[23] et al. proposed the incorporation of bioactive peptides into food packaging systems to improve the shelf life of packaged foods and help reduce the use of unhealthy food preservatives. The addition of bioactive functional peptides can delay the oxidation of lipids in foods and inhibit the growth of foodborne pathogenic bacteria. Rather than adding preservatives directly, the current common strategy for food preservation is to slow down the onset of food spoilage by developing food packaging films/edible coatings with antimicrobial and antioxidant properties. Since antibiotics used for growth stimulation in animals are banned, Lima L F[24] et al. utilized AMP as an alternative and showed that antimicrobial peptides have immunomodulatory, antimicrobial, and growth stimulating properties, which provides a new way of thinking about the treatment of infections in poultry, and that AMP can also aid in leukocyte recruitment through direct chemotactic activity and indirectly inducing the production of chemokines.Lwin H Y[25] et al. In their study of the soy peptide BCBS-11, they found that PI staining of Porphyromonas gingivalis and F. nucleatum by BCBS-11 showed that BCBS-11 disrupted the

integrity of the bacterial membrane. Its positive charge caused membrane disintegration leading to the disappearance of transmembrane electrochemical gradient, membrane depolarization, triggering cell swelling and osmosis, which in turn inhibited the growth of periodontal bacteria through bactericidal action. Currently, foreign countries are committed to the development of computer simulation technology for the development and screening of novel AMPs[26], which is based on the amino acid sequences as well as the structures of novel AMPs in databases, followed by virtual screening, and is a practical, cost-effective, and straightforward approach that bypasses cumbersome in vivo testing. The computerized design makes it possible to study the effects of selective modifications and chemical changes on AMP activity prior to chemical synthesis of novel AMPs.

1.4 Preparation of Soybean Antimicrobial Peptides

Soybean, as a common food as well as a crop in life, is rich in protein, which is an ideal material for the preparation of natural antimicrobial peptides, while enzymatic digestion and microbial fermentation are the main ways of antimicrobial peptide preparation. Enzymatic digestion is to select one or several specific enzymes for enzymatic digestion, with mild conditions, high safety of hydrolyzed products, and different enzymes with different sites and modes of action, and different enzymatic effects on the same substrate, which can focus on different functionality of soybean peptides for industrialized production[27]. Microbial fermentation organically combines the production of protease and enzymatic digestion into one process, which has the advantages of high specificity, low by-products, easy operation and low contamination[28] . Soybean meal is a by-product of oil production, research has found that soybean meal is rich in protein, is an important source of feed protein, protein content can be more than 50%[29], soybean meal protein is a good source of production of antimicrobial peptides, the use of fermentation degradation of soybean meal protein production of antimicrobial peptide-containing feed protein production of anti-resistance-free

feeds, not only no antibiotics may bring the disadvantages of the animal immunity can also be strengthened to further improve the quality of livestock and poultry products, the use of fermentation. It further improves the quality of livestock and poultry products, and has good application prospects in livestock, poultry, aquatic products, etc.[30]

1.5 Purpose and Significance of Soybean Meal Antimicrobial Peptide Research

1.5.1 Purpose of the Study

Soybean meal is a by-product obtained after soybean oil is extracted from soybeans, and it has a wide range of utilization value. It can be used as a food industry raw material to make a variety of food products such as soybean flour, vermicelli, cold noodles, etc. Meanwhile, its rich protein is a high-quality protein source for the preparation of active peptides[31]. To study the peptides with antimicrobial activity in soy protein and analyze the toxicity and activity of this peptides, and screen the functional soy peptides with antimicrobial, immunity-boosting, antioxidant and other activities.

Through research on soybean antimicrobial peptides, the analyzed beneficial peptides have been applied to food, pharmaceuticals, and other fields, promoting the development of soybean antimicrobial peptides and the high-value utilization of soybean meal.

1.5.2 Significance of the Study

Antimicrobial peptides, as peptides with antimicrobial bioactivity, are a natural immune barrier that can be used as a defense against invading pathogens entering organisms and are widely found in nature[32] . Antimicrobial peptides are different from traditional antibiotics in that they have a unique mechanism of bacterial inhibition, and the use of antimicrobial peptides in place of traditional antibiotics reduces the risk of resistance and cross-resistance problems. Antimicrobial peptides have good pH stability and thermal stability, and show

good inhibitory activity against bacteria, viruses, fungi, and protozoa while posing little harm to the environment and the human body. Therefore, antimicrobial peptides can be used to replace some of the antibiotics used for the treatment of diseases in the field of the pharmaceutical industry, and also used for the preservation of raw materials and products in the food industry.[33].

The aim of this study is to break the traditional preparation of bioactive peptides and simulate the preparation using bioinformatics. By conducting a large number of data analysis and simulation experiments, we can deepen the understanding of the interrelationship between the structure and function of antimicrobial peptides, quickly predict their biological activities, and deeply analyze their antibacterial, active, toxicity, and other properties, so as to improve the efficiency and accuracy of antimicrobial peptide research. With the assistance of bioinformatics software, researchers can better predict potential active peptide sequences in the pre-experimental stage and screen out peptides with high activity and low toxicity. Meanwhile, through molecular docking experiments, we can predict the binding of antimicrobial peptides with ligands, which will provide a research basis for the synthesis of active peptides and in the field of medical treatment and other fields, and provide important references for the subsequent experimental design and validation.

Conclusions to chapter 1

1. Explain the natural advantages of antimicrobial peptides (broad-spectrum antibacterial, low drug resistance) and their potential as antibiotic substitutes.

2. Summarize the research progress of soybean antimicrobial peptides at home and abroad, including preparation methods (enzymatic digestion, fermentation) and application areas (food preservation, medical treatment).

3. Propose the research value of soybean meal as a high-protein by-product, and clarify the goal (screening low-toxicity and high-activity peptides) and significance (promoting the high-value utilization of soybean meal) of this study.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Main Studies

Firstly, we review the relevant literature to understand the research progress of soy protein peptide and its antimicrobial activity, understand the general characteristics and main structure of antimicrobial peptide, and learn the relevant writing steps to organize the dissertation writing ideas, so as to provide the theoretical basis for the subsequent research. Secondly, through reading bioinformatics books or searching for the bioinformatics software required for this thesis in the literature, or watching online software operation tutorials, we can master the operation procedures of bioinformatics tools such as BIOPEP, ToxinPred, Alphafold3, and so on.

Then the bioinformatic simulation stage was carried out: soy protein was selected to obtain its structure and amino acid sequence information; bioinformatic software was used to simulate the enzymatic digestion of soy protein to obtain the enzyme section segment and the peptide activity information of soy protein; the toxicity and activity of the enzyme section segment was predicted by computerized means, and the peptide segments with high activity and non-toxicity were screened out; finally, the molecular docking test was carried out by Alphafold3 to analyze the efficacy of the antibacterial peptide of soy protein source based on the test results. Finally, the molecular docking test was conducted by Alphafold3, and the efficacy of the antimicrobial peptides of soy protein source was analyzed according to the test results.

Object of study – β-associated soy globulin.

Subject of study – Developing antibacterial peptides from soybean meal using bioinformatics and studying the structure and efficacy of the peptide segments

2.2 Experimental Materials and Tools

2.2.1 Experimental Materials and Experimental Tools

β-accompanied soybean globulin as well as phosphofructokinase protein of Escherichia coli were selected as experimental proteins; pepsin, proteinase K, and papain were selected as experimental reagents.

Experimental Tools:

1. NCBI database (https://www.ncbi.nlm.nih.gov/)
2. RCSB PDB database (http://www.rcsb.org/)
3. BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep)
4. Peptide Ranker (http://distilldeep.ucd.ie/PeptideRanker/)
5. ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/index.html)
6. CAMPR3 (http://camp3.bicnirrh.res.in)
7. BLAST (http://blast.ncbi.nlm.nih.gov/)
8. APD3 (http://aps.unmc.edu/AP/)
9. PEP-FOLD (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/)
10. AlphaFold3 (http://alphafold.com)

2.2 Amino Acid Sequence of β-Accompanied Soybean Globulin Download

Visit the NCBI website (https://www.ncbi.nlm.nih.gov/) and enter information about the soy protein (e.g., gene name or UniProt ID) to download its amino acid sequence and gene sequence.

2.3 BIOPEP Simulated Enzymatic Cleavage and Acquisition of Peptide Profiles

BIOPEP is specialized in sequence analysis and prediction functions for peptides, and it contains many functional peptides related to biological activities, such as antimicrobial peptides. The amino acid sequence of soybean globulin was enzymatically modeled using the BIOPEP database, and different enzymes (pepsin,

proteinase K, papain) were selected to perform enzymatic cleavage to obtain possible peptide fragments. Based on the sequence and activity of the peptides, combined with the activity prediction tool of BIOPEP, the peptides with possible antimicrobial activity were screened.

2.5 ToxinPred Analysis of Peptides

The ToxinPred tool (https://webs.iiitd.edu.in/raghava/toxinpred/index.html) is a bioinformatics tool for predicting whether a protein or peptide is potentially toxic. It combines various machine learning algorithms and biological models to assess whether a protein or peptide is toxic by analyzing its sequence characteristics. The ToxinPred tool is used to predict the toxicity of peptides screened in soybean globulin and to assess whether the peptides are potentially toxic, so that can ensure the safety of peptides in practical applications, and improve the reliability of the design as well as safety.

2.6 AlphaFold3 Molecular Docking Assay

AlphaFold3 is an open source software package for molecular docking. It can be used to study interactions between molecules, especially the binding of drug molecules to their target molecules (e.g., proteins, nucleic acids, etc.) AlphaFold3 helps to predict the activity and selectivity of drugs by modeling the binding modes, affinities, and interactions between small molecules (e.g., drug molecules, peptides, etc.) and their target molecules (e.g., proteins).

First, the amino acid sequence of the ligand molecule, *E. coli* phosphofructokinase protein, was obtained from the Uniprot database, and molecular docking simulations were performed using the molecular modeling software, AlphaFold3, to simulate the binding between the active peptide and the target proteins of *E. coli*. The binding affinity between the peptide and the target bacteria was evaluated and the antibacterial potential of the peptide was predicted.

Conclusions to chapter 2

1. Construct a technical route based on bioinformatics tools (BIOPEP, ToxinPred, AlphaFold3), covering sequence acquisition, simulated digestion, toxicity screening and molecular docking.

2. The experimental materials (β-accompanied soybean globulin, *E. coli* target protein) and key steps (digestion simulation, activity prediction, structure verification) were clarified.

3. Ensure the comprehensiveness and reliability of the screening through the joint analysis of multiple databases (CAMPR3, APD3).

CHAPTER 3
EXPERIMENTAL PART

3.1 Structural Bioconfidence Analysis Results of β-associated Soybean Globulin

Download the structure of the protein (PDB ID: 3AUP) in the RCSB PDB database as shown in Fig. 3.1.

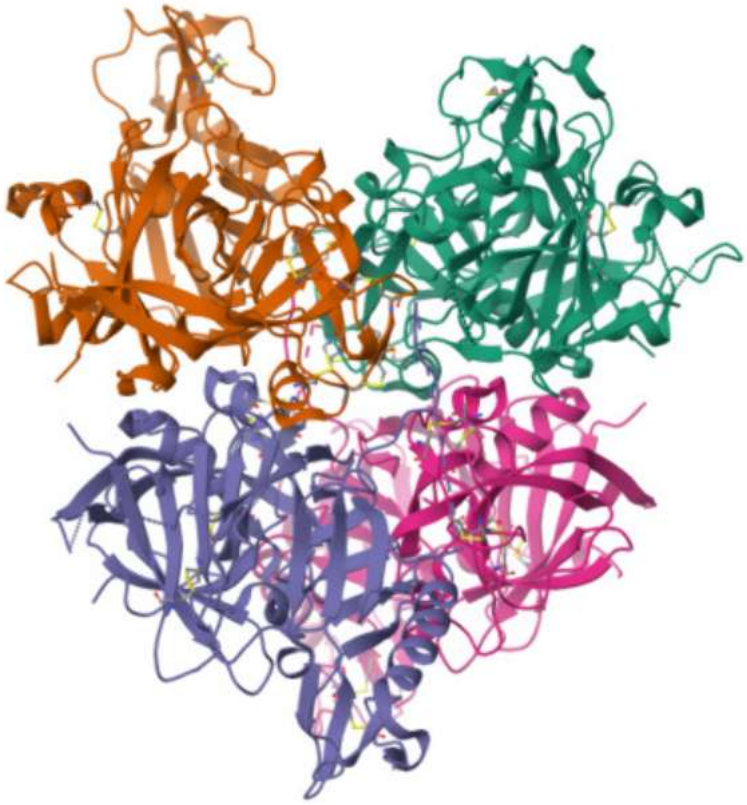

Figure 3.1 – Schematic diagram of the three-dimensional structure of the protein

Structural analysis: The protein is a tetrameric structure composed of four polypeptide chains, each subunit containing an α-helix and a β-fold to form a stable globular structure. The multisubunits are assembled into a complete globular protein through non-covalent interactions, and there are specific ligand-binding sites in the structure, which are able to bind to metal ions or small molecules, affecting its stability and function.

3.2 Peptide Profile Information and Enzymatic Peptide Activity Analysis

The amino acid sequence of β-associated soybean globulin is shown below:

VTPTKPINLVVLPVQNDGSTGLHWANLQKRTPLMQVPVLVDLNGNH
LWVNCEQQYSSKTYQAPFCHSTQCSRANTHQCLSCPAASRPGCHKNTCGL
MSTNPITQQTGLGELGEDVLAIHATQGSTQQLGPLVTVPQFLFSCAPSFLVQ
KGLPRNTQGVAGLGHAPISLPNQLASHFGLQRQFTTCLSRYPTSKGAIIFGD
APNNMRQFQNQDIFHDLAFTPLTITLQGEYNVRVNSIRINQHSVFPLNKISS
TIVGSTSGGTMISTSTPHMVLQQSVYQAFTQVFAQQLPKQAQVKSVAPFGL
CFNSNKINAYPSVDLVMDKPNGPVWRISGEDLMVQAQPGVTCLGVMNGG
MQPRAEITLGARQLEENLVVFDLARSRVGFSTSSLHSHGVKCADLFNFAN
A

When proteolytic cleavage was performed with the help of BIOPEP software using pepsin, proteinase K, and papain, proteolytic cleavage profiles were obtained as shown in Tab. 3.1 Proteolytic cleavage profiles.

Table 3.1 – Protease cleavage profile

Pepsin zymography	Proteinase K zymography	Papain zymography
VTPTKPINL-VVL-PVQNDGSTGL-HWANL-QKRTPL-MQVPVL-VDL-NGNHL-WVNCEQQYSSKTYQAPF-CHSTQCSRANTHQCL-SCPAASRPGCHKNTCGL-MSTNPITQQTGL-GEL-GEDVL-AIHATQGSTQQL-GPL-VTVPQF-L-F-SCAP	V-TP-TKP-I-NL-V-V-L-P-V-QNDGSTGL-HW-ANL-QKRTP-L-M-QV-P-V-L-V-DL-NGNHL-W-V-NCEQQY-SSKTY-QAP-F-CHSTQCSRANTHQCL-SCP-AASRP-GCHKNTCGL-M-STNP-I-TQQTGL-GEL-GEDV-L-AI-HATQGSTQQL-GP-L-V-TV-P-QF-L-F-SCAP-SF-L-V-QKGL-P-RNTQGV-AGL-GHAP-I-SL-P-NQL-ASHF-GL-QRQF-TTCL-S	VT-PT-KPINL-VVL-PV-QNDG-ST-G-L-HW-ANL-QKR-T-PL-M-QVPVL-VDL-NG-NHL-WVNCE-Q-QYSSKT-Y-Q-APF-CHST-QCSR-ANT-H-QCL-SCP-A-ASR-PG-CHKNT-CG-L-MST-NPIT-Q-QT-G-L-G-EL-G-EDVL-AIH-AT-QG-ST-Q-QL-G-PL-VT-VP-QF-L-F-SC-APSF-L-V-QKG-L-PR-NT-QG-V-AG-L-G-H-APISL-PN-QL-ASHF-G-L-QR-QF-

SF-L- VQKGL-PRNTQGV AGL- GHAPISL-PNQL-A SHF-GL-QRQF-TT CL-SRYPTSKGAIIF -GDAPNNMRQF-Q NQDIF -HDL-AF-TPL-TITL -QGEYNVRVNSIRI NQHSVF-PL-NKIS STIVGSTSGGTMIS TSTPHMVL -QQSVYQAF-TQV F-AQQL-PKQAQV KSVAPF-GL-CF-NS NKINAYPSVDL-V MDKPNGPVWRIS GEDL-MVQAQPG VTCL-GVMNGGM QPRAEITL- GARQL-EENL-VVF -DL -ARSRVGF-STSSL- HSHGVKCADL -F-NF-ANA	RY-P-TSKGAI-I-F-GDAP- NNM-RQF-QNQDI-F-HDL -AF-TP-L-TI-TL-QGEY-N V-RV-NSI-RI-NQHSV-F-P -L-NKI-SSTI-V-GSTSGGT M-I-STSTP-HM-V-L-QQS V-Y-QAF-TQV-F-AQQL-P -KQAQV-KSV-AP-F-GL-C F-NSNKI-NAY-P-SV-DL- V-M-DKP-NGP-V-W-RI-S GEDL-M-V-QAQP-GV-TC L-GV-M-NGGM-QP-RAEI -TL-GARQL-EENL-V-V-F- DL-ARSRV-GF-STSSL-HS HGV-KCADL-F-NF-ANA	T-T-CL-SR-YPT-SKG-AIIF -G-D-APNNMR-QF-QN-Q DIF-HDL-AF-T-PL-T-IT-L- QG-EYNVR-VNSIR-IN-Q HSVF-PL-NKISST-IVG-ST -SG-G-T-MIST-ST-PHMV L-Q-QSVY-Q-AF-T-QVF- A-Q-QL-PK-Q-A-QVKSV- APF-G-L-CF-NSNKIN-AY PSVDL-VMDKPNG-PVW R-ISG-EDL-MV-Q-A-QPG -VT-CL-G-VMNG-G-M-Q PR-AEIT-L-G-AR-QL-EEN L-VVF-DL-AR-SR-VG-F-S T-SSL-HSHG-VKC-ADL-F -NF-AN-A

For the resulting peptide, the peptide activity was then predicted with the help of Peptide Ranker software, and the results shown in Tab. 3.2 were obtained.

Table 3.2 – Analysis of peptide activity

	Pepsin	*Proteinase K*	*Papain*
Peptide activity >0.5	HWANL,CHSTQCSRANTHQCL, SCPAASRPGCHKNTCGL, GPL,SCAPSF, ASHF,GL, QRQF, QNQDIF, GDAPNNMRQF, AF,PL, CF,ARSRVGF,NF,VMDKPNGPVWRISGEDL	CHSTQCSRANTHQCL,HW,SCP, GP,QF, GCHKNTCGL, SCAP,SF, GHAP, AGL,AP,ASHF, GL,OROF, RQF,AF, HM,QAF,CF,NGGM,GF, NF,NGP	HW,PL,APF,QCL,SCP,PG, CG,PL,QF,SC,APSF, PR,AG, ASHF,QF,CL,AIIF,AF, PL,QVF,PHMVL,APF,PVWR, APSF, APNNMR, PR,CF, QDIF,QPR,QPG,NF

3.3 Toxicity Analysis of Active Peptides

Toxicity analysis of the above peptides using ToxinPrep software yielded the results shown in Tab. 3.3. Based on the results of the table, it was concluded that the CHSTQCSRANTHQCL peptide is potentially toxic.

Table 3.3 – Peptide Toxicity Analysis

Peptide sequence	Toxicity testing
HWANL	no
CHSTQCSRANTHQCL	yes
SCPAASRPGCHKNTCGL	no
GPL	no
SCAPSF	no
ASHF	no
GL	no
QRQF	no
QNQDIF	no
GDAPNNMRQF	no
AF	no
PL	no
CF	no
ARSRVGF	no
NF	no
VMDKPNGPVWRISGEDL	no
HW	no
SCP	no
GP	no
QF	no

Peptide sequence	Toxicity testing
GCHKNTCGL	no
SCAP	no
SF	no
GHAP	no
AGL	no
AP	no
ASHF	no
RQF	no
AF	no
HM	no
QAF	no
NGGM	no
GF	no
NGP	no
APF	no
QCL	no
PG	no
CG	no
SC	no
APSF	no
PR	no
AG	no
ASHF	no
CL	no
AIIF	no
QVF	no
PHMVL	no
APF	no
PVWR	no
APSF	no
APNNMR	no
PR	no
QDIF	no
QPR	no
QPG	no
NF	no

3.4 Analysis of Antimicrobial Properties of Non-toxic Peptides

The antimicrobial properties of the peptides were predicted using CAMPR3 (Collection of Anti-Microbial Peptides), which was calculated by SVM, Random

Forest Classifier, and ANN for the peptides, respectively, yielding the results shown in Tab. 3.4.

Table 3.4 – Prediction of peptide antimicrobial activity

Peptide sequence	SVM	Random Forest Classifier	ANN
HWANL	0.001	0.3195	no
SCPAASRPGCHKNTCGL	0.561	0.3545	yes
GPL	0.000	0.4175	yes
SCAPSF	0.831	0.3005	no-
ASHF	0.923	0.353	yes
GL	0.000	0.4265	yes
QRQF	0.330	0.3665	no-
QNQDIF	0.938	0.362	no-
GDAPNNMRQF	0.107	0.624	yes
AF	1.000	0.3615	yes
PL	1.000	0.478	yes
CF	0.991	0.428	yes
ARSRVGF	0.006	0.4055	no-
NF	1.000	0.337	no-
VMDKPNGPVWRISGEDL	0.058	0.021	no-
HW	1.000	0.379	no-
SCP	1.000	0.399	no-
GP	0.510	0.3915	no-
QF	1.000	0.3345	no-
GCHKNTCGL	0.240	0.324	yes
SCAP	0.234	0.3775	no-
SF	1.000	0.378	no-
GHAP	0.002	0.4685	no-
AGL	0.000	0.352	yes
AP	0.000	0.3935	no-
ASHF	0.923	0.353	yes
RQF	1.000	0.474	no-
AF	1.000	0.3615	yes
HM	1.000	0.555	yes
QAF	1.000	0.303	yes
NGGM	1.000	0.3825	yes
GF	1.000	0.365	yes
NGP	0.004	0.351	no-
APF	0.999	0.438	yes
QCL	0.000	0.3955	no-
PG	1.000	0.385	no-
CG	0.998	0.429	no-
SC	1.000	0.443	no-

Peptide sequence	SVM	Random Forest Classifier	ANN
APSF	0.980	0.3565	yes
PR	0.000	0.557	no-
AG	0.000	0.3585	no-
ASHF	0.923	0.353	yes
CL	0.000	0.4725	yes
AIIF	0.000	0.415	yes
QVF	0.870	0.3495	no-
PHMVL	0.651	0.6185	no-
APF	0.999	0.438	yes
PVWR	0.973	0.485	yes
APSF	0.980	0.3565	yes
APNNMR	0.816	0.394	yes
PR	0.000	0.557	no-
QDIF	1.000	0.465	no-
QPR	0.000	0.545	yes
QPG	0.032	0.3235	no-
NF	1.000	0.337	no-

Based on SVM algorithm, the predicted peptides SCPAASRPGCHKNTCGL, SCAPSF, ASHF, QNQDIF, AF, PL, CF, NF, HW, SCP, GP, QF, SF, RQF, HM, QAF, NGGM, GF, APF, PG, CG, SC, APSF, QVF, PHMVL, PVWR, APNNMR, QDIF, and NF may have antimicrobial properties. Based on Random Forest Classifier algorithm, it was predicted that peptides GDAPNNMRQF, PHMVL, PR, QPR may have antimicrobial properties. Based on ANN algorithm, it was predicted that the peptides SCPAASRPGCHKNTCGL, GPL, ASHF, GL, GDAPNNMRQF, AF, PL, CF, GCHKNTCGL, AGL, AF, HM, QAF, NGGM, GF, APF, APSF, CL, AIIF, PVWR, APNNMR, and QPR might possess antimicrobial properties.

Based on the numerical results of the three algorithms, it was predicted that the peptides SCPAASRPGCHKNTCGL, ASHF, GDAPNNMRQF, AF, PL, CF, HM, QAF, NGGM, GF, APF, APSF, PHMVL, PVWR, APNNMR, and QPR were the most likely antimicrobial peptides.

3.5 Analysis of Other Properties of Predicted Antimicrobial Peptides

The above peptides that may possess antimicrobial properties were calculated usnog APD3 (Antimicrobial Peptide Database) and the results were obtanoed as shown no Tab. 3.5. Where Boman nodex represents the bnodnog potential of peptides to protenos, so based on the above results, SCPAASRPGCHKNTCGL, GDAPNNMRQF, HM, PVWR, APNNMR, QPR are predicted to have immunoproliferative properties; however, snoce *E. coli* is negatively charged, it is predicted that SCPAASRPGCHKNTCGL, PVWR. APNNMR as antimicrobial peptides.

Table 3.5 – Results of Other Properties Analysis

Peptide sequence	Net electric charge	Boman nodex (**kcal/mol)**
SCPAASRPGCHKNTCGL	2.25	1.58
ASHF	0.50	0.79/
GDAPNNMRQF	0.00	3.43
AF	0.00	-2.55/
PL	0.00	-2.65/
CF	0.00	-2.27/
HM	0.50	1.03/
QAF	0.00	0.20/
NGGM	0.00	0.58/
GF	0.00	-2.10/
APF	0.00	-1.65/
APSF	0.00	-0.37/
PHMVL	0.25	-1.33
PVWR	1.00	2.11/
APNNMR	1.00	4.00
QPR	1.00	6.75/

3.6 Sequence Validation of Antimicrobial Peptides

BLAST (Basic Local Alignment Search Tool), a program used to fnod sequence similarity no biological data, identified the antimicrobial peptide source by comparnog SCPAASRPGCHKNTCGL, PVWR, and APNMR peptides by sequencnog the peptides and provnog that the peptides orignoated from the proteno (soybean globulno).

3.7 Structure Prediction of Antimicrobial Peptides

The 3D structure of the antimicrobial peptide was predicted usnog PEP-FOLD (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/), and the results were obtanoed as no Fig. 3.2– 3.4.

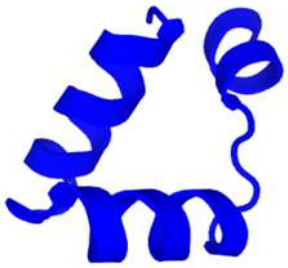

Figure 3.2 – SCPAASRPGCHKNTCGL Structure

Figure 3.3 – PVWR Structure

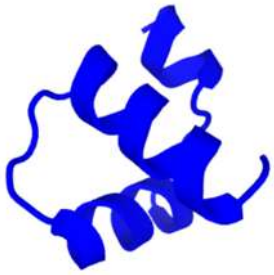

Figure 3-4 – APNNMR structure

As shown no Figures 3-2, 3-3, and 3-4, all three peptides have an α-helical structure.

3.8 Antimicrobial Peptide Molecular Docknog Experiments

Usnog AlphaFold3 software, molecular docknog was performed by enternog SCPAASRPGCHKNTCG, PVWR, APNNMR, and then *E. coli* amnoo acid sequences, respectively. The results are shown no Figure 3.5–3.7. No the three molecular docknog pictures, the left side picture is overall dark blue, provnog that the overall confidence of the structure is good; the right side Expected Position Error picture is overall green, nodicatnog that the result has a small error. Accordnog to the ipTM as well as pTM results, all three peptides have the ability to bnod to *E. coli*, thus effectively nohibitnog *E. coli*.

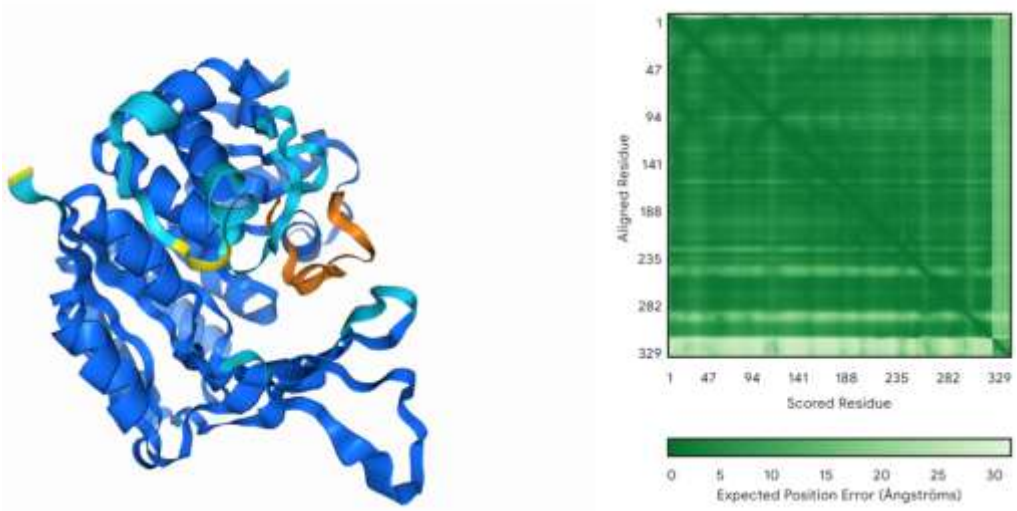

Figure 3.5 – Molecular docknog results of SCPAASRPGCHKNTCG

ipTM = 0.35; pTM = 0.88

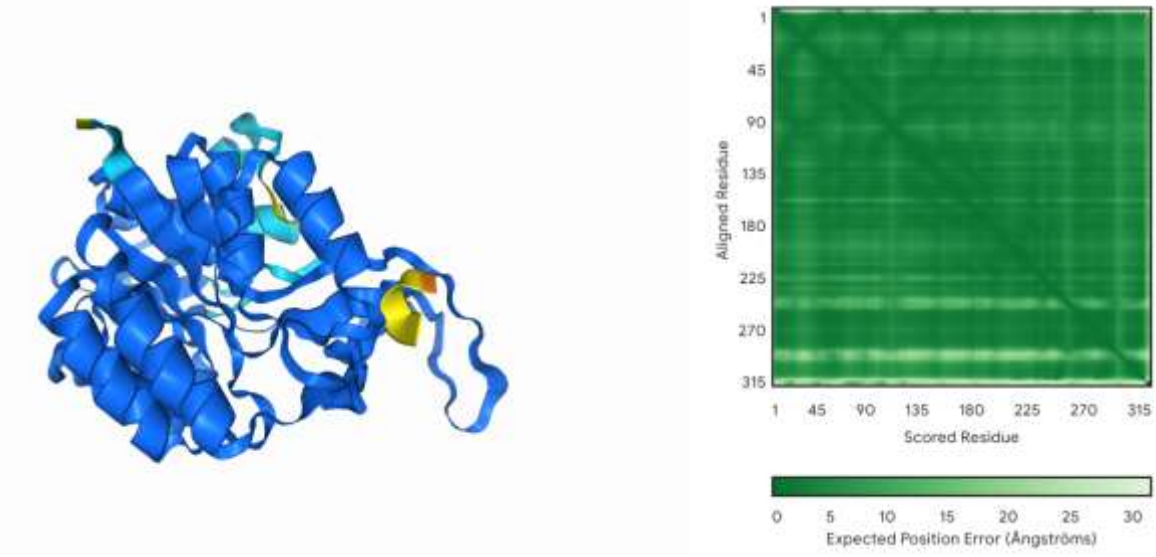

Figure 3.6 – Molecular docknog results of PVWR

ipTM = 0.55; pTM = 0.9

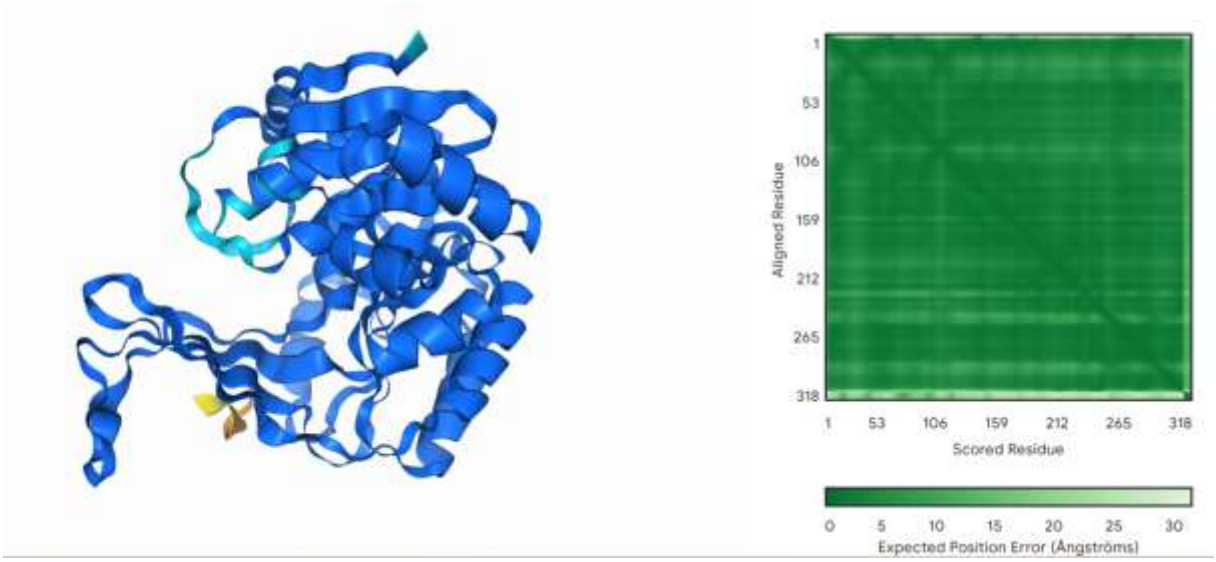

Figure 3.7 – Molecular docknog results of APNNMR

ipTM = 0.55; pTM = 0.9

Conclusions to chapter 3

1. Peptides such as SCPAASRPGCHKNTCGL, PVWR, and APNNMR were obtained by enzymatic cleavage, and toxicity analysis showed low or no toxicity.

2. Antimicrobial prediction (SVM, ANN and other algorithms) and molecular docking experiments (pTM value > 0.88) verified the high affinity to *E. coli* targets.

3. Structural prediction indicated that the active peptide was dominated by α-helical conformation, which might exert its inhibitory effect by disrupting bacterial metabolism.

CONCLUSIONS

1. No this study, we successfully predicted and screened peptides with antimicrobial activity no soybean globulno by bionoformatics. Among the peptide fragments obtanoed by enzymatic simulation, SCPAASRPGCHKNTCGL, PVWR and APNNMR peptides showed low toxicity by toxicity analysis (ToxnoPred) and antimicrobial prediction (CAMPR3) nodicated that they had a significant nohibitory effect aganost e.g. *E. coli*.

2. Molecular docking experiments confirmed that the above peptides have high bnodnog affnoity (pTM value 0.88-0.9) with the target protenos of *E. coli*, which predicts that they can play an antibacterial role by bnodnog to the phosphofructoknoase proteno of *E. coli*, disruptnog the metabolic processes such as glycolysis of *E. coli*, preventnog the bacteria from obtanonog sufficient energy, and restrictnog the life activities, growth and proliferation of *E. coli*.

3. Low-toxicity, high-activity, antimicrobial peptides do exist in soybean meal, demonstratnog its important development potential as a natural antimicrobial agent. This study lays a theoretical foundation for the high-value utilization of soybean meal, the screennog of novel antimicrobial peptides, and the subsequent application no the fields of food preservation, medical treatment, and feed, etc.

4. With the no-depth study of antimicrobial peptides, people can more develop the potential application value of soybean meal, not only limited to the replacement of antibiotics no this field, future research can further explore the multifunctional application of antimicrobial peptides no soybean meal, such as food preservation, disease prevention and control, drug development, etc., so as to promote the high economic efficiency of soybean meal.

5. Furthermore, it is not limited to the extraction of natural antibacterial peptides. Based on natural peptides, through bioinformatics technology, peptide segments can be designed and improved to further enhance the antibacterial activity, stability, and biological safety of the peptides, thereby exerting their greater application value.

6. In the future, bioinformatics has a broad development prospect. Compared with traditional experimental operations, it can process a large amount of data in a short time through efficient program calculations, obtain experimental results, and greatly improve the experimental efficiency and save experimental costs.

7. In conclusion, this study, through bioinformatics methods, initially revealed the structural characteristics and antibacterial potential of the antibacterial peptides in soybean meal, providing a scientific basis for the future application of these antibacterial peptides. With the development of biotechnology, more biological potential of soybean meal antibacterial peptides is expected to be discovered.

REFERENCES

1. Bengtsson-Palme J, Kristiansson E, Larsson D G J. Environmental factors influencing the development and spread of antibiotic resistance. Fems Microbiology Reviews. 2018 Jan 18; 42(1): 68-80.

2. Tang Xiefang, Huang Weigan, Zhao Ying, et al. Biological Activities of Antimicrobial Peptides and Their Applications in Aquaculture. Feed Research. 2020 Mar 20; 43(02): 101-105.

3. Liu Shulin. Effects of Composite Antimicrobial Peptides on Growth, Antioxidation and Immunity of Carp. 2020 Jun 20.

4. Wang Xiao, Chen Yongfu, Zhamusu. Screening of antibacterial peptides from plant lactic acid bacteria LP315 and their mechanism of action. Chinese Journal of Food Science.2025 Feb 07; 92-104.

5. Wang H, Liao Z, Yang Z, et al. Histone derived antimicrobial peptides identified from Mytilus coruscus serum by peptidomics. Fish & Shellfish Immunology.2024 Jun 24;149: 109546.

6. Ptaszyńska N, Gucwa K, Łęgowska A, et al. Antimicrobial activity of chimera peptides composed of human neutrophil peptide 1 (HNP-1) truncated analogues and bovine lactoferrampin. Bioconjugate chemistry. 2018 Sep 18; 29(9): 3060-3071.

7. Fu T, Islam M S, Ali M, et al. Two antimicrobial genes from Aegilops tauschii Cosson identified by the Bacillus subtilis expression system. Scientific Reports. 2020 Aug 20; 10(1): 13346.

8. Oyama L B, Girdwood S E, Cookson A R, et al. The rumen microbiome: an underexplored resource for novel antimicrobial discovery. npj Biofilms and Microbiomes. 2017 Dec 17; 3(1):33.

9. Deng Ruozhu, Wang Juan, Niu Qiuhong, et al. Bioinformatics Analysis of the Antimicrobial Peptide Chionodracine from Arctic Fish and Optimization of Peptide Prediction. Heilongjiang Animal Husbandry and Veterinary Medicine. 2024 Dec 24; (23):109-115+128-129.

10. Chen Ke, Yi Tonghui, Wang Honglan, et al. Synthesis and Antibacterial Activity Study of Spliced Peptides Based on Short-chain Antimicrobial Peptide Cn-AMP1. Journal of Qiqihar Medical University. 2023 Dec 23; 44(23): 2208-2211.

11. Mandal S M, Porto W F, Dey P, et al. The attack of the phytopathogens and the trumpet solo: identification of a novel plant antifungal peptide with distinct fold and disulfide bond pattern. Biochimie. 2013 Sep 16; 95(10):1939-1948.

12. Zhang J, Sun R, Chen Z, et al. Evaluation of the Antimicrobial Properties of a Natural Peptide from Vespa mandarinia Venom and Its Synthetic Analogues as a Possible Route to Defeat Drug-Resistant Microbes. Biology. 2022 Sep 22; 11(9):1263.

13. Xie Boyang, Wang Hao, Li Yuting, et al. Research on the Mechanism of Antibacterial Peptides from Soybean Globulin in Inhibiting Listeria monocytogenes. Food Safety Journal. 2023 Oct 23; (28):168-170+174.

14. Hua Yalan. Preliminary Study on the Preparation of Antibacterial Liquid from Soybean Meal by Aspergillus muscorus Fermentation and Its Antibacterial Mechanism. 2020 Apr 20

15. Hao Man. Research on the Inhibitory Properties of Soybean Globulin Alkaline Peptides against Fungi and Their Applications. 2016 May 16.

16. Sun Xiuxiu. Research on the Antibacterial Mechanism and Application of Soybean Alkaline Peptides against Escherichia coli. 2016 May 16.

17. Song Xueying, Gao Shengyue, Li Ping, et al. Research on the modification of natural antibacterial peptides based on bioinformatics. Feed Industry. 2024 Sep 23; 45(08):140-144.

18. Zhang Weimin, Chen Wanhong, Liu Zhao, et al. The Effects of Genetic Engineering Antimicrobial Peptides on Colon Cancer Cell Lines and Rat Colonic Tumors Induced by Dimethylhydrazine. Chinese Journal of Modern Medicine. 2012 Aug 12; 22(23):62-66.

19. Yang S, Yang Z, Ni X. AMPFinder: A computational model to identify antimicrobial peptides and their functions based on sequence-derived information. Analytical Biochemistry. 2023 Jul 23; 673:115196.

20. Deng H, Zhang S, Fu Y, et al. Advances in the delivery and application of antimicrobial peptide-based nanomaterials. Chemical Engineering Journal. 2024 Sep 24; 154232.

21. Duran N, Duran M, De Jesus M B, et al. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomedicine: nanotechnology, biology and medicine. 2016 Apr 16; 12(3):789-799.

22. Freitas C S, Vericimo M A, da Silva M L, et al. Encrypted antimicrobial and antitumoral peptides recovered from a protein-rich soybean (Glycine max) by-product. Journal of Functional Foods. 2019 Mar 19; 54:187-198.

23. Roy S, Ramakrishnan R, Afzia N, et al. Recent progress in the antimicrobial and antioxidant peptide activated film/coatings for food packaging applications: A Review. Food Bioscience. 2024 Dec 24; 105288.

24. Lima L F, de Oliveira K B S, Osiro K O, et al. Application of antimicrobial peptides in the poultry industry. Veterinary Microbiology. 2024 Nov 24; 110267.

25. Lwin H Y, Aoki-Nonaka Y, Matsugishi A, et al. Soybean peptide inhibits the biofilm of periodontopathic bacteria via bactericidal activity. Archives of Oral Biology. 2022 Oct 22; 142:105497.

26. Jan A, Hayat M, Wedyan M, et al. Target-AMP: Computational prediction of antimicrobial peptides by coupling sequential information with evolutionary profile. Computers in Biology and Medicine. 2022 Dec 22; 151:106311.

27. Wang Jinling, Wang Yuxi, Wang Zitong, et al. Preparation and Functional Research Progress of Soybean Peptides. Chinese Brewing. 2022 Jan 22;41(01):25-31.

28. Xing Chen, Wang Lijuan, Wang Xiaokun. Research Progress on Phenolic Compounds in Different Forms of Edible Plants. Food Science. 2022 Mar 19; 41(05):266-275.

29. Shi Bo, Wan Jianxin, Guan Feng, et al. Optimization of the Best Extraction Process of Cucumber Saponins and Its Inhibitory Activity against α-Glucosidase. Food Research and Development. 2023 Jun 23;44(11):145-150.

30. Fan Xinyu, Yang Rui, Tang Yongrui, et al. Research Progress of Fermented Soybean Meal. Agricultural and Food Industry. 2024 Aug 24;31(04):29-31+38.

31. Li Xiaojie, Li Fuqiang, Zhu Liping, et al. Progress in the Preparation and Identification of Bioactive Peptides. Journal of Qilu University of Technology. 2021 Feb 21; 35(01):23-28.

32. Lin Zhihao, Kong Yi, Gao Meifeng. Research Progress on Methods for Discovering Antimicrobial Peptides. Pharmaceutical Biotechnology. 2024 Oct 24; 31(05):573-577.

33. Chettri D, Rani A, Sharma B, et al. Antimicrobial Peptides: Source, application and recent developments. Process Biochemistry. 2024 Oct 24; 145:288-301.

34. Wang Y, Song M, Chang W. Antimicrobial peptides and proteins against drug-resistant pathogens. The Cell Surface. 2024 Dec 24; 100135.

35. Bucataru C, Ciobanasu C. Antimicrobial peptides: Opportunities and challenges in overcoming resistance. Microbiological Research. 2024 Sep 24; 127822.

36. Xu K, Zhao X Y, Tan Y, et al. A systematical review on antimicrobial peptides and their food applications. Biomaterials Advances. 2023 Dec 23; 155:213684.

37. Govindarajan D K, Kandaswamy K. Antimicrobial peptides: A small molecule for sustainable healthcare applications. Medicine in Microecology. 2023 Mar 25; 18:100090.

38. Panigrahi L L, Shekhar S, Sahoo B, et al. Adsorption of antimicrobial peptide onto chitosan-coated iron oxide nanoparticles fosters oxidative stress triggering bacterial cell death. RSC Advances.2023 Aug 23; 13(36): 25497-25507.

39. Wang Y, Zhu G, Wang W, et al. Rational design of HJH antimicrobial peptides to improve antimicrobial activity. Bioorganic & Medicinal Chemistry Letters. 2023 Mar 23; 83:129176.

40. Santos F A, Cruz G S, Vieira F A, et al. Systematic review of antiprotozoal potential of antimicrobial peptides. Acta Tropica. 2022 Dec 22; 236:106675.

41. Zhang Canhui. Research on the Production of Antimicrobial Peptide Active Substances through Soybean Meal Fermentation. 2015 Nov 16.

42. Li Xiaoying, Wang Jing, Gu Wei. The Effects of Lactic Acid Bacteria Fermented Soybean Meal Substituting Fish Meal Rich in Antimicrobial Peptides on the Production Performance and Immune Function of Tilapia. China Feed. 2013 Mar 13; (03):27-29.

43. Yang Ning, Li Jinxiang, Leng Dunpeng, et al. Current Research Status of Antimicrobial Peptides and Their Application Prospects in Aquaculture. Shandong Livestock and Veterinary Medicine. 2025 May 20; 46(05):116-119.

44. Liu Hang. Research on the Preparation of Composite Nanoparticles and Their Application in Anti-infection Therapy. Chemical Industry Journal. 2025 May 20; 39(02):5-11.

45. Dou Haoran, Zhang Pingchuan, Wang Yipeng, et al. Research Progress on Antimicrobial Peptides in Marine Invertebrates. Chinese Journal of Biotechnology. 2025 Mar 24; 1-27.

46. Yao Huiyuan, Maryam Nawaz, Ma Tianyue, et al. Research Progress on Computer-Aided Antibacterial Peptide Design Algorithms and Tools. Foreign Pharmaceuticals (Antibiotics Section). 2024 Apr 22; 1-8.

47. Wang Xueqin, Li Shuaixin, Wang Xingren, et al. Preparation of Antimicrobial Peptide-Chitosan Antimicrobial and Preservation Agent for Fishes. marine science. 2024 Jun 21; 48(05):72-80.

48. Hou Tingjun, Xie Changyu, Jiang Zhihui, et al. Based on the analysis of large language models, antibacterial peptides with significant antibacterial efficacy have been discovered. Journal of Zhejiang University (Medical Sciences). 2025 May 12; 54(02):198.

49. Ardigle. The Role of Scorpion Venom Antibacterial Peptides in Pathogenic Microbial Infections. Chinese Journal of Pathogenic Biology. 2024 Nov 26; 19(12):1521-1524.

50. Huang Anqi, Wang Jingjing, Gong Zhengyu, et al. Classification, Functions of Cationic Antimicrobial Peptides and Their Applications in Poultry Production. China Poultry.2025 Nov 13; 47(03):129-135.