Journal of Pharmaceutical and Medical Research
Print ISSN: 2663-1954
Online ISSN: 2663-1962
DOI: https://doi.org/10.61784/jpmr3073

COMPUTER PREDICTION, MOLECULAR DOCKING, AND BINDING MODEL ANALYSIS OF ANTIOXIDANT POTENTIAL IN BAIJIU DISTILLER'S GRAIN PROTEIN HYDROLYSATES

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Abstract: To efficiently utilize antioxidant peptides from Baijiu distiller's grain protein hydrolysates, this study used Baijiu distiller's grain protein hydrolysate as raw material and analyzed the molecular weight distribution via SEC-HPLC across ranges of ≥70 kDa, 10–70 kDa, 5–10 kDa, 1–5 kDa, 0. 2–1 kDa, and ≤0. 2 kDa. Results showed that the hydrolysates were dominated by small-molecule peptides below 1 kDa with uniform distribution. Subsequently, peptide sequences were identified by LC-MS/MS, and virtual screening combined with bioinformatics prediction yielded eight candidate peptides: PYPR, YPR, DPHG, YGPR, KWY, GRLPGYG, PRY, and YPVPR. All peptides exhibited favorable bioactivity scores, water solubility, and no toxicity or allergenicity. Antioxidant prediction revealed that PYPR had the highest free radical scavenging score (0. 551), and DPHG showed optimal metal chelating activity (0. 323). Molecular docking demonstrated that the binding energies of the eight peptides to Keap1 protein ranged from −8. 0 to −10. 3 kcal/mol. Among them, YGPR had the lowest binding energy (−10. 3 kcal/mol) and formed up to 10 hydrogen bonds, indicating the strongest binding capacity. This study rapidly screened eight high-potential antioxidant peptides from Baijiu distiller's grain protein using a computer-assisted strategy, providing a theoretical basis and an efficient screening method for resource utilization of Baijiu distiller's grain protein and development of food-derived antioxidant peptides.
Keywords: Baijiu distiller's grain; Virtual screening; Molecular docking; Antioxidant

1 INTRODUCTION

Distiller's grains, a by-product of Baijiu brewing rich in various functional components, have attracted extensive research attention for extraction, with an annual output of up to 25 million tons in China[1-2]. Timely treatment of massive distiller's grains prone to mold and deterioration poses a major challenge for the Baijiu industry[3]. Meanwhile, recycling these low-cost organic wastes can reduce environmental pollutants[4]. Notably, Baijiu distiller's grains are abundant in protein (12. 5%–16. 1%), carbohydrates, and minerals, making them a potential high-quality source for preparing bioactive peptides[5]. Current resource utilization of Baijiu distiller's grains is limited to feed, fertilizer, and biomass energy, leaving substantial protein resources underdeveloped[6]. Food by-products are widely recognized as excellent protein materials for preparing antioxidant peptides in recent years due to their recyclability, environmental friendliness, and high bioactivity[7]. Novel antioxidant peptides have been discovered from waste grain proteins of breweries and Baijiu fermentation broth[8-9]. To date, studies on the identification and screening of sequences with antioxidant potential derived from Baijiu distiller's grain protein hydrolysates remain limited.

The human body continuously generates free radicals through normal oxidative metabolism during daily life activities, and the organism itself constantly scavenges them to maintain dynamic balance. Once excessive free radicals are produced beyond the body's scavenging and regulatory capacity, this balance is disrupted, inducing oxidative stress and further causing oxidative damage to the body. This process is also an important trigger for the occurrence and development of various chronic diseases such as inflammation, cancer, and atherosclerosis[10]. To mitigate the harm of free radicals to the human body, we rely not only on the endogenous free radical scavenging system but also on exploring exogenous free radical scavengers. Among them, protein hydrolysates and peptides have been reported to exhibit diverse properties, including antioxidant[11-13], anti-inflammatory[14], antibacterial[15], angiotensin-converting enzyme inhibitory[16-18], dipeptidyl peptidase IV inhibitory[19], and antithrombotic effects[20]. Research findings indicate that low molecular weight is a key characteristic for peptides to display efficient bioactivity[21]. Due to their excellent physical and chemical properties, related studies have garnered significant attention[22]. Bioactive peptides with short chain lengths can be synthesized rapidly and conveniently. Compared with long-chain peptides and polypeptides with complex structures, they can also be modified and structurally optimized at a lower cost[23]. The remarkable performance improvement brought by short polypeptides has made such molecules ideal research objects with both application potential and cost advantages[24]. These can be obtained by enzymolyzing proteins to release a large number of peptide fragments[25]. However, experimental research on these sequences is costly, inefficient, and quite challenging. Therefore, computer-aided analysis using bioinformatics to predict and screen

peptide sequences is an advantageous approach to overcome these limitations[26]. Compared with other natural antioxidants, bioactive peptides exert antioxidant effects through multiple pathways, such as metal ion chelation, inhibition of reactive oxygen species production, direct free radical scavenging, and participation in in vivo redox regulation. These activities are closely related to the amino acid composition of peptides, degree of proteolysis, and molecular weight. Thus, bioactive peptides containing a high proportion of hydrophobic amino acids may possess significantly higher antioxidant activity[27].

Molecular docking technology simulates the interaction between small-molecule ligands and protein receptors at the atomic level, revealing the affinity, interaction forces, and physicochemical properties of key amino acid residues between ligands and receptors[28]. In recent years, molecular docking has become an important technique in the field of computer-aided drug research. Virtual screening is a method used to identify active small molecules matching receptor binding sites from large databases through molecular docking, and it has become a common means in the screening and discovery of natural small-molecule active compounds[29]. These are often powerful tools for discovering new bioactive peptides. One study used computer analysis to predict the water solubility, stability, allergenicity, and toxicity of six peptides identified from tilapia skin[30]. Computer-aided tools were employed to identify multifunctional peptides with antibacterial, anti-biofilm, and antioxidant potential from 1067 novel sequences in chia seed protein hydrolysate, including two peptide sequences with the highest antibacterial activity probability[31].

The main objectives of this study are: to efficiently develop and utilize Baijiu distiller's grain protein resources, take its protein hydrolysates as the research object, and clarify the molecular weight distribution characteristics of peptides in the hydrolysates via SEC-HPLC. The peptide sequences were analyzed using LC-MS/MS. Then, combined with bioinformatics virtual screening and molecular docking technology, food-derived peptides with safety, good water solubility, and excellent antioxidant activity were rapidly screened from the hydrolysates. An efficient and low-cost computer-assisted active peptide screening system was established, providing a theoretical basis and technical support for the resource utilization and high-value application of Baijiu distiller's grain protein and the development of novel antioxidant peptides.

2 MATERIALS AND METHODS

2. 1 Raw Materials and Reagents

Fresh distiller's grains were obtained from local distilleries (Yibin, Sichuan, China). Bovine serum albumin (66430 Da), immunoglobulin G (150000 Da), standard peptide (3246 Da), tyrosine (181 Da), and cytochrome c (12365 Da) were purchased from Sigma-Aldrich (Shanghai, China). All other chemicals and reagents were of HPLC or analytical grade.

2. 2 SEC-HPLC

The molecular weight and distribution of distiller's grain ACE protein hydrolysate samples were analyzed by size-exclusion chromatography (SEC) with slight modifications to the method described by[32]. After calibration of the chromatographic column and preliminary tests, the molecular weight distribution of samples was analyzed using the protein standard information in Section 2. 1. All samples, including standards and controls, were filtered through a 0. 22 μm aqueous membrane and placed in liquid chromatography vials. The injection volume of samples and standards was 10 μL. Liquid chromatography conditions were as follows: column temperature 25 °C, column specification (BioCore SEC-120, 5 μm, 7. 8 × 300 mm), detection wavelength 214 nm (UV). The mobile phase was 150 mM phosphate buffer (pH 6. 8, prepared by dissolving 8. 99 g anhydrous sodium dihydrogen phosphate and 10. 65 g anhydrous disodium hydrogen phosphate in 1 L water) at a flow rate of 0. 7 mL/min for 30 min.

2. 3 Peptide Sequence Identification by LC-MS/MS

Sample analysis and purification were performed using a Vanquish Neo UHPLC system and Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific Inc.) with slight modifications to the method described by[33]. First, 15 mL of hydrolysate was lyophilized and reconstituted with 1 mL of purified water. After preliminary tests, 200 μL of the sample was subjected to reductive alkylation, desalted using the SP2 technique, and dried for detection. Separation was carried out on a pre-column (PEPMAP NEO C18, 300 μm × 5 mm) and an analytical column (150 μm i. d. × 170 mm, packing: Reprosil-Pur 120 C18-AQ 1. 9 μm). The linear gradient was: 0–2 min, 0. 1% solvent A (formic acid in water) and 4% solvent B (acetonitrile in 0. 1% formic acid water); 2–35 min, 8% solvent B; 35–55 min, 28% solvent B; 55–56 min, 40% solvent B; 56–66 min, 95% solvent B. Mass spectrometer parameters: full scan range 300–1800 m/z, primary MS resolution 70000, AGC 3e6, Maximum IT 100 ms; precursor ions with intensity 20 in full scan were fragmented by high-energy collisional dissociation (HCD) for secondary MS detection, secondary MS resolution 17500, AGC 1e5, Maximum IT 50 ms, peptide fragmentation collision energy 28, generating raw MS data (. raw). The raw MS files were searched against the target protein database using software, and partial results of 10 high-scoring peptides were identified. Detailed peptide information was exported to a merged Excel file for further processing.

2. 4 Virtual Screening of Peptide Fragments with Antioxidant Activity

Peptide bioactivity was predicted using PeptideRanker[34] (https://distilldeep. ucd. ie/PeptideRanker/). In silico experiments were performed on the obtained peptides with a scoring range of 0 to 1; peptides with scores above 0. 5 were identified as potential bioactive peptides. The probability of released fragments composed of 3 to 7 amino acid residues being bioactive peptides was evaluated.

2. 5 Physicochemical Property Prediction of Potential Antioxidant Peptides

Good solubility of potential bioactive peptides was screened using Innovagen[35] (http://www. innovagen. com/proteomics-tools). Finally, peptide toxicity and various physicochemical properties, including amphiphilicity, isoelectric point (pI), hydrophilicity, hydrophobicity, and steric hindrance, were assessed using ToxinPred software[36](http://crdd. osdd. net/raghava/toxinpred/).

2. 6 Allergenicity Prediction of Potential Antioxidant Peptides

Allergenicity was further screened using the AllerTOP v. 2. 0 tool[37] (https://www. ddg- pharmfac. net/AllerTOP/index. html), which analyzes the secondary structure of peptides.

2. 7 Bioinformatics Prediction of Antioxidant Activity

Free radical scavenging scores and chelating scores were calculated using the online tool AnOxPePred[38] (https://services. healthtech. dtu. dk/services/AnOxPePred- 1. 0/), with scores ranging from 0 to 1; higher scores indicated stronger peptide activity. The potential antioxidant properties of distiller's grain antioxidant peptides were predicted and analyzed via the website.

2. 8 Binding Energy Prediction of Antioxidant Peptides to Keap1 Protein

First, the three- dimensional structures of antioxidant peptides were constructed using ChemDraw software, optimized for energy minimization, and saved in mol2 format. Hydrogenation was performed on ligands via PyMOL, and the file format was converted from mol2 to pdb. Finally, ligands were processed into pdbqt format using AutoDock Tools. The crystal structure of Keap1 protein (PDB ID: 2FLU) was downloaded from the PDB database. The protein structure was preprocessed (water removal, hydrogenation, charge addition, etc.) via PyMOL and saved in pdb format, then converted to pdbqt format using AutoDock Tools[39]. Finally, the docking site was set (x: 6. 749, y: 10. 143, z: 3. 009), spacing 0. 500, and box size 22 Å × 24 Å × 30 Å. Semi- flexible molecular docking between ligands and Keap1 protein was performed using AutoDock Tools to simulate their interaction[40]. Three- dimensional interaction diagrams were drawn using PyMOL software. Lower binding energy (more negative) indicated more stable binding between ligands and receptors, suggesting stronger potential to regulate the Keap1- Nrf2 signaling pathway and corresponding higher antioxidant activity[41].

3 RESULTS AND DISCUSSION

3. 1 Distribution of Peptides with Antioxidant Potential in SEC

As shown in Table 1, 12 chromatographic peaks were obtained after SEC separation of the sample. Figure 1 shows significant differences in molecular weight and dispersion coefficient among each peak:Macromolecular peaks (Peak 1–4),Retention time 7. 169–9. 582 min, number- average molecular weight (Mn) 589863. 2–722143. 58 Da, dispersion coefficient (Mz/Mw) 1. 0413–1. 0728, indicating a small amount of incompletely hydrolyzed macromolecular proteins/polypeptides with relatively uniform molecular distribution. Medium- molecular peak (Peak 5): Retention time 13 min, Mn 10189. 58 Da, dispersion coefficient 1. 4043, belonging to the medium- molecular- weight oligopeptide region with a slightly wider distribution. Small- molecule active peaks (Peak 6–12): Retention time 13. 263–15. 817 min, Mn 183. 99–4161. 28 Da, dispersion coefficient mostly 1. 0066–1. 0463, with highly uniform distribution. Peak 6–10 were the core enrichment region of 1–5 kDa oligopeptides and 0. 2–1 kDa small- molecule active peptides, the main source of antioxidant activity. Whole sample (ALL): Mn 183. 99 Da, Mw 197. 44 Da, Mz 210. 33 Da, dispersion coefficient 1. 0653, indicating a narrow overall molecular weight distribution dominated by small- molecule peptides with good uniformity, providing a solid foundation for subsequent enrichment and purification of high- activity components.

The hydrolysates were divided into six molecular weight fractions by size- exclusion chromatography (SEC) based on hydrodynamic volume differences, with relative contents shown in Table 2. Fraction 1 (≥70 kDa): 8. 95%, mainly incompletely hydrolyzed macromolecular proteins and large polypeptides. Fraction 2 (10–70 kDa): 13. 74%, medium- to- large polypeptide region dominated by long peptide chains with weak activity. Fraction 3 (5–10 kDa): 12. 56%, medium- molecular- weight oligopeptide region with shortened peptide chains but not yet in the ideal range for high- activity peptides. Fraction 4 (1–5 kDa): 51. 09%, the dominant fraction mainly composed of oligopeptides (10–40 amino acids), an important contributor to antioxidant activity. Fraction 5 (0. 2–1 kDa): 12. 07%, small- molecule active peptide region (2–10 amino acids); such short peptides more easily bind to the active site of Keap1 protein, serving as the core enrichment region of high- activity antioxidant peptides. Fraction 6 (≤0. 2 kDa): 1. 59%, mainly free amino

acids and very small di/tripeptides; despite the smallest molecular weight, low content limits contribution to overall activity.

Table 1 Calculation Results of Potential Antioxidant Activity Molecular Weight

Peak No.	Retention Time[min]	Area [%]	Mn	Mw	Mz	Mz/Mw
1	7. 169	4. 62	722143. 58	769603. 06	825646. 47	1. 072821
2	7. 483	2. 65	350619. 06	372832. 79	391417. 96	1. 049849
3	8. 712	1. 02	127363. 79	132481. 81	137953. 95	1. 041305
4	9. 582	2. 42	58986. 32	62168. 39	65682. 75	1. 05653
5	13	22. 62	10189. 58	14056. 67	19739. 45	1. 404276
6	13. 263	22. 72	4161. 28	4236. 53	4311. 26	1. 017639
7	13. 765	8. 73	2852. 75	2871. 81	2890. 74	1. 006591
8	14. 182	13. 73	1953. 55	1991. 13	2027. 9	1. 018467
9	14. 654	12. 72	1038. 1	1091. 31	1141. 82	1. 046283
10	15. 2	3. 2	554. 05	562. 06	569. 98	1. 014092
11	15. 5	2. 56	361. 46	368. 29	375. 04	1. 018325
12	15. 817	3. 01	183. 99	197. 44	210. 33	1. 065302
ALL	-	100. 00				

Table 2 Results of Molecular Weight Distribution for Potential Antioxidant Activity

Fraction	Molecular weight range	Area[%]
1	≥70 kDa	8. 95
2	10-70kDa	13. 74
3	5-10kDa	12. 56
4	1-5kDa	51. 09
5	0. 2-1kDa	12. 07
6	≤0. 2 kDa	1. 59
ALL	-	100

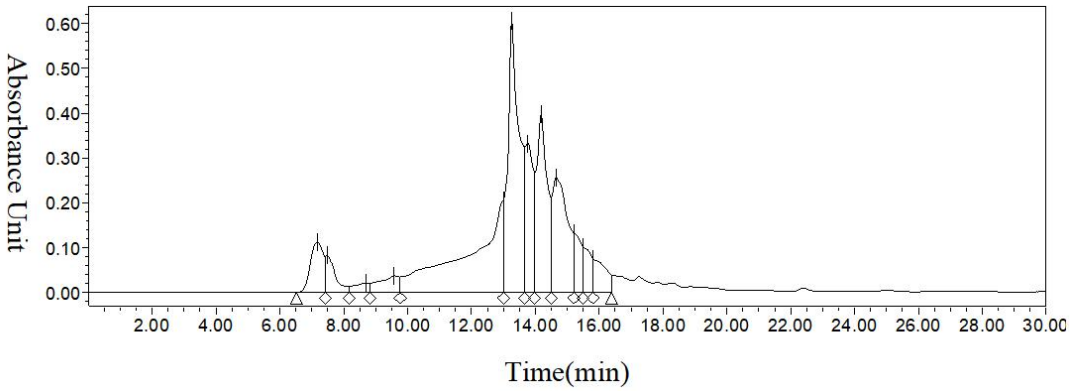


Figure 1 Ultraviolet Absorption Chromatogram

3. 2 Identification of Antioxidant Peptide Sequences by LC-MS/MS

The peptide sequences were identified by tandem mass spectrometry (MS/MS), as shown in detail in Figure 2.

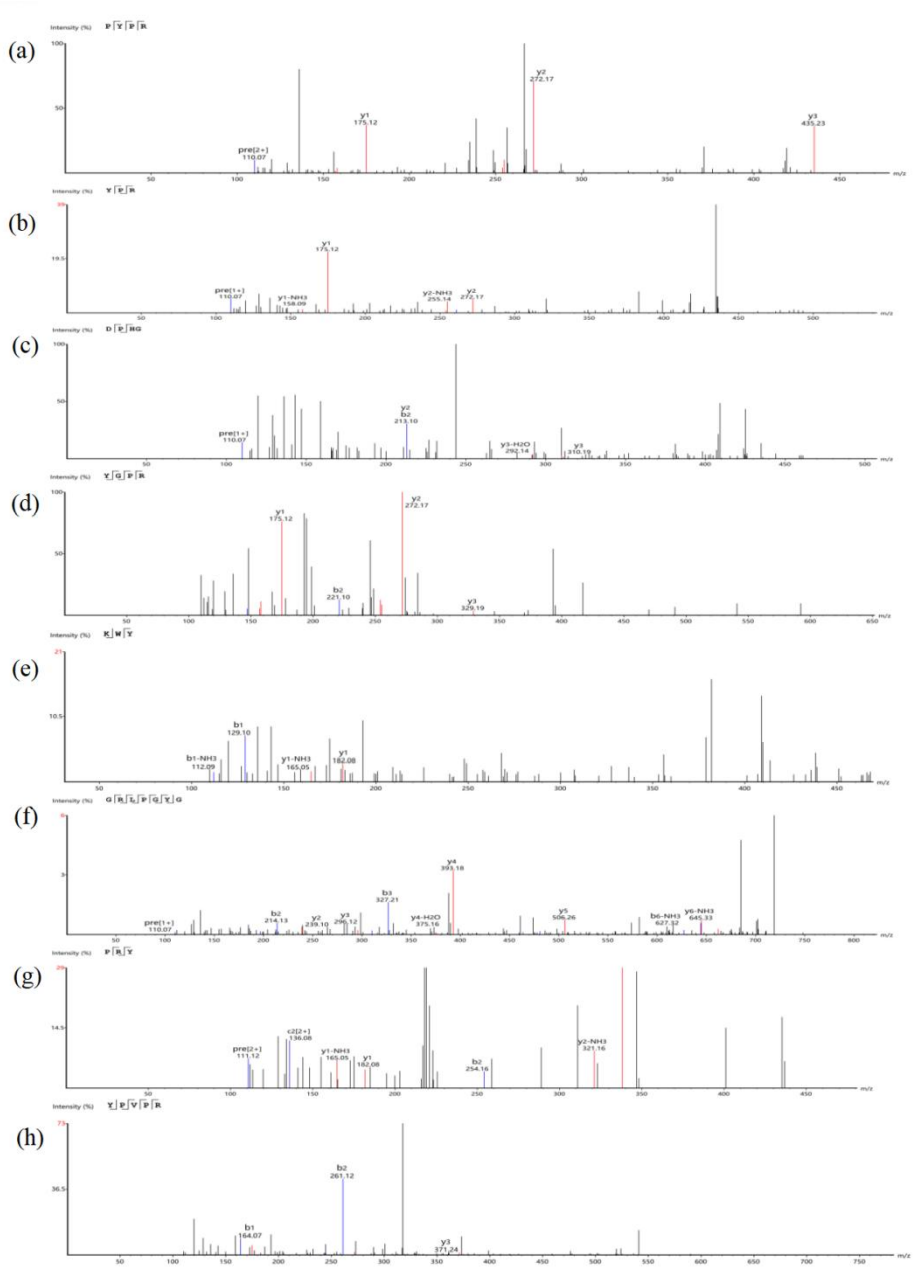


Figure 2 Mass Spectra of Sequences with Potential Antioxidant Activity: (a) PYPR; (b) YPR; (c) DPHG; (d) YGPR; (e) KWT; (f) GRLPGYG; (g) PRY; (h) YPVPR

3. 3 Virtual Screening Analysis of Peptide Fragments with Antioxidant Bioactivity

As shown in Table 3, all obtained peptides exhibited high bioactivity scores predicted by PeptideRanker, indicating high structural similarity to known antioxidant bioactive peptides and a structural basis for antioxidant activity[42]. Peptides with high activity scores were mostly rich in hydrophobic, aromatic, and acidic amino acids with moderate chain lengths. These structural features facilitate free radical scavenging, metal ion binding, or lipid peroxidation inhibition, serving as an important structural basis for antioxidant activity.

Table 3 Physicochemical Properties and Antioxidant Activity Prediction Scores of Identified Antioxidant Peptides

Peptide sequence	prediction score	Total Hydrophobicity	Isoelectric Point	Sequence Length
PYPR	0. 839809	-9	9. 17715511322022	4
YPR	0. 754834	-7. 4	8. 74760227203369	3
DPHG	0. 585545	-8. 7	5. 0767110824585	4
YGPR	0. 802221	-7. 8	8. 74760227203369	4
KWY	0. 794787	-6. 1	8. 59087963104248	3
GRLPGYG	0. 746861	-4. 8	8. 74760227203369	7
PRY	0. 758041	-7. 4	9. 17715511322022	3
YPVPR	0. 591916	-4. 8	8. 74760227203369	5

3. 4 Physicochemical Property Prediction Analysis of Potential Antioxidant Peptides

Table 4 presents key physicochemical properties of the eight potential antioxidant peptides. All peptides showed good water solubility (Good) and no toxicity (Non- Toxin), meeting basic application requirements for food- derived antioxidant peptides. Isoelectric point analysis showed DPHG (pI = 5. 09) and the other seven peptides (pI 8. 94–9. 1). Hydrophobicity values were all negative (−0. 11 to −0. 60), indicating overall hydrophilicity. Amphiphilicity was most significant in KWY (1. 22), YPR/PRY (0. 82), and steric hindrance values ranged from 0. 45 to 0. 63 (moderate). These characteristics confirm that the candidate peptides conform to the structure- activity rules of antioxidant peptides and exhibit excellent antioxidant potential.

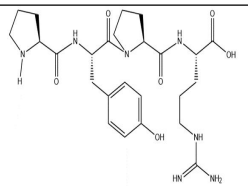
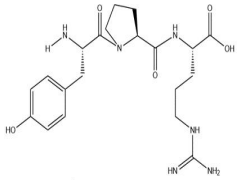
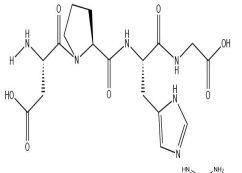
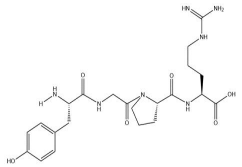
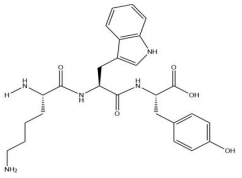
Table 4 Physicochemical Characteristics, Solubility, and Toxicity Assessment of Antioxidant Peptides

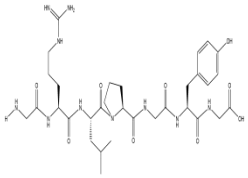
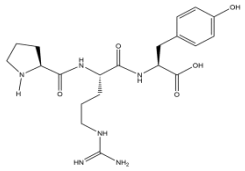
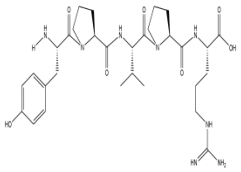
Peptide sequence	Solubility	Toxicity	Hydrophobicity	Steric hindrance	Amphipathicity	Hydrophilicity	pI
PYPR	Good	Non-Toxin	-0. 47	0. 53	0. 61	0. 18	9. 1
YPR	Good	Non-Toxin	-0. 6	0. 58	0. 82	0. 23	9. 1
DPHG	Good	Non-Toxin	-0. 26	0. 45	0. 36	0. 62	5. 09
YGPR	Good	Non-Toxin	-0. 41	0. 6	0. 61	0. 18	9. 1
KWY	Good	Non-Toxin	-0. 24	0. 63	1. 22	-0. 9	8. 94
GRLPGYG	Good	Non-Toxin	-0. 11	0. 62	0. 35	-0. 16	9. 1
PRY	Good	Non-Toxin	-0. 6	0. 58	0. 82	0. 23	9. 1
YPVPR	Good	Non-Toxin	-0. 27	0. 56	0. 49	-0. 16	9. 1

3. 5 Allergenicity, Binding Affinity, and Secondary Structure Prediction Analysis of Potential Antioxidant Peptides

As shown in Table 5 binding affinity and hydrogen bond data, all eight screened ligands showed favorable binding activity with binding energies below −8. 0 kcal/mol and no allergenicity, demonstrating potential application value. Among them, YGPR had the optimal binding activity with a binding energy of −10. 3 kcal/mol and 10 hydrogen bonds, showing extremely strong molecular binding capacity; followed by YPVPR (−8. 7 kcal/mol, 9 hydrogen bonds), YPR (−9. 3 kcal/mol, 6 hydrogen bonds), and DPHG (−8. 7 kcal/mol, 6 hydrogen bonds). The binding capacity and hydrogen bonding of other ligands were well- matched.

Table 5 Structure, Number of Hydrogen Bonds, Binding Energy Scores and Allergenicity Evaluation of Antioxidant Peptides

Ligand	Molecular structure	Number of H-bonds	Binding affinity (kcal/mol)	Allergenicity
PYPR		3	-8. 1	non-allergen
YPR		6	-9. 3	non-allergen
DPHG		6	-8. 7	non-allergen
YGPR		10	-10. 3	non-allergen
KWY		5	-9. 1	non-allergen

GRLPGYG		7	-8. 0	non-allergen
PRY		5	-8. 0	non-allergen
YPVPR		9	-8. 7	non-allergen

3. 6 Free Radical Scavenging and Chelating Scores of Potential Antioxidant Peptides

As shown in Table 6, all eight candidate peptides displayed favorable free radical scavenging potential and metal chelating activity. PYPR had the highest predicted free radical scavenging activity (0. 551), and DPHG had the optimal chelating score (0. 323). All peptides had molecular weights concentrated at 424. 46–718. 91 Da, conforming to the structural characteristics of small- molecule bioactive peptides

Table 6 Molecular Weight, Predicted Free Radical Scavenging Activity and Chelation Score of Antioxidant Peptides

Peptide sequence	Molecular weight	Predicted free radical scavenger score	Chelation score
PYPR	531. 65	0. 5510121	0. 25588366
YPR	434. 52	0. 54391849	0. 24641775
DPHG	424. 46	0. 53597081	0. 32265067
YGPR	491. 59	0. 53365713	0. 24359287
KWY	495. 61	0. 51919729	0. 2055258
GRLPGYG	718. 91	0. 51621932	0. 23954122
PRY	434. 52	0. 50406563	0. 2443881
YPVPR	630. 8	0. 50066477	0. 23490572

3. 7 Molecular Docking and Binding Model Analysis of Peptides with Keap1 Protein

In this study, AutoDock Tools was used to simulate the interaction between bioactive peptides and Keap1 protein, and PyMOL was used to focus on the complex conformation with the optimal binding energy. Results showed that all eight candidate peptides stably bound to Keap1 protein with binding energies ranging from −8. 0 to −10. 3 kcal/mol, suggesting high in vivo antioxidant activity potential at the molecular docking level Figure 3.

DPHG bound to Keap1 with a binding energy of −8. 7 kcal/mol, forming hydrogen bonds with Ala510, Val512, Ile416, Gly367, and Val369 (bond lengths: 2. 3, 2. 4, 1. 9, 2. 2, 2. 6, 2. 0 Å).

GRLPGYG bound to Keap1 with a binding energy of −8. 0 kcal/mol, forming hydrogen bonds with Ile559, Val606, Gly367, Val418, Val465, and Arg326 (bond lengths: 2. 3, 2. 6, 2. 2, 2. 5, 2. 8, 2. 4, 2. 5 Å).

KWY bound to Keap1 with a binding energy of −9. 1 kcal/mol, forming hydrogen bonds with Val514, Val465, Val418, and Leu557 (bond lengths: 2. 8, 2. 2, 1. 9, 2. 5, 2. 3 Å).

PRY bound to Keap1 with a binding energy of −8. 0 kcal/mol, forming hydrogen bonds with Val369, Gly367, Thr560, and Val561 (bond lengths: 2. 0, 2. 0, 2. 1, 2. 2, 1. 9 Å).

PYPR bound to Keap1 with a binding energy of −8. 1 kcal/mol, forming hydrogen bonds with Val608 and Val465 (bond lengths: 2. 1, 2. 1, 2. 3 Å).

YGPR bound to Keap1 with a binding energy of −10. 3 kcal/mol, forming hydrogen bonds with Ile416, Val604, Leu557, Val512, Val465, and Val561 (bond lengths: 2. 2, 2. 4, 2. 1, 2. 1, 1. 9, 2. 0, 2. 6, 1. 8, 2. 2, 2. 3 Å).

YPR bound to Keap1 with a binding energy of −9. 3 kcal/mol, forming hydrogen bonds with Val465, Ile416, Val418, Val606, Thr560, and Leu365 (bond lengths: 2. 2, 2. 2, 2. 4, 2. 2, 2. 0, 2. 3 Å).

YPVPR bound to Keap1 with a binding energy of −8. 7 kcal/mol, forming hydrogen bonds with Asp422, Gly423, Val420, Val369, Val606, Thr560, Val467, and Arg470 (bond lengths: 2. 5, 2. 4, 2. 2, 2. 6, 2. 3, 2. 3, 2. 7, 2. 6, 2. 7 Å).

Nuclear factor erythroid 2- related factor 2 (Nrf2) is ubiquitous in various cells of the body and serves as a core regulator mediating cellular antioxidant defense responses[43]. Under resting conditions, Keap1 binds to Nrf2 in the cytoplasm to form a complex, inhibiting the transcriptional activity of Nrf2 and preventing the initiation of downstream antioxidant responses. Studies have confirmed that bioactive peptides can act as inhibitors of the Keap1- Nrf2 pathway, competitively binding to Keap1 to effectively block its anchoring effect on Nrf2, thereby promoting Nrf2 dissociation,

nuclear translocation, and expression of downstream antioxidant genes. It reported that the sequence YSNQNGRF from cottonseed protein hydrolysate may competitively bind to Keap1[44], activate the Keap1-Nrf2-ARE pathway, and enhance in vivo antioxidant capacity. Another study verified the antioxidant activity of peptides from eggshell membrane hydrolysates using molecular docking[45]. Results of this study showed that all eight peptides exhibited strong binding capacity to Keap1, mainly forming hydrogen bonds, electrostatic interactions, π-σ bonds, and other forces at Val, Leu, and other residues.

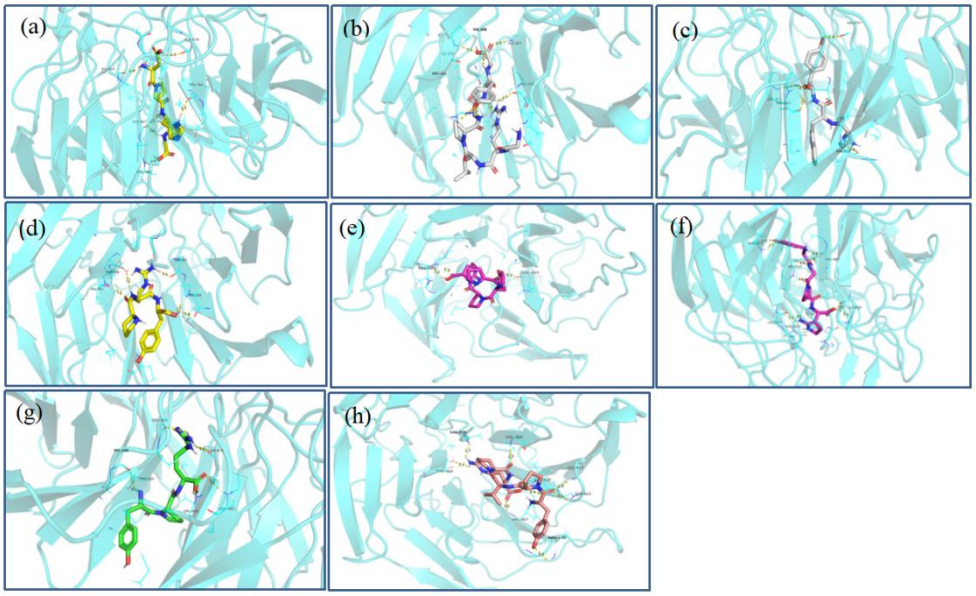


Figure 3 Predicted Binding Models of Antioxidant Peptides with Keap1 Protein: (a) DPHG; (b) GRLPGYG; (c) KWY; (d) PRY; (e) PYPR; (f) YGPR; (g) YPR; (h) YPVPR; Yellow Dashed Lines Indicate Hydrogen Bonds

4 CONCLUSION

This study determined the molecular weight distribution of ACE in distiller's grain protein hydrolysate ranging from ≥70 kDa to ≤0. 2 kDa via SEC-HPLC. The hydrolysate was dominated by small-molecule oligopeptides and short peptides, with molecular weights concentrated in the 0. 2–1 kDa range and uniform distribution, making it a high-quality raw material for preparing antioxidant peptides. Peptide sequences were analyzed by LC-MS/MS, and eight safe, water-soluble, non-toxic, and non-allergenic candidate antioxidant peptides (PYPR, YPR, DPHG, YGPR, KWY, GRLPGYG, PRY, YPVPR) were identified via bioinformatics prediction and virtual screening, with structures conforming to food-derived bioactive peptide characteristics. Antioxidant prediction and molecular docking confirmed that the peptides possessed excellent free radical scavenging, metal chelating, and strong Keap1 binding capacity, providing a reference for prioritizing in vitro or in vivo verification as high-activity antioxidant peptides. This study established an integrated computer-assisted screening system combining isolation and identification, virtual screening, and molecular docking from protein hydrolysates, enabling efficient and low-cost mining of bioactive peptides from food by-product proteins, and offering a novel technical approach for high-value utilization of Baijiu distiller's grains.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

FUNDING

The authors gratefully acknowledge the financial support from the Graduate Innovation Fund Project of Sichuan University of Science & Engineering (Grant No. D10503356).

AUTHOR CONTRIBUTIONS

Yue Zheng: Writing – original draft, Conceptualization, Methodology, Data curation, Visualization. Liang Dong: Writing –review & editing, Supervision, Funding acquisition, Project administration, Conceptualization. Wenjia Chen: Validation, Methodology, Conceptualization. Junxian Li: Validation, Methodology, Conceptualization. Rui Liu: Validation, Methodology, Conceptualization.

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