Article

Not peer-reviewed version

Preparation and Characterization of Antarctic Krill Peptides with Multiple Bioactivities

Rui Liu , Wen-Xiao Zhao , Wen-Jie Cao , Jian Li , Xiao-Jie Yuan , Yu-Zhong Zhang , Qi-Long Qin , Xiao-Yan Song , Xi-Ying Zhang , Hai-Lun He , Xiu-Lan Chen *, Zheng Luo *

Posted Date: 22 September 2025

doi: 10.20944/preprints202509.1812.v1

Keywords: Antarctic krill bioactive peptides; antioxidant capacity; ACE-inhibitory (ACEI) activity; antimicrobial activity; α-glucosidase inhibition; DPP-IV (DPP4) inhibition

 doi:10.20944/preprints202509.1812.v1

Article

Preparation and Characterization of Antarctic Krill Peptides with Multiple Bioactivities

Rui Liu [1], Wen-Xiao Zhao [1], Wen-Jie Cao [1], Jian Li [1], Xiao-Jie Yuan [1], Yu-Zhong Zhang [1,2,3], Qi-Long Qin [1], Xiao-Yan Song [1], Xi-Ying Zhang [1], Hai-Lun He [4], Xiu-Lan Chen [1,3,*] and Zheng Luo [5,*]

[1] State Key Laboratory of Microbial Technology, Marine Biotechnology Research Center, Shandong University, Qingdao 266237, China;
[2] Frontiers Science Center for Deep Ocean Multispheres and Earth System & College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China;
[3] Joint Research Center for Marine Microbial Science and Technology, Shandong University and Ocean University of China, Qingdao 266237, China;
[4] School of Life Sciences, Central South University, Changsha 410013, China;
[5] Department of Geriatric Medicine, Qilu Hospital, Shandong University, Jinan 250012, China.
* Correspondence: cxl0423@sdu.edu.cn; lizzyrichie@163.com

Abstract

Bioactive peptides have been widely used in food, medicine, healthcare products and other fields due to their diverse bioactivities. **Antarctic krill** (*Euphausia superba*) is a huge Antarctic biological resource with high nutritional value, which is **now commonly** used for krill oil production. **However,** its potential as a high-quality protein source remains **underexploited.** This study established a technological process for preparing AKPs based on the optimization of hydrolysis parameters and determined their physicochemical properties and biological activities. Additionally, multiple bioactive peptides in AKPs were identified. The prepared AKPs are rich in low-molecular-weight **peptides**, and contain 20 amino acids including 9 human essentials. The AKPs have high **antioxidant activity, with scavenging rates** for free radicals $ABTS^+$, DPPH· and ·OH reaching **93.33% (at** 10 mg/mL**), 99.58% (at** 30 mg/mL**) and 92.33% (at** 40 mg/mL**),** respectively. The AKPs exhibited significant inhibition of angiotensin-converting enzyme (ACE), evidenced by an IC_{50} value of 0.27 mg/mL, suggesting antihypertensive potential. Furthermore, at a concentration of 40 mg/mL, the AKPs also suppressed the activities of α-glucosidase and dipeptidyl peptidase IV (DPP-IV) by 12.15% and 16.89%, respectively, demonstrating hypoglycemic effects. The antibacterial activity of the AKPs against *Escherichia coli* was also observed. Further analysis revealed that the AKPs contain a spectrum of bioactive peptides, comprising 39 distinct sequences with functions in antioxidant, antihypertensive, and hypoglycemic activities. These included 11 antioxidants, 19 ACE inhibitors, 2 α-glucosidase inhibitors, and 7 DPP-IV inhibitors. **The** prepared AKPs **have** multiple bioactivities, and may have wide applications. This research presents a viable strategy for the valorization of defatted Antarctic krill powder, enhancing its commercial value and paving the way for high-value applications.

Keywords: Antarctic krill bioactive peptides; antioxidant capacity; ACE-inhibitory (ACEI) activity; antimicrobial activity; α-glucosidase inhibition; DPP-IV (DPP4) inhibition

Introduction

Bioactive peptides are short polymer chains of amino acids that can exert a positive physiological effect on human health upon ingestion. They are small molecules comprising between 2 and 20 amino acid residues linked by covalent peptide bonds [1]. In recent years, with the development of biotechnology and food science, bioactive peptides have been successfully incorporated into various products,ranging from foodstuffs [1,2], and medicines [2,3], to health supplements [1,4], and other

 doi:10.20944/preprints202509.1812.v1

fields. Active peptides exhibit various biological functions, such as antioxidation [5–7], anti-inflammation [8,9], anti-bacteria [10–12], immune regulation [13–15], lowering blood pressure [16,17], and lowering uric acid [18], thereby emerging as a focus of research and application. Bioactive peptides originate from diverse sources, and animal [19,20] and plant proteins [21,22] have become significant raw materials for the preparation of bioactive peptides due to their advantages of high safety and wide-availability. Bioactive peptides are usually obtained through enzymatic hydrolysis technology, which holds the advantages of fewer by-products, high specificity, and ease of control compared with physical and chemical techniques [23]. The commonly employed enzymes for the preparation of bioactive peptides include proteases from animal, plant and microbial sources, such as pepsin, trypsin, papain, alkaline protease, neutral protease, and others [24–26].

Antarctic krill (*Euphausia superba*) is a ubiquitous zooplankton species that forms massive swarms in the frigid marine ecosystems of the Antarctic region, making it a keystone species in this environment. Antarctic krill is rich in high-quality protein, lipids, chitin and other components, of which protein and lipids account for 50%-60% and 10%-15% of its dry weight, respectively. Antarctic krill is a huge reserve of Antarctic biological resource, with current approximations suggesting a standing stock of approximately 379 million tons [27]. This immense population establishes it as the most abundant source of world in animal protein. As a biological resource that is not fully utilized, Antarctic krill has attracted significant attention, and its outstanding nutritional value is favorable. Antarctic krill is now mainly utilized in the production of krill oil, which possesses high nutritional value due to its rich active ingredients, such as Omega-3 fatty acids [28], astaxanthin [28,29], eicosa pentaenoic acid (EPA) [28–30], and docosahexaenoic acid (DHA) [28–30]. Defatted Antarctic krill powder (DAKP) is a by-product of krill oil production, which, however, has high protein content. Moreover, Antarctic krill protein contains all essential amino acids, and its amino acid composition is highly consistent with human requirements [31,32]. Therefore, using DAKP as the raw material to prepare bioactive peptides holds great potential. So far, there have been only a few reports on Antarctic krill peptides (AKPs). Two reports showed that the AKPs prepared by alkaline protease or by Alcalase and flavorzyme had antioxidant activity [33,34]. Zhao et al. reported that the AKPs prepared by Protame had antimicrobial activity [35]. Zheng et al. reported that the AKPs prepared by neutral protease had α-glucosidase-inhibitory [36]. Zhao et al. reported that the AKPs prepared by trypsin had angiotensin-converting enzyme (ACE)-inhibitory activity [37]. Wei et al. prepared AKPs by papain, Corolase PP, flavourzyme, and Alcalase, and observed that the AKPs all showed inhibitory effect on both dipeptidyl peptidase IV (DPP-IV) activity and ACE activity [38]. However, the synthesis of Antarctic krill peptides (AKPs) exhibiting multifunctional bioactivities remains unreported in scientific literature.

This work was designed to prepare AKPs with multiple bioactivities from DAKP. Using Alcalase as the catalytic agent, optimal conditions for three key hydrolysis parameters—hydrolysis temperature, enzyme-to-substrate (E/S) ratio, and duration—were systematically determined, and a technological process of preparing AKPs by hydrolysis of DAKP with Alcalase was developed. Characterization of the AKPs revealed a high content of low-molecular-weight peptides, a complete amino acid profile, and a broad spectrum of bioactivities: antioxidant, ACE-inhibitory, α-glucosidase-inhibitory, DPP-IV-inhibitory, and antibacterial. The findings reveal that the AKPs prepared from DAKP have multiple bioactivities and good nutritional function.

Materials and Methods

Experimental Materials

The defatted Antarctic krill powder (DAKP) used in this study was generously supplied by the Yellow Sea Fisheries Research Institute under the Chinese Academy of Fishery Sciences. Alcalase was purchased from Novozymes (Tianjin, China). All chemical reagents used were of analytical grade. The following materials were procured from commercial suppliers: Aprotinin, cytochrome C, salicylic acid, pyrogallol, 2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS), potassium

 doi:10.20944/preprints202509.1812.v1

persulfate, L-reduced glutathione, ACE, hippuric acid, Hip-His-Leu (HHL), Starch, DPP-Ⅳ and Gly-Pro-para-nitroaniline hydrochloride (Gly-Pro-PNA) from Sigma-Aldrich(St Louis, MO, USA); α-amylase, α-glucosidase and 4-nitrophenyl-α-D-glucopyranoside (PNPG) from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China); bacitracin and H_2O_2 from Aladdin (Shanghai, China); synthetic peptides GGYR and GGG from Shanghai Qiangyao Biological Technology Co., Ltd. (Shanghai, China); ascorbic acid (Vc) from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); hyaluronic acid (HA) from Shandong Freda Bioeng Co., Ltd. (Jinan, China); and DPPH• from Tokyo Chemical Industry (Tokyo, Japan).

Determination of Optimal Hydrolysis Conditions

The optimal conditions for preparing AKPs from DAKP using Alcalase, including hydrolysis temperature, enzyme-to-substrate (E/S) ratio, and duration, were established through single-factor experimentation. Alcalase activity was quantified using the Folin-Ciocalteu assay according to established methodology as previously reported [39]. Enzyme activity units (U) were expressed as the quantity of enzyme necessary to liberate 1 μg of tyrosine per minute from casein under conditions of 55°C and pH 9.0.

To determine the optimal hydrolysis temperature, DAKP was subjected to enzymatic hydrolysis at an E/S ratio of 5000 U/g for a duration of 6 h across a range of temperatures (35, 45, 55, 65, and 75°C). To determine the optimal E/S ratio, DAKP was subjected to enzymatic hydrolysis at 55°C for a duration of 6 h using varying E/S ratio ranging from 500 to 6000 U/g (specifically: 500, 1000, 2000, 3000, 4000, 5000, and 6000 U/g). To determine the optimal duration, DAKP was subjected to enzymatic hydrolysis at an E/S ratio of 5000 U/g and a temperature of 55°C, with the reaction duration varying from 1 to 6 h. All reactions were carried out at pH 9.0 with 10 M NaOH solution. Following hydrolysis, the reaction was terminated by heating the mixtures at 100°C for 15 minutes. The samples were subsequently centrifuged at 16,970 × g for 30 minutes under 4°C conditions. Following centrifugation, both the supernatant and precipitate were lyophilized and weighed. The lyophilized supernatant, constituting the AKPs, was reconstituted in ddH_2O to a concentration of 5 mg/mL, and this solution was subsequently analyzed for peptide molecular weight distribution using high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) equipped with a TSK gel G2000 SWXL column (7.8 × 300 mm; Tosoh, Tokyo, Japan) in accordance with a previously established methodology [39]. Meanwhile, the mass of the lyophilized precipitate was utilized to determine the hydrolysate yield by applying the formula: Hydrolysate yield (%) = $(W_a - Wb) / W_a \times 100$, where W_a corresponds to the initial mass of DAKP before hydrolysis, and Wb represents the mass of the freeze-dried precipitate after hydrolysis.

Preparation of AKPs

A mixture containing 150 g DAKP and Alcalase (E/S ratio of 5000 U/g) in 1 L ddH_2O was constantly stirred (180 rpm) at 55℃ for 6 h. During the enzymatic hydrolysis process, the pH of the reaction mixture was maintained at 9.0 through the continuous addition of 10 M NaOH. After hydrolysis, the AKPs were obtained from the reaction mixture by a series of purification steps, including activated carbon treatment, centrifugation, filtration and centrifugal spray drying according to the method previously described [40].

Characterization of the Prepared AKPs

To determine its water solubility, the AKPs powder was reconstituted in ddH_2O to generate solutions at concentrations of 10% (w/v), 20% (w/v), and 30% (w/v). The molecular weight distribution of peptides in the AKPs sample (5 mg/mL) was characterized using high-performance liquid chromatography (HPLC) as outlined earlier. The free and total amino acid compositions of the prepared AKPs were determined with an automatic amino acid analyzer (HITACHI 835, Tokyo, Japan) in accordance with an established protocol [39].

 doi:10.20944/preprints202509.1812.v1

Quantification of Protein Content.

The protein content in DAKP samples was quantified colorimetrically both prior to and following hydrolysis with Alcalase [40].

Assays of the Bioactivities of the Prepared AKPs

The antioxidant activity of the AKPs was assessed based on their ability to scavenge $ABTS^+$, DPPH· and ·OH. The $ABTS^+$ scavenging activity was determined using the procedure established by by Liu et al [41] and the DPPH· and ·OH scavenging activity was determined using the procedure established by Cheng et al [39]. **The anti-hypertensive activity of the AKPs** was evaluated by measuring its ACE-inhibitory rate, which was conducted following the protocol described by Zhang et al. [42]. The inhibitory activities against α-amylase, α-glucosidase, and DPP-IV were evaluated based on established protocols from Zhou et al. [43], Daou et al. [44], and Liu et al. [45], respectively.

The antibacterial activity of the AKPs was evaluated by measuring its inhibition effects on *Escherichia coli* and *Staphylococcus aureus* using the oxford cup method reported by Zhang et al [46] with some modification. Briefly, the cultured *E. coli* and *S. aureus* cell suspensions were diluted with Lysogeny-Broth (LB) liquid culture medium to an OD_{600} value of 0.01. Then, 1 mL of the solutions were spread on the LB solid medium in plates, and excess solution was removed after 1 min. After the solid culture in the plates dried, three oxford cups were placed in equal distances in the divided areas of each plate. Subsequently, 100 μL of each of the following was added to the cups: AKPs solution (prepared in sterile water), kanamycin (1 mg/mL, serving as a positive control), and sterile water (serving as the negative control).The plates were maintained at 37°C under controlled conditions using an incubator, and the inhibition zones were observed and measured after 12 h, 24 h, 36 h and 48 h incubation.

LC-MS/MS Analysis of the Prepared AKPs

The amino acid sequences of peptides in the prepared AKPs were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, which was performed by Beijing Biotech-Pack Scientific Co., Ltd. (Beijing, China). Briefly, the AKPs sample was analyzed using an Easy-nLC 1200 system coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Mass spectrometric data were acquired in data-dependent acquisition mode. Raw files were processed with Byonic software and searched against a species-specific protein database. Only high-confidence peptide-spectrum matches were retained for subsequent protein identification.The identified peptide sequences from the AKPs were screened against specialized bioactive peptide databases to predict potential functions. Sequences were matched against: (1) the AHTPDB (https://webs.iiitd.edu.in/raghava/ahtpdb/, accessed on 23 March 2025) for ACE-inhibitory peptides; (2) the AODB (https://aodb.idruglab.cn/, accessed on 17 March 2025) for antioxidant peptides; (3) the APD (https://aps.unmc.edu, accessed on 13 March 2025) for antimicrobial peptides; and (4) the BIOPEP-UWM database (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep, accessed on 23 March 2025) for peptides inhibiting α-amylase, α-glucosidase, or DPP-IV.

Results and Discussion

Optimization of the Conditions of DAKP Hydrolysis by Alcalase for AKPs Production

According to the product instructions, Alcalase has optimal pH ranging between 7.0 and 10.0, and optimal temperature from 30°C to 65°C. To establish the optimal enzymatic hydrolysis conditions for preparing AKPs from DAKP using Alcalase, three key parameters—hydrolysis temperature, E/S ratio, and duration—were systematically optimized. All hydrolysis reactions were conducted at pH 9.0.When hydrolysis of DAKP was conducted across a temperature range of 35 °C to 55 °C, both the hydrolysate yield and the content of low-molecular-weight peptides (＜1000 Da

 doi:10.20944/preprints202509.1812.v1

and＜500 Da) reached their maximum values at 55 °C. When DAKP was hydrolyzed at temperatures from 35℃ to 55℃, both the hydrolysate yield and the proportion of low-molecular-weight peptides (＜1000 Da and ＜500 Da) reached their maximum values at 55 °C (Figure 1a, Table 1), indicating that 55℃ is the optimal temperature for the hydrolysis of DAKP by Alcalase. When DAKP was hydrolyzed by Alcalase at different E/S ratios, the hydrolysate yield increased quickly from 500 U/g to 2000 U/g, and then slowly from 2000 U/g to 6000 U/g (Figure 1b). The highest proportion of low-molecular-weight peptides (＜1000 Da and ＜500 Da) in the hydrolysate was observed when the E/S ratio was 5000 U/g (Table 2). At 55°C and pH 9.0, with the E/S ratio of 5000 U/g, the hydrolysate yield and the proportion of low-molecular-weight peptides (＜1000 Da and ＜500 Da) in the hydrolysate rose steadily with duration from 1 h to 6 h (Figure 1c and Table 3). Based on these experimental results and cost-effective consideration, the optimal conditions for DAKP hydrolysis by Alcalase to prepare AKPs were determined to be 55℃, 5000 U/g, and 6 h.

Alcalase has been reported to be used to prepare AKPs. Zhang et al. prepared AKPs with hydrolysis temperature, pH, E/S ratio and duration of 50℃, 8.5, 2% (w/w) and 6 h [34], Ji et al. prepared AKPs by the conditions 50℃, pH 7.0, 500 (U/g, E/S) and 4 h [38], and Zheng et al. prepared AKPs by the conditions 50℃, pH 10, 4000 (U/g, E/S) and 4 h [36]. In addition, as a commercial enzyme, Alcalase is also widely used in the hydrolysis of various animal and plant proteins to prepare bioactive peptides, including proteins from chicken (55℃, 8.0, 0.5% (w/w, protein basis) and 8 h) [47], *Erythrina edulis* (50℃, 8.3, 1:200 (v/v), and 2 h) [48], rice bran (55℃, 8.0, 20 μL /g, and 50 min) [49], seahorse (60℃, 9.0, 1:100 (w/w), and 6 h) [50], bovine α-lactalbumin (55℃, 8.5, 1:20 (v/v), and 4 h) [51] and others. Thus, when Alcalase was used, the hydrolysis parameters differ for different proteins, probably due to the discrepancy in protein sequences and structures.

Table 1. Molecular weight distribution of Alcalase-hydrolyzed AKPs at different temperatures (6 h, E/S ratio: 5000 U/g, pH 9.0) .

Molecular Weight Range (Da)	Content (%)			
	35℃	45℃	55℃	65℃
＞10000	0.04	0.02	0.01	0.01
5000-10000	0.09	0.12	0.04	0.08
3000-5000	1.17	0.79	0.58	0.65
1000-3000	19.36	17.10	14.9	15.64
500-1000	31.03	30.79	30.63	30.81
＜1000	79.34	81.97	**84.47**	83.62
＜500	48.31	51.18	**53.84**	52.81

Table 2. Molecular weight distribution of Alcalase-hydrolyzed AKPs under different E/S ratios (55°C, pH 9.0, 6 h).

Molecular Weight Range (Da)	Content (%)						
	500 U/g	1000 U/g	2000 U/g	3000 U/g	4000 U/g	5000 U/g	6000 U/g
＞10000	0.04	0.03	0.04	0.01	0.01	0.00	0.00
5000-10000	0.73	0.71	0.34	0.36	0.28	0.10	0.11
3000-5000	2.68	2.54	1.59	1.60	0.28	0.48	0.93
1000-3000	21.84	20.00	16.53	17.24	14.84	12.73	12.89
500-1000	27.37	27.34	26.91	25.07	25.96	27.70	28.90
＜1000	74.71	76.72	81.50	80.79	84.59	**86.69**	86.07
＜500	47.34	49.38	54.59	55.72	58.63	**58.99**	57.17

Table 3. Molecular weight distribution of Alcalase-hydrolyzed AKPs across different duration (E/S ratio: 5000 U/g, 55°C, pH 9.0).

	Content (%)

 doi:10.20944/preprints202509.1812.v1

Molecular Weight Range (Da)	1 h	2 h	3 h	4 h	5 h	6 h
＞10000	0.04	0.04	0.01	0.01	0.01	0.01
5000-10000	0.98	0.40	0.50	0.29	0.27	0.24
3000-5000	2.78	1.67	1.80	1.39	1.28	1.17
1000-3000	23.23	20.51	19.88	18.75	17.97	17.41
500-1000	30.48	25.48	25.04	24.22	25.22	26.16
＜1000	72.97	77.38	77.81	79.56	80.47	**81.17**
＜500	42.49	51.90	52.77	55.34	55.25	**55.01**

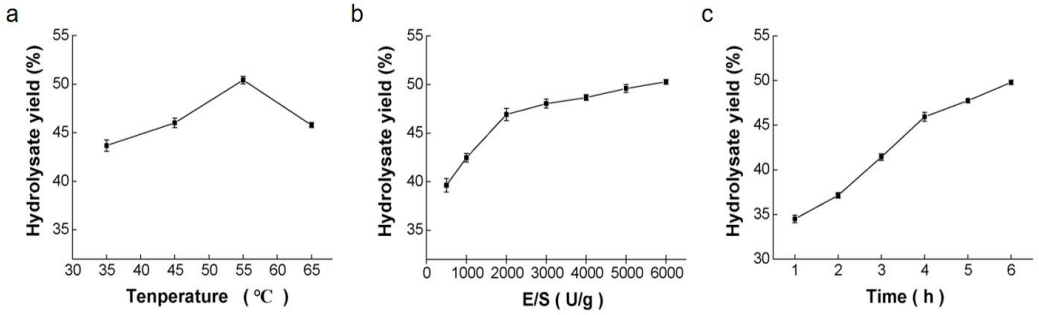

Figure 1. Parameter optimization for the hydrolysis of DAKP by Alcalase. (a) The influence of hydrolysis temperature on yield was investigated. Hydrolysis was carried out at an E/S ratio of 5000 U/g and pH 9.0 for 6 h. (b) The influence of the E/S ratio on yield was investigated. Hydrolysis was performed at 55°C and pH 9.0 for 6 h. (c) The influence of duration on yield was investigated. Hydrolysis was conducted at an E/S ratio of 5000 U/g, 55°C, and pH 9.0. All data represent the mean ± SD from three independent experiments.

Preparation and Characterization of AKPs

Based on the optimized hydrolysis conditions established above, a laboratory-scale process was developed for the preparation of AKPs using Alcalase (Figure 2). With this process, the hydrolysis efficiency was 49.22 ± 0.56% based on determination of the weight of the DAKP substrate before and after enzymatic hydrolysis, and the hydrolysis efficiency was 65.40 ± 0.04% based on determination of the protein content of the DAKP substrate before and after enzymatic hydrolysis. The AKPs produced via this method obtained as a milky white powdered form (Figure 3), which was completely dissolved in water up to 30% concentration (w/v) (Figure 4). As shown in Figure 5 and Table 4, peptides with a molecular mass under 500 Da constituted 52.73% of the AKPs, while those under 1000 Da accounted for 83.90%. This indicates that the AKPs are predominantly composed of low-molecular-weight peptides containing fewer than 10 amino acid residues. Given that bioactive peptides generally consist of 2-20 amino acid residues [1], these results suggest that the AKPs may harbor a diverse range of bioactive peptides with potential multiple biological functions.

 doi:10.20944/preprints202509.1812.v1

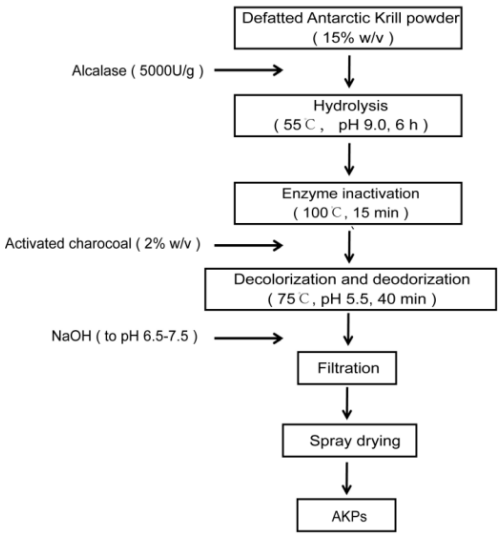

Figure 2. A flow chart of the preparation of AKPs from DAKP hydrolysis with Alcalase.

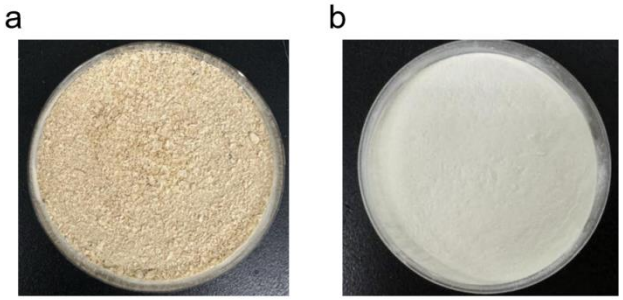

Figure 3. DAKP (a) and the prepared AKPs powder (b).

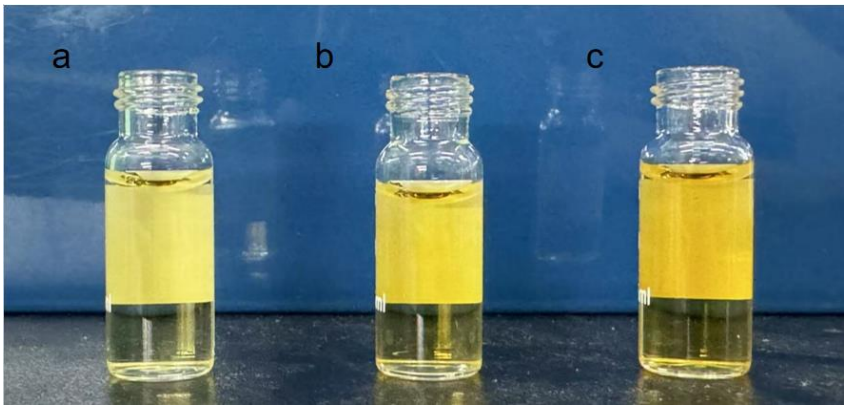

Figure 4. Solubility of the prepared AKPs powder in aqueous solution at different concentrations: (a) 10% (w/v), (b) 20% (w/v), and (c) 30% (w/v).

 doi:10.20944/preprints202509.1812.v1

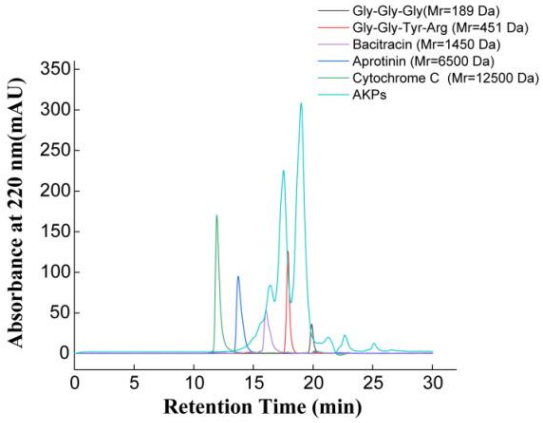

Figure 5. HPLC gel filtration chromatogram showing the molecular weight distribution of the prepared AKPs.

Table 4. Distribution of molecular weight ranges in the prepared AKPs, as classified by HPLC.

Molecular Weight Range (Da)	Concentration (%)
＞10000	0
5000-10000	0.07
3000-5000	0.70
1000-3000	15.33
500-1000	31.17
＜1000	83.90
＜500	52.73

The composition of free and total amino acids of the AKPs was detected to evaluate its nutritional value. The prepared AKPs exhibited free amino acid and total amino acid contents of 3.998% and 65.842%, respectively, respectively (Table 5). Acid hydrolysis revealed 17 detectable amino acids. Given the conversion of Asn and Gln to Asp and Glu, along with the degradation of Trp during the acid hydrolysis process,it is reasonable to conclude that the original composition comprised all 20 standard amino acids. Among the total amino acids, acidic amino acids (Glu + Gln and Asp + Asn) were predominant, with combined contents of 9.76 ± 0.60% and 6.86 ± 0.33%, respectively. The contents of Pro and Lys were also elevated, measuring 7.96 ± 0.46% and 6.86 ± 0.33%, respectively. Furthermore, 8 essential amino acids for humans were detected in the AKPs, constituting 22.74 ± 0.27% of the total composition. Although tryptophan (Trp) was degraded during acid hydrolysis and therefore not quantitatively detected, the AKPs can be considered to contain all nine essential amino acids required by humans. These results affirm the high nutritional quality of the AKPs.

Table 5. Amino acid profile of Alcalase-hydrolyzed AKPs[a].

Amino Acids Types	Free Amino Acids Content (g/100 g)	Total Amino Acids Content (g/100 g)
Asp[b]	0.019 ± 0.001	6.856 ± 0.315
Thr	**0.036 ± 0.001**	**2.744 ± 0.125**
Ser	0.084 ± 0.002	2.472 ± 0.167
Glu[b]	0.198 ± 0.006	9.758 ± 0.602
Gly	0.168 ± 0.005	2.743 ± 0.129
Ala	0.231 ± 0.006	3.675 ± 0.177
Cys	0.185 ± 0.003	1.815 ± 0.182
Val	**0.201 ± 0.006**	**3.334 ± 0.137**
Met	**0.141 ± 0.003**	**1.335 ± 0.238**

 doi:10.20944/preprints202509.1812.v1

Ile	**0.000**	**2.442 ± 0.115**
Leu	**0.045 ± 0.002**	**4.189 ± 0.204**
Tyr	0.509 ± 0.017	2.435 ± 0.123
Phe	**1.361 ± 0.026**	**3.56 ± 0.287**
Lys	**0.297 ± 0.009**	**5.341 ± 0.202**
His	0.000	1.396 ± 0.039
Arg	0.434 ± 0.011	3.785 ± 0.176
Pro	0.089 ± 0.004	7.964 ± 0.457
Trp[c]	-	-
Total	3.998 ± 0.006	65.842 ± 0.216

[a]Essential amino acids for humans are indicated in bold. All data presented in the table represent the mean ± standard deviation (SD) derived from triplicate experiments. [b]The measured values of Asp and Glu incorporate potential contributions from Asn and Gln, as these residues were converted to Asp and Glu, respectively, during acid hydrolysis. [c]Trp could not be detected due to its degradation under acid hydrolysis conditions.

Analysis of Antioxidant Activity of Prepared AKPs

The in vitro antioxidant activity of the prepared AKPs was assessed through their $ABTS^{+}$, DPPH· and ·OH scavenging abilities. The scavenging ratio of $ABTS^{+}$ by the AKPs was measured with Vc, HA, and L-reducing glutathione as three positive controls, while those of DPPH· and ·OH by the AKPs was measured with Vc and HA as two positive controls. As shown in Figure 6, the AKPs exhibited remarkable scavenging ability to all the tested free radicals. Across the investigated concentration range of AKPs, the radical scavenging rates for all targeted free radicals showed a concentration-dependent increase, indicating a clear dose-response relationship in the antioxidant activity of AKPs. At a concentration of 10.00 mg/mL, the $ABTS^{+}$ scavenging ratio of AKPs reached a maximum of 93.33 ± 0.09%, which was comparable to that of Vc and L-reduced glutathione, and significantly exceeded that of HA (Figure 6a). When the concentration was increased to 30.00 mg/mL, the ·OH scavenging ratio of AKPs attained 99.58 ± 0.12%, a level nearly identical to Vc and markedly superior to HA (Figure 6b). Similarly, the DPPH· radical scavenging ratio of AKPs achieved 92.33 ± 1.25% at 40.00 mg/mL, essentially matching the performance of Vc and considerably higher than that of HA (Figure 6c).

The EC_{50} values for the radical-scavenging activity of AKPs were determined to be 0.84 ± 0.03 mg/mL ($ABTS^{+}$), 4.69 ± 0.18 mg/mL (·OH), and 7.96 ± 0.54 mg/mL (DPPH·). These findings indicate that AKPs exhibit potent antioxidant properties, with particularly notable efficacy in scavenging $ABTS^{+}$ radicals.

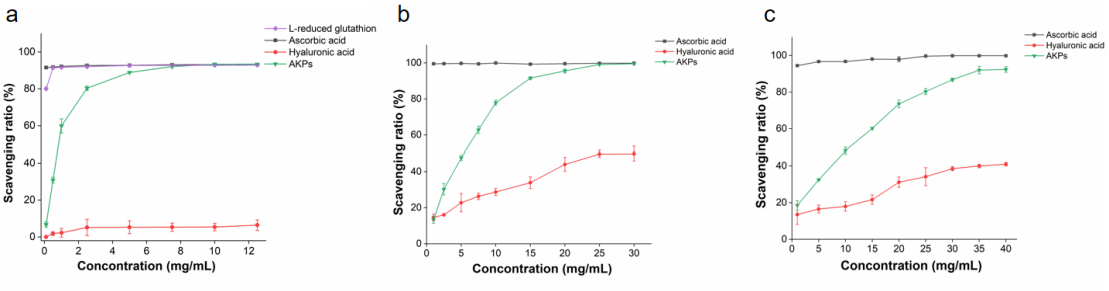

Figure 6. Antioxidant activity of the prepared AKPs. (a) $ABTS^{+}$ scavenging activity. (b) ·OH scavenging activity. (c) DPPH• scavenging activity. All data presented in the graphs represent the mean ± standard deviation (SD) derived from triplicate experiments.

Free radicals are produced along with various physiological processes and have important physiological functions [52,53]. However, if free radicals are excessive or not eliminated in time, they will cause oxidative stress in the body and then lead to a variety of diseases [54–56]. Therefore,

 doi:10.20944/preprints202509.1812.v1

antioxidants with radical-scavenging ability are becoming popular. There have been two reports on the antioxidant activity of AKPs prepared by different enzymes. According to Zhang et al., AKPs prepared by Alcalase hydrolysis exhibited ·OH and DPPH· scavenging ratio of 65.99 ± 1.22% and 55.32 ± 1.08%, respectively, at a concentration of 5 mg/mL. These values were significantly superior to those of peptides hydrolyzed using trypsin, neutrase, pepsin, or papain [34]. In another study, Lan et al. demonstrated that oligopeptides with a high Fischer ratio, prepared through sequential hydrolysis of Antarctic krill powder with Alcalase and Flavorzyme, also displayed radical scavenging activity. The reported EC_{50} values were 0.91 mg/mL ($ABTS^+$), 0.83 mg/mL ($O_2^{-}\cdot$), 4.90 mg/mL (DPPH·), and 4.62 mg/mL (·OH) [33]. The AKPs prepared in this study also had free radical scavenging activity, with EC50 values of 0.84 ± 0.03 mg/mL ($ABTS^+$), 4.70 ± 0.18 mg/mL (·OH), and 9.01 ± 0.54 mg/mL (DPPH·), which are comparable to the above data reported by Lan et al. [33].

Analysis of ACE-Inhibitory Activity of Prepared AKPs

To determine the ACE-inhibitory activity of the prepared AKPs, their inhibition rates were determined at varying concentrations. As shown in Figure 7, the ACE-inhibitory rate of the AKPs rose sharply across the concentration range of 0 to 1 mg/mL, and reached 91.85 ± 0.75% at 2.5 mg/mL. The calculated IC_{50} for ACE activity by the AKPs was found to be 0.27 ± 0.03 mg/mL. This notably low IC_{50} value indicates strong ACE-inhibitory activity, suggesting the presence of bioactive peptides with potent antihypertensive potential within the AKP fraction.

Previous studies have confirmed the ACE-inhibitory activity of AKPs, with efficacy varying by enzymatic preparation method. Trypsin-hydrolyzed AKPs showed an inhibition rate of 38.82 ± 0.71% at 1 mg/mL [37], while those produced with Corolase PP reached approximately 48% at 10 mg/mL, demonstrating superior activity compared to variants generated using Alcalase, Flavourzyme, or papain [38]. Additionally, AKPs derived from peeled krill tail meat via Thermoase PC10F hydrolysis exhibited an IC_{50} value of 1.944 mg/mL [57]. Our result in this study showed that the ACE-inhibitory rate of the AKPs prepared with Alcalase reached 91.85 ± 0.75% at 2.5 mg/mL, and its IC50 was 0.27 ± 0.03 mg/mL, much higher than those of the reported AKPs, indicating that it may have good antihypertensive potential.

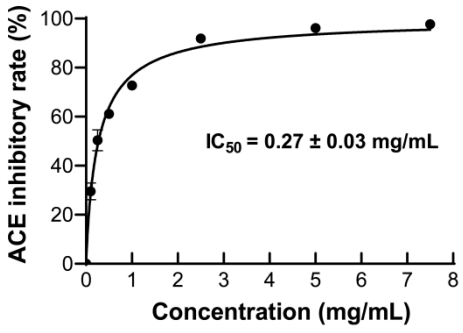

Figure 7. The ACE-inhibitory activity of AKPs at varying concentrations. All data presented in the graphs represent the mean ± standard deviation (SD) derived from triplicate experiments.

Analysis of Anti-Diabetic Activity of Prepared AKPs

The mechanisms of action of anti-diabetic peptides include α-amylase inhibition [58], α-glucosidase inhibition [59], DPP-IV inhibition [60], and incretin inhibition [61]. Research has indicated that α-glucosidase, α-amylase, and DPP-IV represent significant therapeutic targets for type 2 diabetes management [62]. To evaluate the anti-diabetic potential of the prepared AKPs, their inhibitory effects on α-amylase, α-glucosidase, and DPP-IV activities were assessed. At a

 doi:10.20944/preprints202509.1812.v1

concentration of 40 mg/mL, the AKPs exhibited inhibitory rates of 12.15% against α-glucosidase and 16.89% against DPP-IV, while no significant inhibition of α-amylase activity was observed.

AKPs have demonstrated α-glucosidase-inhibitory and DPP-Ⅳ-inhibitory activities in previous studies, while their potential α-amylase-inhibitory activity remains unreported. According to Zheng et al., AKPs prepared using neutral protease exhibited an α-glucosidase inhibition rate of 43.82%, outperforming those prepared with trypsin, Protamex, Alcalase,papain, or Flavoenzyme [36]. Ji et al. demonstrated that AKPs prepared with an animal-derived proteolytic enzyme displayed DPP-IV-inhibitory activity, achieving an IC50 value of 1.6272 mg/mL [63]. In another study, Lang et al. found that AKPs prepared with a composite protease (containing endonuclease, exonuclease, and flavour protease) reached a DPP-IV-inhibitory rate of 66.81 ± 2.50% at 100 mg/mL, significantly higher than variants obtained using neutral protease, alkaline protease, flavour protease, or animal hydrolase [64]. Ji et al. demonstrated that AKPs prepared using Corolase PP, Alcalase, Flavourzyme, and papain all exhibited DPP-Ⅳ-inhibitory activity. Among these, the Corolase PP-derived AKPs displayed the strongest inhibition, reaching approximately 40% at a concentration of 10 mg/mL and that flavourzyme-derived AKPs showed the lowest inhibition rate, approximately 18% at 10 mg/mL [38]. In addition, there have been many reports on enzymatic hydrolysis of various foodborne proteins with different enzymes to produce hydrolysates with anti-diabetic activity. For instances, Chen et al. prepared Russian sea cucumber body wall protein hydrolysate with papain, and its IC50 value against α-amylase was 1.10 ± 0.04 mg/mL [65]. Admassu et al. prepared red seaweed hydrolysate by pepsin, and its inhibitory rate against α-amylase was 50.34% at 1.86 mg/mL [66]. Fadimu et al. prepared Lupin protein hydrolysate by Alcalase that demonstrated potent inhibitory activity against α-amylase and α-glucosidase, with IC_{50} values of 1.66 mg/mL and 1.65 mg/mL, respectively [67]. Carrera-Alvarado et al. prepared chicken blood hydrolysate by mixed enzyme (2% Alcalase and 5% Protana Prime), and its DPP-Ⅳ inhibitory rate was 60.55% at 10 mg/mL [68]. By comparison, the α-glucosidase and DPP-IV inhibitory activities observed in the Alcalase-derived AKPs in this study were lower than those cited in previous reports. This reduced efficacy may result from the limited quantity and/or relative scarcity of bioactive peptides targeting these enzymes within the peptide mixture.

Analysis of Antibacterial Activity of Prepared AKPs

The antibacterial activity of the AKPs was evaluated against the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *S. aureus* by measuring the inhibition zones, with kanamycin and ddH_2O as positive and negative controls, respectively. On the *E. coli* culture plate, while kanamycin (1 mg/mL) showed an inhibitory zone of similar size 21.8 ± 2.0 mm from 12 h to 48 h incubation, the AKPs (40 mg/mL) formed an inhibitory zone with its size being increased from 17.9 ± 0.9 mm to 28.6 ± 0.3 mm with the incubation time from 12 h to 48 h, which was bigger than that of kanamycin after 36 h incubation (Figure 8a). In contrast, on the *S. aureus* culture plate, while kanamycin (1 mg/mL) showed an inhibitory zone of similar size 18.4 ± 3.0 from 12 h to 48 h incubation, the AKPs (40 mg/mL) showed no inhibitory zone (Figure 8b). These results demonstrate that the AKPs exhibit significant antibacterial activity against *E. coli*, but show no detectable efficacy against *S. aureus*.

There have been many reports on the preparation of antimicrobial hydrolysates by hydrolysis of different protein sources with various proteases, and the hydrolysates have antimicrobial activity against many bacteria, including both Gram-positive bacteria—such as *Staphylococcus aureus*, *Micrococcus luteus*, and *Enterococcus faecalis*—and Gram-negative species, namely *Escherichia coli*, *Pseudomonas aeruginosa, Vibrio parahaemolyticus*, *Klebsiella pneumoniae*, and *Salmonella enterica* [69–71]. However, there has been only one report on the antimicrobial activity of AKPs. Zhao et al. prepared AKPs by using protamex, and purified an antimicrobial polypeptide fraction with molecular weight ranging from 245-709 D by cation exchange chromatography. The minimum inhibition concentration (MIC) of this polypeptide fraction against *S. aureus* was 5.0 mg/mL [35]. In contrast, in this study, the prepared AKPs with Alcalase showed antimicrobial activity against *E. coli*, but no against *S. aureus.* The antimicrobial activity of peptides is influenced by their amino acid composition, sequence

 doi:10.20944/preprints202509.1812.v1

arrangement, and three-dimensional structural conformation [72]. The hydrolysis of Antarctic krill by protamex and Alcalase may generate antimicrobial peptides with different amino acid sequences and spatial conformations, leading to their different antibacterial behaviors.

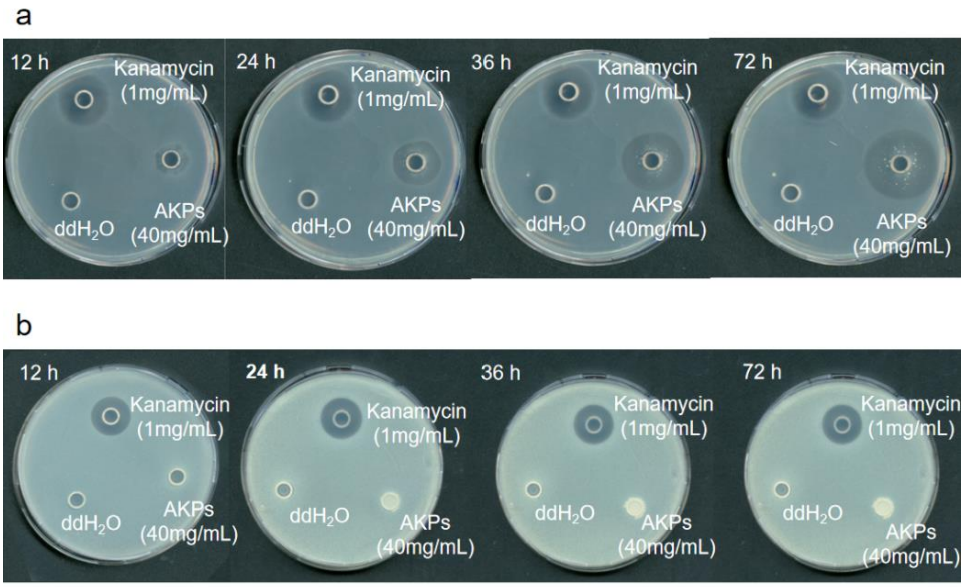

Figure 8. Antibacterial activity of AKPs against *E. coli* (a) and *S. aureus* (b). The plates shown are representative of three independent experiments.

Identification of Peptides with Bioactivity from the Prepared AKPs

The peptide sequences within the prepared AKPs were identified using LC-MS/MS to determine those with potential bioactivity. Consequently, 5089 distinct peptide sequences were identified within the AKPs. To identify potential ACE-inhibitory peptides, a search was conducted against the AHTPDB database. This screening revealed 19 such bioactive peptides within the AKPs, including 5 dipeptides, 6 tripeptides, 4 tetrapeptides, 2 pentapeptides，and 2 hexapeptide (Table 7). To identify potential antioxidant peptides, a search was conducted against the AODB database.This screening revealed 11 such bioactive peptides within the AKPs, including 11 antioxidant peptides were identified, including 1 dipeptide, 6 tripeptides, 1 tetrapeptide, 1 heptapeptide, and 1 octapeptide (Table 8). To identify potential α-amylase-inhibitory peptides, α-glucosidase-inhibitory peptides, or DPP-Ⅳ-inhibitory peptides, a search was conducted against the BIOPEP-UWM. This screening revealed 2 α-glucosidase-inhibitory peptides and 7 DPP-Ⅳ-inhibitory peptides within the AKPs, but no α-amylase-inhibitory peptides were identified. The 2 α-glucosidase-inhibitory peptides are 1 tetrapeptide and 1 heptapeptide (Table 9), and the 7 DPP-Ⅳ-inhibitory peptides include 6 dipeptides and 1 tripeptide (Table 10). To identify potential antimicrobial peptides, a search was conducted against the APD database and no antimicrobial peptides were detected within the AKPs. These results indicate that the multiple bioactivities observed are attributable to the diverse repertoire of bioactive peptides within the prepared AKPs.

A search of the AODB database confirmed that no antioxidant peptides have been previously documented as originating from Antarctic krill. Consequently, the 14 antioxidant peptides we identified in the AKPs represent sequences novel to this source, though they are known to be derived from other proteins. A search of the AHTPDB database confirmed that only 4 ACE-inhibitory peptides have been previously documented as originating from Antarctic krill, including VW [57,91], LKY [57,91], ITRY [91], and VFER [91]. Consequently, the 24 ACE-inhibitory peptides we identified in the AKPs represent sequences novel to this source, distinguishing them from all previously reported krill-derived ACE-inhibitory peptides. A search of the BIOPEP-UWM database did not

 doi:10.20944/preprints202509.1812.v1

detect any α-amylase-inhibitory peptides within the prepared AKPs, a finding consistent with the experimental results and indicative of their general absence in this preparation. Although the prepared AKPs exhibited low levels of α-glucosidase-inhibitory and DPP-IV-inhibitory activity in vitro, 2 α-glucosidase-inhibitory peptides and 7 DPP-IV-inhibitory peptides were nevertheless identified. The limited bioactivity observed is likely due to the low abundance of these peptides in the AKP mixture. A search of the APD revealed no previously documented antimicrobial peptides originating from Antarctic krill. Correspondingly, no such peptides were identified within the prepared AKPs through our bioinformatic screening. Despite these findings, the AKP preparation displayed observable in vitro antibacterial activity(Figure 8 and Table 6), suggesting the presence of antimicrobial peptides that could not be identified through database search. Thus, further studies are required to isolate and identify these antimicrobial peptides.

Table 6. Antibacterial activity of AKPs against *E. coli* and *S. aureus*.

strain	sample	Inhibition zone diameter （mm）			
		12 h	**24 h**	**36 h**	**48 h**
E. coli	kanamycin (1 mg/mL)	21.8 ± 2.0	21.8 ± 2.0	21.8 ± 2.0	21.8 ± 2.0
	H_2O	-	-	-	-
	AKPs（40 mg/mL)	17.9 ± 0.9	20.8 ± 0.1	23.6 ± 0.2	28.6 ± 0.3
S. aureus	kanamycin (1 mg/mL)	18.4 ± 3.0	18.4 ± 3.0	18.4 ± 3.0	18.4 ± 3.0
	H_2O	-	-	-	-
	AKPs（40 mg/mL)	-	-	-	-

All data presented in the table represent the mean ± standard deviation (SD) derived from triplicate experiments.

Table 7. Summary ofi dentified peptides with antioxidant activity from the prepared AKPs.

Sequence	Molecular Weight (Da)	Source	Antioxidant Activity	References
LT	232.28	Black-Bone Silky Fowl (*Gallus Gallus Domesticus Brisson*)	The DPPH· scavenging activity of below 20%.	[73]
FSL	365.42	Egg White Protein	ORAC-FL value: <0.022 μmol TE/μmol peptide.	[74]
LAL	315.41	Bovine Whey Protein (β-Lactoglobulin)	A FRAP value of 5.73 ± 0.010 mmol Fe^{3+} mol^{-1} was recorded for the peptide.	[75]
SFN	366.37	Bovine Whey Protein (β-Lactoglobulin)	The peptide showed antioxidant activity of 1.17 ± 0.05 mmol Fe^{3+}.mol^{-1} (FARP)	[75]

 doi:10.20944/preprints202509.1812.v1

WAF	422.48	Palm Kernel Cake Hydrolysates	DPPH· scavenging: 71 ± 2.22% (IC_{50} = 1.360 μM); Metal chelation: 41 ± 1.08% (IC_{50} = 0.002 μM).	[76]
YMY	475.56	Synthesis Peptide	-	[77]
YYG	401.41	Synthesis Peptide	-	[77]
FLPE	504.57	Corn	-	[78]
LKYPI	632.79	Casein-Derived Bioactive Peptides	-	[79]
IIAPPER	794.93	*Gryllodes Sigillatus*	$ABTS^{\cdot+}$ scavenging: EC_{50} = 15.62 ± 0.1 mg/mL;DPPH· scavenging: EC_{50} = 1.01 ± 0.02 mg/mL;Fe^{2+} chelation: EC_{50} = 0.142 ± 0.05 mg/mL;Reducing power: 0.148 ± 0.01 (700 nm);LOX inhibition: IC_{50} = 8.21 ± 0.04 mg/mL;COX inhibition: IC_{50} = 8.16 ± 2.22 mg/mL	[80]
TEAPLNPK	868.97	*Scomberomorus Niphonius*	-	[81]

Table 8. Summary of identified peptides with ACE-inhibitory activity from the prepared AKPs.

Sequence	**Molecular Weight (Da)**	**Source**	**IC_{50} (μmol L^{-1})**	**References**
PT	216.22	Milk hydrolysate	-	[82]
PT	216.24	Pork sarcoplasmic proteins	-	[83]
PT	216.24	Chicken (*Gallus gallus*)	-	-
PT	216.24	Bovine (*Bos taurus*) β-caseins	-	-
PT	216.24	Bovine lactoferrin (*Bos taurus*)	-	[84]
PT	216.24	Cereals storage protein	-	[85]
LV	230.31	-	-	[86]

 doi:10.20944/preprints202509.1812.v1

LL	244.33	-	-	[87]
LL	244.33	-	-	[86]
VF	264.32	Fish (Sardine)	43.7	[88]
VF	264.32	Milk	-	[89]
IF	278.34	-	930	[90]
IF	278.35	-	<20000	[86]
IF	278.35	Soybean Sauce	65.8	[22]
IF	278.35	Soybean (Salt-free soy sauce)	65.8	[91]
IF	278.35	Royal jelly	1.67-930	[91]
IF	278.35	Pork sarcoplasmic proteins	-	[83]
IF	278.35	Chicken (*Gallus gallus*)	-	-
IF	278.35	Bovine β-caseins	-	-
IF	278.35	Cereals (Wheat (Gamma-gliadin from wheat))	-	[84]
IF	278.35	-	-	[87]
IF	278.35	Cereals storage protein	-	[85]
IF	278.35	-	-	[92]
IF	278.35	-	-	[93]
IF	278.35	-	-	[94]
IF	278.35	-	-	[86]
PPL	325.41	Milk	>1000	[95]
LLP	341.00	Amaranth (*Amaranthus hypochondriacus*)	-	[96]
LLP	341.00	Cereals (Maize (*Zea mays*))	-	[97]
LLP	341.00	Cereals (Maize (*Zea mays*))	57	[98]
LLP	341.44	Alpha-zein	57	[97]
LLP	341.45	Cereals (Rye)	57	[99]
LLP	341.45	-	<20000	[86]
LLP	341.45	Cereals storage protein	-	[85]
LLP	341.45	Cereals (Wheat (α/β-wheat gliadin))	-	[84]
LLP	341.45	-	-	[94]

Table 8. (*Continued*).

Sequence	Molecular Weight (Da)	Source	IC_{50} (μmol L^{-1})	References
LLP	341.45	-	57	[100]

 doi:10.20944/preprints202509.1812.v1

FGF	369.42	-	-	[93]
FGF	369.42	-	-	[94]
FGF	369.42	-	-	[86]
TVY	381.00	Milk (bovine casein)	15	[101]
TVY	381.43	-	<20000	[86]
TVY	381.43	Milk derived	15	-
TVY	381.43	Milk casein	15	[102]
TVY	381.43	-	-	[94]
TVY	381.43	-	15	[100]
GVPK	399.49	Milk-Cheese (Goat milk protein and cheeses)	-	[103]
YYG	401.42	-	-	[93]
YYG	401.42	-	-	[94]
YYG	401.42	-	-	[86]
FPF	409.49	-	-	[93]
FPF	409.49	-	-	[94]
FPF	409.49	-	-	[86]
SLPQ	443.50	Milk (Goat milk hydrolysate)	330	[91]
SLPQ	443.50	Milk-Cheese (Goat milk protein and cheeses)	330 μg/ml	[103]
SLPQ	443.50	-	-	[94]
SLPQ	443.50	-	330	[100]
SLPQ	444.00	Milk (goat)	330	[104]
VFPS	448.51	Cereals (Finnish)	0.46	[105]
VFPS	448.52	Synthesized	0.46	[106]
IYLL	520.65	Soybean (Soy bioactive peptides)	42	[107]
IYLL	520.67	Soybean	42	-
IYLL	520.67	Soybean	42	[88]
KPVAL	526.68	Casein	-	[108]
VIPEL	569.69	Pork meat	799.24	[109]
VIPEL	569.70	-	799.24	[110]
VIPEL	569.70	-	46.56 - >1000	[88]
VIPEL	570.00	Pork (*Sus domesticus*)	799.24	[109]

 doi:10.20944/preprints202509.1812.v1

MYP GIA	650.78	-	641.02	[110]
MYP GIA	650.79	-	46.56 - >1000	[88]
MYP GIA	650.79	-	641.02	[100]
MYP GIA	650.79	Pork (*Sus domesticus*)	641.02	[111]
MYP GIA	651.00	Pork meat	641.02	[111]
IVG RPR	696.85	-	302	[110]
IVG RPR	696.85	Fish (Dried bonito hydrolysate)	-	[112]
IVG RPR	696.85	Fish (Dried bonito hydrolysate)	-	[113]

Table 9. Summary of identified peptides with α-glucosidase-inhibitory activity from the prepared AKPs.

Sequence	Molecular Mass (Da)	IC_{50} (μmol L^{-1})
YYPL	554.63	3700
IIAPPER	794.94	28.75

Table 10. Summary of identified peptides with DPP-Ⅳ-inhibitory activity from the prepared AKPs.

Sequence	Molecular Mass (Da)	IC_{50} (μmol L^{-1})
PPL	325.40	390.14
LL	244.33	-
LV	230.30	-
VF	264.32	-
LT	232.28	-
PT	216.23	-
NN	246.22	-

Conclusions

The present investigation involved the enzymatic hydrolysis of defatted Antarctic krill powder (DAKP) using Alcalase to generate Antarctic krill peptides (AKPs) exhibiting a range of biological activities. Based on the optimized hydrolysis parameters, a scalable process was established for the preparation of the AKPs. The AKPs produced via the optimized process exhibited a high proportion (83.9%) of low-molecular-weight peptides (< 1000 Da) and contained all 20 standard amino acids, including 9 essential amino acids required by humans. The prepared AKPs had high antioxidant activity, showing strong scavenging ability on $ABTS^{+}$, ·OH, and DPPH·, with the highest scavenging rate of 93.33 ± 0.09%, 99.58 ± 0.12%, and 92.33 ± 1.25%, respectively. The prepared AKPs demonstrated potent ACE-inhibitory activity, exhibiting an IC_{50} value of 0.27 ± 0.03 mg/mL. The prepared AKPs also had α-glucosidase-inhibitory and DPP-Ⅳ-inhibitory activities, with the inhibitory rates of 12.15% and 16.89%, respectively, when the concentration was 40 mg/mL. In addition, the prepared AKPs showed antibacterial activity against *E. coil*, and the diameter of the inhibition zone reached 21.8 ± 2.0 mm at 48 h. Based on LC-MS/MS and database searching, 39 distinct functional peptides were identified in the prepared AKPs, exhibiting antioxidant, antihypertensive, and hypoglycemic activities. These included 11 antioxidants, 19 ACE inhibitors, 2 α-glucosidase inhibitors, and 7 DPP-IV inhibitors. Notably, neither antimicrobial nor α-amylase-inhibitory peptides were observed.

 doi:10.20944/preprints202509.1812.v1

Nevertheless, it is plausible that additional bioactive peptides with uncharacterized sequences remain undetected in the AKPs, necessitating further isolation, structural characterization, and activity assessment. The findings of this study demonstrate that AKPs prepared from DAKP using Alcalase exhibit favorable nutritional properties and diverse biological activities, suggesting their potential for broad application in functional foods, nutraceuticals, and related products. This work also presents a viable strategy for the high value-added utilization of DAKP.

References

1. Chakrabarti S, Guha S, Majumder K. 2018. Food-Derived Bioactive Peptides in Human Health: Challenges and Opportunities. *Nutrients* 10:1738
2. Sreelekshmi PJ, Devika V, Aiswarya LS, Jeevan SR, Ramanunni K, et al. 2023. Recent Advances in Bioactive Peptides as Functional Food for Health Promotions and Medicinal Applications. *Protein and Peptide Letters* 30:626-39
3. Agyei D, Ahmed I, Akram Z, Iqbal HMN, Danquah MK. 2017. Protein and Peptide Biopharmaceuticals: An Overview. *Protein and Peptide Letters* 24:94-101
4. Acquah C, Agyei D, Obeng EM, Pan S, Tan KX, Danquah MK. 2020. Aptamers: an emerging class of bioaffinity ligands in bioactive peptide applications. *Critical Reviews in Food Science and Nutrition* 60:1195-206
5. Ng W-J, Wong F-C, Abd Manan F, Chow Y-L, Ooi A-L, et al. 2024. Antioxidant Peptides and Protein Hydrolysates from Tilapia: Cellular and In Vivo Evidences for Human Health Benefits. *Foods* 13:2945
6. Zhang Y, Lian H, Yang L, Tong L, Wu Y, et al. 2024. Preparation and characterization of novel antioxidant peptides from protein hydrolysate of Ophiocordyceps gracilis. *Process Biochemistry* 146:571-86
7. Li L, Yang Y, Ma C-m, Wang B, Bian X, et al. 2025. Structure, antioxidant activity, and neuroprotective effect of black soybean (*Glycine* max *(L.) merr*.) protein hydrolysates. *Food Chemistry* 463:141390
8. Abril AG, Pazos M, Villa TG, Calo-Mata P, Barros-Velazquez J, Carrera M. 2022. Proteomics Characterization of Food-Derived Bioactive Peptides with Anti-Allergic and Anti-Inflammatory Properties. *Nutrients* 14:4400
9. Guha S, Majumder K. 2019. Structural-features of food-derived bioactive peptides with anti-inflammatory activity: A brief review. *Journal of Food Biochemistry* 43:e12531
10. Chakka AK, Elias M, Jini R, Sakhare PZ, Bhaskar N. 2015. In-vitro antioxidant and antibacterial properties of fermentatively and enzymatically prepared chicken liver protein hydrolysates. *Journal of Food Science and Technology-Mysore* 52:8059-67
11. Jiang L, Wang B, Li B, Wang C, Luo Y. 2014. Preparation and identification of peptides and their zinc complexes with antimicrobial activities from silver carp (*Hypophthalmichthys molitrix*) protein hydrolysates. *Food Research International* 64:91-98
12. Liu Z, Dong S, Xu J, Zeng M, Song H, Zhao Y. 2008. Production of cysteine-rich antimicrobial peptide by digestion of oyster (*Crassostrea gigas*) with alcalase and bromelin. *Food Control* 19:231-35
13. Li S, An M, Zhao Y, Zhao W, Li P, Du B. 2024. Immunomodulatory peptides from sturgeon cartilage: Isolation, identification, molecular docking and effects on RAW264.7 cells. *Food Chemistry-X* 24:101863
14. Wang Z, Fang Y, Zeng Y, Yang X, Yu F-M, Wang B. 2024. Immunomodulatory peptides from thick-shelled mussel (*Mytilus coruscus*): Isolation, identification, molecular docking and immunomodulatory effects on RAW264.7 cells. *Food Bioscience* 59:103874
15. Montserrat-de la Paz S, Villanueva-Lazo A, Millan F, Martin-Santiago V, Rivero-Pino F, Millan-Linares MC. 2023. Production and identification of immunomodulatory peptides in intestine cells obtained from hemp industrial by-products. *Food Research International* 174:113616
16. Wang S, Zhang L, Wang H, Liu J, Hu Y, Tu Z. 2024. Angiotensin converting enzyme (ACE) inhibitory peptide from the tuna (*Thunnus thynnus*) muscle: Screening, interaction mechanism and stability. *International Journal of Biological Macromolecules* 279:135469
17. Lee SY, Hur SJ. 2017. Antihypertensive peptides from animal products, marine organisms, and plants. *Food Chemistry* 228:506-17

 doi:10.20944/preprints202509.1812.v1

18. Mehmood A, Iftikhar A, Chen X. 2024. Food-derived bioactive peptides with anti-hyperuricemic activity: A comprehensive review. *Food Chemistry* 451:139444
19. Quah Y, Tong S-R, Bojarska J, Giller K, Tan S-A, et al. 2023. Bioactive Peptide Discovery from Edible Insects for Potential Applications in Human Health and Agriculture. *Molecules* 28:1233
20. Zamora-Sillero J, Gharsallaoui A, Prentice C. 2018. Peptides from Fish By-product Protein Hydrolysates and Its Functional Properties: an Overview. *Marine Biotechnology* 20:118-30
21. Chatterjee C, Gleddie S, Xiao C-W. 2018. Soybean Bioactive Peptides and Their Functional Properties. *Nutrients* 10:1211
22. Garcia MC, Puchalska P, Esteve C, Marina ML. 2013. Vegetable foods: A cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrence bioactivities. *Talanta* 106:328-49
23. Shuai X, Gao L, Geng Q, Li T, He X, et al. 2022. Effects of Moderate Enzymatic Hydrolysis on Structure and Functional Properties of Pea Protein. *Foods* 11:2368
24. Peng R, Shi Q, Su J. 2015. Preparation of bioactive peptides from lotus seed protein by enzy-matic hydrolysis. *BioTechnology: An Indian Journal* 11:401-04
25. Mohammad SS, Barbosa M, Gamallo O, Junior JBB. 2023. The production of bioactive peptides by optimization of enzymatic hydrolysis process of protein from tilapia fish skin waste (Oreochromis niloticus, Linnaeus 1758) using alcalase 2.4.L. *Current Bioactive Compounds* 19:10
26. Weng Z, Chen Y, Liang T, Lin Y, Cao H, et al. 2023. A review on processing methods and functions of wheat germ-derived bioactive peptides. *Critical Reviews in Food Science and Nutrition* 63:5577-93
27. Veytia D, Corney S, Meiners KM, Kawaguchi S, Murphy EJ, Bestley S. 2020. Circumpolar projections of Antarctic krill growth potential. *Nature Climate Change* 10:568-75
28. Yang S, He Q, Shi L, Wu Y. 2023. Impact of Antarctic krill oil supplementation on skeletal muscle injury recovery after resistance exercise. *European Journal of Nutrition* 62:1345-56
29. Xie D, Gong M, Wei W, Jin J, Wang X, et al. 2019. Antarctic Krill (*Euphausia superba*) Oil: A Comprehensive Review of Chemical Composition, Extraction Technologies, Health Benefits, and Current Applications. *Comprehensive Reviews in Food Science and Food Safety* 18:514-34
30. Choi JY, Jang JS, Son DJ, Im H-S, Kim JY, et al. 2017. Antarctic Krill Oil Diet Protects against Lipopolysaccharide-Induced Oxidative Stress, Neuroinflammation and Cognitive Impairment. *International Journal of Molecular Sciences* 18:2554
31. Tang S, Wang JJ, Li Y, Malakar PK, Zhao Y. 2024. Recent advances in the use of antarctic krill (*Euphausia superba*) as a sustainable source of high-quality protein: A comprehensive review. *Trends in Food Science & Technology* 152:104684
32. Yao M, Gai X, Zhang M, Liu X, Cui T, et al. 2023. Two proteins prepared from defatted Antarctic krill (*Euphausia superba*) powder: Composition, structure and functional properties. *Food Hydrocolloids* 145:109009
33. Lan C, Zhao Y-Q, Li X-R, Wang B. 2019. High Fischer ratio oligopeptides determination from Antartic krill: Preparation, peptides profiles, and in vitro antioxidant activity. *Journal of Food Biochemistry* 43:e12827
34. Zhang S-Y, Zhao G-X, Suo S-K, Wang Y-M, Chi C-F, Wang B. 2021. Purification, Identification, Activity Evaluation, and Stability of Antioxidant Peptides from Alcalase Hydrolysate of Antarctic Krill (*Euphausia superba*) Proteins. *Marine Drugs* 19:347
35. Zhao L, Yin B, Liu Q, Cao R. 2013. Purification of antimicrobial peptide from Antarctic Krill (*Euphausia superba*) and its function mechanism. *Journal of Ocean University of China* 12:484-90
36. Zheng K, Wu Y, Dai Q, Yan X, Liu Y, et al. 2024. Extraction, identification, and molecular mechanisms of α-glucosidase inhibitory peptides from defatted Antarctic krill (*Euphausia superba*) powder hydrolysates. *International Journal of Biological Macromolecules* 266:131126
37. Zhao Y-Q, Zhang L, Tao J, Chi C-F, Wang B. 2019. Eight antihypertensive peptides from the protein hydrolysate of Antarctic krill (*Euphausia superba*): Isolation, identification, and activity evaluation on human umbilical vein endothelial cells (HUVECs). *Food Research International* 121:197-204
38. Ji W, Zhang C, Ji H. 2017. Two Novel Bioactive Peptides from Antarctic Krill with Dual Angiotensin Converting Enzyme and Dipeptidyl Peptidase IV Inhibitory Activities. *Journal of Food Science* 82:1742-49

 doi:10.20944/preprints202509.1812.v1

39. Cheng J-H, Zhang X-Y, Wang Z, Zhang X, Liu S-C, et al. 2021. Potential of Thermolysin-like Protease A69 in Preparation of Bovine Collagen Peptides with Moisture-Retention Ability and Antioxidative Activity. *Marine Drugs* 19:676
40. Cao W-J, Liu R, Zhao W-X, Li J, Wang Y, et al. 2024. Potential of Marine Bacterial Metalloprotease A69 in the Preparation of Peanut Peptides with Angiotensin-Converting Enzyme (ACE)-Inhibitory and Antioxidant Properties. *Marine Drugs* 22:305
41. Liu F-F, Li Y-Q, Wang C-Y, Liang Y, Zhao X-Z, et al. 2022. Physicochemical, functional and antioxidant properties of mung bean protein enzymatic hydrolysates. *Food Chemistry* 393:133397
42. Zhang X, Zhao W-X, Wang Y, Cheng J-H, Bao K, et al. 2023. Production of marine bacterial metalloprotease A69 and evaluation of its potential in preparing soybean peptides with angiotensin-converting enzyme-inhibitory activity. *Journal of the Science of Food and Agriculture* 103:7153-63
43. Zhou H, Safdar B, Li H, Yang L, Ying Z, Liu X. 2023. Identification of a novel α-amylase inhibitory activity peptide from quinoa protein hydrolysate. *Food Chemistry* 403:134434
44. Daou M, Elnaker NA, Ochsenkuhn MA, Amin SA, Yousef AF, Yousef LF. 2022. In vitro α-glucosidase inhibitory activity of *Tamarix nilotic* shoot extracts and fractions. *Plos One* 17:e0264969
45. Liu R, Zhou L, Zhang Y, Sheng N-J, Wang Z-K, et al. 2017. Rapid Identification of Dipeptidyl Peptidase-IV (DPP-IV) Inhibitory Peptides from *Ruditapes philippinarum* Hydrolysate. *Molecules* 22:1714
46. Zhang Y-Q, Zhang S, Sun M-L, Su H-N, Li H-Y, et al. 2022. Antibacterial activity of peptaibols from *Trichoderma longibrachiatum* SMF2 against gram-negative *Xanthomonas oryzae* pv.*oryzae*, the causal agent of bacterial leaf blight on rice. *Frontiers in Microbiology* 13:1034779
47. Xiao C, Toldra F, Zhao M, Zhou F, Luo D, et al. 2022. *In vitr* an in silico analysis of potential antioxidant peptides obtained from chicken hydrolysate produced using Alcalase. *Food Research International* 157:111253
48. Intiquilla A, Jimenez-Aliaga K, Guzman F, Alvarez CA, Zavaleta AI, et al. 2019. Novel antioxidant peptides obtained by alcalase hydrolysis of *Erythrina edulis* (pajuro) protein. *Journal of the Science of Food and Agriculture* 99:2420-27
49. Vijitpunyaruk T, Theerakulkait C. 2014. Preparation of alcalase hydrolysed rice bran protein concentrate and its inhibitory effect on soybean lipoxygenase activity. *International Journal of Food Science and Technology* 49:501-07
50. Shi J, Su R-q, Zhang W-t, Chen J. 2020. Purification and the secondary structure of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the alcalase hydrolysate of seahorse protein. *Journal of Food Science and Technology-Mysore* 57:3927-34
51. Gao J, Gong H, Mao X. 2020. Dipeptidyl Peptidase-IV Inhibitory Activity and Related Molecular Mechanism of Bovine α-Lactalbumin-Derived Peptides. *Molecules* 25:3009
52. Sila A, Bougatef A. 2016. Antioxidant peptides from marine by-products: Isolation, identification and application in food systems. A review. *Journal of Functional Foods* 21:10-26
53. Hu Y-m, Lu S-z, Li Y-s, Wang H, Shi Y, et al. 2022. Protective effect of antioxidant peptides from grass carp scale gelatin on the H2O2-mediated oxidative injured HepG2 cells. *Food Chemistry* 373:131539
54. Liu H, Liang J, Xiao G, Vargas-De-La-Cruz C, Simal-Gandara J, et al. 2022. Active sites of peptides Asp-Asp-Asp-Tyr and Asp-Tyr-Asp-Asp protect against cellular oxidative stress. *Food Chemistry* 366:130626
55. Zhang J, Li M, Zhang G, Tian Y, Kong F, et al. 2021. Identification of novel antioxidant peptides from snakehead (*Channa argu*) soup generated during gastrointestinal digestion and insights into the anti-oxidation mechanisms. *Food Chemistry* 337:127921
56. Hamzeh A, Rezaei M, Khodabandeh S, Motamedzadegan A, Noruzinia M, Mac Regenstein J. 2019. Optimization of Antioxidant Peptides Production from the Mantle of Cuttlefish (*Sepia pharaonis*) Using RSM and Fractionation. *Journal of Aquatic Food Product Technology* 28:392-401
57. Hatanaka A, Miyahara H, Suzuki KI, Sato S. 2009. Isolation and Identification of Antihypertensive Peptides from Antarctic Krill Tail Meat Hydrolysate. *Journal of Food Science* 74:H116-H20
58. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. 2021. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical Biology & Drug Design* 98:539-60

 doi:10.20944/preprints202509.1812.v1

59. Hossain U, Das AK, Ghosh S, Sil PC. 2020. An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications. *Food and Chemical Toxicology* 145:111738
60. Kwok AJ, Mashar M, Khavandi K, Sabir I. 2014. DPP-IV inhibitors: Beyond glycaemic control? *Trends in Cardiovascular Medicine* 24:157-64
61. Tan Q, Akindehin SE, Orsso CE, Waldner RC, DiMarchi RD, et al. 2022. Recent Advances in Incretin-Based Pharmacotherapies for the Treatment of Obesity and Diabetes. *Frontiers in Endocrinology* 13:838410
62. Fuentes LR, Richard C, Chen L. 2021. Sequential alcalase and flavourzyme treatment for preparation of α-amylase, α-glucosidase, and dipeptidyl peptidase (DPP)-IV inhibitory peptides from oat protein. *Journal of Functional Foods* 87:104829
63. Ji W, Zhang C, Ji H. 2017. Purification, identification and molecular mechanism of two dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from Antarctic krill (*Euphausia superba*) protein hydrolysate. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 1064:56-61
64. Lang M, Song Y, Li Y, Xiang X, Ni L, Miao J. 2021. Purification, identification, and molecular mechanism of DPP-IV inhibitory peptides from defatted Antarctic krill powder. *Journal of Food Biochemistry* 45:e13872
65. Chen G, Ge X, Sun Y, Sui W, Jin Y, et al. 2025. Identification of two novel α-amylase inhibitory activity peptide from Russian sea cucumber body wallprotein hydrolysate. *International Journal of Biological Macromolecules* 295:139499
66. Admassu H, Gasmalla MAA, Yang R, Zhao W. 2018. Identification of Bioactive Peptides with α-Amylase Inhibitory Potential from Enzymatic Protein Hydrolysates of Red Seaweed (*Porphyra* spp). *Journal of Agricultural and Food Chemistry* 66:4872-82
67. Fadimu GJ, Farahnaky A, Gill H, Olalere OA, Gan C-Y, Tuyen T. 2022. In-Silico Analysis and Antidiabetic Effect of α-Amylase and α-Glucosidase Inhibitory Peptides from Lupin Protein Hydrolysate: Enzyme-Peptide Interaction Study Using Molecular Docking Approach. *Foods* 11:3375
68. Carrera-Alvarado G, Toldra F, Mora L. 2022. DPP-IV Inhibitory Peptides GPF, IGL, and GGGW Obtained from Chicken Blood Hydrolysates. *International Journal of Molecular Sciences* 23:14140
69. Akbarmehr A, Peighambardoust SH, Ghanbarzadeh B, Sarabandi K. 2023. Physicochemical, antioxidant, antimicrobial, and in vitro cytotoxic activities of corn pollen protein hydrolysates obtained by different peptidases. *Food Science & Nutrition* 11:2403-17
70. Hamed F, Elgaoud I, Eljoudi S, Deracinois B, Flahaut C, et al. 2024. *Diplodus* Protein Hydrolysates: Antioxidant and Antibacterial Properties and Identification of Biopeptides. *Waste and Biomass Valorization* 15:4309-23
71. Karami Z, Muangnapoh C, Suthienkul O, Suriyarak S, Duangmal K. 2024. Exploring novel peptides in adzuki bean and mung bean hydrolysates with potent antibacterial activity. *International Journal of Food Science and Technology* 59:4829-40
72. Zhang R, Xu L, Dong C. 2022. Antimicrobial Peptides: An Overview of their Structure, Function and Mechanism of Action. *Protein and Peptide Letters* 29:641-50
73. Liu W, Gu R, Lin F, Lu J, Yi W, et al. 2013. Isolation and identification of antioxidative peptides from pilot-scale black-bone silky fowl (*Gallus gallus domesticus* Brisson) muscle oligopeptides. *Journal of the Science of Food and Agriculture* 93:2782-88
74. Dávalos A, Miguel M, Bartolomé B, López-Fandiño R. 2004. Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. *Journal of Food Protection* 67:1939-44
75. Tian M, Fang B, Jiang L, Guo H, Cui J, Ren F. 2015. Structure-activity relationship of a series of antioxidant tripeptides derived from β-Lactoglobulin using QSAR modeling. *Dairy Science & Technology* 95:451-63
76. Zarei M, Ebrahimpour A, Abdul-Hamid A, Anwar F, Abu Bakar F, et al. 2014. Identification and characterization of papain-generated antioxidant peptides from palm kernel cake proteins. *Food Research International* 62:726-34
77. Saito K, Jin DH, Ogawa T, Muramoto K, Hatakeyama E, et al. 2003. Antioxidative properties of tripeptide libraries prepared by the combinatorial chemistry. *Journal of Agricultural and Food Chemistry* 51:3668-74
78. Wang C, He H, Zhang J-L, Li X, Ma Z-l. 2016. High performance liquid chromatography (HPLC) fingerprints and primary structure identification of corn peptides by HPLC-diode array detection and HPLC-electrospray ionization tandem mass spectrometry. *Journal of Food and Drug Analysis* 24:95-104

 doi:10.20944/preprints202509.1812.v1

79. del Mar Contreras M, Sanchez D, Angeles Sevilla M, Recio I, Amigo L. 2013. Resistance of casein-derived bioactive peptides to simulated gastrointestinal digestion. *International Dairy Journal* 32:71-78
80. Zielinska E, Baraniak B, Karas M. 2018. Identification of antioxidant and anti-inflammatory peptides obtained by simulated gastrointestinal digestion of three edible insects species (*Gryllodes sigillatus*, *Tenebrio molitor*, *Schistocerca gragari*). *International Journal of Food Science and Technology* 53:2542-51
81. Huang Y, Ruan G, Qin Z, Li H, Zheng Y. 2017. Antioxidant activity measurement and potential antioxidant peptides exploration from hydrolysates of novel continuous microwave-assisted enzymolysis of the *Scomberomorus niphonius* protein. *Food Chemistry* 223:89-95
82. van Platerink CJ, Janssen H-GM, Haverkamp J. 2008. Application of at-line two-dimensional liquid chromatography-mass spectrometry for identification of small hydrophilic angiotensin I-inhibiting peptides in milk hydrolysates. *Analytical and Bioanalytical Chemistry* 391:299-307
83. Castellano P, Aristoy M-C, Angel Sentandreu M, Vignolo G, Toldra F. 2013. Peptides with angiotensin I converting enzyme (ACE) inhibitory activity generated from porcine skeletal muscle proteins by the action of meat-borne *Lactobacillus*. *Journal of Proteomics* 89:183-90
84. Iwaniak A, Dziuba B. 2009. MOTIFS WITH POTENTIAL PHYSIOLOGICAL ACTIVITY IN FOOD PROTEINS - BIOPEP DATABASE. *Acta Scientiarum Polonorum-Technologia Alimentaria* 8:59-85
85. Cavazos A, de Mejia EG. 2013. Identification of Bioactive Peptides from Cereal Storage Proteins and Their Potential Role in Prevention of Chronic Diseases. *Comprehensive Reviews in Food Science and Food Safety* 12:364-80
86. Wu JP, Aluko RE, Nakai S. 2006. Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative structure-activity relationship study of di- and tripeptides. *Journal of Agricultural and Food Chemistry* 54:732-38
87. Tian F, Zhou P, Lv F, Song R, Li Z. 2007. Three-dimensional holograph vector of atomic interaction field (3D-HoVAIF): a novel rotation-translation invariant 3D structure descriptor and its applications to peptides. *Journal of Peptide Science* 13:549-66
88. He H-L, Liu D, Ma C-B. 2013. Review on the Angiotensin-I-Converting Enzyme (ACE) Inhibitor Peptides from Marine Proteins. *Applied Biochemistry and Biotechnology* 169:738-49
89. Meisel H, Schlimme E. 1990. Milk proteins: precursors of bioactive peptides. *Trends in Food Science & Technology* 1:41-43
90. Cheung HS, Wang FL, Ondetti MA, Sabo EF, Cushman DW. 1980. BINDING OF PEPTIDE-SUBSTRATES AND INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME - IMPORTANCE OF THE COOH-TERMINAL DIPEPTIDE SEQUENCE. *Journal of Biological Chemistry* 255:401-07
91. Puchalska P, Marina Alegre ML, Garcia Lopez MC. 2015. Isolation and Characterization of Peptides with Antihypertensive Activity in Foodstuffs. *Critical Reviews in Food Science and Nutrition* 55:521-51
92. Pripp AH, Ardo Y. 2007. Modelling relationship between angiotensin-(I)-converting enzyme inhibition and the bitter taste of peptides. *Food Chemistry* 102:880-88
93. Wang X, Wang J, Lin Y, Ding Y, Wang Y, et al. 2011. QSAR study on angiotensin-converting enzyme inhibitor oligopeptides based on a novel set of sequence information descriptors. *Journal of Molecular Modeling* 17:1599-606
94. Zhou P, Yang C, Ren Y, Wang C, Tian F. 2013. What are the ideal properties for functional food peptides with antihypertensive effect? A computational peptidology approach. *Food Chemistry* 141:2967-73
95. Vercruysse L, Van Camp J, Morel N, Rouge P, Herregods G, Smagghe G. 2010. Ala-Val-Phe and Val-Phe: ACE inhibitory peptides derived from insect protein with antihypertensive activity in spontaneously hypertensive rats. *Peptides* 31:482-88
96. Silva-Sanchez C, de la Rosa APB, Leon-Galvan MF, de Lumen BO, de Leon-Rodriguez A, de Mejia EG. 2008. Bioactive peptides in amaranth (*Amaranthus hypochondriacus*) seed. *Journal of Agricultural and Food Chemistry* 56:1233-40
97. Yano S, Suzuki K, Funatsu G. 1996. Isolation from alpha-zein of thermolysin peptides with angiotensin I-converting enzyme inhibitory activity. *Bioscience Biotechnology and Biochemistry* 60:661-63

 doi:10.20944/preprints202509.1812.v1

98. Miyoshi S, Ishikawa H, Kaneko T, Fukui F, Tanaka H, Maruyama S. 1991. STRUCTURES AND ACTIVITY OF ANGIOTENSIN-CONVERTING ENZYME-INHIBITORS IN AN ALPHA-ZEIN HYDROLYSATE. *Agricultural and Biological Chemistry* 55:1313-18
99. Hu Y, Stromeck A, Loponen J, Lopes-Lutz D, Schieber A, Gaenzle MG. 2011. LC-MS/MS Quantification of Bioactive Angiotensin I-Converting Enzyme Inhibitory Peptides in Rye Malt Sourdoughs. *Journal of Agricultural and Food Chemistry* 59:11983-89
100. Tan J, Tian F, Lv Y, Liu W, Zhong L, et al. 2013. Integration of QSAR modelling and QM/MM analysis to investigate functional food peptides with antihypertensive activity. *Molecular Simulation* 39:1000-06
101. Tauzin J, Miclo L, Gaillard JL. 2002. Angiotensin-I-converting enzyme inhibitory peptides from tryptic hydrolysate of bovine s2-casein. *Febs Letters* 531:369-74
102. Wang Z-l, Zhang S-s, Wang W, Feng F-q, Shan W-g. 2011. A Novel Angiotensin I Converting Enzyme Inhibitory Peptide from the Milk Casein: Virtual Screening and Docking Studies. *Agricultural Sciences in China* 10:463-67
103. Abd El-Salam MH, El-Shibiny S. 2013. Bioactive Peptides of Buffalo, Camel, Goat, Sheep, Mare, and Yak Milks and Milk Products. *Food Reviews International* 29:1-23
104. Geerlings A, Villar IC, Hidalgo Zarco F, Sanchez M, Vera R, et al. 2006. Identification and characterization of novel angiotensin-converting enzyme inhibitors obtained from goat milk. *Journal of Dairy Science* 89:3326-35
105. Loponen J. 2004. Angiotensin converting enzyme inhibitory peptides in Finnish cereals: a database survey. *Agricultural and Food Science* 13:39-45
106. Terashima M, Oe M, Ogura K, Matsumura S. 2011. Inhibition Strength of Short Peptides Derived from an ACE Inhibitory Peptide. *Journal of Agricultural and Food Chemistry* 59:11234-37
107. Wang WY, De Mejia EG. 2005. A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. *Comprehensive Reviews in Food Science and Food Safety* 4:63-78
108. del Mar Contreras M, Carron R, Jose Montero M, Ramos M, Recio I. 2009. Novel casein-derived peptides with antihypertensive activity. *International Dairy Journal* 19:566-73
109. Escudero E, Angel Sentandreu M, Arihara K, Toldra F. 2010. Angiotensin I-Converting Enzyme Inhibitory Peptides Generated from in Vitro Gastrointestinal Digestion of Pork Meat. *Journal of Agricultural and Food Chemistry* 58:2895-901
110. Sagardia I, Roa-Ureta RH, Bald C. 2013. A new QSAR model, for angiotensin I-converting enzyme inhibitory oligopeptides. *Food Chemistry* 136:1370-76
111. Majumder K, Wu J. 2009. Angiotensin I Converting Enzyme Inhibitory Peptides from Simulated in Vitro Gastrointestinal Digestion of Cooked Eggs. *Journal of Agricultural and Food Chemistry* 57:471-77
112. Martinez-Maqueda D, Miralles B, Recio I, Hernandez-Ledesma B. 2012. Antihypertensive peptides from food proteins: a review. *Food & Function* 3:350-61
113. Wang J-H, Liu Y-L, Ning J-H, Yu J, Li X-H, Wang F-X. 2013. Is the structural diversity of tripeptides sufficient for developing functional food additives with satisfactory multiple bioactivities? *Journal of Molecular Structure* 1040:164-70