Natural Resources for Human Health

visagaa publishing house

Review

View Article online

Check for updates

Received 15 February 2026
Revised 24 March 2026
Accepted 16 June 2026
Available Online 11 August 2026

Edited by Kannan RR Rengasamy

KEYWORDS:
Antimicrobial
Cytotoxic
Bioactive peptide
SPPS

Natr Resour Human Health 2026; 6 (4): 379–388
https://doi.org/10.53365/nrfhh/224626
eISSN: 2583-1194

Synthesis and Bioactivity Evaluation of Peptides Derived from *Crassostrea rivularis* as Bioactive Functional Food Components

Anderson Arnold Aloanis[1], Vlagia Indira Paat[1], Stefan Marco Rumengan[1], Jessika Maya Jovanka Najoan[1], Rani Maharani[2]

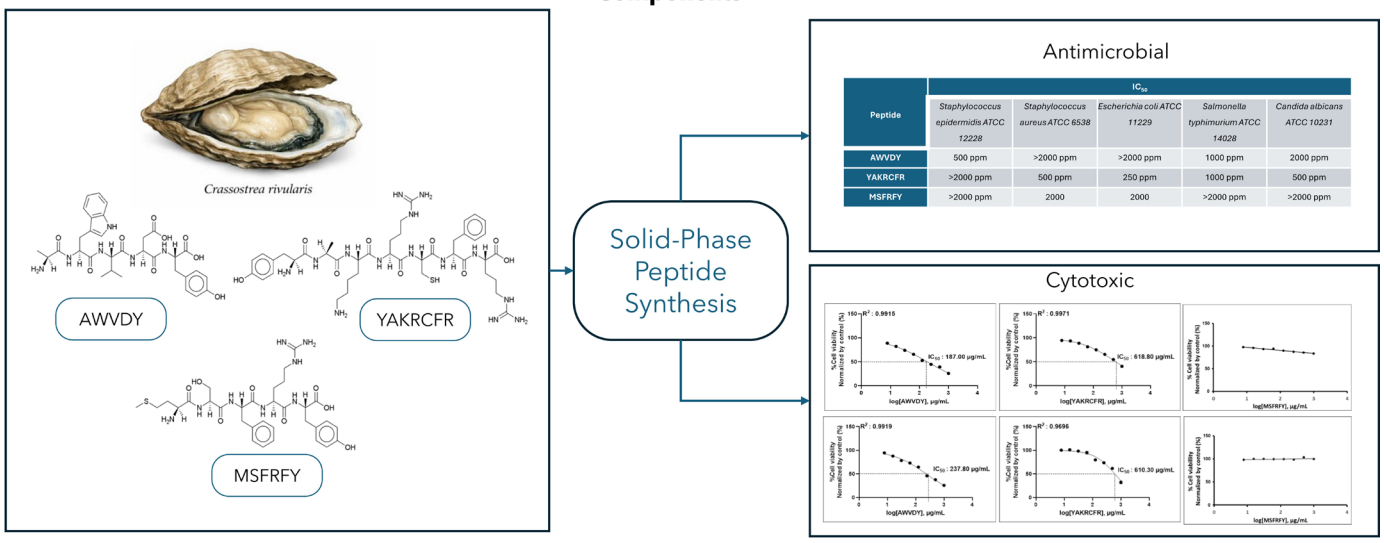
[1]Chemistry, Universitas Negeri Manado, Indonesia
[2]Chemistry, Universitas Padjadjaran, Indonesia

ABSTRACT: Peptides derived from marine organisms represent promising candidates for functional food components because of their diverse bioactivities and favorable safety profiles. This study aimed to synthesize three peptides, AWVDY, YAKRCFR, and MSFRFY, from *Crassostrea rivularis* and assess their antimicrobial and non-toxic potential as bioactive ingredients. Peptide synthesis was carried out using the solid-phase method based on the Fmoc/tBu strategy, employing resin loadings of 0.469, 0.424, and 0.635 mmol/g for AWVDY, YAKRCFR, and MSFRFY, respectively. Chain elongation utilized HATU/HOAt/DIPEA for YAKRCFR and DIC/Oxyma for AWVDY and MSFRFY, followed by TFA-mediated cleavage. Structural characterization was confirmed by LC-MS and NMR analyses. Cytotoxicity assays using A549 and CV-1 cell lines showed that AWVDY exhibited moderate cytotoxicity toward A549 cells and weak cytotoxicity toward CV-1 cells, whereas YAKRCFR and MSFRFY were non-cytotoxic. Antimicrobial evaluation against *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, and *Escherichia coli* revealed selective inhibition by AWVDY and broader activity by YAKRCFR, while MSFRFY exhibited minimal activity. These results indicate that YAKRCFR possesses the most favorable combination of antimicrobial activity and safety, whereas AWVDY shows moderate cytotoxicity but retains selective antimicrobial potential.

* Corresponding author.
E-mail address: andersonaloanis@unima.ac.id (Anderson Arnold Aloanis)

GRAPHICAL ABSTRACT

Synthesis and Bioactivity Evaluation of Peptides Derived from *Crassostrea rivularis* as Bioactive Functional Food Components



Peptide	IC_{50}				
	Staphylococcus epidermidis ATCC 12228	*Staphylococcus aureus ATCC 6538*	*Escherichia coli ATCC 11229*	*Salmonella typhimurium ATCC 14028*	*Candida albicans ATCC 10231*
AWVDY	500 ppm	>2000 ppm	>2000 ppm	1000 ppm	2000 ppm
YAKRCFR	>2000 ppm	500 ppm	250 ppm	1000 ppm	500 ppm
MSFRFY	>2000 ppm	2000	2000	>2000 ppm	>2000 ppm

1. INTRODUCTION

The search for bioactive peptides derived from natural resources has gained significant momentum in recent years, driven by the need for novel therapeutic agents with multifunctional biological properties (Zhao et al., 2024). Among these, short linear peptides obtained through enzymatic hydrolysis of food proteins have attracted particular attention because of their biocompatibility, structural diversity, and ease of synthesis (Purohit et al., 2024). Among marine organisms, oysters are recognized as rich sources of bioactive peptides that have shown antimicrobial, immunomodulatory, antioxidant, and antihypertensive properties (Admassu et al., 2018; Ucak et al., 2021).

Recent reports have highlighted isolated peptides from oyster (*Crassostrea rivularis*) as promising antioxidant candidates. Peptides AWVDY (Ala-Trp-Val-Asp-Tyr), YAKRCFR (Tyr-Ala-Lys-Arg-Cys-Phe-Arg), and MSFRFY (Met-Ser-Phe-Arg-Phe-Tyr) showed antioxidant activity against various free radicals (Huang et al., 2023). Their activity has been attributed to the presence of amino acid residues such as tyrosine, cysteine, aspartic acid, and phenylalanine, which are capable of donating hydrogen atoms or electrons to neutralize free radicals (Shi et al., 2022; Zhu et al., 2024; Zou et al., 2016). However, beyond antioxidant function, the structural features of these peptides, including cationic residues (Lysine, Aspartic acid, Tyrosine, and Arginine) and the sulfur-containing cysteine, suggest that these peptides may also exert antimicrobial properties mediated by membrane damage, oxidative stress generation, or modulation of microbial signaling pathways (Clark et al., 2021; Kumar et al., 2018).

Peptides are increasingly recognized as valuable components in food supplements, especially those with antioxidant and antimicrobial properties and no detectable toxicity. Antioxidant peptides exert protective effects against oxidative damage by neutralizing free radicals, preserving cellular homeostasis, and decreasing susceptibility to chronic diseases (Chandimali et al., 2025). At the same time, antimicrobial peptides offer natural protection against harmful bacteria and fungi, contributing to improved gut health and overall immunity (Aquino-Domínguez et al., 2025). Because these peptides are typically derived from natural food sources and undergo careful safety evaluation, they tend to exhibit low or no toxicity, making them suitable for long-term consumption. With their dual function and excellent safety profile, antioxidant and antimicrobial peptides are becoming key candidates for next-generation functional food supplements (Zhao et al., 2024).

In this study, we report the evaluation of YAKRCFR, AWVDY, and MSFRFY for their antimicrobial potential against representative Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*), Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*), and fungus (*Candida albicans*) (Aloanis et al., 2024). The peptides were obtained through Fmoc SPPS, purified by decantation and semipreparative HPLC. The purity was analyzed with

RP-HPLC and characterized by HR-ToF-MS, ^1H-NMR, and ^13C-NMR (Aloanis, Herlina, et al., 2025b; Aloanis, Najoan, et al., 2025). Their biological activities were assessed through antimicrobial and toxicity assays, with the aim of establishing AWVDY, YAKRCFR, and MSFRFY as multifunctional peptides of marine origin (Figure 1).

2. MATERIAL AND METHODS

2.1. Material

Resin 2-Chlorotrityl chloride (CTC), Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Met-OH, Fmoc-Ser(tBu)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, hexafluorophosphate N-oxide (HATU), 1-Hydroxy-7-azabenzotriazole (HOAt), diisopropylcarbodiimide (DIC), Oxyma Pure, acetonitrile (MeCN), N,N-dimethylformamide (DMF), N,N-Diisopropylethylamine (DIEA), water (H2O), dichloromethane (DCM), piperidine, triisopropylsilane (TIS), methanol (MeOH), and trifluoroacetic acid (TFA) were used. Resin, amino acids, and coupling agents were obtained from GL Biochem (Shanghai, China), while solvents and reagents were obtained from Merck.

2.2. Method

2.2.1. Loading and capping resin

CTC resin (0.4 g, 0.6 mmol) was swollen in dichloromethane (DCM) for 30 min in an SPPS reactor, and the solvent was removed under reduced pressure. A solution of Fmoc-Arg(Pbf)-OH (used for the YAKRCFR sequence) or Fmoc-Tyr(tBu)-OH (used for the AWVDY and MSFRFY sequences) (0.6 mmol), together with N,N-diisopropylethylamine (DIEA, 612.189 µL, 3.6 mmol) in 4 mL of DCM, was added to the pre-swollen resin and agitated for 4 h to facilitate coupling. Following attachment of the first amino acid, the reaction solvent was drained. Residual active sites were capped twice with DIEA/MeOH/DCM (1:3:16, 5 mL) for 5 min to prevent undesired acylation during subsequent coupling steps. The resin was thoroughly washed with DCM, dried, and the substitution level (resin loading) was determined.

2.2.2. Fmoc deprotection

Removal of the Fmoc protecting group was carried out using 20% (v/v) piperidine in DMF (10 mL) for 30 min. The resin was then thoroughly rinsed with DMF followed by DCM and allowed to dry. Successful deprotection was verified using the chloranil assay, in which the development of a blue or red coloration signified the presence of liberated amine functionalities.

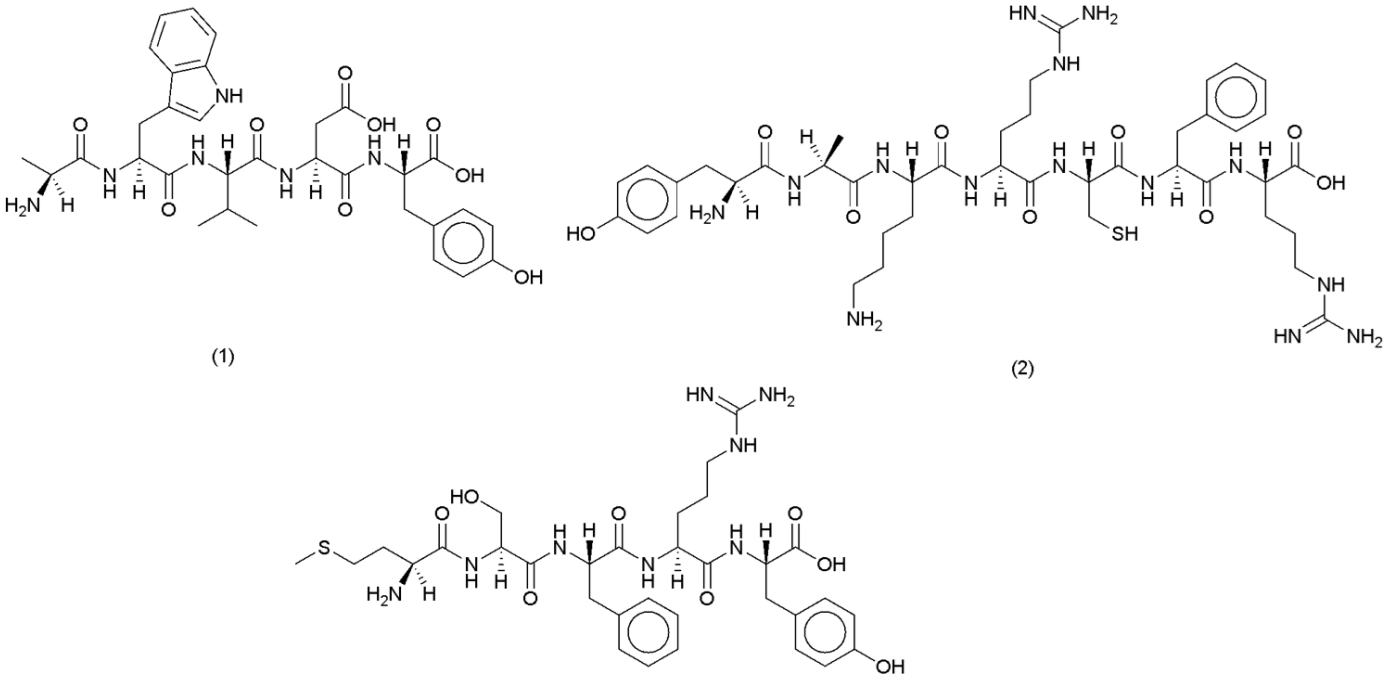


Figure 1. Isolated peptides from oyster *(Crassostrea rivularis)*: (1) AWVDY, (2) YAKRCFR, and (3) MSFRFY.

2.2.3. Amino acid coupling

Amino acid elongation was performed using either Fmoc-amino acid/HATU/HOAt/DIEA (3:6:6:6 equivalent) or Fmoc-amino acid/DIC/Oxyma (3:3:3 equivalent) in 4 mL DMF. The activated amino acid solution was introduced to the resin-bound free amine and agitated for 4 h when employing the HATU/HOAt system or 2 h with the DIC/Oxyma protocol. Upon completion of each coupling step, the resin was filtered and sequentially washed with DMF and DCM. Coupling efficiency was monitored by the chloranil assay; the absence of color change indicated successful amide bond formation.

2.2.4. Peptide cleavage

Cleavage of the linear peptide from the resin was carried out using a cocktail composed of TFA/TIS/H_2O (95:2.5:2.5, v/v/v). A 5 mL aliquot of the cleavage mixture was added to the reactor, and the suspension was gently agitated for 2 h at room temperature. The filtrate was collected, and the resin was washed three times with 5 mL DCM. The cleavage procedure was repeated twice to ensure complete peptide recovery. The combined filtrates were concentrated under reduced pressure to afford the crude linear peptide.

2.2.5. Peptide purification and characterization

The crude peptides AWVDY, YAKRCFR, and MSFRFY were purified using a decantation method. The concentrated peptide solution was precipitated with diethyl ether and maintained at low temperature for 8 h to facilitate complete precipitation. The diethyl ether bound residual impurities from the cleavage reaction and dissolved them. The purified peptide settled and was recovered by decantation. This step was repeated twice. Peptide purity was evaluated through reversed-phase chromatographic analysis using a C18 stationary phase (5 µm, 4.6 × 250 mm, Jupiter). Molecular weight determination was achieved by high-resolution time-of-flight mass spectrometry, while structural elucidation was performed using proton and carbon-13 nuclear magnetic resonance spectroscopy (Aloanis, Herlina, et al., 2025b; Aloanis, Najoan, et al., 2025).

2.2.6. Antimicrobial assay

AWVDY, YAKRCFR, and MSFRFY were analyzed against *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella typhimurium, and Escherichia coli.* Antimicrobial activity was evaluated using a broth microdilution technique in 96-well microplates containing Mueller–Hinton medium. Peptides were initially dissolved in 2% (v/v) DMSO to obtain a stock concentration of 2000 ppm, followed by serial dilution to the desired concentrations. Test samples, positive controls, and 2% DMSO (negative control) were incubated at 37 °C for 18 h. After incubation, optical density was measured at 600 nm using a microplate reader, and the minimum inhibitory concentration (MIC) was determined based on the percentage of growth inhibition (Aloanis et al., 2024).

2.2.7. Cytotoxic assay

The cytotoxic effects of AWVDY, YAKRCFR, and MSFRFY were evaluated in vitro against A549 and CV-1 cell lines using the resazurin-based viability assay. Cells were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (v/v) fetal bovine serum and antibiotics (50 µL per 50 mL medium), and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Doxorubicin and cisplatin served as positive controls, whereas 2% DMSO was used as the negative control. For the assay, cells were seeded in 96-well plates at a density of 2×10^4 cells per well in 100 µL culture medium and allowed to adhere for 24 h. The peptides were then administered at eight different concentrations (7.81–1000 µg/mL) prepared in 2% DMSO. Following 96 h of exposure, cell viability was determined based on the reduction of resazurin to the fluorescent metabolite resorufin by metabolically active cells. Absorbance was measured at 570 nm using a multimode microplate reader. IC_{50} values were calculated from concentration–response curves using linear regression analysis.

According to the cytotoxicity classification guidelines of the U.S. National Cancer Institute (NCI), the cytotoxic activity was categorized as follows: $IC_{50} \leq 20$ µg/mL indicates *high cytotoxicity*; IC_{50} between 21 and 200 µg/mL indicates *moderate cytotoxicity*; IC_{50} between 201 and 500 µg/mL indicates *weak cytotoxicity*; and $IC_{50} > 501$ µg/mL is considered to indicate *no cytotoxic effect* (Duengo et al., 2024).

3. RESULTS AND DISCUSSION

3.1. Peptide synthesis

Preparation of the linear peptide was conducted on a solid-phase support using the Fmoc/tBu strategy (Aloanis et al., 2024; Aloanis, Herlina, et al., 2025b, 2025a; Aloanis, Najoan, et al., 2025). The process began with the loading of the first amino acid. Fmoc-Arg(Pbf)-OH/Fmoc-Tyr(tBu)-OH (0.6 mmol) was coupled onto the resin in the presence of DIPEA (3.6 mmol) using 4 mL DCM as the solvent, after which the suspension was agitated for 30 min at room temperature. Resin loading analysis was carried out to calculate the extent of first amino acid incorporation onto the solid phase. The resin loading values of AWVDY, YAKRCFR, and MSFRFY were 0.469, 0.424, and 0.635 mmol/g, respectively. Unreacted sites were capped with a solution of MeOH:DCM:DIPEA (15:80:5), applied twice for 15 min each. Fmoc deprotection was carried out by exposing the resin to 20% piperidine in DMF for

two consecutive 5 min treatments, thereby generating the free amine required for the next coupling step.

The peptide chain was elongated step by step using standard activation with HATU/HOAt/DIPEA in DMF for YAKRCFR and DIC/Oxyma in DMF for AWVDY and MSFRFY. The second amino acid of YAKRCFR, Fmoc-Phe-OH (1.272 mmol) was coupled using HATU as the activating reagent (1.272 mmol), HOAt (1.272 mmol), and DIPEA (2.544 mmol) in 5 mL DMF, and the reaction was maintained at room temperature for 4 h. The second amino acid of AWVDY (Fmoc-Asp(OtBu)-OH) (1,407 mmol), and that of MSFRFY (Fmoc-Arg(Pbf)-OH) (1.905 mmol), were coupled in the presence of DIC (1.876 mmol for AWVDY and 2.540 mmol for MSFRFY), and Oxyma (1.876 mmol for AWVDY and 2.540 mmol for MSFRFY) in 5 mL DMF. Deprotection of the Fmoc group was achieved by treatment with 20% piperidine in DMF for 2 × 5 min to continue the elongation. The synthesis continued under identical coupling conditions followed by Fmoc removal.

After assembly of the full peptide sequence, deprotection with a piperidine cocktail followed by release of the peptide from the resin was achieved with a TFA:TIS:H_2O (95:2.5:2.5) cocktail, applied twice for 2 h each at room temperature. The crude product was exposed to diethyl ether to induce precipitation and subsequently stored in the cold (below 20 °C) for 6 h to enhance precipitation, followed by decantation. This precipitation-decantation process was repeated 3–4 times until the ether layer appeared clear, after which the peptide was dried by leaving it at room temperature for several minutes until the diethyl ether had completely evaporated. The MSFRFY peptide was obtained with good purity and was therefore progressed to the characterization stage. In contrast, the AWVDY and YAKRCFR peptides were further purified using semipreparative HPLC to obtain pure compounds.

3.2. AWVDY (1)

AWVDY (97.2%). IR (cm^{-1}) 3271, 2964, 1630, 1514, 1438, 1186, 1138; ^{1}H NMR (600 MHz, C_2D_6OS) δ (ppm) Ala: 3.75 (m, 1H), 1.32 (d, *J* 6.7, CH_3); Trp: 4.69 (m, 1H), 3.16 (m, 2H), 7.16 (s, 1H), 7.06-7.79 (m, 4H); Val: 4.24 (m, 1H), 1.98 (m, 1H), 0.82 (d, *J* 6.8, 3H, CH_3), 0.79 (d, *J* 6.8, 3H, CH_3); Asp : 4.58 (m, 1H), 2.87 (m, 1H), 2.67 (m, 1H); Tyr: 4.29 (m, 1H), 2.97 (m, 1H), 2.82 (m, 1 H), 6.96 (d, *J* 8, 2H), 6.64 (d, *J* 8, 2H); ^{13}C NMR (125 MHz, C_2D_6OS) δ (ppm) 171.0, 172.6, 171.7, 170.6, 170.3, 169.7, 155.8, 136.0, 130.0, 127.2, 127.2, 123.8, 120.8, 118.5, 118.2, 114.9, 111.1, 109.7, 57.3, 54.0, 53.6, 49.4, 48.0, 36.0, 30.1, 28.5, 27.3, 19.2, 17.6, 17.3; HR-MS (ESI) *m/z*, calcd. for $[C_{32}H_{41}N_6O_9 + H]^+$: 653.2935, found: 653.2924.

3.3. YAKRCFR (2)

YAKRCFR (86.5%). IR (cm^{-1}) 3270, 1627, 1517, 1452, 1180, 1080, 836, 799, 698, 603; ^{1}H NMR (600 MHz, C_2D_6OS) δ (ppm) Tyr: 4.08 (m, 1H), 3.44 (overlaped), 3.12 (m, 1H), 7.14 (m, 4 H); Ala: 3.90 (m, 1H), 1.72 (m, 2H), 1.24 (m, 2H), 1.52 (m, 2H), 2.68 (m, 2H); Arg: 3.91 (m, 1H), 1.72 (m, 2H), 1,52 (m, 2H), 3.04 (m, 2H); Cys: 4.38 (m, 1H), 2.84 (m, 2H); Phe: 4.38 (m, 1H), 3.42 (overlaped), 3.12 (m, 1H), 7.27 (m, 4H), 6.7 (m, 1H); Arg: 4.12 (m, 1H), 1.72 (m, 2H), 1,52 (m, 2H), 2.96 (m, 2H); ^{13}C NMR (125 MHz, C_2D_6OS) δ (ppm) 174.2, 172.8, 172.6, 172.2, 171.0, 170.3, 168.6, 158.4, 157.4, 157.0, 144.2, 138.4, 129.5, 128.8, 128.6, 126.7, 115.8, 65.4, 56.2, 55.1, 54.4, 53.7, 53.7, 49.0, 44.3, 42.0, 41.0, 38.5, 37.1, 31.1, 30.8, 29.1, 28.8, 26.6, 26.3, 25.2, 22.7, 18.3; HR-MS (ESI) *m/z*, calcd. for $[C_{42}H_{67}N_{14}O_9S + H]^+$: 943.4936, found: 943.4925.

3.4. MSFRFY (3)

MSFRFY (95.3%) ^{1}H NMR (600 MHz, C_2D_6OS) δ (ppm) Met: 3.58 (m, 1H), 1.91 (m, 2H), 2.44 (m, 2H), 1.99 (s, 3H); Ser: 4.19 (m, 1H), 3.56 (m, 1H), 3.01 (m, 1H); Phe: 4.41 (m, 1H), 3.00 (m, 1H), 2.8 (m, 1H), 7.19 (m, 5H); Arg: 3.87 (m, 1H), 1.49 (m, 1H), 1.65 (m,1H), 1.42 (m, 2H), 2.96 (m, 2H), 4.47 (m, 1H), 2.98 (m, 1H); 2.81 (m, 1H), 7.19 (m, 5H); Tyr: 4.22 (m, 1H), 3.03 (m, 1H); 2.79 (m, 1H), 6.98 (d, *J* 8.5, 2H), 6.65 (d, *J* 8.5, 2H); ^{13}C NMR (125 MHz, C_2D_6OS) δ (ppm) 173.4, 171.1, 170.8, 170.5, 169.4, 168.0, 156.8, 155.7, 137.7, 137.6, 130.1, 129.0, 129.0, 128.0, 127.8, 127.6, 126.1, 126.1, 114.8, 61.6, 54.9, 54.4, 54.3, 54.1, 52.1, 51.5, 43.6, 37.3, 36.9, 36.0, 31.0, 29.5, 28.0, 24.3, 14.3; HR-MS (ESI) *m/z*, calcd. for $[C_{41}H_{56}N_9O_9S + H]^+$: 850.3922, found: 850.3917.

3.5. Antimicrobial assay

The antimicrobial properties of AWVDY, YAKRCFR, and MSFRFY were assessed using a broth microdilution method against representative Gram-positive and Gram-negative bacteria, as well as fungal strains. The antimicrobial evaluation revealed distinct activity profiles among the tested peptides. AWVDY exhibited moderate inhibitory activity against S. epidermidis (IC_{50} = 500 ppm) and S. typhimurium (IC_{50} = 1000 ppm), but showed weak or negligible activity against S. aureus, E. coli, and C. albicans (IC_{50} > 2000 ppm). In contrast, YAKRCFR demonstrated stronger potency, particularly

against S. aureus (IC_{50} = 500 ppm), E. coli (IC_{50} = 250 ppm), and C. albicans (IC_{50} = 500 ppm), while maintaining moderate activity against S. epidermidis and S. typhimurium (IC_{50} = 1000 ppm). Meanwhile, MSFRFY displayed consistently weak activity, with IC_{50} values ≥ 2000 ppm across all tested strains, indicating limited antimicrobial potential (Table 1).

3.6. Cytotoxic assay

The cytotoxic effects of AWVDY, YAKRCFR, and MSFRFY on A549 and CV-1 cells were assessed using the resazurin assay, with absorbance measured at 570 nm. The resulting absorbance values were subsequently used to determine the percentage of viable cells. Furthermore, the IC_{50} values were determined using linear regression of the log concentration versus the percentage of cell viability.

Doxorubicin (16.24 μM) was used as the positive control against the A549 cell line. The results indicated that AWVDY and YAKRCFR exhibited IC_{50} values of 187.00 and 618.80 μg/mL, respectively. On the other hand, peptide MSFRFY showed toxicity above 1000 μg/mL (Figure 2). Based on the U.S. NCI cytotoxicity criteria, AWVDY demonstrated moderate cytotoxic activity, whereas YAKRCFR and MSFRFY showed no toxicity.

Cisplatin (16.24 μM) was used as the positive control for the CV-1 cell line. The results showed that AWVDY and YAKRCFR had IC_{50} values of 237.80 and 610.30 μg/mL, respectively. In contrast, the peptide MSFRFY exhibited an IC_{50} greater than 1000 μg/mL (Figure 3). According to the U.S. NCI cytotoxicity criteria, AWVDY displayed weak cytotoxic activity, while YAKRCFR and MSFRFY were classified as non-cytotoxic (Duengo et al., 2024).

AWVDY's cytotoxic activity against A549 and CV-1 cell lines can be attributed to several structural features that optimize membrane disruption. The presence of Tryptophan (W), the most membrane-active aromatic amino acid with hydrophobic properties, serves as a powerful anchor for membrane insertion (Khemaissa et al., 2021). This peptide maintains an optimal hydrophobic content (A, W, and V), which is balanced by the inclusion of Aspartic acid (Asp) for membrane selectivity (Gagat et al., 2024). The compact size of five residues further enhances its ability to penetrate cellular membranes efficiently.

YAKRCFR displays IC_{50} = 618.80 μg/mL against A549, which reflects its structural design that favors antimicrobial rather than cytotoxic mechanisms. Although this peptide contains Phenylalanine (F) with the highest hydrophobicity index, its high cationic content, with three positively charged residues (two Arginine and one Lysine), positions it as an antimicrobial peptide rather than a highly cytotoxic agent (Clark et al., 2021; Gagat et al., 2024). The presence of Arginine's guanidinium group provides electrostatic attraction to anionic bacterial membranes, while Lysine's ε-amino group exhibits enhanced hydrophobicity that facilitates membrane insertion (Andreev et al., 2014). This structural composition, combined with the peptide's longer length of seven residues, suggests that YAKRCFR operates primarily through electrostatic membrane disruption characteristic of cationic antimicrobial peptides, rather than the hydrophobic insertion mechanism typical of potent cytotoxic peptides.

MSFRFY, which showed non-cytotoxicity (IC_{50} > 1000 μg/mL), is primarily explained by the disruption of hydrophobic character caused by the presence of Serine (S), a highly hydrophilic amino acid that significantly impairs membrane insertion capability. Despite containing Phenylalanine (F) with the highest hydrophobicity index, the overall peptide sequence lacks sufficient hydrophobic character due to this polar residue positioned early in the sequence. Additionally, the presence of only a single cationic charge is insufficient to drive membrane disruption through the electrostatic mechanism, and the suboptimal spatial arrangement of hydrophobic residues prevents effective membrane interaction (Gagat et al., 2024). This combination of factors renders MSFRFY incapable of achieving the membrane penetration necessary for cytotoxic activity.

The experimental findings align well with established principles in peptide cytotoxicity research. Studies on hydrophobic amino acids and membrane activity have

Table 1
Antimicrobial activities of AWVDY, YAKRCFR, and MSFRFY.

Peptide	IC_{50}				
	Staphylococcus epidermidis ATCC 12228	*Staphylococcus aureus ATCC 6538*	*Escherichia coli ATCC 11229*	*Salmonella typhimurium ATCC 14028*	*Candida albicans ATCC 10231*
AWVDY	500 ppm	>2000 ppm	>2000 ppm	1000 ppm	2000 ppm
YAKRCFR	>2000 ppm	500 ppm	250 ppm	1000 ppm	500 ppm
MSFRFY	>2000 ppm	2000	2000	>2000 ppm	>2000 ppm

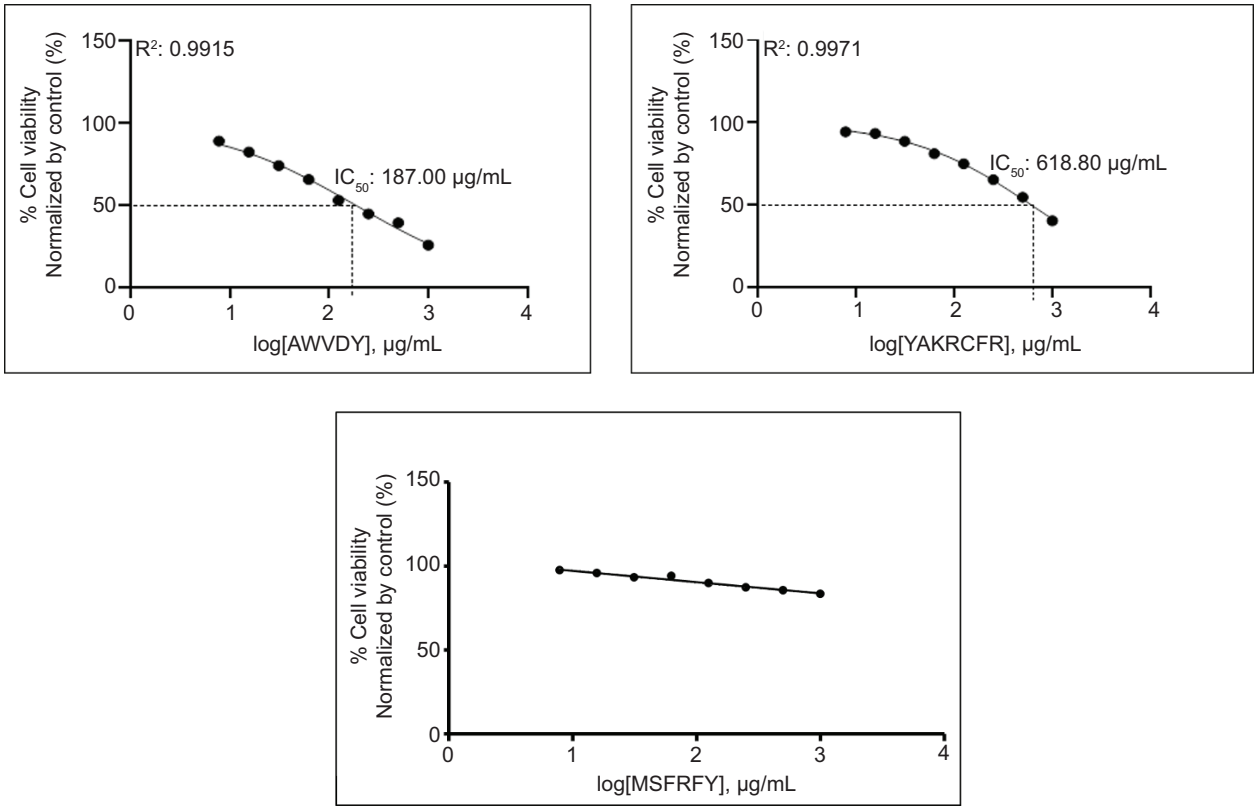


Figure 2. Graph of log concentration vs. % A549 cell viability of AWVDY, YAKRCFR, and MSFRFY.

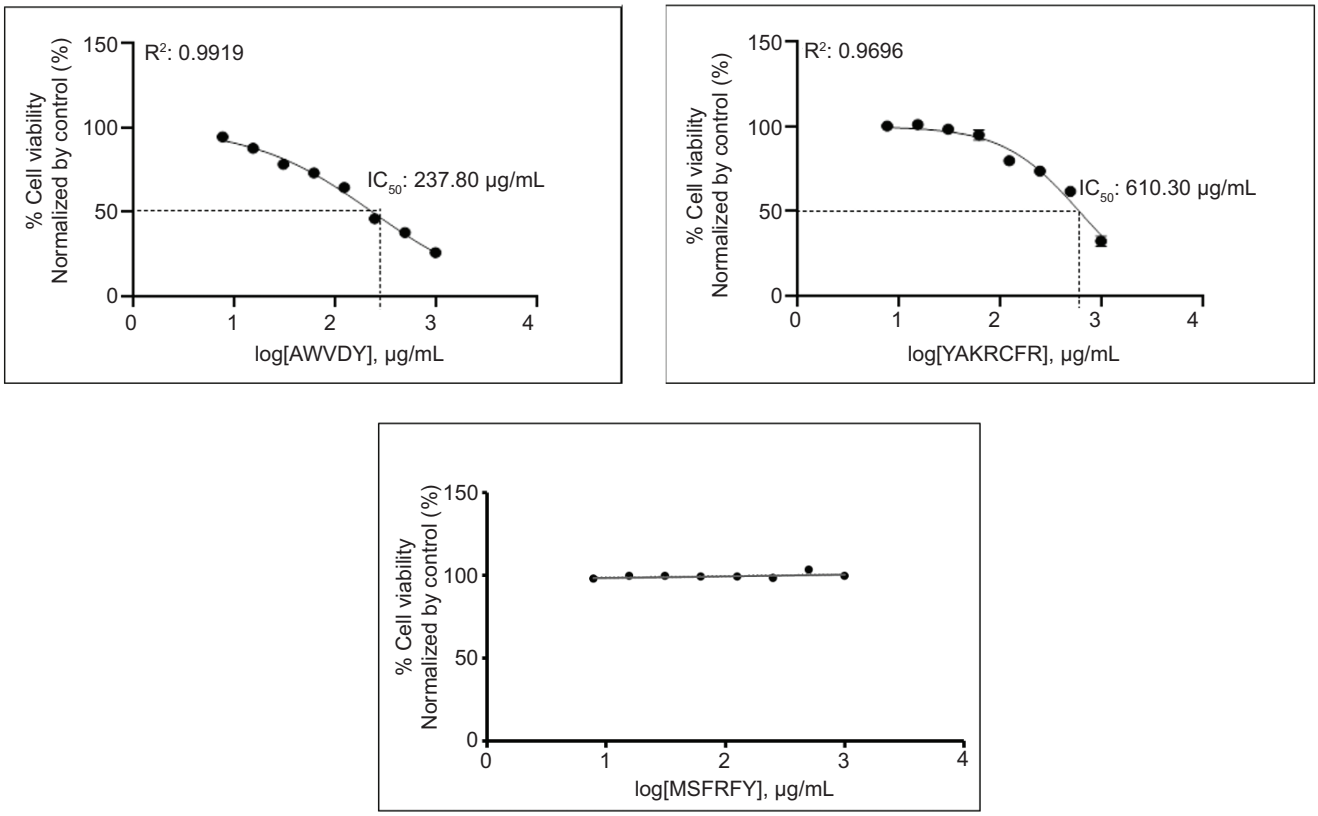


Figure 3. Graphic log concentration vs. % CV-1 cell viability of AWVDY, YAKRCFR, and MSFRFY.

consistently demonstrated that aromatic residues, particularly Phenylalanine and Tryptophan, play crucial roles in membrane disruption. The aliphatic index, which measures the content of Alanine, Valine, Isoleucine, and Leucine, has been identified as a positive factor for increased cytotoxicity in numerous studies (Ghandehari et al., 2015). Furthermore, research indicates that cationic residues (Lysine and Arginine) combined with approximately 30% hydrophobic content are characteristic of peptides exhibiting antimicrobial and cytotoxic properties. Specifically, Glycine (Gly), Lysine (Lys), and Leucine (Leu) have been reported as predominant amino acids in peptides with demonstrated anticancer abilities (Chiangjong et al., 2020). These findings also emphasize that the balance between hydrophobicity and charge determines whether a peptide exhibits primarily cytotoxic or antimicrobial activity, with higher cationic content favoring antimicrobial mechanisms. The effectiveness of these peptides as antimicrobials indicates their potential as natural bioactive components that can be utilized in the development of functional foods with antimicrobial activity (Cumbane et al., 2025).

Based on these findings, several design principles emerge for optimizing peptide bioactivity. For enhanced cytotoxic activity, peptides should incorporate high-hydrophobicity amino acids such as Tryptophan, Phenylalanine, Isoleucine, and Leucine, while maintaining an overall hydrophobic amino acid content. The inclusion of highly polar residues such as Serine and Threonine should be minimized, as these significantly disrupt the hydrophobic character necessary for membrane insertion. For peptides designed primarily for antimicrobial activity, such as YAKRCFR, the design strategy differs significantly. These peptides maintain high cationic content with two to three Lysine or Arginine residues to enable electrostatic attraction to negatively charged bacterial membranes. A moderate hydrophobic content is sufficient for membrane insertion without causing excessive cytotoxicity to mammalian cells. This balance favors antimicrobial over cytotoxic activity and may provide a therapeutic window for treating infections or for use as food supplements without significant host cell damage. AWVDY represents an optimal cytotoxic peptide structure with the right balance of hydrophobicity (contributed by Trp and Val), structural support (Ala), membrane selectivity (Asp), and dual functionality (Tyr). Its design principles can serve as a template for developing novel anticancer peptides, while YAKRCFR's structure exemplifies the antimicrobial peptide archetype. The stark contrast in bioactivity between AWVDY and MSFRFY, despite both containing aromatic residues, underscores the critical importance of overall sequence composition and the positioning of hydrophilic residues in determining peptide bioactivity. These insights provide valuable guidance for the rational design of bioactive peptides with tailored therapeutic applications.

Beyond therapeutic applications, these marine-derived peptides demonstrate significant potential for incorporation into functional foods and nutraceuticals. The peptides examined in this study, particularly YAKRCFR and MSFRFY, with their non-cytotoxic profiles against normal cells (CV-1 cell line), are especially suitable for food applications because of their favorable safety profiles. In accordance with regulatory frameworks, peptides sourced from edible foods are generally considered GRAS, supported by extensive toxicological and safety reviews. The classification of YAKRCFR and MSFRFY as non-cytotoxic, while maintaining antimicrobial activity, suggests that they could be considered for potential application as natural food preservatives, particularly in efforts to help manage spoilage in perishable products such as meat, seafood, and dairy products (Obafemi et al., 2025; Teshome et al., 2022).

4. CONCLUSIONS

The study successfully synthesized and characterized three peptides, AWVDY, YAKRCFR, and MSFRFY, derived from *Crassostrea rivularis* using solid-phase peptide synthesis. Structural confirmation through LC-MS and NMR, combined with antimicrobial and cytotoxicity evaluations, provided comprehensive insight into their bioactive potential. Among the peptides, YAKRCFR demonstrated the most favorable biological profile, exhibiting broad antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi while maintaining a non-cytotoxic profile toward normal and cancerous cell lines. AWVDY showed selective antimicrobial properties and moderate cytotoxicity, attributed to its balanced hydrophobic composition and membrane-interactive residues, suggesting potential utility in targeted therapeutic applications. In contrast, MSFRFY displayed minimal antimicrobial activity and no detectable cytotoxicity, largely due to its reduced hydrophobic character and limited membrane-disruptive capacity. YAKRCFR and MSFRFY, with their demonstrated safety and moderate antimicrobial activity, have considerable potential to be utilized in functional food formulations. AWVDY, with its moderate cytotoxic profile, may serve as a structural template for the further development of selective anticancer peptides. This research underscores the potential of marine-derived peptides as multifunctional bioactive agents and provides a foundation for future studies focusing on peptide optimization, stability in food matrices, and evaluation of their activity in real food systems.

ACKNOWLEDGMENTS

The authors would like to acknowledge Directorate of Research, and Community Services (DPPM), Ministry of High Education, Science, and Technology, Indonesia through National Competitive Grants, Fundamental Research (PFR) 2025 grant (contract number 086/C3/DT.05.00/PL/2025).

FUNDING

This research is funded by Directorate of Research, and Community Services (DPPM), Ministry of High Education, Science, and Technology, Indonesia through National Competitive Grants, Fundamental Research (PFR) 2025 grant (contract number 086/C3/DT.05.00/PL/2025).

AUTHOR CONTRIBUTIONS

A.A.A.: Research concept and design, data analysis and interpretation, writing the article, final approval of the article; V.I.P.: research concept and design, collection and/or assembly of data, data analysis and interpretation, writing the article; S.M.R.: collection and/or assembly of data, data analysis and interpretation; J.M.J.N.: collection and/or assembly of data, data analysis and interpretation, writing the article; R.M.: writing the article, critical revision of the article, final approval of the article.

CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to research, authorship and/or publication of this article.

ORCID

Anderson Arnold Aloanis	0000-0002-6934-1503
Vlagia Indira Paat	0009-0001-1012-0095
Stefan Marco Rumengan	0009-0002-7883-5772
Jessika Maya Jovanka Najoan	0009-0006-5783-2037
Rani Maharani	0000-0001-8156-9773

REFERENCES

Admassu, H., Gasmalla, M.A.A., Yang, R., & Zhao, W. (2018). Bioactive peptides derived from seaweed protein and their health benefits: Antihypertensive, antioxidant, and antidiabetic properties. Journal of Food Science, 83(1), 6–16. https://doi.org/10.1111/1750-3841.14011

Aloanis, A.A., Herlina, T., Hardianto, A., & Maharani, R. (2024). Total synthesis of Cyclosenegalin A. ChemistryOpen. https://doi.org/10.1002/open.202400175

Aloanis, A.A., Herlina, T., Hardianto, A., & Maharani, R. (2025a). Alanine-rich cyclopeptides: Natural resources, bioactivity, total synthesis, and spectroscopy identification. ChemistrySelect, 10(1), e202404379. https://doi.org/10.1002/slct.202404379

Aloanis, A.A., Herlina, T., Hardianto, A., & Maharani, R. (2025b). Structural design and synthesis of Cyclosenegalin A analogue. Journal of Heterocyclic Chemistry, 62(9), 781–790. https://doi.org/10.1002/jhet.70020

Aloanis, A.A., Najoan, J.M.J., Paat, V.I., Rumengan, S.M., & Rumampuk, R.J. (2025). Total synthesis and molecular docking study of peptide AWVDY as an anti-inflammation agent. JKPK (Jurnal Kimia Dan Pendidikan Kimia), 10(2), 234–254. https://doi.org/10.20961/jkpk.v10i2.103623

Andreev, K., Bianchi, C., Laursen, J.S., Citterio, L., Hein-Kristensen, L., Gram, L., Kuzmenko, I., Olsen, C.A., & Gidalevitz, D. (2014). Guanidino groups greatly enhance the action of antimicrobial peptidomimetics against bacterial cytoplasmic membranes. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1838(10), 2492–2502. https://doi.org/10.1016/j.bbamem.2014.05.022

Aquino-Domínguez, A.S., Gómez-López, M., & Aguilar-Ruiz, S.R. (2025). Harmonious allies: The synergy of antimicrobial proteins and microbes in breast milk to protect neonatal health. Hygiene, 5(2), 19. https://doi.org/10.3390/hygiene5020019

Chandimali, N., Bak, S.G., Park, E.H., Lim, H.-J., Won, Y.-S., Kim, E.-K., Park, S.-I., & Lee, S.J. (2025). Free radicals and their impact on health and antioxidant defenses: A review. Cell Death Discovery, 11, 19. https://doi.org/10.1038/s41420-024-02278-8

Chiangjong, W., Chutipongtanate, S., & Hongeng, S. (2020). Anticancer peptide: Physicochemical property, functional aspect and trend in clinical application (Review). International Journal of Oncology, 57(3), 678–696. https://doi.org/10.3892/ijo.2020.5099

Clark, S., Jowitt, T.A., Harris, L.K., Knight, C.G., & Dobson, C.B. (2021). The lexicon of antimicrobial peptides: A complete set of arginine and tryptophan sequences. Communications Biology, 4(1), 605. https://doi.org/10.1038/s42003-021-02137-7

Cumbane, P.J., Machava, A.F., Niyragira, A., Quessaugy, K., Machute, L., & Ruco, T. (2025). Mineral and trace element quantification, phytochemical composition, antioxidant, and antimicrobial activities of *Gladiolus dalenii* van Geel corms: Potential applications in the food and pharmaceutical industries. Natural Resources for Human Health, 5(3), 310–320. https://doi.org/10.53365/nrfhh/203183

Duengo, S., Hidayat, A. T., Musa, W. J. A., & Maharani, R. (2024). Total synthesis and anticancer evaluation of BZR-cotoxin IV. Trends in Sciences, 21(11), Article 11. https://doi.org/10.48048/tis.2024.8370

Gagat, P., Ostrówka, M., Duda-Madej, A., & Mackiewicz, P. (2024). Enhancing antimicrobial peptide activity through modifications of charge, hydrophobicity, and structure. International Journal of Molecular Sciences, 25(19), 10821. https://doi.org/10.3390/ijms251910821

Ghandehari, F., Behbahani, M., Pourazar, A., & Noormohammadi, Z. (2015). In silico and in vitro studies of cytotoxic activity of different peptides derived from vesicular stomatitis virus G protein. Iranian Journal of Basic Medical Sciences, 18(1), 47–52.

Huang, H., Cen, J., Yang, D., Li, L., Li, C., Yang, X., Wang, Y., Hu, X., Wang, J., & Cai, Q. (2023). Isolation and characterization

of antioxidant peptides from oyster (*Crassostrea rivularis*) protein enzymatic hydrolysates. Food Science & Nutrition, 11(1), 261–273. https://doi.org/10.1002/fsn3.3058

Khemaissa, S., Sagan, S., & Walrant, A. (2021). Tryptophan, an amino-acid endowed with unique properties and its many roles in membrane proteins. Crystals, 11(9), 1032. https://doi.org/10.3390/cryst11091032

Kumar, P., Kizhakkedathu, J.N., & Straus, S.K. (2018). Antimicrobial peptides: Diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. Biomolecules, 8(1), 4. https://doi.org/10.3390/biom8010004

Obafemi, Y.D., Obiukwu, A.C., & Oranusi, S.U. (2025). Revisiting the application, current trends, and prospect of bacteriocins in food preservation. Discover Food, 5(1), 165. https://doi.org/10.1007/s44187-025-00472-w

Purohit, K., Reddy, N., & Sunna, A. (2024). Exploring the potential of bioactive peptides: From natural sources to therapeutics. International Journal of Molecular Sciences, 25(3), Article 3. https://doi.org/10.3390/ijms25031391

Shi, C., Liu, M., Zhao, H., Lv, Z., Liang, L., & Zhang, B. (2022). A novel insight into screening for antioxidant peptides from hazelnut protein: Based on the properties of amino acid residues. Antioxidants, 11(1), 127. https://doi.org/10.3390/antiox11010127

Teshome, E., Forsido, S.F., Rupasinghe, H.P.V., & Olika Keyata, E. (2022). Potentials of natural preservatives to enhance food safety and shelf life: A review. The Scientific World Journal, 2022, 9901018. https://doi.org/10.1155/2022/9901018

Ucak, I., Afreen, M., Montesano, D., Carrillo, C., Tomasevic, I., Simal-Gandara, J., & Barba, F.J. (2021). Functional and bioactive properties of peptides derived from marine side streams. Marine Drugs, 19(2), 71. https://doi.org/10.3390/md19020071

Zhao, T., Su, G., Zhang, L., Chen, J., Zhang, Y., Liu, W., Zhao, M., Zhang, J., & Huang, Q. (2024). A comprehensive review of specific activity and intrinsic connections of food-derived bioactive peptides for human health. Food Frontiers, 5(3), 1145–1165. https://doi.org/10.1002/fft2.373

Zhu, Z., Xu, Z., Li, Y., Fan, Y., Zhou, Y., Song, K., & Meng, L. (2024). Antioxidant function and application of plant-derived peptides. Antioxidants, 13(10), 1203. https://doi.org/10.3390/antiox13101203

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. Molecules (Basel, Switzerland), 21(1), 72. https://doi.org/10.3390/molecules21010072