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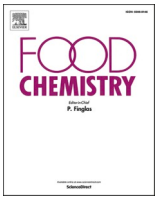

Discovery of novel umami peptides and their bitterness masking effects from yellowfin tuna (*Thunnus albacares*) via peptidomics, multisensory evaluation, and molecular docking approaches

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ABSTRACT

In this study, we identified and screened nine novel umami peptides derived from yellowfin tuna (*Thunnus albacares*) utilizing peptidomics combined with various machine learning based umami screening methodologies, including UMPred-FRL, TPDM, Umami-MRNN, and iUmami-SCM. The results of the multisensory evaluation indicated that SI-5 and VE-8 exhibited a high intensity of umami flavor, while AP-6, LD-5, and LT-5 effectively masked bitterness without compromising umami intensity. Furthermore, molecular docking techniques revealed interactions between the identified umami peptides and T1R1/T1R3 umami receptors as well as TAS2R14 bitter receptors, highlighting critical binding residues. Notably, LT-5 demonstrated a significant capacity to reduce quinine concentration, thereby enhancing the bitter-masking effect and elevating the bitterness threshold. These findings highlighted the potential of tuna-derived peptides as natural umami enhancers and bitterness inhibitors, providing valuable prospects for flavor improvement in food products.

1. Introduction

Umami, commonly referred to as the "fifth taste," is essential for enhancing the flavor profile of a wide range of foods (Zhang et al., 2019). It contributes significantly to sensory enjoyment and depth of flavor, rendering dishes more satisfying and well-balanced. This savory taste is essential for enriching the overall dining experience. The umami sensation, primarily elicited by amino acids such as glutamate and certain peptides, is vital for human nutrition and food satisfaction. Foods rich in umami typically exhibit complex and robust flavor profiles, thereby intensifying the sensory experience of taste. Moreover, foods that are rich in umami have been associated with increased satiety, thereby contributing to the regulation of appetite and the management of food intake (Abeywickrema et al., 2022; Masic & Yeomans, 2014). Umami peptides, which are naturally found in ingredients such as seafood and mushrooms, have been shown to stimulate salivary production (Shi et al., 2024; Yu et al., 2023). Enzymatic treatments can generate flavor-enhancing peptide derivatives enriched with hydroxyl or amino groups (Zhang et al., 2019). This process not only enhances the perception of taste but also facilitates digestion by improving the breakdown of food and the absorption of nutrients. Consequently, umami contributes to both the enjoyment of flavor and the efficiency of nutrient absorption (Shi et al., 2024; Yu et al., 2023).

Umami peptides possess taste-modulating properties, enhancing the perception of food flavors. For instance, umami peptides can intensify salty flavors while masking bitterness (Cai et al., 2024; Li et al., 2023; Zhang et al., 2023). Umami is particularly valuable in improving the flavor of low-sodium foods. Umami compounds can substitute for salt, reducing dietary sodium intake and lowering the risk of hypertension and cardiovascular disease in adults (Crowe-White et al., 2023). By enhancing the overall taste experience, umami ensures reduced-sodium foods remain flavorful and appealing, benefiting those managing their salt intake for health reasons (Ma et al., 2024; Shi et al., 2024). Additionally, umami peptides can diminish the intensity of bitter compounds. Tokita and Boughter studied the interaction between bitter and umami flavors by exposing C57BL/6J mice to sucrose mixed with quinine hydrochloride (QHCl) or umami stimuli and recording neuronal activity, confirming umami's masking effect on bitterness (Tokita &

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Boughter, 2012). Fraction (F05), a key umami-active component in Korean soy sauce, reduced perceived bitterness and inhibited the intracellular Ca^{2+} response to caffeine in human bitter taste receptor cells expressing hTAS2R43 and hTAS2R46 (Kim et al., 2017). A previous study demonstrated that umami peptides suppress bitterness by interacting with bitter receptors (Kim et al., 2015). Identifying and mitigating bitterness-causing compounds or utilizing bitterness-masking agents can significantly enhance the sensory quality of foods.

Peptidomics is a scientific discipline focused on low molecular weight proteins and active peptides, examining their patterns of change and enabling large-scale characterization. Peptidomics analysis allows efficient identification and screening of umami peptides in foods. For instance, high-resolution mass spectrometry and ab initio sequencing in peptidomics enable precise measurement of peptides in food products, facilitating the identification of potential umami peptides (Gao et al., 2024; Ju et al., 2024). Peptidomics analysis qualitatively and quantitatively reveals intact, non-targeted peptide fingerprints within a given matrix (Gu et al., 2020). Peptidomics technology is an extension of proteomics. Recently, integrating peptidomics with machine learning has proven to be a reliable, accurate, and efficient method for identifying umami peptides. This approach improves screening efficiency and establishes a robust scientific basis for subsequent sensory evaluations (Gong et al., 2025; Gu et al., 2025; Ju et al., 2024).

Yellowfin tuna (*Thunnus albacares*) is a pivotal species in high-quality fisheries and exhibits a broad distribution across tropical and subtropical marine environments. This species is celebrated for its exceptional flavor profile and rich nutritional composition (Sun et al., 2024). In this study, novel umami peptides exhibiting bitter-masking effects were identified from yellowfin tuna through enzymatic hydrolysis and subsequently characterized using peptidomics protocols. Employing multisensory evaluation and molecular docking techniques, it was determined that an increased concentration of these peptides significantly inhibited the perception of bitterness. Notably, the binding sites of AP-6 and LT-5 on bitter receptors were found to overlap with critical binding sites for quinine, resulting in competitive inhibition. Although umami peptides have been extensively investigated for their properties in enhancing umami flavor, their potential role in modulating bitterness has received limited research attention. Furthermore, there has been insufficient exploration of the interactions between umami peptides and bitter taste receptors (e.g., TAS2R) through bioinformatics approaches combined with sensory validation. In this study, we systematically investigated the potential of umami peptides extracted from yellowfin tuna to mask bitter taste through a combination of peptidomics, machine learning-based predictions, molecular docking, and sensory analysis. These findings not only offer novel insights for the development of natural agents that mask bitter flavors but also contribute to the broader application of functional peptides within the food industry. Ultimately, this research aims to enhance food quality and improve taste experiences.

2. Materials and methods

2.1. Materials and chemicals

Yellowfin tuna (*Thunnus albacares*), provided by Zhejiang Ocean Family Co., Ltd. in Zhoushan, China, was transported to our laboratory within three hours via a cold chain at −20 °C and promptly stored at −40 °C until needed. Chymopapain and flavourzyme were sourced from Shanghai Solarbio Bioscience & Technology Co., Ltd. in Shanghai, China. Wuhan Dan Gang Biotechnology Co., Ltd. (Wuhan, China) synthesized potential umami peptides with >95 % purity, including APEEHP, DDLTVT, DFKSPDDPSRY, FDQDDWE, LDPFE, LTEAP, SIEDP, VEPEILPD, and WDDMEK.

2.2. Preparation of enzymatic hydrolysates from yellowfin tuna

Collect the flesh from the belly and back of the yellowfin tuna were removed after thawing under running water for 30 min. Mince the processed fish with a meat grinder and weigh 50 g surimi. Fish mince was mixed with purified water at a ratio of 1:4 (*w/v*) and homogenized for 10 min to obtaining a homogeneous mixture of liquid suspensions. The homogenate was hydrolyzed with papain at 50 °C for 150 min, using an enzyme concentration of 2500 U/g. After heating the enzymatic solution for 10 min at 100 °C, it was allowed to cool to ambient temperature. The inactivated hydrolysate was further hydrolyzed with flavor protease at 50 °C for 150 min, using an enzyme concentration of 2500 U/g (Noman et al., 2018; Xu et al., 2022). A high-speed centrifuge was used to filter the enzymatic hydrolysate through an 80-mesh sieve and centrifuge it for 10 min at 4 °C and 12,000 g. After being collected, the supernatant was lyophilized and kept for later use at −18 °C.

2.3. Peptidomics-based identification of peptides from enzymatic hydrolysates

The Orbitrap Exploris 480 mass spectrometer coupled with an EASY-nanoLC1200 system (Thermo Fisher Scientific, MA, USA) was used to analyze the peptides. The hydrolysates were redissolved in solvent A (0.1 % formic acid in water). A 25 cm analytical column (75 μm inner diameter, 1.9 μm resin, Dr. Maisch) was loaded with a 1 μL peptide sample, and a gradient separation process of 60 min was used. At 2.2 % buffer B (80 % ACN with 0.1 % FA), the gradient began, increased to 90 % over 54.5 min, reached 99 % in 0.5 min, and was maintained for 5 min. The column temperature was kept at 40 °C, and the flow rate was fixed at 350 nL/min. 2 kV was used as the electrospray voltage. The mass spectrometer switched between MS and MS/MS modes automatically while operating in data-dependent acquisition (DDA) mode. At a resolution of 60,000, full-scan MS spectra (*m/z*: 200–1500) were obtained using the Orbitrap. With a maximum injection time of 25 ms, the normalized automatic gain control (AGC) objective was set at 300 %. Higher-energy collision dissociation (HCD) with a normalized collision energy of 30 % was used to break up precursor ions in the collision cell. A normalized AGC target of 50 %, a maximum injection time of 22 ms, a dynamic exclusion duration of 30 s, and an MS/MS resolution of 15,000 were all established.

Tandem mass spectra were analyzed using PEAKS Studio version 10.6 (Bioinformatics Solutions Inc., Waterloo, Canada). The database used was *Thunnus albacares* (version 2024, 360 entries), downloaded from UniProt. No digestion enzyme was specified. With a parent ion mass tolerance of 10 ppm and a fragment ion mass tolerance of 0.02 Da, the PEAKS DB search was conducted. Methionine oxidation and asparagine and glutamine deamidation were designated as variable changes. Proteins having at least one distinct peptide and peptides with a -10lgP score $\geq$ 20 were kept.

2.4. Screening of the potential umami peptides

Machine learning processes large datasets to extract insights through predictive analytics, generating actionable information and facilitating decision-making across diverse applications. Machine learning algorithms were applied to evaluate additional peptide characteristics, enhancing the accuracy of umami peptide selection. In this study, the potential umami peptides were screened by the following protocols: UMPred-FRL (https://pmlabstack.pythonanywhere.com/UMPred-FRL) is a sequence-based meta-predictor that enhances the accuracy of umami peptide predictions through feature representation learning. TPDM (http://tastepeptides-meta.com/TPDM) is a computational tool designed to identify and characterize peptides with umami or bitter flavors, utilizing co-linked receptor ligand residues and molecular fingerprinting data. Umami-MRNN (https://umami-mrnn.herokuapp.com/) evaluates the umami characteristics and thresholds of

anticipated peptides by combining two neural network models. iUmami-SCM (https://camt.pythonanywhere.com) identifies and characterizes umami peptides based on peptide sequence information. These predictors were used to assess the flavor profile of the peptides. The following requirements must be fulfilled by the predicted peptides: UMPred-FRL > 0.5, recognition as "umami" by both TPDM and Umami-MRNN, and an iUmami-SCM score > 588. The effectiveness and dependability of these computational approaches in accelerating the discovery of novel umami peptides have been confirmed by numerous investigations (Li, Hua, et al., 2023).

The identified umami peptides were analyzed for their physicochemical properties, allergenicity, and toxicity using various bioinformatics tools. Hydrophobicity was assessed using PepDraw (http s://www.pepdraw.com), while solubility was evaluated with the proteomics tool provided by Innovagen (http://www.innovagen.com). Toxicity and allergenicity were predicted using ToxinPred (https://webs .iiitd.edu.in/raghava/toxinpred/design.php) and AlgPred 2.0 (https:// webs.iiitd.edu.in/raghava/algpred2/batch.html), respectively.

2.5. Sensory evaluation

Sensory analysis was performed by a trained panel consisting of individuals aged 20 to 30. Five men and five women from Institute of Seafood, Zhejiang Gongshang University were on the panel. Panelists underwent a 4-week training session prior to the examination to ensure they could correctly identify five basic tastes: umami (monosodium glutamate), salty (NaCl), bitter (quinine), sour (citric acid), and sweet (sucrose). Table S1 summarizes the descriptive terms and corresponding evaluation results. At an average temperature of 25 ± 1 °C, samples prepared in a 1 mg/mL solution were moved to tiny glass cups and subjected to a methodical evaluation by panelists. The Ethics Committee of Zhejiang Gongshang University gave its permission for all sensory analyses carried out in this study.

2.5.1. Sensory characteristics of identified umami peptides

The identified peptides were dissolved in ultrapure water at a concentration of 1 mg/mL. The taste characteristics of the samples were evaluated and scored according to the sensory evaluation results (Table S1).

2.5.2. Determination of bitterness masking effects of identified umami peptides

A standard solution was prepared with quinine. Ultrapure water and 1 mM quinine were mixed at a 3:5 ratio as a positive control. Solutions of AP-6, LD-5, and LT-5 at concentrations of 0.1 mg/mL, 0.3 mg/mL, and 0.5 mg/mL, respectively, were prepared by mixing with 1 mM quinine in the same ratio (Kan et al., 2023). The bitterness intensity of the solutions was assessed through sensory evaluation. The bitterness intensity of the positive control was set to 10, while pure water was 0.

2.5.3. Determination of bitterness reduction threshold of identified umami peptides

Dissolve AP-6, LD-5, and LT-5 peptides in ultrapure water to a concentration of 1 mg/mL, and add quinine to a final concentration of 0.1 mg/mL. Determine the bitterness reduction threshold of peptides using a triangle test. Gradually add quinine to the solution until the sensory group can feel the bitterness. The bitterness reduction threshold for each peptide is calculated as the average of the final and second to last concentrations of bitterness detected by sensory group members.

2.6. Bitterness intensity assay in vitro

An electronic tongue, SA402B (INSENT, Japan) equipped with a taste sensor. Bitterness intensity was determined based on the potential difference between the coated sensor and the reference electrode. The bitterness intensity value reflects the degree of bitterness. The bitterness intensity of potential peptides and positive controls was measured at a concentration of 0.3 mg/mL. The bitterness intensity of peptide-quinine mixtures (1 mM quinine, mixed at a 3:5 ratio) was measured using an electronic tongue. The bitterness intensity was evaluated based on the potential difference between the conventional reference electrode and the bitterness sensor. The percentage of TAS2R14 blockade was calculated using the electronic tongue method described below:

$$\text{The TAS2R14 blocking activity } (\%) = (A - B)/A^{*}100\%$$

A represents the bitterness value of quinine reaction solution, and B represents the bitterness value of a mixed solution of peptides and quinine.

2.7. Molecular docking

Homology modeling was used to create the three-dimensional structure of T1R1/T1R3 (PDB ID: 5K5S). The receptor sequence was retrieved from the AlphaFold database via the RCSB Protein Data Bank (https://www.rcsb.org/). AutoDock Vina and AutoDock Tools 1.5.6 were employed to perform molecular docking of receptor-ligand interactions. Discovery Studio 2019 was utilized to generate two-dimensional representations of the docking results.

The three-dimensional structure of TAS2R14 was obtained from the BitterDB database (BitterDB ID: 14). TAS2R14 has a molecular weight of 33.61 kDa. Hydrogen atoms were the first atoms added to TAS2R14 during preparation. AutoDock Vina and AutoDock Tools 1.5.6 were also used to dock receptor ligands with ligand-free TAS2R14. The docking pocket of TAS2R14 was defined with coordinates x: 5.449, y: −6.607, and z: −4.309. All other parameters were set to default values. The affinity of peptides for TAS2R14 was predicted based on the CDOCKER energy value (in kcal/mol). Screened umami peptides were docked with TAS2R14, and peptides with docking energies below −5 kcal/mol were selected for subsequent bitter taste masking experiments. PROCHECK was used to evaluate the resultant homology models' quality. Ramachandran plots for umami and bitter flavor receptors are presented in the supplementary materials (Laskowski et al., 1993).

2.8. Statistical analysis

Study results are expressed as mean ± standard deviation (mean ± SD), with each experiment performed in triplicate. The statistical analysis was conducted using SPSS (Statistical Product and Service Solutions. Error bars, representing the standard deviation of experimental results, were generated using Origin 2024 software.

3. Results and discussion

3.1. Identification of peptides in yellowfin tuna based on peptidomics

Peptidomics is a computational and analytical technique, belonging to a branch and complement of proteomics, which allows the classification, screening and identification of complex peptide fractions in foods (Chen et al., 2024; Wang et al., 2024). Mass spectrometry is the core tool of peptiomics. Data interpretation and analysis of peptiomics is a crucial step in the entire research process (Portmann et al., 2023). Mass spectrometry-based peptidomics offers a highly effective approach for the identification of peptides, which are involved in various functional roles, including umami peptides, antioxidant peptides, and antihypertensive peptides (Cournoyer et al., 2025; Gao et al., 2024). Bioinformatic and statistical methods are used to screen, quantify, identify and annotate mass spectrometry data to identify biologically significant peptides and their functions. Approximately 1800 peptides were identified in each sample using nanoLC-MS/MS analysis. The peptide sequences were determined by database matching (Uniprot *Thunnus albacares*) through Peaks software, based on fragmentation into

b- and y-ions. Research indicates that umami peptides typically have a low molecular weight, generally below 1500 Da (Zhang et al., 2019) These identified peptides were further filtered based on their molecular weights (<1500 Da), and subsequently subjected to umami potential prediction. Based on the identification results, peptides within this molecular weight range were preferentially selected for further analysis to assess their umami potential. Computer simulations and machine learning algorithms, including iUmami-SCM and UMPred-FRL, were then used to predict the umami potential of the selected peptides.

3.2. Screening of potential umami peptides from yellowfin tuna

The remaining peptides were then screened for umami properties using UMPred-FRL, TPDM, Umami-MRNN, and iUmami-SCM (Charoenkwan et al., 2020; Charoenkwan et al., 2021; Cui et al., 2023; Qi et al., 2023). In these screening tools, the umami properties of peptides were assessed through various methodologies employed by different models. UMPred-FRL classified peptides as umami if their prediction probability exceeded 0.5. TPDM evaluated the potential for umami in polypeptides based on a probability fraction. Umami-MRNN verifies the umami taste of peptides by predicting and establishing specific thresholds; iUmami-SCM categorizes peptides as umami based on an S(*P*) score greater than 588. Consequently, as indicated in Table 1, a screening process was used to select and qualify nine peptides. Fig. S1 displays their MS/MS spectrum. The potential umami peptide with the highest S(P) score (663.6) among the nine that were found was AP-6. There were distinct umami fragments in every putative umami peptide. Additionally, the BIOPEP-UWM database was used to assess the umami components of yellowfin tuna peptides (Iwaniak et al., 2024).

Peptide hydrophobicity and water solubility are key factors, as they greatly affect receptor interactions and biological activity (Ramos & Kempka, 2024). Innovative server and PepDraw were applied to estimate the physicochemical characteristics of umami peptides, with a focus on their solubility in water and hydrophobic nature. As demonstrated in Table 1, all nine identified umami peptides exhibited favorable hydrophobicity and water solubility. Peptides with high water solubility are likely to demonstrate improved bioavailability (Lafarga et al., 2015). Meanwhile, the nine umami peptides showed non-toxicity after screening with the toxinpred tool. All of them showed Non-Allergen after Algpred 2.0, which indicates that the nine umami peptides can be important candidates for subsequent work on umami peptides as well as bitter-reducing peptides.

3.3. The umami peptide source analysis

Additionally, source study of the precursor proteins of the nine umami peptides was carried out. As shown in Fig. 1, LD-5 originates from cytochrome *c* oxidase subunit III (COX3). Generally, COX3 is an essential part of the mitochondrial electron transport chain's cytochrome *c* oxidase complex in fish (Farhadi et al., 2023). The multimeric complex of COX in mitochondria, which acts as a terminal enzyme in the electron transport chain, catalyzing the reduction of dioxygen (O2) to water. LT-5, AP-6, and WD-6 are derived from beta-actin. Beta-actin plays a crucial role in providing structural support to fish muscle fibers and facilitating cell migration. These functions are essential for fish swimming, predation, and adaptation to environmental changes (Cornejo et al., 2010). VE-8 originates from fructose-bisphosphate aldolase. In yellowfin tuna muscle tissue, fructose-bisphosphate aldolase is crucial for meeting the high energy demands of the fish. Fish continuously produce ATP to sustain rapid movement, with fructose-bisphosphate aldolase supplying ATP for muscle activity via glycolysis. DF-11, DD-6, SI-5, and FD-7 are derived from alpha-enolase. Alpha-enolase is essential for glycolysis, catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate, thereby supplying ATP for rapid muscle movement. As a highly active species, yellowfin tuna relies on alpha-enolase activity to meet its substantial energy demands (Keller et al., 2000; Nakagawa & Nagayama, 1989). The primary focus of this study was on umami peptides derived from yellowfin tuna. Consequently, the identification of the corresponding precursor proteins served to confirm that these peptides are indeed endogenous to tuna muscle proteins. This information not only reinforces the authenticity and specificity of the peptide source but also provides valuable insights into the regions of proteins that are susceptible to generating umami peptides.

3.4. Sensory evaluation and bitter taste inhibition effect of identified umami peptides

3.4.1. Sensory evaluation of the identified umami peptides

The taste profiles of umami peptides were assessed in this study through sensory evaluation. Among the umami peptides examined, SI-5 exhibited the highest umami score, as illustrated in Fig. 2A. In contrast, the umami scores of other peptides, including VE-8 and WD-6, decreased successively until reaching FD-7, which recorded the lowest umami score. These findings indicate significant differences in the umami

Table 1
Amino acid sequence and physicochemical properties of the identified umami peptides.

Peptides	Abbreviations	Amino acid No.	The ratio of D to E	Formula weight/Da	Hydrophobicity[1] /kcal/mol	Water solubility[2]	S(P) score[3]	Corresponding umami fragments[4]	Toxicity[5]	Allergic[6]
APEEHP	AP-6	6	0.33	678.69	18.27	Good	663.6	PE, EE	Non-Toxin	Non-Allergen
DDLTVT	DD-6	6	0.33	662.69	13.97	Good	644.0	DD, DL	Non-Toxin	Non-Allergen
DFKSPDDPSRY	DF-11	11	0.27	1326.37	22.21	Good	591.2	DD	Non-Toxin	Non-Allergen
FDQDDWE	FD-7	7	0.58	953.9	19.42	Good	619.5	DD	Non-Toxin	Non-Allergen
LDPFE	LD-5	5	0.40	619.66	12.35	Good	588.3	D	Non-Toxin	Non-Allergen
LTEAP	LT-5	5	0.20	529.58	11.17	Good	590.0	TE, E	Non-Toxin	Non-Allergen
SIEDP	SI-5	5	0.40	559.57	14.65	Good	595.0	ED, E	Non-Toxin	Non-Allergen
VEPEILPD	VE-8	8	0.38	911.01	16.25	Good	607.1	VE, PE	Non-Toxin	Non-Allergen
WDDMEK	WD-6	6	0.50	822.88	18.85	Good	643.8	DD, E	Non-Toxin	Non-Allergen

Note: "1" represents the prediction of hydrophobicity (PepDraw); "2" represents the prediction of water solubility (Proteomics tools); "3" represents umami score prediction (iUmami-SCM); "4" represents the prediction of umami feature fragments (BIOPEP-UWM) "5" represents prediction of toxicity; "6" representing the prediction of allergenicity.

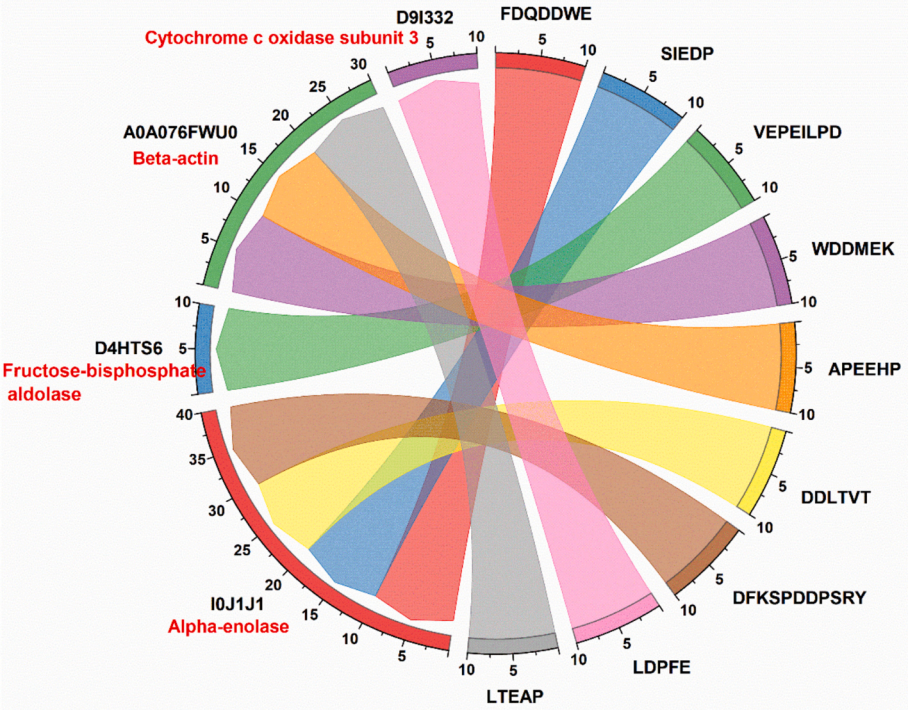

Fig. 1. Phylogenetic tree of potential umami peptide sequences identified in *Thunnus albacares* (Cytochrome c oxidase subunit 3, Beta-actin, Fructose-bisphosphate aldolase, Alpha-enolase).

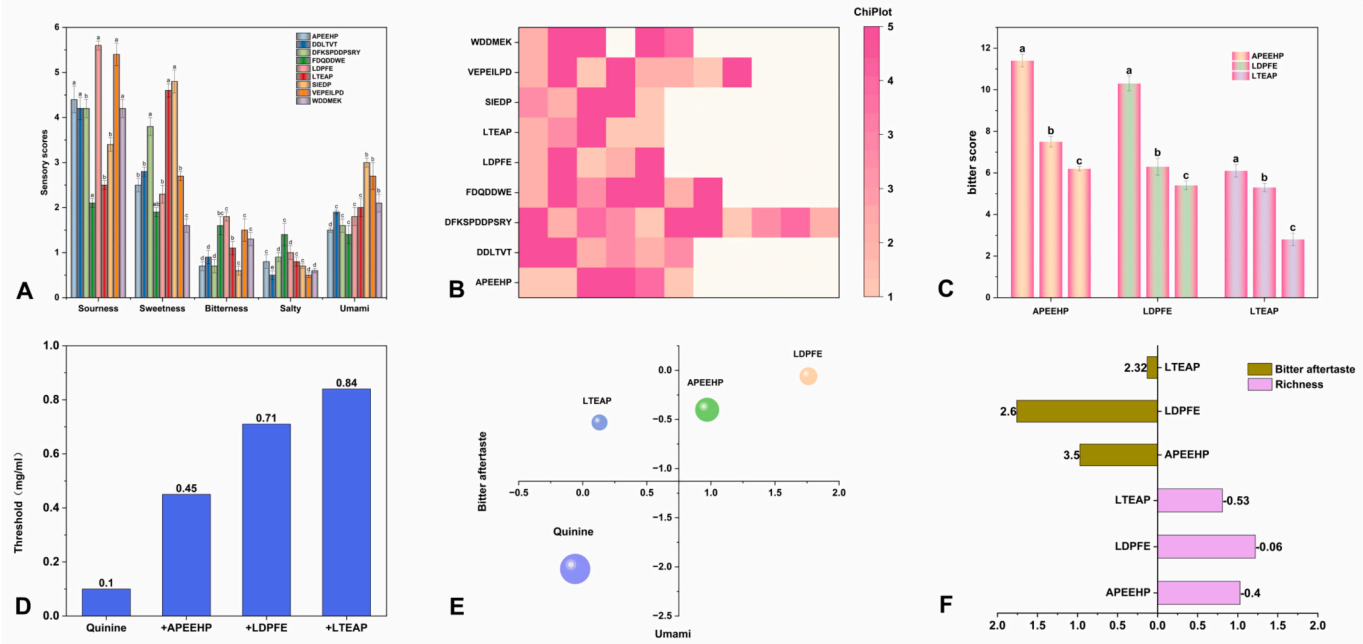

Fig. 2. (A) Sensory evaluation of identified umami peptides; (B) Amino acid fingerprints of nine identified umami peptides, indicating that the type of amino acids affects umami intensity. The darker the red color, the stronger the perceived umami taste; (C) Correlation between synthetic peptide concentration and its bitterness inhibitory effect; (D) Effect of synthetic peptides on quinine taste detection threshold; (E) Bubble chart of umami peptides, illustrating their umami intensity, bitterness (Bubble size) and bitter aftertaste; (F) Bitterness (Tag number) and bitter aftertaste of umami peptide system with umami (Tag number) and richness. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

intensity of various umami peptides. This phenomenon can be attributed to the capacity of individual amino acids to enhance distinct umami flavors, as illustrated in Fig. 2B, which delineates the contribution of each amino acid to umami perception. SI-5 exhibited the highest intensity of umami, likely due to its elevated proportion of umami-related amino acids with lower relative molecular weights. For instance, LD-5 demonstrated a higher umami intensity compared to LD-6 at an equivalent mass concentration, despite both having identical percentages of acidic amino acids. Earlier studies have found that aspartic acid (Asp) and glutamic acid (Glu) are the main amino acids for umami production

(Cui et al., 2024). As shown in Table 1, Asp and Glu were present in all the umami peptides analyzed in this study, with FD-7 containing the highest percentage of these amino acids (57.7 %). In addition, the result also revealed significant differences in sour, sweet, bitter, and salty profiles among the nine umami peptides. Specifically, LD-5 and SI-5 displayed notable acidity. This study employs static sensory assessment; however, time intensity (TI) analysis may offer more nuanced insights into the temporal characteristics of taste perception, encompassing onset, persistence, and aftertaste. Future research utilizing TI technology could enhance our understanding of the dynamic taste changes associated with umami peptides. Shi et al. integrated visual and gustatory sensations of food products for a comprehensive sensory evaluation. This approach also serves as an effective method for characterizing the taste profile of umami peptides (Shi et al., 2025).

3.4.2. Effect of screened umami peptides on the intensity of quinine bitterness

As shown in Fig. 2C, LT-5 exhibited the most pronounced bitter taste inhibition. The bitterness score decreased to 2.3 points in a 0.3 mg/mL mixture of LT-5 and quinine solution. LT-5 demonstrated greater effectiveness in suppressing bitter intensity at the same concentration compared to the other two umami peptides. The results also indicated that TAS2R14 blocking activity increased with rising peptide concentration, aligning with findings from previous studies (Kan et al., 2023; Xiang et al., 2024). Based on the capacity of umami peptides to mitigate bitterness, future researchers may explore the development of food products that effectively mask the undesirable flavors inherent in certain ingredients.

3.4.3. Effect of screened umami peptides on quinine bitterness thresholds

The peptides AP-6, LD-5, and LT-5 not only influenced the intensity of bitter substances but also affected their taste thresholds. As illustrated in Fig. 2D, LT-5 raised the taste threshold of quinine to 0.84 mg/mL, potentially due to its ability to reduce bitterness intensity. In all three umami peptide solutions, the quinine taste threshold was significantly elevated. Previous studies suggest that peptides with relatively low molecular weights are generally more effective as bitter taste receptor inhibitors, which may explain LT-5's superior ability to inhibit bitterness (An et al., 2024; Kan et al., 2023). The glutamyl peptides investigated by Kim et al. demonstrated the ability to reduce bitterness through non-competitive inhibition of the salicyloside-induced intracellular Ca^{2+} response. This approach illustrates the gustatory interactions between umami and bitterness at the cellular level. The bitter-reducing peptides utilized in this study all contain glutamyl peptide fragments, which effectively inhibit bitter taste receptors, thereby competitively inhibiting bitter substances (Kim et al., 2015).

3.5. In vitro validation of bitter masking effects by e-tongue

Furthermore, the bitter-masking effect of umami peptides was confirmed in vitro using an electronic tongue. As shown in Table 2, when the concentration of added umami peptide was 0.3 mg/mL, the inhibition rates of AP-6, LD-5 and LT-5 were 20.6 %, 41 % and 47.1 %, respectively. The application of this umami peptide effectively reduced the perceived bitterness, demonstrating high efficiency as a physical masking strategy (Bertelsen et al., 2018; Xu et al., 2019). That is to say, LD-5 and LT-5 exhibit higher quinine masking activity compared to AP-6. Therefore, LD-5 and LT-5 are identified as the most potent bitter receptor blockers, effectively reducing bitter intensity.

Table 2
In vitro bitterness intensity assay results.

Peptides	Bitter intensity	blocking activity(%)
Quinine	4.41	/
APEEHP	3.50	20.6
LDPFE	2.60	41.0
LTEAP	2.32	47.4

As shown in Fig. 2E, after mixing 0.3 mg/mL peptide solution with 1 mM quinine solution in a 3:5 ratio, none of the three solutions exhibited umami flavor. This indicates that the bitter substances can mask the umami flavor produced by the low concentration of umami peptides. Meanwhile, the three peptides mixed with quinine exhibited varying bitter aftertaste profile. The pure quinine solution showed no bitter aftertaste. Solutions containing added peptides exhibited distinct bitter aftertaste characteristics. LD-5 exhibited the most pronounced bitter aftertaste. As indicated by the electronic tongue experiment, low concentrations of LD-5 mixed with quinine significantly enhanced the bitter aftertaste. Additionally, the electronic tongue was employed to investigate the beneficial interaction between umami peptides and bitter substances. The results demonstrated a gradual decrease in bitterness intensity with increasing concentrations of the LD-5 umami peptide. Table S2 displays the outcomes of the electronic tongue experiment. This phenomenon may result from peptides altering the overall taste profile during gustatory perception. Mixing may result in a more complex or prolonged bitter flavor. Previous studies have shown that peptides or proteolytic products influence taste perception (Bigiani & Rhyu, 2023). Fig. 2F demonstrates that in peptide-quinine mixtures, bitterness and bitter aftertaste do not exhibit a direct linear relationship. The LD-5 system shows the strongest bitter aftertaste compared to AP-6 and LT-5, but exhibits less bitterness than the AP-6 system. This suggests that there is no direct linear relationship between bitter and bitter aftertastes in this system. The labels in Fig. 2F indicate that the umami flavor of the peptide-quinine system is positively correlated with its richness. A more pronounced umami taste within this system corresponds to an increased level of richness. This suggests that the richness of the system is significantly influenced by the umami characteristics of peptides.

3.6. Molecular docking

3.6.1. Molecular docking of umami receptor T1R1/T1R3

To clarify the mechanism of interaction between umami peptides and T1R1/T1R3 receptors, molecular docking was utilized in this investigation. The model was successfully established (Fig. S2) and subsequently validated (Fig. S3), prove that the model is reasonable. The active amino acid residues of the receptor are illustrated in Fig. 4, highlighting the magnitude of the interaction force between the receptor and the ligand. These peptides primarily bind to the umami receptor through hydrogen bonding, which contributes to 11.5 %–46.2 % of the total interaction force. In contrast, hydrophobic and electrostatic interactions account for 0 %–42.3 % and 0 %–7.7 %, respectively, of the overall interaction force. This is consistent with previous research findings that indicate hydrogen bonding serves as a significant binding force in the association of umami peptides with umami receptors (Gu et al., 2025). The docking energies and binding locations of the nine umami peptides with T1R1/T1R3 are shown in Table 3. FD-7 > LT-5 > DF-11 > WD-6 > LD-5 > AP-6 > DD-6 ≥ SI-5 > VE-8. The FD-7 umami peptide, with a binding energy of −9 kcal/mol, exhibits stronger receptor affinity compared to the other umami peptides. Previous studies suggest that lower binding energies indicate greater stability of umami peptides in binding to receptors, potentially facilitating umami perception. The high affinity of FD-7 may be attributed to the presence of key acidic amino acid recognition residues (Ile-237 and Ser-240) in T1R1/T1R3, as this peptide has the highest proportion of acidic amino acids among the peptides studied. Acidic amino acids constitute 57.1 % of FD-7. However, consistent with other studies, this work suggests that there is no direct relationship between docking energy, peptide length, or umami intensity (Yu et al., 2021; Zhu et al., 2021).

In order to analyze the complex and explore the presentation mechanism of umami peptides, 3D and 2D images of nine umami peptides docked with T1R1/T1R3 were constructed (Fig. 3). Hydrogen bonds and electrostatic interactions were identified between umami

Table 3
Docking energy and binding site of the umami peptide with T1R1 / T1R3.

Peptides	Abbreviations	Docking energy (kcal/mol)	Binding site
APEEHP	AP-6	−7.5	Leu-242, Ser-244, Asn-178, Arg-172, Ser-175, Asn-189
DDLTVT	DD-6	−7.4	Pro-559, Cys-236, Glu-231, Arg-227
DFKSPDDPSRY	DF-11	−8.2	Lys-177, Asn-178, Glu-251, Asp-215, Cys-482, Ser-175, Asp-480, Glu-191, Glu-241, Lys-225, Glu-229
FDQDDWE	FD-7	−9.0	Cys-236, Pro-559, Ile-235, Gly-557, Ile-237, Arg-227, Ser-240, Asp-236, Glu-558
LDPFE	LD-5	−7.8	Glu-251, Asp-215, Arg-172, Asn-178, Asp-248
LTEAP	LT-5	−8.7	Tyr-218, Ser-272, Asp-275, Asn-102, Asp-216, Gln-245, Asn-176, Arg-220, Asp-217, Val-104
SIEDP	SI-5	−7.4	Ile-237, Ser-240, Glu-231, Cys-236, Asp-234, Gly-557
VEPEILPD	VE-8	−7.3	Asn-178, Glu-251, Cys-482, Leu-242, Arg-172, Thr-221
WDDMEK	WD-6	−8.1	Ser-240, Glu-232, Glu-228, Arg-227, Ile-237, Glu-231, Asp-238

peptides and umami receptors. In this experiment, hydrogen bonding and van der Waals forces were observed between the nine umami peptides and T1R1/T1R3. The findings are consistent with previous research, which indicated that the C-terminal residues of umami peptides have a greater likelihood of interacting with the T1R1/T1R3 active site compared to their N-terminal residues (Wang et al., 2022). In accordance with these published results, our findings suggest that hydrophobic and electrostatic interactions play a crucial role in the formation of umami peptide-receptor complexes (Gao et al., 2022). Furthermore, there are also pi-anion and alkyl-pi-alkyl interactions observed between certain umami peptides and receptors. These interactions bind to Glu, the umami receptor, and occur exclusively when umami peptides contain aromatic groups.

3.6.2. *Molecular docking of bitter receptor TAS2R14*

TAS2R14, an important bitter taste receptor in humans, belongs to the G protein-coupled receptor (GPCR) family. TAS2R14 recognizes and responds to various bitter taste molecules. It is primarily expressed in the taste receptor cells on the tongue. Binding of TAS2R14 to its bitter ligand activates the G-protein signaling pathway, resulting in an increase in intracellular calcium ion concentration and triggering the transmission of bitter neural signals to the brain (Carey et al., 2022). The homology modeling of the bitter taste receptor TAS2R14 were successfully modeled (Fig. S4) and validated (Fig. S5). Nine umami peptides were molecularly docked with the bitterness receptor TAS2R14. Three umami peptides (APEEHP, LDPFE, and LTEAP) exhibiting docking energies below −5.0 kcal/mol were selected for further investigation into mechanisms of bitterness reduction. As illustrated in Fig. 5, the optimal docking position between TAS2R14 and the peptide is depicted. In the molecular docking analysis, it was observed that both AP-6 and LD-5 occupy the cavity of the bitter taste receptor TAS2R14. This positioning may explain the higher binding energy associated with these compounds compared to LT-5 (Tokmakova et al., 2023). Table 4 indicates that AP-6 had docking energies comparable to or lower than those of LT-5. Notably, LT-5 was confirmed as the most effective agent in subsequent in vitro tests aimed at reducing bitter taste, aligning with sensory evaluation results. When docked to bitter taste receptors, AP-6 and LT-5 demonstrate more prominent binding sites compared to LD-5. Following its docking with TAS2R14, the Trp89 residue plays a significant role in forming Pi-Cation interactions. The interaction between peptide AP-6 and TAS2R14 involves residues Asp-168, Ile-262, Ser-250,

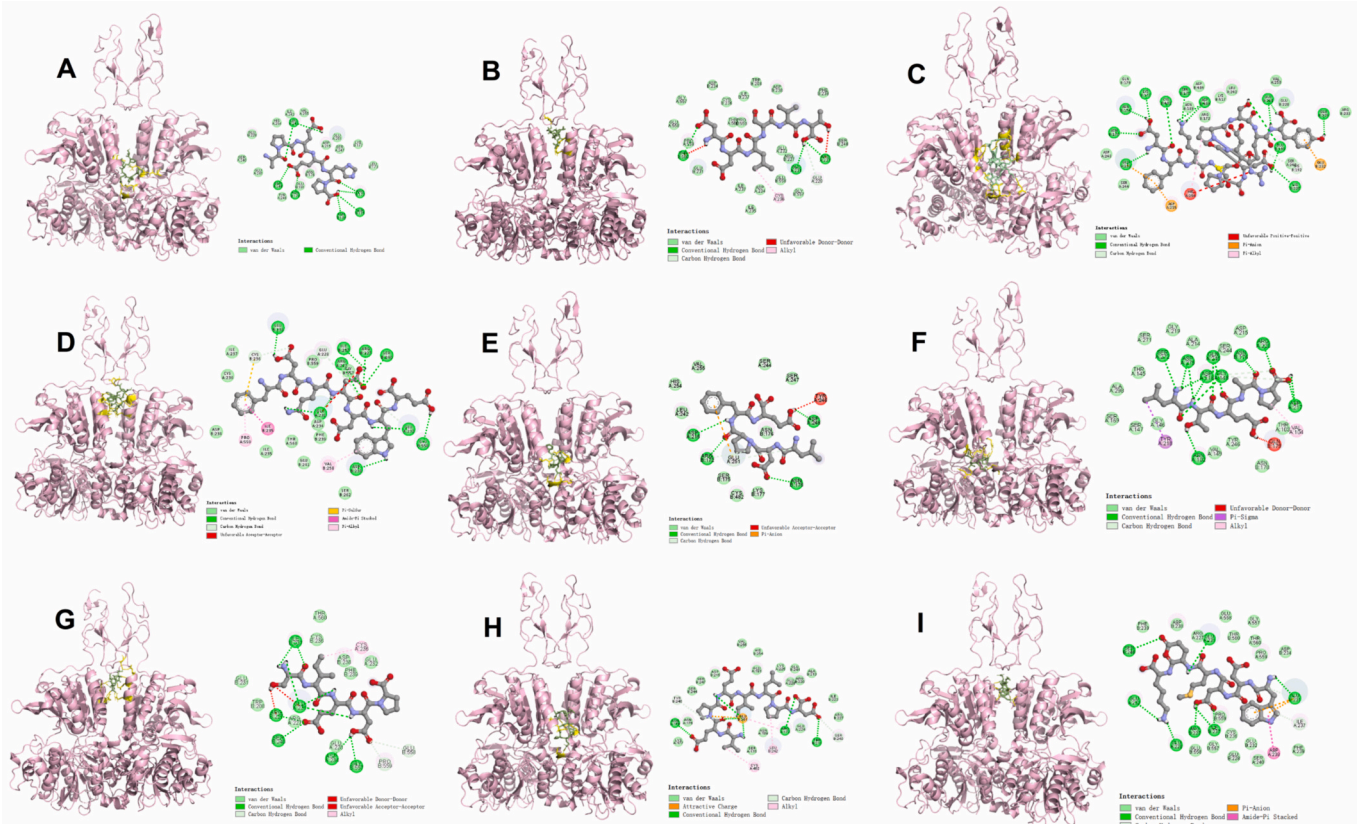

Fig. 3. (A–I) Molecular docking analysis between umami taste receptor T1R1/T1R3 and identified umami peptides.

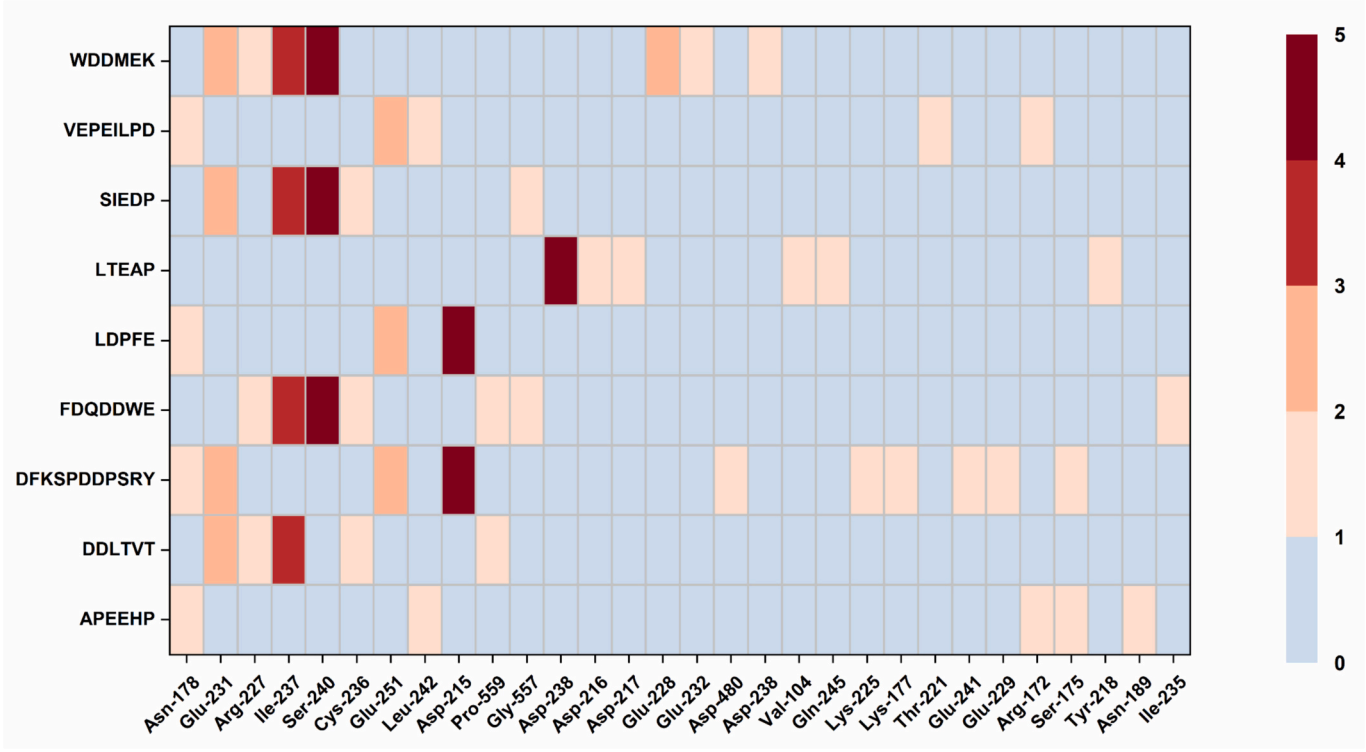

Fig. 4. Summary of the results of the docking of nine umami peptides with T1R1/T1R3 active amino acid residues (red colors indicate higher forces formed between the active sites of umami ligands and umami receptors). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

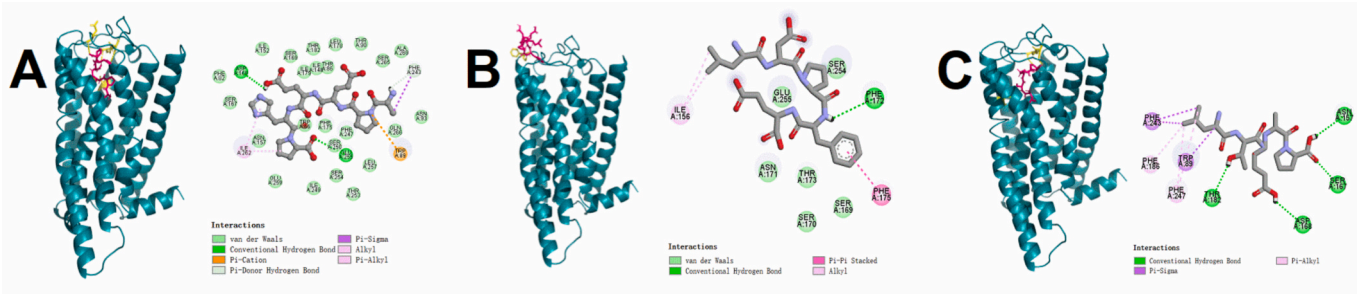

Fig. 5. (A–C) Molecular docking analysis between umami taste receptor TAS2R14 and identified umami peptides.

Table 4
Docking energy and binding site of the umami peptide with TAS2R14.

Peptides	Abbreviations	Docking energy (kcal/mol)	Binding site
APEEHP	AP-6	−7.6	Asp-168, Ile-262, Ser-250, Trp-89, Phe-243
LDPFE	LD-5	−6.2	Ile-156, Glu-255, Ser-254, Phe-172, Phe-175
LTEAP	LT-5	−7.6	Phe-243, Phe-186, Phe-247, Trp-89, Thr-182, Asp-168, Asn-157, Ser-167

Trp-89, and Phe-243. Residues Asp-168, Trp-89, and Phe-243 serve as common binding sites for both LT-5 and AP-6 systems. These amino acid residues may represent critical binding sites for the interaction of bitter substances with their corresponding receptors. Furthermore, Ile-262 and Trp-89 are essential residues implicated in quinine's binding to TAS2R14; they potentially play a crucial role in modulating TAS2R14's affinity for bitter compounds. As a complementary approach, we subsequently performed molecular docking of AP-6, LD-5, and LT-5 with the bitter taste receptors TAS2R10 and TAS2R46. As illustrated in Table S3, all three peptides demonstrated strong binding affinity to both of these bitter taste receptors and exhibited excellent binding energy.

3.6.3. Intermolecular interaction force analysis

Fig. S6 and Fig. S7 provide comprehensive information regarding the interactions between umami peptides and the T1R1/T1R3 as well as TAS2R14 receptors. These figures illustrate various types of interactions, including aromatic interactions, hydrogen bonding, ionization, hydrophobicity, and solvent accessibility, which are emphasized through color changes. Aromatic interactions were evaluated by analyzing the localized electrostatic interactions between protons on the side rings and the π-electron density present on the face rings (Li et al., 2020). A total of nine umami peptides were identified within T1R1/T1R3, with aromatic groups predominantly exposed on the surface of the binding pocket. This observation suggests that the aromatic ring of umami peptides may engage in π-π stacking interactions with surface aromatic groups of the receptor, thereby stabilizing ligand positioning within the binding

pocket. Furthermore, T1R1/T1R3 form numerous hydrogen bonds with all nine peptides, significantly enhancing binding stability. In contrast to umami receptors, when these three umami peptides interact with bitter receptors, a greater number of aromatic groups are found embedded within these receptors. This discrepancy may be attributed to differences in molecular structure between umami and bitter receptors. Additionally, there is a substantial presence of hydrogen bonds formed between bitterness receptor TAS2R14 and these three umami peptides.

The 3D hydrophobicity map indicates that the peptides exhibit a greater number of hydrophilic regions interacting with the umami receptor, while displaying more hydrophobic regions in interaction with the bitter taste receptor. For example, the blue region between LT-5 and T1R1/T1R3 demonstrates a significantly higher proportion of hydrophilic areas compared to hydrophobic ones. In contrast, LT-5 and TAS2R14 reveal an increased presence of hydrophobic regions. Furthermore, interactions between T1R1/T1R3 and the nine umami peptides indicate that the binding regions possess high solvent-accessible surface (SAS) areas. The strong binding forces generated by van der Waals interactions are likely responsible for this phenomenon. These findings offer valuable insights into ligand stability analysis and binding modes within the pocket.

4. Conclusions

In conclusion, nine umami peptides were identified through the application of peptidomics and screening tools following the enzymatic hydrolysis of yellowfin tuna. Among these, three umami peptides demonstrated significant bitter inhibition effects. Sensory evaluation demonstrated that SI-5, VE-8, and AP-6 possessed distinct umami characteristics. Further results from electronic tongue assessments and sensory evaluations indicated that AP-6, LD-5, and LT-5 displayed the ability to reduce bitterness while maintaining umami intensity. The interactions between umami peptides and T1R1/T1R3 taste receptors were further examined using molecular docking studies. According to the docking data, binding sites involve Glu-231, Ile-237, Ser-240, Glu-251, Asp-238, and Asp-215 in T1R1/T1R3 were essential for these interactions. Subsequently, the interaction between the bitter receptor TA2R14 and umami peptides was validated through molecular docking analysis. The findings suggested that residues Asp-168 and Trp-89 may be key amino acids responsible for facilitating the binding of bitter receptor blockers to TA2R14. These findings hold significant value for advancing our understanding of both the mechanisms underlying umami perception as well as those involved in bitterness reduction by umami peptides. Overall, the results highlight the potential of tuna-derived peptides as natural umami enhancers and bitterness inhibitors, providing valuable prospects for flavor improvement in food products.

CRediT authorship contribution statement

Lai Wei: Writing – original draft, Investigation, Data curation. **Cui Shi:** Writing – original draft, Investigation, Data curation. **Dongcheng Li:** Methodology, Investigation. **Xuan Yuan:** Methodology, Investigation. **Xinyi Yu:** Software, Data curation. **Botao Liang:** Methodology, Data curation. **Jiajia Wu:** Formal analysis, Data curation. **Yiqi Zhang:** Formal analysis, Data curation. **Zhiyuan Dai:** Resources, Data curation. **Yanbin Lu:** Writing – review & editing, Supervision, Project administration. **Jing Ye:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.145028.

Data availability

Data will be made available on request.

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