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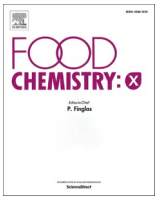

Enhancement of nutritional, organoleptic, and umami qualities of chicken soup induced by enzymatic pre-treatment of chicken

Ziyan Yue [a], Jing Lai [a], Qiqiong Li [b], Qiuyu Yu [a], Yuchun He [a], Jiali Liu [a], Yingchun Zhu [a,*]

[a] College of Food Science and Engineering, Shanxi Agricultural University, Taigu, Shanxi 030801, China
[b] State Key Laboratory of Food Science and Resources, Nanchang University, Nanchang, Jiangxi 330047, China

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ABSTRACT

To enhance both the quality and cooking efficiency of chicken soup, the effect of enzymatic pre-treatment of chicken on the quality of the chicken soup was investigated in this study. Results indicated that the application of enzymatic pre-treatment markedly enhanced the sensory attributes, physicochemical properties, and nutritional value, compared with the control sample. Specifically, the chicken soup with enzymatic pre-treatment exhibited a significant increase in the concentration of water-soluble proteins, 5′-nucleotides, and umami amino acids ($P < 0.05$). There were 52 umami peptides identified in enzymatic pre-treatment chicken soup, and eight abundant umami peptides were chosen for molecular docking. The analysis revealed that the primary active sites for interaction between the umami peptide and the T1R1/T1R3 receptor were ASP-30, GLU-27, and MET-342. Therefore, enzymatic pre-treatment of chicken facilitates high-value-added chicken soup products and provides a new approach to improve the quality and efficiency of chicken soup.

1. Introduction

Chicken is rich in high-quality proteins, essential vitamins, and minerals, and it has gained popularity as a healthful option among consumers, due to its affordability, minimal fat and cholesterol levels, and absence of religious dietary constraints (Cao et al., 2021). The Chinese three-yellow chicken, a breed indigenous to China, is commonly enjoyed for its richly flavored skin, delightful taste, and texture (Andaleeb et al., 2023). Additionally, chicken stew, a traditional chicken culinary way in the Chinese diet, is known for maximizing nutritional value and flavor of chicken meat. It was considered a tonic with medicinal value and is particularly suitable for young children, the elderly, and the physically weak (Wu et al., 2023). In the culinary customs of China, chicken soup was employed as a cuisine to ward off colds, reduce inflammation, and enhance the immune system (Li et al., 2022). Moreover, the choice of processing technique significantly influences the soup's overall taste and improves its overall palatability.

Recently, research has indicated that meat products of enzymolysis generally hold high nutritional worth and biological efficacy (Wu et al., 2022). Enzymatic hydrolysis protein, in contrast to chemical hydrolysis, boasts benefits like gentle reaction conditions, manageable processes, high effectiveness, and superior selectivity (Zhang et al., 2024). The enzymatic hydrolysis process is commonly employed for breaking peptide bonds to generate bioactive peptides, harnessing a diverse range of proteolytic enzymes. This approach stands out as an environmentally sustainable technique for peptide production (Wang et al., 2023). Choosing suitable enzymes can enhance meat breakdown and intensify the flavor profile of the resulting product (Tang et al., 2023). Guo et al. (2024) observed that the process of enzymatic hydrolysis augmented the liberation of free amino acids and umami nucleotides within chicken breast hydrolysates. In addition, enzymatic hydrolysis produces more nutrients such as amino acids and nucleotides, significantly improving the product's taste (Liao et al., 2024). Kong et al. (2017) found that the concentration of umami flavor compounds in the chicken enzymatic hydrolysate exceeded that in untreated chicken soup. It also accelerates the reaction rate, thereby enhancing production efficiency (Xiang et al., 2023).

Based on the above research, scholars are dedicated to exploring the mechanisms by which enzymatic hydrolysis improves product flavor, with a particular emphasis on its impact on taste. Food's umami taste is imparted by free amino acids, organic acids, nucleotides, organic bases, and small peptide compounds (Yao et al., 2024). Umami peptides are made up of a few amino acids and demonstrate a pronounced umami flavor (Shiyan et al., 2021). Recently, the increased demand for umami

* Corresponding author.
E-mail address: yingchun0417@163.com (Y. Zhu).

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enhancers in the food industry has prompted research on umami substances. Fresh flavor peptides can be enzymatically hydrolyzed to enhance fresh flavor (Liao et al., 2024). The techniques of homology simulation and docking of molecular are used to clarify the connections between umami compounds and flavor receptor proteins to gain a deeper understanding of the taste mechanism of these peptides (Dang et al., 2019). The major umami receptor is thought to be the T1R1/T1R3 receptor due to its high affinity for binding umami compounds (Zhang et al., 2019). Nevertheless, there is still limited research on the improvement of the quality of enzymatic pre-treatment chicken soup, particularly explained through the molecular docking of umami peptides with the umami receptor T1R1/T1R3.

The main objective of this study is to comprehensively investigate the effects of enzymatic pre-treatment on the sensory qualities, physical and chemical properties, and nutritional composition of chicken soup. Therefore, in this study, the flesh from Chinese three-yellow chickens was enzymatic pre-treatment and stewed separately for 1, 2, and 3 h, respectively, with untreated chicken soup stewed for 3 h serving as the control reference. This was done to analyze whether enzymatic hydrolysis could shorten the stewing time, thus improving the efficiency of chicken soup preparation. Additionally, the study delved into the isolation of umami peptides and molecular docking, revealing the mechanism behind the enhanced umami flavor in chicken soup. After the chicken soup was enzymatically pre-treated, umami compounds were isolated, and determined, and their taste perception mechanism was assessed using molecular docking with the T1R1/T1R3 interface. The findings are intended to provide reliable data support for the production of high-quality chicken soup.

2. Materials and methods

2.1. Materials and reagents for experiments

24 of Chinese three-yellow chickens were obtained from Donghai Farm (Taigu, Jinzhong, China), which were 300 days old. These chickens, which weighed approximately 1000 ± 50 g when prepared, were used as the experimental sample. Weighing, anesthesia with a captured bolt, exsanguination, preparation for slaughter, hand defeathering, and evisceration-the removal of the head, neck, and claws-were all performed on each chicken and, which was then quickly chilled them to 4 °C and transported to the laboratory on ice. Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) provided the chemicals and reagents.

2.2. Preparation of enzymatic pre-treatment chicken soup

The whole chicken was thoroughly cleaned and then chopped into pieces of around 3 ± 0.2 cm^3. Enzymatic treatment of the chicken pieces was carried out with flavored protease under specific enzymatic conditions (0.2 % flavor protease (1.5 × 10^5 U/ g) was added, the enzyme digestion time was 60 min, and the enzyme digestion temperature was 60 °C). The chicken soup was prepared with the addition of salt and ginger at a carcass/ salt/ ginger/ water weight ratio of 100:1.5:3:300 (w/ w/ w/ w) (Lai et al., 2022). Samples were taken for determination and analysis after 1 h, 2 h, and 3 h of stewing, respectively, and were named Enzymatic pre-treatment (1 h, 2 h, and 3 h). The Samples that were stewed for 3 h without enzymatic treatment were considered the control group. Each experimental group stewed soup three times, using one chicken each time.

2.3. Sensory assessment

The taste assessment took place at Shanxi Agricultural University's College of Food Science and Engineering's lab (Jinzhong, Shanxi, China). Ethical permission for sensory research was not required in Shanxi Agricultural University. All participants signed an informed consent before the sensory evaluation. The rights and privacy of all participants were protected during the execution of the study. Ten postgraduate experienced in nutrition science - 6 females and 4 males, between the ages of 20 and 40 - participated the study. To eliminate the effect of fluctuations in temperature on aroma perception, every 60 mL container of chicken soup was served in a clear plastic container and tagged with a randomized three-digit number. The soup was also maintained at a constant 45 °C. 6 flavor descriptors were screened through 10 sensory personnel, namely "Color", "Texture", "Oily", "Taste", "Aroma" and "general acceptance". The panel of judges used a 15 point magnitude to rate the following aspects of the assessment: color (1 for dark and dull to 15 for pale yellow), texture (1 for cloudy to 15 to clear), oily (1 for greasy to 15 for non-greasy), taste (1 for extremely unsuitable to 15 for highly appealing), aroma (1 for highly not good to 15 for very desirable), and general acceptance (1 for low to 15 for excellent).

2.4. Zeta potential and particle size determination

The measurement of particle size and Zeta potential followed the procedure described by (Yu et al., 2022) with slight alterations. 1 mL of the sample was carefully transferred into a sterile container, ensuring no air bubbles were formed and no impurities were introduced. The Nano-ZS90 potentiometric analyzer (Malvern Instruments Ltd., Worcestershire, UK) was used to evaluate the Zeta energy and size of particles within the chicken soup.

2.5. Determination of rheological properties

The viscosity and shear strain of the chicken soup were determined at 25 °C using a DHR1 device (TA Instruments Ltd., Crawley, UK). For this experiment, a 40 mm identical panel configuration was employed, with a fixed 1000 μm gap between the parallel plates. After that, the soup was exposed to a shear velocity that increased gradually from 0 s^{-1} to 300 s^{-1} following a preset protocol. Stress from shear and apparent viscosity were measured throughout this period (Yu et al., 2022).

2.6. Determination of nutritional components

The quantity of protein that dissolves in water in the chicken soup was determined using the Folin phenolic test. The liquid-soluble protein concentration (μg/mL) was measured employing the linear correlation model ($y = 0.0019\times + 0.0166$, $R^2 = 0.99380$) with bovine serum albumin serving as the standard deviation. The method of extracting methanol and chloroform was used to assess the fat content. The entire quantity of glucose was determined using the phenol-sulfuric acidic technique. The process outlined by Lai et al. (2022) was used to measure the soluble solid matter content.

2.7. Determination of 5′-nucleotides and free amino acids

The content of 5′-nucleotides in the samples was assessed by following the protocol outlined by (Zhang et al., 2018), 2 mL of chicken soup were centrifuged (4 °C for 15 min, 10,000 r/min), and the supernatant was collected. The supernatant was then filtered through a 0.22 μm hydrophobic nylon filter membrane. The quantification was conducted using High-Performance Liquid Chromatography (HPLC) on an Agilent 1260ALS system (Agilent Technologies, Santa Clara, CA, USA). The separation of nucleotides was performed using a Venusil ASB C18 column (4.6 mm × 250 mm × 5 μm) with the temperature set at 30 °C. The mobile phase consisted of methanol, distilled water, and phosphoric acid in a ratio of 16:400:1 (v:v:v), with a flow rate of 1 mL/min. Quantification of 5′-nucleotides was conducted using an external calibration curve and expressed in mg/L.

The free amino acids in the samples were measured using the method described by Lai et al. (2022). 1 mL of the sample solution was taken and

diluted with an equal volume of concentrated hydrochloric acid, followed by the addition of 6 mL of 6 mol/L HCl to bring the final volume to 8 mL. Next, 3–4 drops of phenol were added to the hydrolysis tube. The hydrolysis tube was then vacuumed, filled with nitrogen, and sealed. It was placed at 120 °C for 22 h for hydrolysis. After completion of hydrolysis, 4.8 mL of 10 mol/L NaOH was added to neutralize the hydrochloric acid. The solution was transferred to a 25 mL volumetric flask and the volume was made up. The solution was filtered using Whatman No.1 double-layer filter paper (Shanghai Jinpan Biotechnology Co., Ltd., Shanghai, China). 1 mL of the filtrate was taken and centrifuged (4 °C for 10 min, 10,000 r/min). Finally, 400 μL of the supernatant was transferred to a bottle for liquid chromatography injection. Chromatographic conditions were as follows: Chromatographic column type was ODS Hypersil (250 mm × 4.6 mm × 5 μm); column temperature was 40 °C; mobile phase A was 0.6 mmol/L sodium acetate; and mobile phase B was the mixture (0.15 mmol/L) of natrium aceticum, methanol, and acetonitrile at the ratio of 1:2:2 (volume ratio); and flow rate was 1.0 mL/min.

2.8. Peptide separation

The chicken soup with enzymatic pre-treatment was vacuum freeze-dried and then processed for 24 h. After the powdered chicken soup was freeze-dried, it was kept in storage at −80 °C. After being freeze-dried, the powdered chicken soup was diluted in distilled water and ultrafiltered using tubes containing 10 kDa ultrafiltration. The solution was then desalinated using Waters SEP-PAK C18 solid-phase extraction columns, and the procedure was repeated using freeze-drying. After carefully removing the supernatant, the powder was re-dissolved in an aqueous solution containing 0.1 % formic acid, centrifuged, and the residue was used for assay.

The mobile phase B is a 0.1 % formic acid solution in methanol (containing 80 % nitrile) and mobile phase A is a 0.1 % formic acid solution in water in the liquid phase. 92 % fluid A was used to equilibrate the liquid chromatographic column (50 μm × 150 mm, Acclaim PepMap TM RSLC, Thermo Scientific Technology Inc.). A chemical column was run through an injection volume of 1 μL. The liquid phase gradient was configured as follows: 0–98 min: the linear gradient of mobile phase B increased from 8 % to 28 %; 98–113 min: the linear gradient of mobile phase B increased from 28 % to 37 %; 113–117 min: the linear gradient of mobile phase B increased from 37 % to 100 %; 117–120 min: mobile phase B was maintained at 100 %. After materials were divided using capillaries high-efficiency liquid chromatography, a Thermo QE HF mass spectrometer (Thermo Fisher Scientific, CA, USA) was employed for the analysis.

2.9. Peptide screening

Peptide fragments from enzymatic pre-treatment were obtained using the active forecasting tool Peptide Ranker (http://distilldeep.ucd.ie/PeptideRanker/) to screen for highly active peptide fragments (> 0.5). After that, non-toxic active peptides were filtered out using the ToxinPred toxicity prediction program (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php). Then, BIOPEP database (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) (umami peptide and umami amino acid) was utilized to predict the possible functions of peptides found by mass spectrometry, investigating the relationship between peptides and sensory characteristics. The frequency of occurrence of bioactive segments within the identified peptides was calculated. The calculation formula is as follows:

$$A = a/N$$

where N is the total number of amino acid residues present in the identified peptides; A is the frequency of bioactive segments within the identified peptides; and a is the count of segments within the identified peptides with specific taste-active qualities.

2.10. Homology modeling and molecular docking

Protein BLAST function in the NCBI Protein Data Bank provided the umami receptor's crystal form T1R1/T1R3. The crystal structure with ID 5X2M showed the greatest sequence similarity to T1R1/T1R3 among the search results. PyMOL software (Schrödinger, LLC., New York, NY, USA) was utilized to simulate the tertiary structures of the umami peptides that were found. After importing the crystal structure 5X2M into PyMOL, the model was cleared of the water molecules and other ligand structures. After that, AutoDockTools4 software (San Diego, CA, USA) was applied to atomically bind the umami polypeptide and umami receptor.

2.11. Statistical analysis

All experiments were repeated three times. IBM SPSS Statistics 22.0 was implemented to do a one-way ANOVA study to look at any significant variations between the sets of participants. A p-value less than 0.05 was considered statistically significant. The raw mass spectrometry files were analyzed with Proteome Discoverer 2.5 to compare against the relevant database, leading to peptide identification. The BIOPEP dataset was consulted to estimate the possible biological purposes of the discovered umami peptides while AutoDockTools4 software was deployed to perform the docking process. For plotting and data processing, Origin 2021 and TBtools software were utilized.

3. Results and discussion

3.1. The impact of enzymatic pre-treatment of chicken on the sensory quality of chicken soup

The sensory characteristics of chicken soup are shown in Fig. 1. The duration of stewing did not significantly affect the sensory attributes of the chicken soups in the three experimental groups following enzymatic pre-treatment ($P > 0.05$). However, after enzyme pre-treatment, the sensory quality of the soup was significantly higher than that of the control group ($P < 0.05$). Following a duration of one to 3 h of stewing, the enzymatic pre-treatment group had superior ratings in taste, aroma, texture, and oily when compared to the reference group. After enzymatic pre-treatment, the increase in taste may be due to the substantial increase of the free amino (Aspartic acid and glutamic acid) acids and 5′-nucleotides (Wei et al., 2018). The increase in amino acid content, caused by the acceleration of enzymatic protein hydrolysis, lead to the production of more flavor compounds, making their aroma more outstanding (Zhang et al., 2017). Enzymatic pre-treatment facilitated the transfer of more fat-soluble and water-soluble compounds from the chicken to the soup, resulting in a better texture and reduced surface oil aggregation compared to the control group (Liao et al., 2024). Conversely, the color rating was inferior. Notably, the general acceptance sensory evaluation for the enzymatic pre-treatment group exhibited a statistically significant enhancement over the control group ($P < 0.05$). This data suggests that the enzymatic treatment of chicken enhances the sensory profile of chicken soup and it was almost unaffected by the stewing time ($P > 0.05$).

3.2. The impact of enzymatic pre-treatment of chicken on the particle size, Zeta potential, and rheological characteristics of chicken soup

The measurement of protein aggregate size typically involves the analysis of particle size. Zeta potential serves as a physical measure that describes the charge density present on the surface of particles within a solution. These two metrics are capable of indicating the functional attributes of proteins (Yu et al., 2022). Viscosity is the internal frictional force exhibited by a liquid during flow, reflecting the characteristics of food (Qi, Li, et al., 2023). These indicators represent the physicochemical properties of the chicken soup. Good physicochemical properties

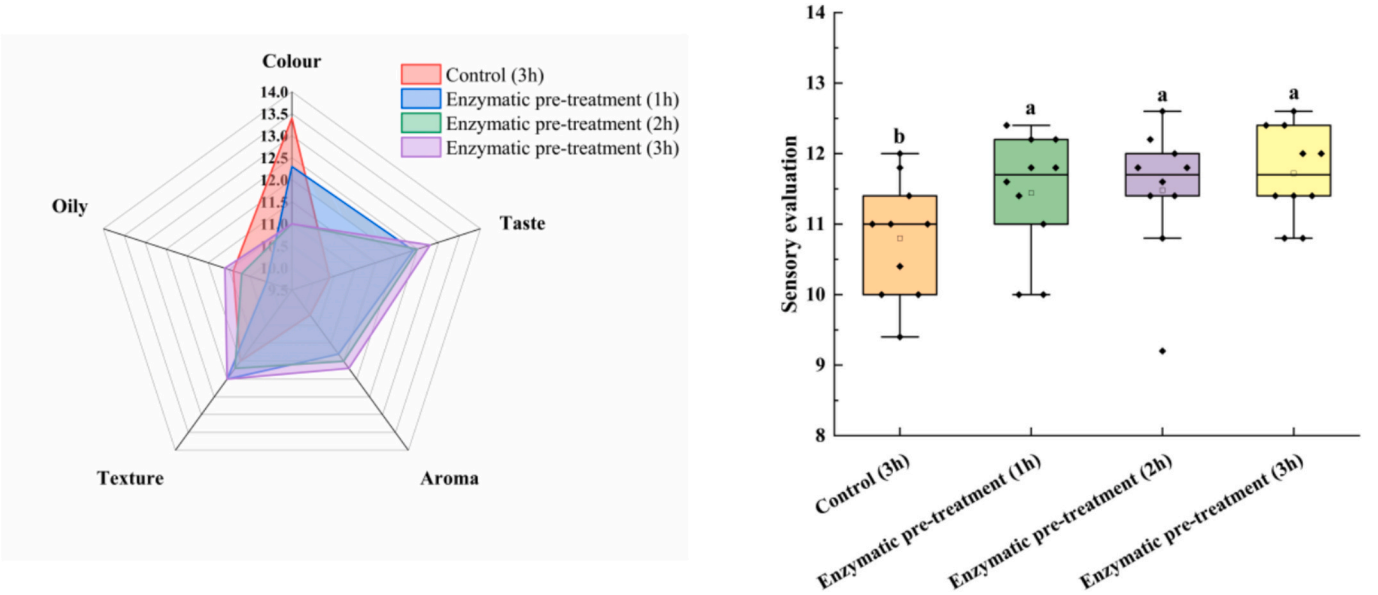

Fig. 1. Sensory evaluation score of the chicken soup. (A) Radar chart of the five sensory attributes: color, texture, oiliness, taste, and aroma; (B) General acceptance score. Enzymatic pre-treatment (1 h, 2 h, and 3 h) represents chicken soup samples stewing for 1 h, 2 h, and 3 h after enzymatic pre-treatment of chicken meat, respectively. The same letter indicates no significant difference ($P \geq 0.05$), while different letters indicate a significant difference ($P < 0.05$).

mean that the chicken soup contained smaller particles, resulting in a smoother texture when tasted (Chaffee et al., 2023).

Particle size and Zeta potential properties in chicken soup are affected by the enzymatic pre-treatment given to the chicken, as shown in Fig. 2A and Fig. 2B. As depicted in Fig. 2A, the average particle sizes of the chicken soup in the enzymatic pre-treatment groups were 299.23 nm, 255.53 nm, and 227.80 nm for 1 h, 2 h, and 3 h of stewing, respectively. These findings suggest that with extended stewing of pre-enzymatic chicken soup, the particulate size progressively moved towards smaller sizes. In contrast to the control chicken soup (321.27 nm), the particulate size in the enzymatic pre-treatment sample was reduced. This decrease in particulate size is believed to result from the flavor protease, which breaks down the larger protein molecules within the soup. In the course of enzymatic hydrolysis, the non-covalent bonds within protein aggregates are broken, impacting the hydrogen bonds and hydrophobic forces among proteins. This disruption results in a decrease in particle dimensions (Wei et al., 2024).

Fig. 2B demonstrated that the Zeta potential readings for all chicken soup samples were negative, suggesting an excess of negative charges on the particle surfaces over positive ones. In contrast to the control group (7.37 mV), the samples subjected to enzymatic pre-treatment exhibited a marked rise ($P < 0.05$) in the absolute Zeta potential value (ranging from 8.57 to 9.66 mV). The enzymatic pre-treatment facilitated the breakdown of protein molecules into peptide fragments and free amino acids, revealing a greater number of negatively charged amino acid residue. The chicken soup specimens following enzymatic prior to treatment had an increase in their absolute Zeta potential as a result of this change in the density of the surface charges of the granules (Ahn et al., 2022).

Fig. 2C illustrates that the viscosity of the chicken soup decreased logarithmically across a shear rate spectrum of 0.1 s^{-1} to 200 s^{-1}, before leveling off between shear rates of 200 s^{-1} and 1000 s^{-1}. This behavior suggests that the chicken soup possesses shear-thinning characteristics and pseudo-plastic behavior. This phenomenon may be caused by the disruption of the water environment around the dry matter in the chicken soup, leading to weakened intermolecular forces. Furthermore, asymmetric particles aligned with the flow direction, reducing the obstruction to convective movement. Shear-thinning action resulted from the alignment changing the chicken soup's viscosity (Guan et al., 2024). The enzymatic pre-treatment group's chicken soup consistently performed better than the control group's at comparable shear speeds. The chicken was treated with enzymes that made it easier for the protein compounds in the components to dissolve, which increased the soup's nutritional value and, as a result, increased the viscosity of the system.

Fig. 2D demonstrates the impact of the enzymatic pre-treatment of chicken on the shear stress within chicken soup. The soup behaves as a non-Newtonian fluid, where shear stress rises in tandem with the shear rate. Within a shear rate range of 0 to 300 s^{-1}, no discernible differences in shear stress were observed between the groups, as their trend lines nearly overlapped. However, beyond 300 s^{-1}, variations in shear stress became apparent. At identical shear rates, the shear stress of the chicken soup in the enzymatic pre-treatment cohort surpassed that of the control cohort. The higher shear stress in the enzymatic pre-treatment group can be attributed to the increased nutrient content and complex material interactions within the soup (Goudoulas & Germann, 2017). In contrast, the control chicken soup had a more uniform distribution and lower flow resistance, resulting in lower shear stress to overcome. In summary, the enzymatic treatment group produced chicken soup with a better texture compared to the control group.

3.3. The impact of enzymatic pre-treatment of chicken on the nutritional value of chicken soup

Table 1 illustrated the influence of the enzymatic pre-treatment on the nutritional ingredients of chicken soup. Following enzymatic pre-treatment, there was a notable rise in the levels of soluble solids and water-soluble proteins in the soup ($P < 0.05$). For instance, the soluble solid concentration in the soup from the enzymatic pre-treatment group, after 3 h of cooking (5.47 g/100 mL), exhibited a 61.79 % enhancement over the control group (3.38 g/100 mL). With the extension of stewing duration, the soluble solid and water-soluble protein contents in the chicken soup also rose. Consequently, it is reasonable to conclude that both enzymatic pre-treatment and extended stewing times facilitate the solubilization of solids from the chicken. For the control group, the content of water-soluble protein was 1772.77 μg/mL after 3 h of cooking. In contrast, the the content of water-soluble protein was 2611.26 μg/mL in the enzymatic pre-treatment group's soup. After the same stewing time, there was a 1.47-fold increase in levels over the control group. Moderate enzymatic pre-treatment disrupts the tight three-dimensional spatial structure of chicken proteins (Ma et al., 2022),

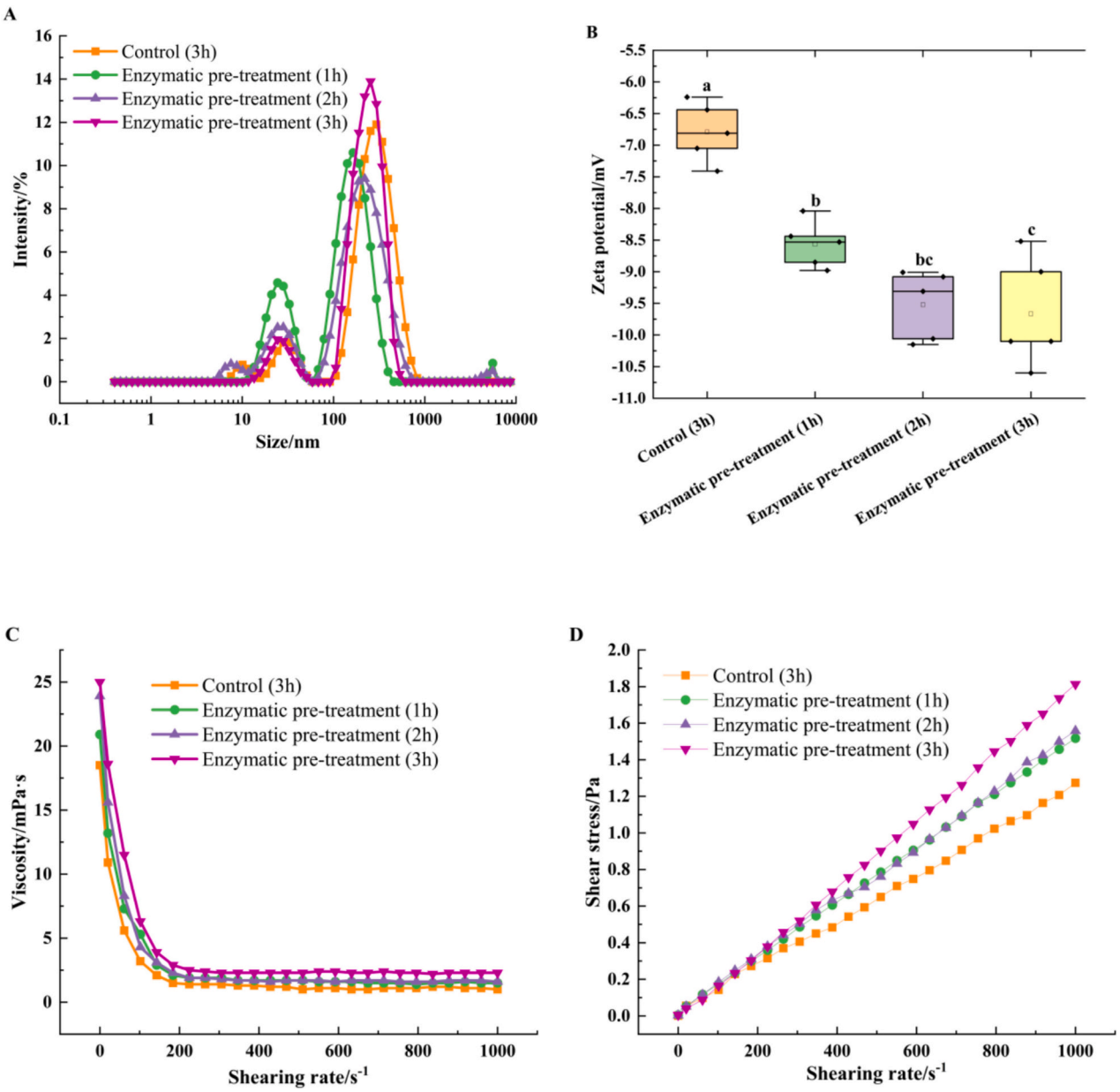

Fig. 2. Effect of enzymatic pre-treatment of chicken meat on the particle size (A), Zeta potential (B), viscosity(C), and shear stress (D) of chicken soup. Enzymatic pre-treatment (1 h, 2 h, and 3 h) represents chicken soup samples stewing for 1 h, 2 h, and 3 h after enzymatic pre-treatment of chicken meat, respectively. The same letter indicates no significant difference ($P \geq 0.05$), while different letters indicate a significant difference ($P < 0.05$).

making non-polar bonds more readily exposed and promoting the dissolution of soluble proteins. Dong et al. (2020) also demonstrated that enzymatic pre-treatment of chicken significantly increases the protein content in chicken soup. The total sugar content did not exhibit a significant discrepancy between the enzymatic pre-treatment group after 1 h of cooking (0.84 mg/mL) and the control group's chicken soup after 3 h of cooking (0.85 mg/mL). As the stewing duration increased, the total sugar concentration in the chicken soup from the enzymatic pre-treatment group rose notably ($P < 0.05$). Upon completion of stewing, the total sugar content in the soup exceeded that of the control group by 0.15 mg/mL. This indicated that moderate enzymatic pre-treatment not only promotes the dissolution of sugar in chicken but also reduces the stewing time. This is due to enzymatic pre-treatment disrupting the structure of glycoproteins, causing the breakage of protein-glycopeptide bonds and releasing sugar (Zeng et al., 2020), leading to an increase in the total sugar content in chicken soup. The fat content in chicken soup is closely related to its flavor (Qi et al., 2023). The control group exhibited the highest fat content, with the chicken soup from the enzymatic pre-treatment group, whether cooked for 2 or 3 h, following closely. However, no substantial differences were observed among the various chicken soup groups.

3.4. The impact of enzymatic pre-treatment of chicken on the amount of free amino acids and 5′-nucleotides in chicken soup

Fig. 3A demonstrates how the 5′-nucleotide content of chicken soup is affected by the enzymatic pre-treatment of chicken. Upon completion of the stewing process, the nucleotide concentration in the enzymatic pre-treatment group was notably greater ($P < 0.05$) compared to the control group. Under the action of enzymes, the protein structure of the chicken was further broken down, promoting the migration of 5′-nucleotides (Liao et al., 2024). Furthermore, the duration of stewing significantly affected the nucleotide content in the chicken soup ($P < 0.05$), where extended stewing led to an increase in the overall flavor

Table 1
Effects of enzymatic pre-treatment of chicken meat on the nutrition content of three-yellow chicken soup.

Parameter	Control (3 h)	Enzymatic pre-treatment (1 h)	Enzymatic pre-treatment (2 h)	Enzymatic pre-treatment (3 h)
Soluble solid matter content (g/ 10 0 mL)	3.38 ± 0.10^{d}	4.05 ± 0.04^{c}	4.37 ± 0.07^{b}	5.47 ± 0.12^{a}
Water-soluble protein content (ug/ mL)	1772.77 ± 40.35^{c}	2015.74 ± 51.15^{b}	2545.13 ± 90.33^{a}	2611.26 ± 28.67^{a}
Total sugar content (mg/ mL)	0.85 ± 0.04^{c}	0.84 ± 0.03^{c}	0.91 ± 0.01^{b}	1.00 ± 0.01^{a}
Crude lipid content (g/ 100 mL)	1.31 ± 0.07^{a}	1.28 ± 0.03^{a}	1.30 ± 0.05^{a}	1.27 ± 0.01^{a}

Note: Different lowercase letters in the same row indicate a significant difference ($P < 0.05$). Enzymatic pre-treatment (1 h, 2 h, and 3 h) represents chicken soup samples stewing for 1 h, 2 h, and 3 h after enzymatic pre-treatment of chicken meat, respectively.

nucleotide levels. Of the nucleotides, only the quantity of 5'-IMP diminished with prolonged stewing, potentially due to the breakdown and conversion of 5'-IMP during the high-temperature cooking process. Wu et al. (2023) reported that prolonged stewing led to increased degradation of 5'-IMP and a decrease in its concentration in chicken soup.

Enzymatic hydrolysis is a crucial technique for producing biologically active peptides and functional proteins (Kong et al., 2017). Fig. 3B and C depicted the influence of the enzymatic pre-treatment of chicken on the free amino acid content and composition of chicken soup. The main component of chicken is protein, which can be broken down by flavor proteases into peptides and free amino acids. Fig. 3B reveals the presence of 21 amino acids within the soup, among which glutamic acid, leucine, alanine, serine, valine, and lysine are the predominant free amino acids. These six amino acids constitute 45 %–48 % of the overall amino acid content. After the enzymatic pre-treatment, the chicken soup had a significantly higher concentration of liberated amino acid residues than the control sample; the soup that had been boiled for 3 h had the greatest amounts.

Amino acids are categorized into four groups according to their flavor profiles: sweet, bitter, umami, and flavorless (Jia et al., 2023). After 3 h of heating, Fig. 3C shows that the amino acid content of the chicken soup is significantly affected by the enzymatic pre-treatment ($P < 0.05$). The 3 h enzymatic preliminary processing sample exhibited three times the concentration of umami and sweet amino acids when compared to the control group. Notably, compared to the control group, the levels of the sweet and umani amino acids, which are alanine and glutamic acid, were 71.35 and 82.52 μg/mL higher, respectively. Furthermore, the elevated content of free amino acids in the enzymatic pre-treatment chicken soup correspondingly resulted in an increase in bitter amino acids. In the enzymatic pre-treatment group, the concentration of bitter amino acids reached 606.70 μg/mL, a figure that significantly surpasses the control group's level of 123.76 μg/mL. Aspartic acid and glutamic acid can accentuate the umami flavor by masking the bitterness of bitter amino acids (Zhang et al., 2019). Additionally, sweet amino acids synergize with umami amino acids, enhancing the flavor and umami effect (Ma et al., 2024).

The FAO/WHO proposes an ideal value of around 40 % for EAA/TAA and greater than 60 % for EAA/NEAA (FAO/WHO, 1973). Table 2 reveals the calculated composition ratios of amino acids across the four chicken soup groups in this experimental study. The EAA/TAA ratio of the control group in the chicken soup was 28.93 %, while the ratio in the enzymatic pre-treatment group was close to 40 %. Moreover, the EAA/NEAA ratios for the chicken soup in the enzymatic pre-treatment group were 83.00 %, 84.12 %, and 84.46 %, each figure substantially exceeding the ratio in the control group's chicken soup (40.72 %) ($P < 0.05$). This suggests that the ratio of free amino acids in the chicken soup is more favorable following enzymatic pre-treatment.

3.5. Identification of proteins of umami peptides from chicken soup

To further identify the peptides with excellent umami taste in the enzymatic pre-treatment chicken soup, the peptide sequences in the chicken soup were identified, using a Thermo QE HF mass spectrometer and capillary high-performance liquid chromatography. There were 2122 peptide fragments found in the soup overall. All peptide segments in the chicken soup were subjected to activity prediction using Peptide Ranker, resulting in 323 peptide sequences with an activity probability score greater than 0.5. Toxicity prediction using ToxinPred indicated that only the sequence GPAGPRGPPGPSGPPGKD exhibited toxicity among all peptide segments. Small-molecule peptides are often the peptide components that contribute to umami flavor (Jia et al., 2023), usually consisting of branched chains with 3–9 carbon atoms. Table S1 reveals that the peptide sequences were filtered according to specific criteria, including an activity probability score above 0.5, non-toxicity, and a molecular weight under 1000 Da. Consequently, a total of 149 peptide sequences were chosen.

The BIOPEP database a technique that helps analyze the relationship between peptides' chemical makeup and sensory attributes, was employed to screen for umami peptides. This approach assists in the development of potential flavor profiles for protein fragments (Iwaniak et al., 2016). The experiment identified 52 peptide sequences containing umami-associated amino acid segments in the enzymatic pre-treatment chicken soup according to the BIOPEP database. As shown in Table 3, there were 8 peptide sequences in which umami amino acid fragments appeared more frequently than other peptide sequences. These sequences were identified as FAGDDAPRA (FA-9), AGDELF (AG-6), FSEDTF (FS-6), DAGELF (DA-6), SQHDVDGF (SQ-8), AGFAGDDAPR (AG-10), GGYEVGF (GG-7), and KFDEAF (KF-6). Out of the eight peptide sequences, AG-6, which includes the umami segments D, E, DE, EL, and EDL, had the highest number of umami active pieces. Subsequently, DA-6 and KF-6 exhibited umami activity segments D, E, DA, EL, DE, and EA, while GG-7 showed the lowest frequency with umami activity segments E, VG, and EV. Toda et al. (2013) reported that aspartic acid (D) could activate the taste receptor T1R1/T1R3, suggesting that peptides AG-6, DA-6, and KF-6 were likely to present an umami taste. The peptide segment with the highest frequency of bitter taste activity was GG-7, followed by KF-6 and DA-6, while FS-6 exhibits the lowest frequency. Analysis of reported umami peptides reveals that certain bitter taste amino acids were components of umami peptides, such as histidine (H), valine (V), arginine (R), leucine (L), isoleucine (I), phenylalanine (F), tryptophan (W), and proline (P) (Jia et al., 2024). The peptides with the highest frequency of acidic taste active segments were AG-6 and KF-6. Acidic amino acids were considered significant umami stimulants and were typically present in the amino acid sequences of umami peptides (Wang et al., 2022). As such, the frequency of the detected peptides or the frequency of taste-active segments within them cannot be used to determine their taste-active potential. To determine the flavor presenting properties of these eight peptide sequences, molecular docking was used for further investigation.

3.6. Umami peptides and the T1R1/T1R3 receptor molecular docking

Research has identified several receptors that are capable of sensing umami taste, including mGluR4, T1R1/T1R3, and mGluR1. T1R1/T1R3 is known to have the highest affinity for umami chemicals and to be the main receptor involved in initiating the umami flavor (Zhang et al., 2019). Molecular docking was used to mimic the synergistic interaction between these peptides and the umami taste receptor T1R1/T1R3, to

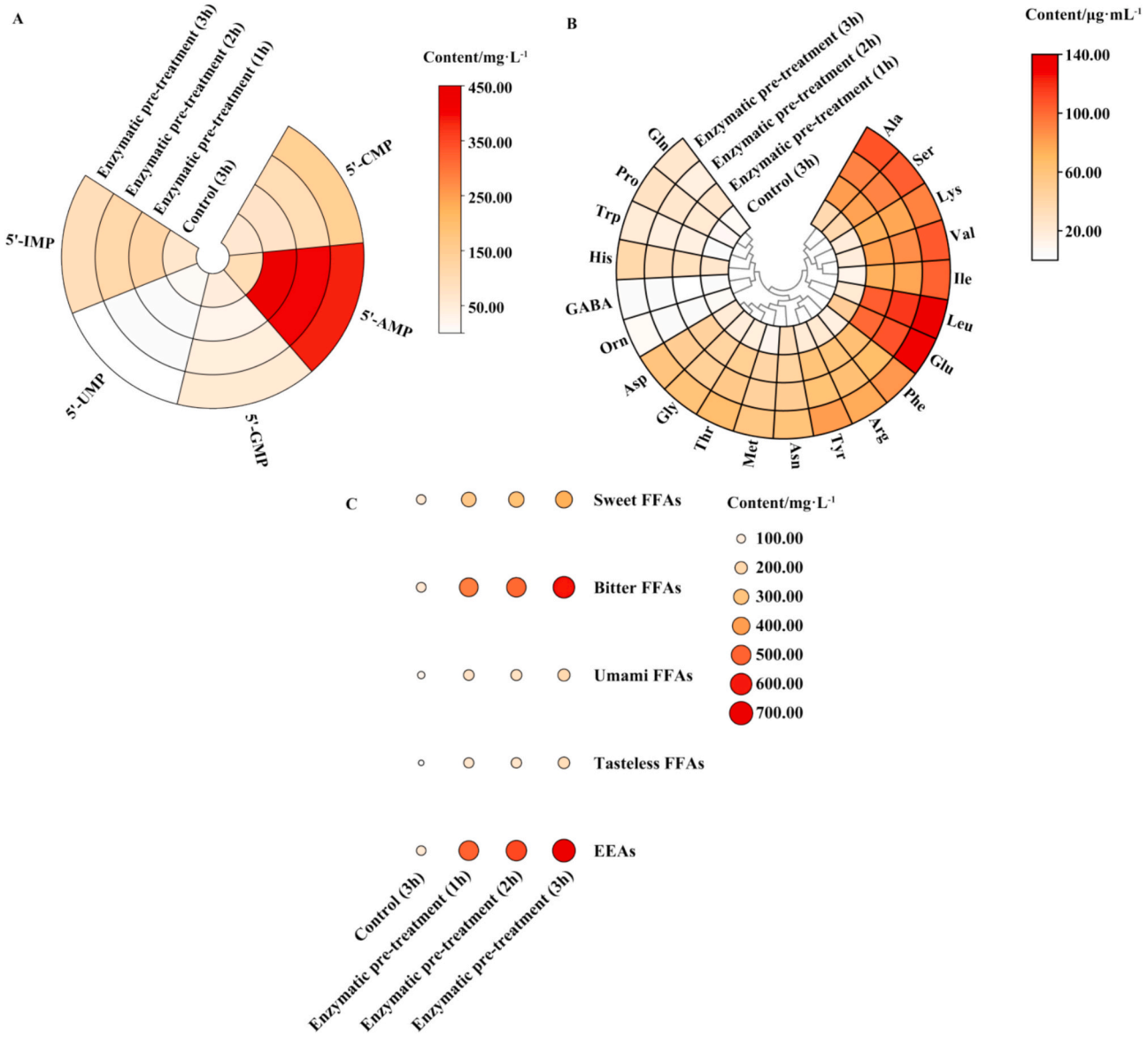

Fig. 3. Effect of enzymatic pre-treatment of chicken meat on the 5′-nucleotides (A), free amino acid content (B), and free amino acid composition (C) of chicken soup. Enzymatic pre-treatment (1 h, 2 h, and 3 h) represents chicken soup samples stewing for 1 h, 2 h, and 3 h after enzymatic pre-treatment of chicken meat, respectively. Umami FAA = Asp + Glu; Sweet FAA = Thr + Ser + Gly + Ala; Bitter FAA = Val + Met + Ile + Leu + Phe + His + Arg; Tasteless FAA = Cys + Tyr + Lys. EAA, essential free amino acids.

Table 2
Effect of enzymatic pre-treatment of chicken meat on free amino acid composition ratio of Chinese three-yellow chicken soup.

Group	Control (3 h)	Enzymatic pre-treatment (1 h)	Enzymatic pre-treatment (2 h)	Enzymatic pre-treatment (3 h)
EAA/TAA	28.94 %	45.36 %	45.69 %	45.79 %
EAA/NEAA	40.72 %	83.00 %	84.12 %	84.46 %

Note: Enzymatic pre-treatment (1 h, 2 h and 3 h) represent chicken soup samples stewing after 1 h, 2 h and 3 h after enzymatic pre-treatment of chicken meat, respectively.

understand their reciprocal engagement, and to get a deeper knowledge of the taste mechanism of umami peptides in chicken soup (Feng et al., 2024). The main criterion for evaluating ligand-receptor interactions was binding energy. The docking energies for eight umami peptides with the T1R1/T1R3 receptor are listed in Table 4. These eight umami peptides were found to efficiently engage in molecular docking with the T1R1/T1R3 receptor, resulting in the formation of umami peptide-umami taste receptor complexes. The docking total energy decreased in the following order: FS-6, AG-6, AG-10, GG-7, AG-10, SQ-8, DA-6, FA-9. During docking, the interaction energies for KF-6, FA-9, AG-10, DA-6, GG-7, AG-6, SQ-8, and FS-6 were 1.63, 0.81, 0.75, −0.03, −0.07, −1.76, −1.81, and − 4.34 kcal/mol, respectively. When the interaction energy between a ligand and a receptor is negative, the binding occurs spontaneously without external forces, making the docking results more reliable (Chen et al., 2024). Among the eight umami peptides, FS-6 exhibited the most negative and lowest binding energy, suggesting that it binds most strongly to the receptor, thereby forming the most stable complex.

The key binding sites where umami peptides interact with the T1R1/T1R3 umami taste receptor are shown in Table S2. Table S2 displays the hydrogen bonds formed by the eight umami peptides with 26 amino acid residues on the T1R1/T1R3 receptor. An effective docking with the

Table 3
Predicted taste properties of amino acid fragments of table polypeptide sequences.

Polypeptide sequences	Frequency of active segment occurrence						Umami activity segments
	Bitterness	Bitterness inhibition	Sweetness	Sourness	Saltiness	Umaminess	
FAGDDAPRA	0.56	0.11	0.56	0.33	0.44	0.44	D、DD、DA
AGDELF	0.67		0.33	0.67	0.33	0.83	D、E、DE、EL、EDL
FSEDTF	0.33	0.17	0.17	0.50	0.33	0.50	ED、D、E、ED
DAGELF	0.83		0.33	0.50	0.17	0.67	D、E、DA、EL
SQHDVDGF	0.75		0.25	0.50	0.25	0.50	D、VD、DG
AGFAGDDAPR	0.60	0.10	0.70	0.30	0.30	0.50	D、DD、DA、AGFAGDDAPR
GGYEVGF	0.86		0.71	0.28		0.43	E、VG、EV
KFDEAF	0.83	0.17	0.33	0.67	0.33	0.67	D、E、DE、EA

Note: Alanine(A), Aspartic acid(D), Glutamic acid(E), Phenylanaline(F), Glycine(G), Histidine(H), Lycine(K), Leucine(L), Proline(P), Glutamine(Q), Arginine(R), Serine(S), Threonine(T), Valine(V), Tyrosine(Y).

Table 4
Docking energy and Hydrogen bond number of peptides and receptors.

Polypeptide sequences	Abbreviations of polypeptide sequences	Total docking energy (kcal/mol)	Docking interaction energy(kcal/mol)	Hydrogen bond number
FAGDDAPRA	FA-9	−8.85	0.81	2
AGDELF	AG-6	−3.83	−1.76	2
FSEDTF	FS-6	−1.93	−4.34	4
DAGELF	DA-6	−6.44	−0.03	4
SQHDVDGF	SQ-8	−6.18	−1.81	4
AGFAGDDAPR	AG-10	−5.88	0.75	8
GGYEVGF	GG-7	−5.02	−0.07	4
KFDEAF	KF-6	−7.60	1.63	1

Note: Alanine(A), Aspartic acid(D), Glutamic acid(E), Phenylanaline(F), Glycine (G), Histidine(H), Lycine(K), Leucine(L), Proline(P), Glutamine(Q), Arginine(R), Serine(S), Threonine(T), Valine(V), Tyrosine(Y).

ligands was indicated by the involvement of amino acid residues ASP-30, GLU-27, and MET-342 in the contacts of two ligand-receptor complexes. This suggests that the umami taste may be primarily triggered by the amino acid residues Glu-65, Arg-393, and Gly-389 located inside the binding cavity of the T1R1/T1R3 subunit. Lysine (K), which occurs five times in the umami peptide sequences, is the most recurrent amino acid residue. It is followed by glutamic acid (E), asparagine (N), and serine (S), which appear four, three, and three times, respectively, in the residues of proteins that bind savory compounds and sensors. This indicates that these residues may represent key recognition sites for the umami receptor.

Docking diagrams were created to clarify the molecular recognition process between umami peptides and the protein receptor T1R1/T1R3. The binding relationship between the peptides and the receptor was shown graphically in these figures. The quantity and length of hydrogen bonds that have been established between the T1R1/T1R3 receptor subunit and the eight umami peptides are shown in Table 4 and Fig. 4. The number of hydrogen bonds in the respective ligand-receptor complexes was 2, 2, 4, 4, 4, 8, 4, and 1 for FA-9, AG-6, FS-6, DA-6, SQ-8, AG-10, GG-7, and KF-6, respectively. The distances of these hydrogen bonds were notably brief (1.6–3.4 Å), signifying a robust binding affinity and stable conformation of the umami peptides with the umami receptor.

Moreover, Fig. 4 indicates that each peptide molecule occupied the hydrophilic cavity within the protein's active site, establishing robust hydrophilic interactions with the neighboring amino acids. Amino acid residues such as GLU-422, GLU-27, GLU-131, GLU-97, LYS-427, LYS-101, LYS-347, LYS-112, LYS-265, SER-60, SER-351, SER-28, ASP-57, ASP-30, and ASP-118 created the pocket cavity where the umami peptides adhered and formed hydrogen bonds with the surrounding amino acid residues. The umami taste receptor's active sites were activated and T1R1/T1R3 umami activity was subsequently increased thanks to the binding sites that these amino acid residues provided for interactions with the umami receptor. Thus, through these key intermolecular forces, the umami peptide ligands tightly bind to the umami receptors, thereby activating umami activity and enhancing the T1R1/T1R3 receptor's functionality, ultimately imparting the chicken soup with optimal umami flavor.

4. Conclusions

In summary, this study examined the physicochemical properties, nutritional makeup, and umami peptides of chicken soup made from enzymatic pre-treatment chicken. The findings indicated that enzymatic pre-treatment boosts the concentration of compositions that are soluble solids, water-soluble protein, 5′-nucleotides, umami amino acid, and other nutrients while also improving sensory qualities and physicochemical features. Compared to the untreated group that was stewing for 3 h, the enzymatic prior treatment group produced chicken soup with higher quality after simmering for 1 h or more. There were 52 umami proteins in enzymatic pre-treatment chicken soup. The eight most common umami peptides were chosen for molecular docking with T1R1/T1R3. According to the molecular docking data, ASP-30, GLU-27, and MET-342 may be the main active sites that umami peptides use to interact with the T1R1/T1R3 receptor, and they may be essential to the umami flavor. These findings demonstrate that enzymatic pre-treatment can enhance the quality of chicken soup and effectively shorten the stewing time, which is promised for advance the development of enzyme-catalyzed meat products in the future. Molecular dynamics simulations will be performed following molecular docking to further validate and optimize the docking results.

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CRediT authorship contribution statement

Ziyan Yue: Writing – original draft, Methodology, Investigation, Data curation. **Jing Lai:** Visualization, Investigation. **Qiqiong Li:** Writing – review & editing. **Qiuyu Yu:** Software, Investigation. **Yuchun He:** Visualization, Resources. **Jiali Liu:** Software, Resources. **Yingchun Zhu:** Writing – review & editing, Validation, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

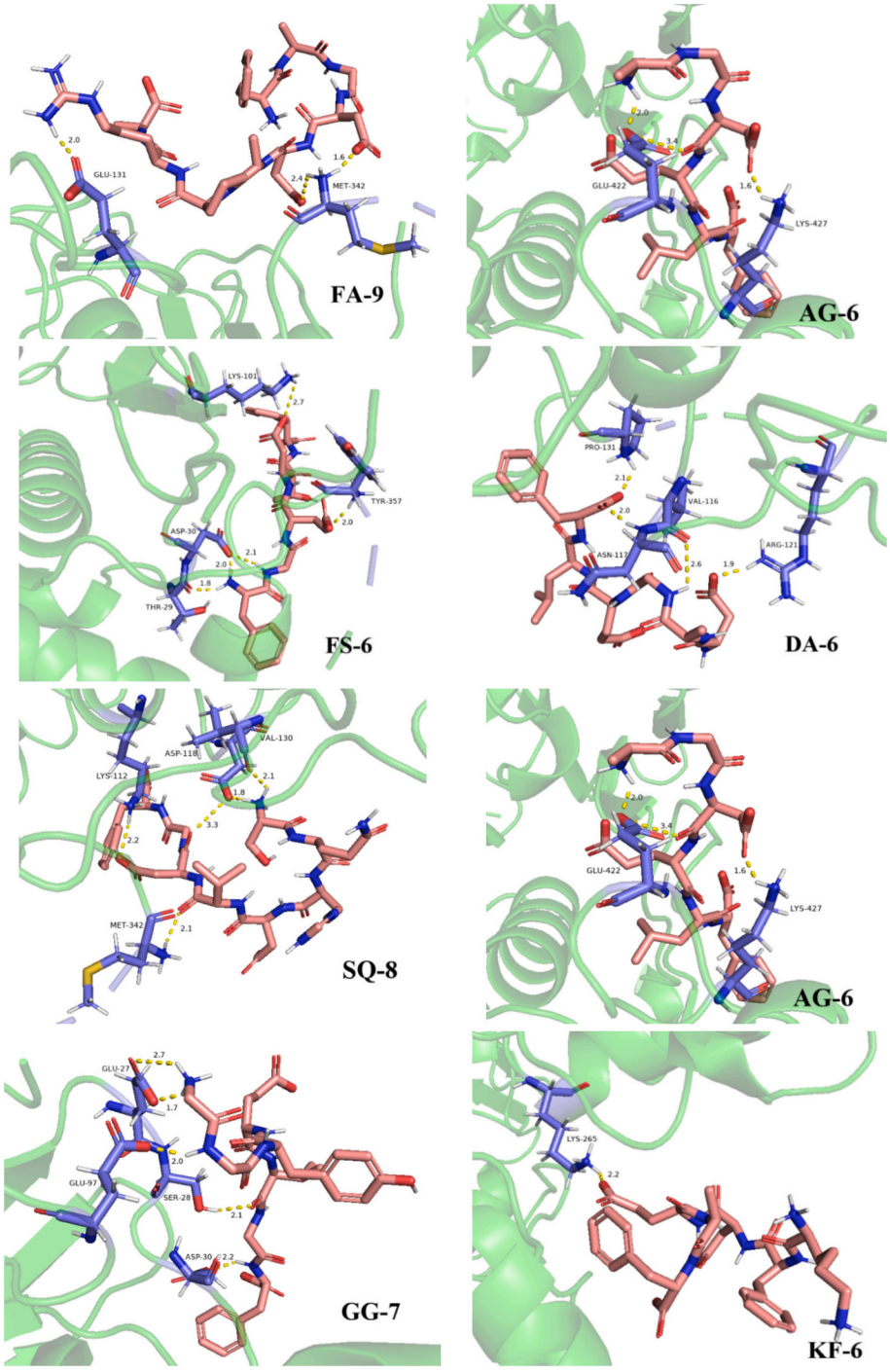

Fig. 4. The mechanism of the binding of peptides to T1R1/T1R3.

the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101914.

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