

Deciphering the bitterness contribution of *Zanthoxylum bungeanum* Maxim and its masking mechanism

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Abstract

BACKGROUND: *Zanthoxylum bungeanum* Maxim. (*Z. bungeanum*) is widely applied in the processing of stewed beef with spices (SBS) to improve sensory quality, yet the processing-induced interactions between its flavonol glycosides and myofibrillar protein (MP) and their role in taste modulation remain poorly understood. In particular, the structural basis and binding behavior underlying protein–flavonol interactions during stewing, as well as their contribution to taste regulation. In this study, metabolomics combined with random forest modeling was employed to screen key taste-active compounds in SBS. The structure–affinity relationship and the mechanism underlying the interaction between flavonol glycosides and MP were investigated using fluorescence spectroscopy, Fourier transform infrared spectroscopy, and molecular docking.

RESULTS: The top 15 key taste compounds were identified by a random forest model as significant contributors to flavor enhancement. A subsequent investigation focused on isoquercetin and astragalin due to their notably high variable importance scores and acknowledged chemical relevance. E-tongue results suggest that MP exhibits strong bitterness-masking activity. The interaction of flavonol glycosides (isoquercetin and astragalin) with MP in higher pH environments caused fluorescence quenching. Molecular docking results indicate that variations in the molecular geometries of isoquercetin and astragalin contribute to their distinct binding interactions with bitter taste receptor TAS2R14.

CONCLUSIONS: These findings provide mechanistic insight into processing-induced protein–flavonol interactions and offer theoretical guidance for optimizing spice-assisted meat processing strategies and taste quality regulation.

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ABBREVIATIONS

MP	myofibrillar protein
SBS	stewed beef with spices
ST	semitendinosus
<i>Z. bungeanum</i>	<i>Zanthoxylum bungeanum</i> Maxim

INTRODUCTION

Stewed beef with spices (SBS) is a highly important traditional meat product in China, known for its unique taste and excellent nutritional value.¹ Traditionally, beef has always been marinated and then cooked with spices, including *Zanthoxylum bungeanum* Maxim. (*Z. bungeanum*). The impact of various spices on the flavor profile of SBS exhibits significant variability. Spices have been found to affect the taste of SBS, including its sweetness, umami, sourness, and bitterness.² It is well known that *Z. bungeanum* has a distinct tingling and anesthetic sensation, which is attributed to numbing substances, such as hydroxy- α -sanshool, hydroxy- β -sanshool, and hydroxy- γ -isosanshool.^{3,4} Recently, the bitterness in *Z. bungeanum* has attracted considerable attention from researchers.⁵ Bitterness is detected by 25 human bitter taste receptors (TAS2Rs), among which TAS2R14 plays a dominant role.⁶ To establish the relationship between these receptors and specific bitter tastants, complex flavor profiles must be comprehensively characterized. In a study by Yang *et al.*,⁷ metabolomic analysis using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS) was employed to identify nonvolatile compounds. Combined with sensory evaluation, a total of 15 key bitter-tasting compounds in *Z. bungeanum* were characterized. Among these, quercetin, arbutin, and isorhamnetin were identified as the major bitter contributors due to their high variable importance in projection (VIP) values and low taste thresholds. To date, researchers have primarily focused on two independent aspects: the characterization of bitter-tasting compounds and the elucidation of the numbing mechanism of *Z. bungeanum*. However, there is a lack of systematic research on the taste contribution of *Z. bungeanum* to SBS. Metabonomic technologies, which have demonstrated potential for identifying and quantifying low-molecular-weight metabolites, are efficient in characterizing the key taste impact of *Z. bungeanum* on SBS.^{8,9} Further research is required to fully understand the taste contribution mechanism, including the application of target-oriented flavor editing techniques.

Plant flavonoids, such as quercetin-3-O-glucoside, quercetin, and isorhamnetin, have been identified as key bitter compounds in *Z. bungeanum*.⁷ As secondary metabolites, plant flavonoids play a crucial role in mediating plant responses to both biotic and abiotic environmental stimuli.¹⁰ Flavonol glycosides, which contain a higher number of 3-OH groups compared to other flavonoids, are abundant in the epidermal cells of plant tissues.¹¹ This structural characteristic may contribute to their perceived bitterness, potentially through specific interactions with TAS2Rs.¹² The abundance of hydroxyl groups in these compounds distinguishes them from other flavonoid subclasses in terms of sensory impact.¹³ Consequently, it is necessary to examine further the function of flavonoids in the taste contribution mechanisms of *Z. bungeanum* on

beef. While flavonoids, particularly quercetin derivatives, have been identified as the key contributors to the bitter taste profile in *Z. bungeanum*, their sensory impact can be significantly modulated by protein interactions.^{7,14} The bitterness of quinine was notably reduced by whey protein isolate compared to its bitterness in water at an equivalent concentration.¹⁵ Additionally, research has explored the interactions between proteins and volatile aroma compounds.^{16,17} The binding between myosin and aldehydes has been shown to involve rapid association and dissociation.¹⁸ The addition of etheric compounds decreases the surface hydrophobicity of myofibrillar protein (MP), potentially aiding in the release of aldehydes with longer carbon chains.¹⁹ This indicates that the binding and release behaviors of flavor compounds are highly dependent on the microenvironment of the protein matrix. Crucially, environmental factors such as pH significantly affect protein functionality and structure, thereby altering these protein binding patterns.^{20–22} Shen *et al.*²² examined the binding of specific pyrazine compounds to MP and established that pH significantly influences these interactions, particularly at lower pH levels (4.9, 5.5). While these studies have advanced our understanding of volatile aroma–protein interactions, it remains unclear how exactly nonvolatile flavonoids regulate the taste of SBS, and more importantly, how flavonol glycosides bind to MP under different pH conditions.

Therefore, the objective of the study reported here was to investigate the taste contribution effect of *Z. bungeanum* to SBS and to assess the impact of the endowed flavonol glycoside–protein binding on its bitter perception. The taste contribution of *Z. bungeanum* on SBS was elucidated using metabolomic analysis based on UHPLC–Q Exactive Focus-mass spectrometry (QE-Focus-MS). Additionally, fluorescence spectroscopy and molecular interaction analysis were employed to reinforce our understanding of MP interactions with *Z. bungeanum*. Overall, the study provides technical support for the scientific formulation of spice blends in SBS. The findings offer crucial insights into the taste contribution of spices on SBS and the mechanisms of taste–protein binding behavior.

MATERIALS AND METHODS

Sample preparation and chemicals

Z. bungeanum was Dahongpao Huajiao, purchased from Jiangsu Chaoyue Agricultural Development Co. Ltd. The powder was then stored in a sealed container until use. Fresh muscle (semitendinosus, ST) was acquired from three 2-year-old grassland yellow cattle of Beijing Zhuochen Animal Husbandry Co. Ltd. The cattle passed through quarantine and had a mass of approximately 400 ± 20 kg. The slaughtered cattle were hung and air-cooled for 48 h (circulating cold air, 2 m s^{-1} ; temperature, $0\text{--}4^\circ\text{C}$). As shown in supporting information, Fig. S1, all the shanks were randomly allocated to five groups (the five groups were: EZ, *Z. bungeanum*; EBS-1, ST boiled in salt; EBS-2, ST boiled in salt and *Z. bungeanum*; EBS-3, ST boiled in salt and compound spices, *Z. bungeanum* contained; EBS-4, ST boiled in salt and compound spices, *Z. bungeanum* not contained). In the positive control group (EZ), the *Z. bungeanum* powder was boiled in water with no ST. In

the negative control group (EBS-1), the beef shanks were boiled in 2000 mL of water, added with 17 g of salt, for 40 min, and then soaked in the stewing fluid for 90 min. The final concentration of *Z. bungeanum* in the sample mixture was maintained at 10 g kg⁻¹. In the other three experimental groups, the beef shanks, 17 g of salt, and four different packets of spice powder were prepared according to the proportion of spices in the SBS and were boiled in 2000 mL of water for 40 min. Then the beef shanks were soaked in stewing fluid for 90 min and pulled out.

Solvent extraction of metabolites

Following Yang *et al.*⁷ with slight modification, the *Z. bungeanum* was desiccated in an air-drying oven set at 65 °C for 4 h and then pulverized using a high-speed crusher. The *Z. bungeanum* powder was sifted via a 60-mesh sieve. The powder was obtained by extracting it at 60 °C for 2 h using an 800 g kg⁻¹ aqueous methanol solution (250 mL), zinc acetate solution (0.5 mL), and potassium ferrocyanide solution (0.5 mL). The solution was centrifuged at 11 200 × *g* for 10 min at 4 °C after being left to stand for 10 min. The supernatant was filtered and concentrated in a 50 mL volumetric flask. Subsequently, 2 mL was drawn using a syringe and filtered through a 0.45 µm organic filter membrane. The filtered solution was then kept at 4 °C for later use.

After being frozen in liquid nitrogen, the beef samples were crushed using a grinder (FSJ-A03D1, Bear Electric Appliance Co. Ltd, China). Beef powder (5 g) was extracted by ultrasonication it at 40 °C for 2 h, using an 800 g kg⁻¹ aqueous methanol solution (20 mL), zinc acetate solution (0.5 mL), and potassium ferrocyanide solution (0.5 mL). The solution was centrifuged at 11 200 × *g* for 10 min at 4 °C. This process was repeated once more. The supernatants were pooled and then filtered through a 0.45 µm organic filter membrane. The filtered solution was then kept at 4 °C for later use.

UHPLC–QE–Focus–MS analysis

Based on the method by Wang *et al.*,²³ the nonvolatile substances of the five groups were identified using UHPLC–QE–Focus–MS. Chromatographic separation was performed on an HSS-C18 column (Waters Acquity UPLC HSS T3; 2.1 mm × 100 mm, 1.8 µm). The mobile phase system consisted of A (formic acid and acetonitrile, 1:1000 v/v) and B (formic acid, 1:1000 v/v). The injection volume was set at 5 µL. The ESI source MS detection operational settings were as follows: capillary temperature, 320 °C; spray voltage, +3800 V/–3400 V; scan range, *m/z* 300–1500 for MS; collision energy, 15/30/45 eV; scanning mode, Full MS/dd-MS2; Full MS scan, 70 000 FWHM; dd-MS2 scan, 17 500 FWHM.

Data processing and machine learning analysis

The raw data from UHPLC–QE–Focus–MS analysis for metabolomics were analyzed using Thermo Compound Discover 2.0 (CD), Chembook (<https://www.chemicalbook.com/ProductIndex.aspx>), ChemSpider (<https://www.chemspider.com/>), HMDB (<http://www.hmdb.ca/>), and METLIN (<http://www.metlin.scripps.edu/index.php>), together with an in-house MS/MS database, utilized for peak annotation. To identify significant differential metabolites, we applied a two-step statistical analysis process, including partial least squares discriminant analysis and Student's *t*-test. Only metabolites simultaneously satisfying VIP > 1 and *P* < 0.05 were defined as candidate biomarkers. Statistical analyses were conducted using SPSS version 20.0 software and R version 13.4 software. Machine learning analysis (K-means and random forest) was conducted and results visualized using MetaboAnalyst 6.0.²⁴

Sensory evaluation

The sensory evaluation was conducted according to the method of Li *et al.*²⁵ with slight modifications. Eleven sensory evaluators (6 females and 5 males; aged from 22 to 35 years) were selected from the Chinese Food Processing and Equipment Innovation Team in the Chinese Institute of Agricultural Sciences. The bitter reference standard was prepared with quinine (20 mg kg⁻¹). The bitter intensity of the reference standard was assigned as 5 points. The bitter intensity of the samples was presented using a 0–10 scale. Higher scores mean a bitter taste. The sensory study was approved by the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (IFST026003).

Extraction of MPs at different pH values

MPs were isolated following the method outlined by Wang *et al.*²⁶ and Shen *et al.*,²² with slight adjustments. Beef muscle was chopped and combined with 4 volumes of 10 mmol L⁻¹ phosphate buffer (PBS) containing 0.1 mol L⁻¹ NaCl, 1 mmol L⁻¹ EGTA, and 2 mmol L⁻¹ MgCl₂ at pH 7.0. The mixture was homogenized twice using an Ultra-Turrax homogenizer at 15 000 rpm for 30 s in an ice bath. The supernatant produced after centrifuging the combination of tissue homogenate (4 °C, 2000 × *g*, for 15 min) was poured off. It was then washed two additional times with the same PBS, utilizing identical blending and centrifugation procedures. The initial precipitate was subsequently rinsed three additional times with 4 volumes of 0.1 mol L⁻¹ NaCl using identical parameters to those previously described. Before the last centrifugation, the suspension was filtered through four layers of cheesecloth to exclude connective tissue. Following the final centrifugation, the MP paste was separated into five portions and subsequently mixed with the appropriate pH PBS (50 mmol L⁻¹) with 0.6 mol L⁻¹ NaCl. The MP solutions were pH-adjusted to 5.0, 5.5, 6.0, 6.5, and 7.0 (± 0.02) using 2 mol L⁻¹ HCl or 2 mol L⁻¹ NaOH. They were then held at 4 °C overnight before additional analysis. The MP solutions were diluted to 1 mg mL⁻¹ with the appropriate pH PBS (50 mmol L⁻¹, 0.6 mol L⁻¹ NaCl). Protein concentration in each solution was determined using the Biuret method with bovine serum albumin (Sigma Chemical Co., USA).

Electronic tongue (E-tongue) analysis

An electronic tongue (Astree, Alpha MOS, Toulouse, France) was used to measure the bitter masking activity of MP. Before the experiment, the taste sensors were dipped into a reference solution (30 mmol L⁻¹ KCl containing 0.3 mmol L⁻¹ tartaric acid). The electrodes were submerged in a 3.3 mmol L⁻¹ KCl solution to ensure optimal sensor performance. The activation process lasted for 15 min, after which the sensors were thoroughly rinsed with ultrapure water to eliminate any remaining activation solution.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were recorded using an attenuated total reflection accessory. The MP dispersion was adjusted to 2 mg mL⁻¹ using PBS at the corresponding pH. Standard solutions of isoquercitrin, astragalin, and acetophenone (0.1 mg mL⁻¹) were prepared in HPLC-grade methanol. Briefly, 100 µL of each standard solution was added to 9.9 mL of the MP dispersion at different pH levels to achieve a final concentration of 1 mg L⁻¹ in the reaction system. The mixtures were vortexed and allowed to react at room temperature (25 °C) for 40 min before measurement. The scan range was set from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.

Fluorescence spectroscopy measurements

Assay solutions were created by combining MP solution (9.9 mL, 4 mg mL⁻¹) with flavonol glycoside solution (100 µL, 0.1 mg mL⁻¹, diluted with methanol) at varying pH levels. Fluorescence spectroscopy measurements were conducted using an F-2500 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence spectra were acquired with the excitation and emission slit widths set at 10 nm, excitation voltage at 400 V, and scanning speed at 300 nm min⁻¹. Fluorescence emission spectra were measured between 300 and 450 nm using an excitation wavelength of 280 nm. Synchronous fluorescence provides specific information about tyrosine (Tyr) or tryptophan (Trp) residues, as stated by Pan *et al.*²⁷

Molecular docking

The protein sequence was obtained from the UniProt database. The structural diagram was created with AlphaFold3 software and PyMOL (Version 2.5, Schrödinger, LLC). The three-dimensional structures of isoquercetin (ID: 5280804) and astragalin (ID: 5282102) were retrieved from PubChem. Molecular docking investigations were conducted on the CB-DOCK2 online server (<https://cadd.labshare.cn/cb-dock2/php/blinddock.php>).²⁸ CB-DOCK2 will perform cavity probing based on artificial neural networks and docking using Autodock Vina. The results of the docked complex were selected based on the binding energy, and force analysis and docking position assessment were performed for subsequent kinetic verification of binding stability. Ligand–receptor force analysis was done by relying on the PLIP

online server (<https://plip-tool.biotec.tu-dresden.de/plip-web>) analysis, and ligand–receptor three-dimensional conformations were displayed using PyMOL software.

Statistical analysis

Experimental results were derived from three independent biological replicates ($n = 3$). All results are presented as mean $\pm$ standard error. One-way analysis of variance was conducted to compare the variations among several recipe groups following the same cooking method. Differences caused by the presence and absence of *Z. bungeanum* were analyzed by an independent-samples *t*-test. Statistical comparisons of the various treatments were conducted using Duncan's method at a significance level of $P < 0.05$. Data analyses were performed using SPSS Version 22.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Global analysis of nonvolatile compounds in different treatment groups

Metabolomics data of sample groups (EZ; EBS-1; EBS-2; EBS-3; EBS-4) were obtained using a UHPLC–QE–Focus–MS system. Data acquisition was conducted in both positive and negative ionization modes to ensure comprehensive metabolite detection. Potential nonvolatile taste metabolites were identified by integrating data from relevant taste literature, focusing on compounds that met both structural criteria and sensory thresholds. Figure 1 shows the results of K-means analysis used to determine

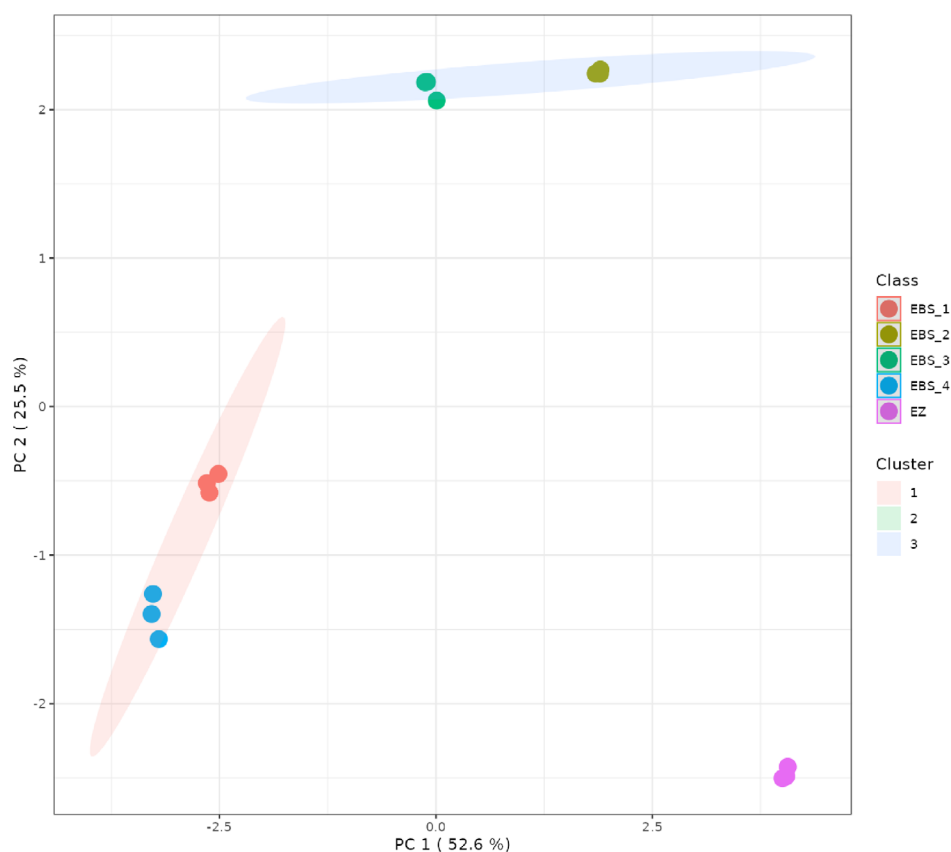


Figure 1. UHPLC–QE–Focus–MS-based metabolomic analysis of five groups, K-means score plot. EZ, *Z. bungeanum*; EBS-1, ST boiled in salt; EBS-2, ST boiled in salt and *Z. bungeanum*; EBS-3, ST boiled in salt and compound spices, *Z. bungeanum* contained; EBS-4, ST boiled in salt and compound spices, *Z. bungeanum* not contained.

how the variables of the nonvolatile compounds contribute to the differences between samples. The nonvolatile compounds in the extractive solution of *Z. bungeanum* differed from those in other samples (EZ). There was a similarity in the nonvolatile profiles of the EBS-2 and EBS-3 samples, suggesting that the addition of *Z. bungeanum* played a key role in the taste contribution of SBS processing.

Identification of key nonvolatile compounds in SBS from *Z. bungeanum*

The VIP value was used to filter out the differential compounds of the different samples. It is generally considered that a higher VIP value suggests that a substance plays a more critical role in distinguishing among samples. The screening conditions were set at $P < 0.05$, and $VIP > 1$ for the nonvolatile difference of substances between EBS-1 and EBS-2, and a total of 242 nonvolatile substances were obtained. As shown in Fig. 2(A-a), these 242 nonvolatile compounds play a key role in distinguishing EBS-1 from EBS-2. According to Fig. 2(A-b), the selection of 207 nonvolatile differential chemicals suggests that these compounds are important in the separation of the two EBS-3 and EBS-4 samples and may predominantly originate from *Z. bungeanum*. To further determine the key nonvolatile tasting compounds imparted by *Z. bungeanum* to SBS, the results of the analysis of differences between the four treatment groups mentioned above were compared, and Venn diagrams were constructed as shown in Fig. 2(B).

There were 119 nonvolatile compounds shared by the results of the analysis of differences among the four groups. To achieve reliable classification, a supervised random forest algorithm based on ensemble learning was employed. Consequently, 15 volatile compounds with high discriminative power were identified, enabling clear differentiation among the five groups (Fig. 2(C)). These 15 compounds were primarily flavonoids and glycosides, phenolic acids, coumarins, and indole-derived compounds. Flavonoids in *Z. bungeanum* were rich in variety, with a low bitterness threshold.^{29,30} It has been established that many of these compounds are well-documented contributors to bitterness and astringency.³¹ The selection of isoquercetin and astragaloside was made for further investigation based on their elevated variable importance scores and their chemical relevance. These two flavonol glycosides are structurally analogous, sharing a quercetin aglycone core. Isoscaparin and astragaloside only differ in their glycosylation at the C3 patterns. The differences in molecular structure may influence their taste contribution behavior by affecting binding ability to protein and bitter taste receptor. Their similar structure makes them ideal candidates for comparative studies on how plant glycosylation impacts bitter taste-enhancing mechanisms. Isoscaparin and astragaloside, as key-taste flavonol glycoside compounds, still need to be selected for further analysis.

Bitterness-masking activities of MP

As shown in Fig. 3, there were significant differences in the bitterness intensity among the five groups. Compared to EZ, the bitterness intensity score indicated that the bitterness intensity significantly decreased ($P < 0.05$) after the addition of beef. In order to better observe the ability of MP to mask the bitterness of isoquercetin and astragaloside, an E-tongue was used to assess the intensity of bitterness. We used quinine as a standard to create a standard curve to correct the bitterness score. The E-tongue taste profiles are displayed in Fig. 3(B). The bitter score of isoquercetin was higher than that of astragaloside. After the addition of MP, the bitterness score decreased, suggesting a potential

bitterness-masking activity of MP. Figure 3(C) shows the principal component analysis of the E-tongue response values before and after the addition of MP to isoquercetin and astragaloside. The taste profiles of the various groups could be well differentiated, as evidenced by the cumulative contribution rate of the first two main components (PC1 and PC2), which reached 97.9%. A small Euclidean distance between two groups was expected to show similar tastes.²⁵ Isoquercetin and astragaloside displayed similar taste after the addition of MP.

FTIR spectroscopy

FTIR spectroscopy was used to identify the amide I bands of MP, calculate the ratios of α -helices, β -turns, β -sheets, and random coils, and subsequently analyze the secondary structure of MP further to investigate the influence of pH on protein structure.³² As presented in Table 1, the α -helix structure of MP decreased, and the β -sheet structure increased with decreasing pH, and MP in low-pH environments (pH 5.0, 5.5) had relatively fewer α -helix and more β -sheet structures. This indicated that low-pH environments induced the denaturation of proteins and a greater degree of aggregation of MP.

To explore the impact of nonvolatile tasting compounds on the secondary structure of MP under various pH settings, isoquercetin and astragaloside were used. As presented in Table 1, the α -helix structure of isoquercetin and astragaloside significantly increased, whereas the β -sheet and random coil structures in both showed a declining trend. The connection between isoquercetin, astragaloside, and MP is facilitated by electrostatic interactions and hydrophobic interactions, which can potentially improve the stability of MP. Consequently, this interaction may result in a minor elevation of the α -helix content. Isoquercetin and astragaloside may play a part in preventing damage to the MP's secondary structure and preserving the integrity of the hydrogen bonding structure, which may be connected to flavonoids' antioxidant properties.³³ Rawel *et al.*³³ discovered that the flavonoid quercetin can bind to soy protein through hydrogen bonding, which contributes to the production of α -helices in the protein structure, as well as interacts with soy protein to scavenge free radicals produced during the storage process and causes the α -helices in the secondary structure to increase. Encapsulation of tryptophan residues had a pronounced effect on fluorescence quenching. This interaction protected the secondary structure of MP from damage and maintained the stability of the hydrogen bonding structure.

Fluorescence spectroscopy measurement

Tryptophan and tyrosine, two fluorescent amino acid residues found in MPs, are more sensitive to the polarity change of the microenvironment. Consequently, endogenous fluorescence can be used to reflect the fluorescence properties of the protein molecule and the change in the microenvironment in which these residues are embedded, and then to explain the change in the tertiary structure of the protein. The results are shown in Fig. 4, and indicate that the fluorescence intensity of MP increased with an increase in pH and that the fluorescence intensity in low-pH environments (pH 5.0, 5.5) was lower than that in high-pH environments (pH 6.0, 6.5, 7.0). Fluorescence spectroscopy was utilized to investigate the connection of flavoring substances with MPs under different pH conditions. Tryptophan residues exhibited a decrease in fluorescence intensity when exposed to a hydrophilic environment, according to Zhang *et al.*³⁴

Additionally, protein aggregation may obscure some non-polar aromatic amino acid residues, resulting in a reduction in

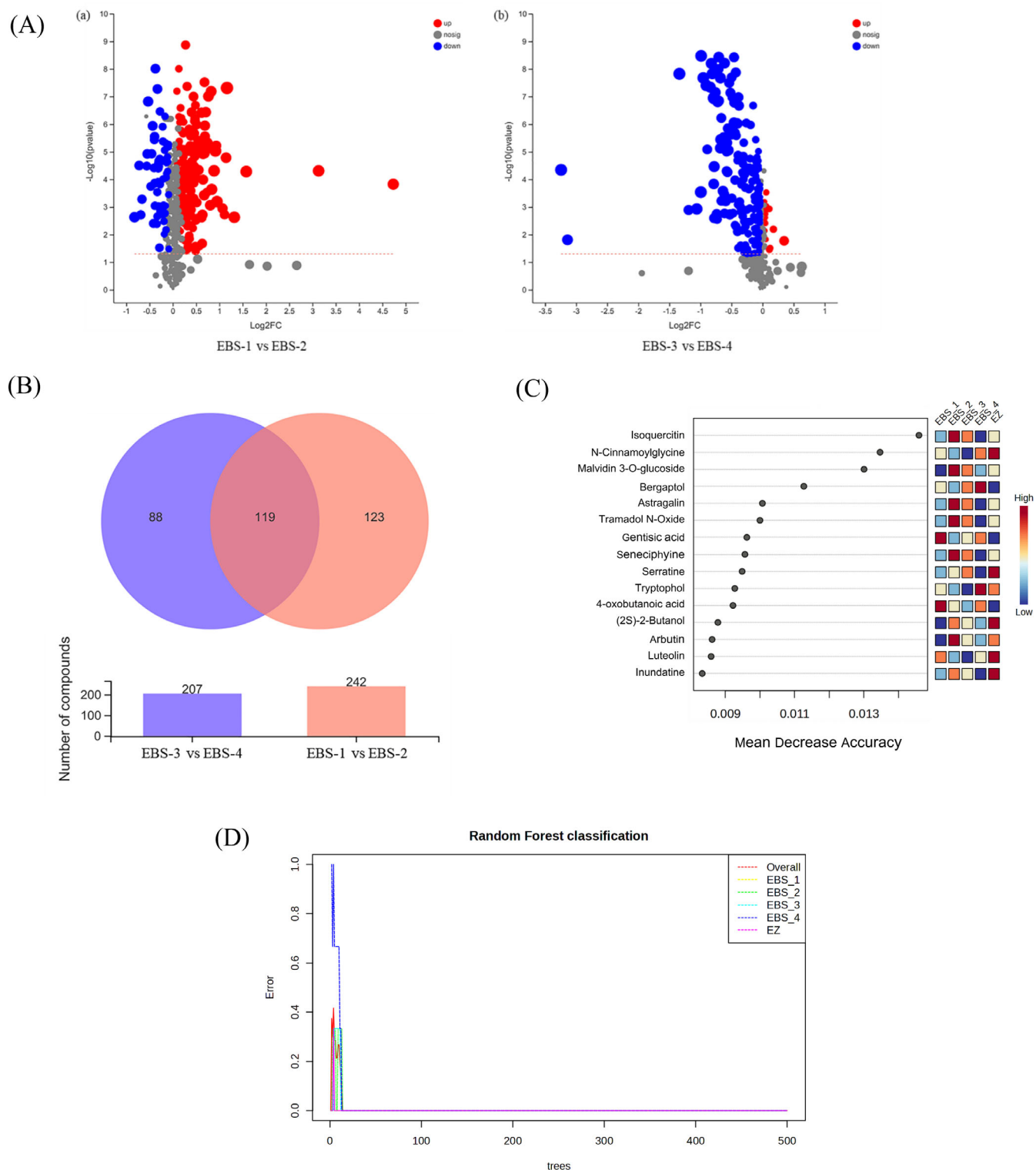


Figure 2. UHPLC-QE-Focus-MS-based metabolomic analysis of four groups: (A) volcano map of differential nonvolatile compounds in different treatment groups; (B) Venn diagram; (C) top 15 key nonvolatile components identified by the random forest model; (D) OBB error results by random forest with 500 trees.

endogenous fluorescence intensity. This finding suggests that a low-pH environment can trigger the unfolding of proteins' tertiary structure. However, it is clear from the aforementioned findings that MPs are more hydrophobic on their surfaces, have larger average particle sizes, and are located nearer to their isoelectric

point in low-pH environments. Du *et al.*³⁵ demonstrated that when the degree of protein denaturation reaches a certain point, the protein molecules interact with one another through hydrophobic interactions, disulfide bonds, and intermolecular interactions. This results in an increase in protein mutual aggregation,

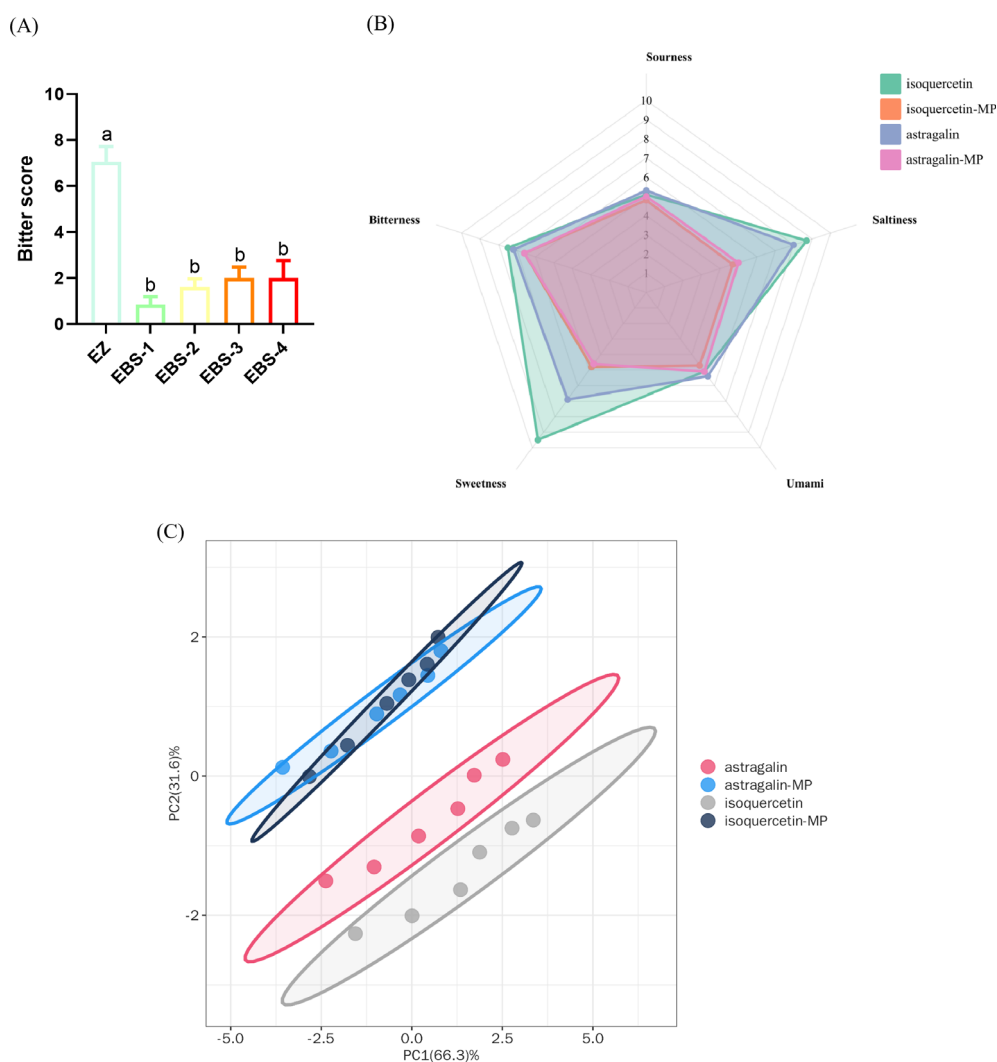


Figure 3. Bitter-masking activity evaluation: (A) sensory evaluation result profile; (B) E-tongue taste profile of isoquercetin and astragalin; (C) principal component analysis score plot. Significant differences among different groups are indicated by different lowercase letters ($P < 0.05$).

which creates new polymers and re-encapsulates the tryptophan residues. Thus, a decline in protein fluorescence intensity due to low pH is possible.

After further investigation, it was discovered that the MP-isoquercetin group and the MP-astragalin group had lower fluorescence intensities than the MP group. This phenomenon was more pronounced at high pH levels and showed less variation from the MP group at other pH levels. As a result, it can be concluded that isoquercetin and astragalin have a quenching impact on fluorescence. This might be because the interaction between flavoring agents and MP encourages protein aggregation, which encapsulates tryptophan residues and causes the fluorescence burst phenomenon. Conversely, proteins in an environment with high pH are more stretched out, which allows for a better reaction between them and the compounds, resulting in a more significant bursting effect. The fluorescence intensity of the protein showed a declining trend as rutin and quercetin increased. Rawel *et al.*³³ discovered that tryptophan levels can be decreased by flavonoids like populin because the hydroxyl substituents on their B and C rings can interact with the tryptophan side chains of proteins.

Molecular docking

The bitter taste of isoquercetin and astragalin was stimulated by the interaction with the receptor protein TAS2R14.⁶ Utilization of molecular docking as a vital strategy to reveal the binding conformation between small-molecule compounds and proteins has long been recognized. Figure 5 displays two- and three-dimensional representations of the outcomes of molecular docking. TAS2R14 and the two compounds, and the accompanying amino acid residues, were visualized in two dimensions. Isoquercetin and astragalin preferentially connect with particular residues at the binding site and vary in conformation. Combined with the overall ligand-protein interaction position analysis, it can be seen that the ligand binds to the protein-binding cavity with a low free energy, forming a certain number of noncovalent interactions. Furthermore, hydrogen bonding controls interactions with the binding pocket of bitter taste receptors.³⁶ Isoquercetin and astragalin showed the same binding free energy with the bitter taste receptor. While their binding sites were not completely identical. The conformation features of the two compounds may result in this phenomenon. Although isoquercetin and astragalin are both flavonol glycosides, the distinct

Table 1. Secondary structure changes in MPs at different pH and flavonol glycosides.

	pH	Control	MP-isoquercetin	MP-astragaloside
β -Sheets	5.0	48.34 $\pm$ 2.80 ^a	29.79 $\pm$ 1.04 ^c	34.68 $\pm$ 1.85 ^b
	5.5	40.46 $\pm$ 1.63 ^a	22.09 $\pm$ 1.57 ^b	23.62 $\pm$ 1.74 ^b
	6.0	38.23 $\pm$ 1.18 ^a	25.61 $\pm$ 1.36 ^b	18.23 $\pm$ 1.18 ^c
	6.5	27.09 $\pm$ 3.33 ^a	19.18 $\pm$ 0.31 ^b	15.24 $\pm$ 1.76 ^c
	7.0	23.09 $\pm$ 1.29 ^a	24.08 $\pm$ 3.02 ^a	15.10 $\pm$ 2.75 ^b
Random coils	5.0	23.18 $\pm$ 2.61 ^a	14.28 $\pm$ 2.06 ^b	10.16 $\pm$ 2.06 ^c
	5.5	22.34 $\pm$ 2.28 ^a	21.50 $\pm$ 2.10 ^a	23.80 $\pm$ 1.85 ^a
	6.0	18.38 $\pm$ 1.77 ^a	22.24 $\pm$ 2.18 ^a	21.38 $\pm$ 2.67 ^a
	6.5	13.76 $\pm$ 2.30 ^b	13.79 $\pm$ 0.60 ^b	24.18 $\pm$ 2.02 ^a
	7.0	13.43 $\pm$ 1.17 ^b	11.13 $\pm$ 1.29 ^b	17.15 $\pm$ 1.91 ^a
α -Helixes	5.0	8.31 $\pm$ 1.29 ^c	21.75 $\pm$ 0.88 ^a	18.86 $\pm$ 0.65 ^b
	5.5	15.36 $\pm$ 0.74 ^b	32.24 $\pm$ 3.57 ^a	31.57 $\pm$ 2.17 ^a
	6.0	22.84 $\pm$ 1.87 ^b	33.72 $\pm$ 4.76 ^a	36.84 $\pm$ 1.19 ^a
	6.5	40.56 $\pm$ 3.74 ^{ab}	45.74 $\pm$ 2.73 ^a	37.72 $\pm$ 1.28 ^b
	7.0	43.89 $\pm$ 2.59 ^a	45.42 $\pm$ 1.20 ^a	47.00 $\pm$ 1.61 ^a
β -Turns	5.0	20.17 $\pm$ 2.67 ^b	34.19 $\pm$ 1.29 ^a	36.30 $\pm$ 0.86 ^a
	5.5	21.84 $\pm$ 1.18 ^a	24.17 $\pm$ 2.47 ^a	21.00 $\pm$ 2.48 ^a
	6.0	20.55 $\pm$ 1.44 ^{ab}	18.42 $\pm$ 1.87 ^c	23.55 $\pm$ 2.70 ^a
	6.5	18.59 $\pm$ 0.72 ^b	21.29 $\pm$ 2.65 ^{ab}	22.85 $\pm$ 0.63 ^a
	7.0	19.59 $\pm$ 2.08 ^a	19.37 $\pm$ 2.80 ^a	20.75 $\pm$ 0.10 ^a

Data are the average value $\pm$ the standard deviation. Different letters in the same pH and different treatment groups indicate significant differences ($P < 0.05$).

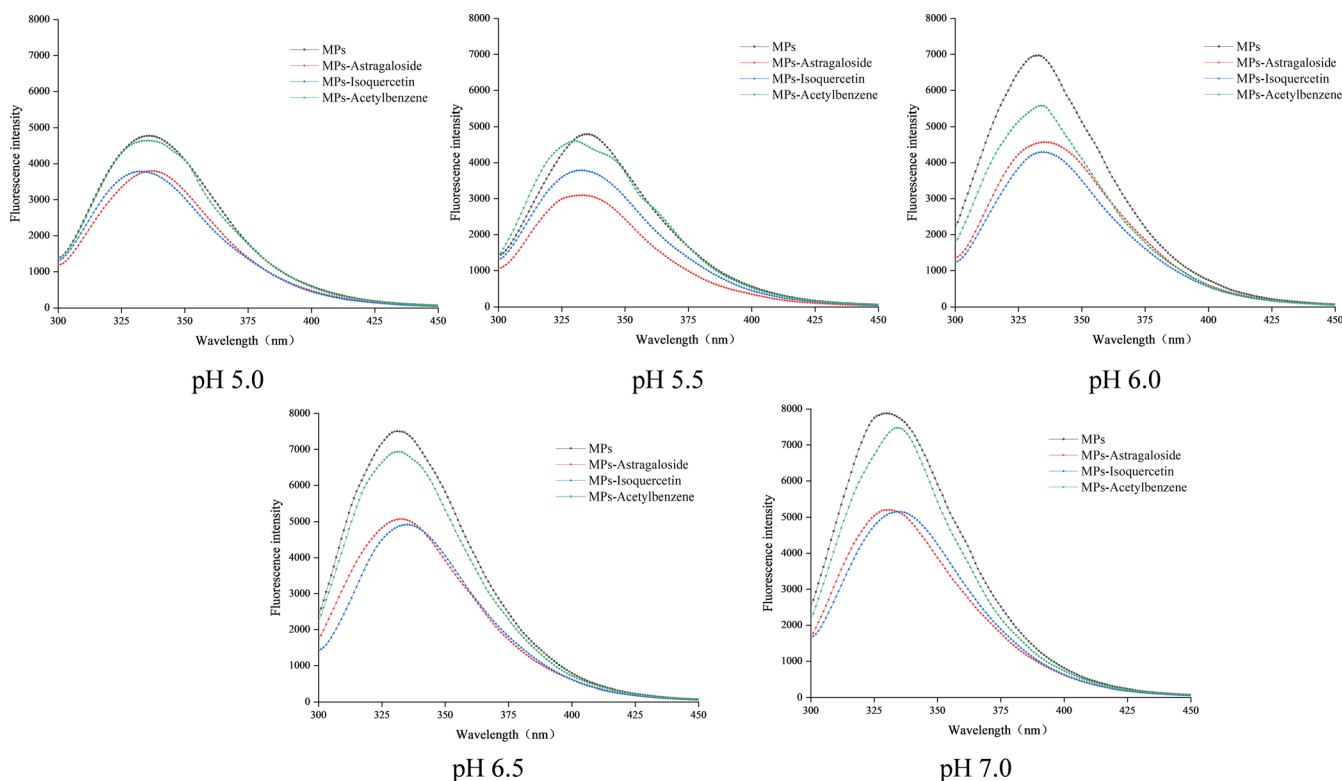
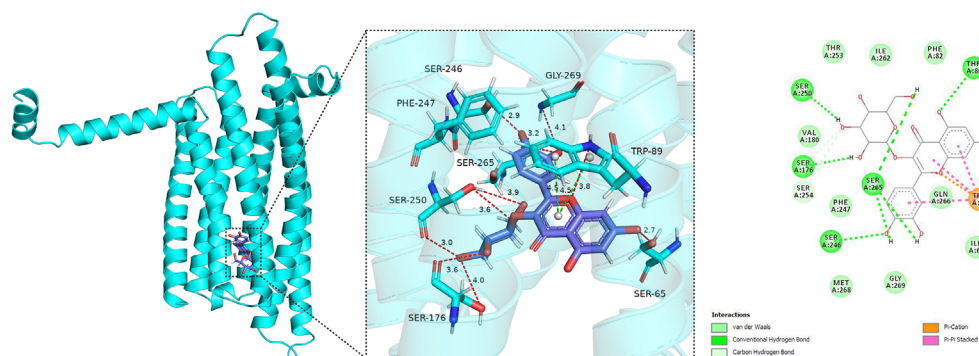


Figure 4. Effects of nonvolatile compounds on the fluorescence of MP at different pH.

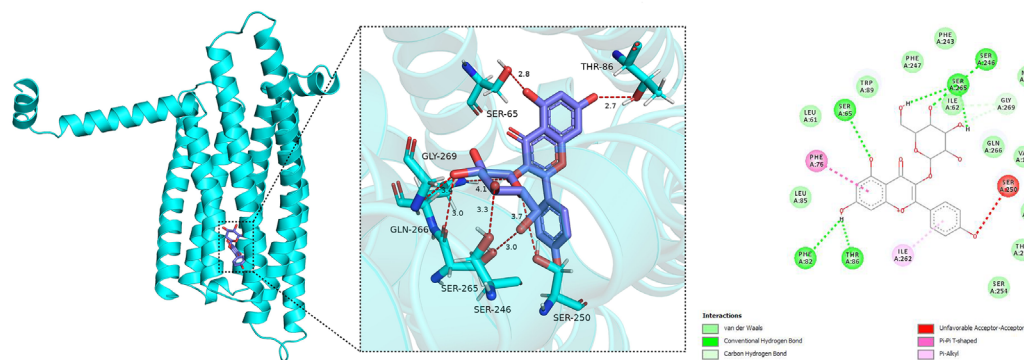
positioning of their sugar moiety composition (isoquercetin with a glucose moiety and astragaloside with a galactose moiety) likely modulates hydrogen bonding or steric effects with adjacent

receptor residues. As shown in Fig. 5, myosin and the two compounds' interactions and the accompanying amino acid residues were visualized in two dimensions. The interactions between

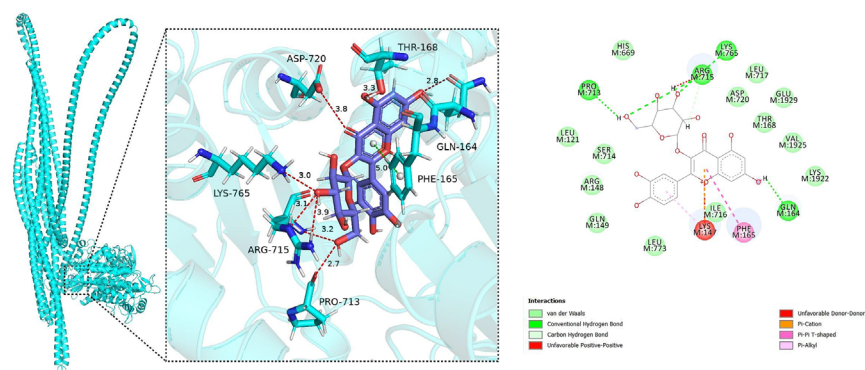
Isoquercetin-TAS2R14



Astragalin-TAS2R14



Isoquercetin-MP



Astragalin-MP

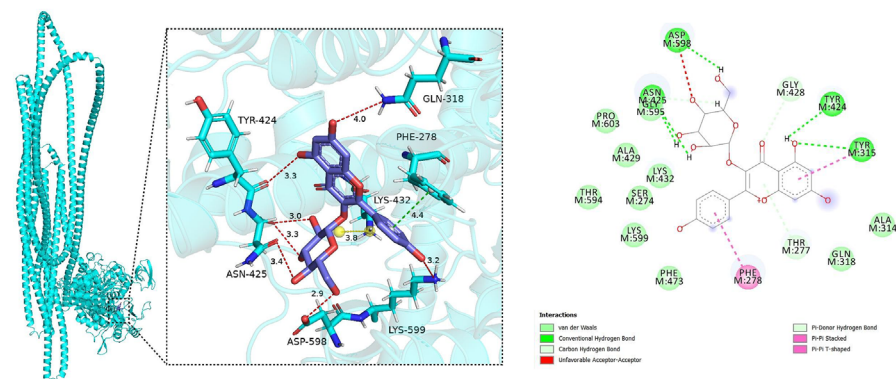


Figure 5. Docking conformations and binding sites of two flavonol glycoside compounds with TAS2R14.

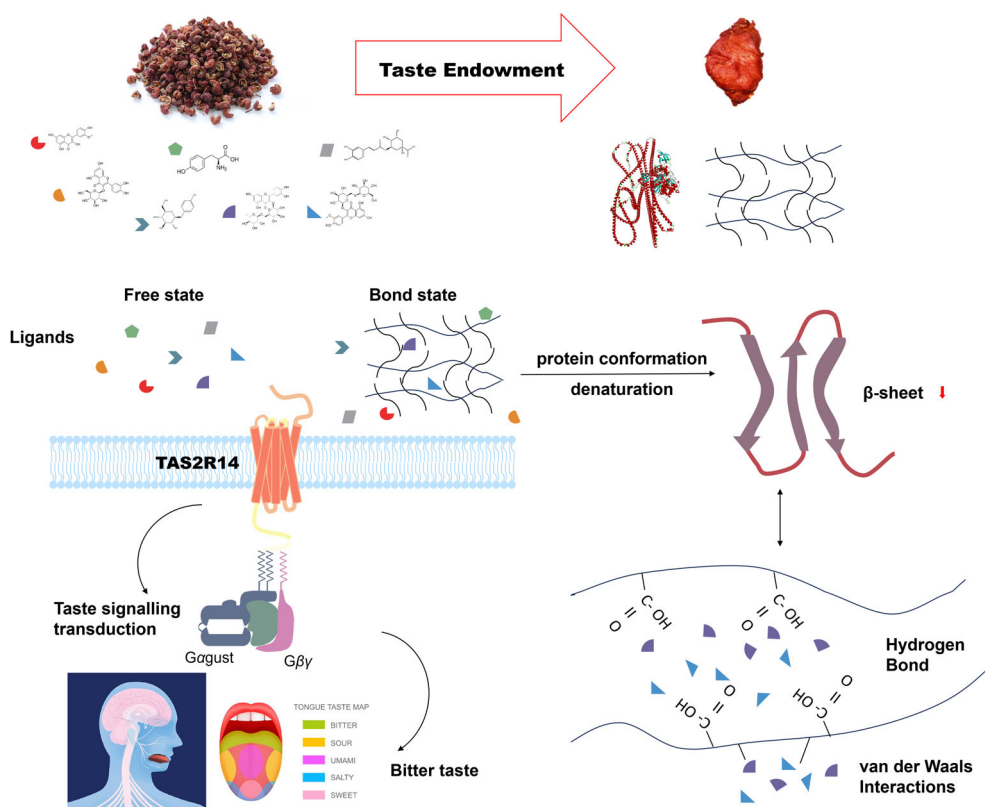


Figure 6. Schematic modeling of the taste contribution.

myosin and isoquercetin and astragalin were mainly hydrogen bonds and van der Waals interactions. However, the two compounds exhibit binding energies different from that of myosin. The differentiation is linked to variations in their molecular geometries, particularly the arrangement of hydroxyl groups. Huang *et al.*³⁷ used molecular docking to demonstrate that citronellol binds to bovine serum albumin through hydrophobic interactions, hydrogen bonds, and van der Waals forces, which is similar to the results in this study.

DISCUSSION

In this study, we have identified the taste compounds contributing to SBS by *Z. bungeanu*, with bitter compounds being predominant. Sensory evaluation results confirmed that *Z. bungeanu* alone exhibits noticeable bitterness. However, the bitterness is significantly reduced with the addition of beef. This taste-masking phenomenon aligns with the complex food matrices, particularly those rich in lipids and proteins, that structurally entrap or chemically bind flavor molecules, thereby reducing their volatility and sensory availability.³⁸ Figure 6 illustrates a possible taste contribution mechanism derived from the experimental results in this study. Tenney *et al.*¹⁵ investigated the effect of whey isolate proteins on the perception of quinine and caffeine bitterness. MP may have a similar bitter-masking ability. To elucidate this phenomenon, E-tongue analysis was conducted to reveal the bitterness-masking ability of MP to the two major bitter compounds: isoquercetin and astragalin. Although isoquercetin and astragalin are flavonol glycosides and share some structural similarities, there are some differences in their binding behavior to MP. These interactions help maintain the secondary structure of

MP by stabilizing hydrogen bonding networks and preventing structural degradation. The addition of astragalin and isoquercetin leads to an increase in α -helix and a decline in β -sheet structures. Such structural transitions suggest that the binding of flavonol glycosides induces a more compact and ordered conformation in MP.^{39,40} This result might be related to the hydrophobic and electrostatic interactions that strengthen the hydrogen bonding of protein structure with the creation of protein–flavonoid complexes. Consistent with FTIR analysis, molecular docking results indicated that hydrogen bonding and van der Waals interactions were stronger than hydrophobic interactions. The abundant hydroxyl groups on the glycoside rings, driven by electrostatic orientation, may form dense hydrogen networks with the amino acid residues of the host protein.^{41,42} Astragalin and isoquercetin have different binding sites, although they both have similar binding energy to TAS2R14. Subtle variations in ligand–receptor interactions have the potential to induce distinct conformational changes in the receptor's transmembrane domain. Binding with MP raises the possibility of steric hindrance, which would inhibit the interaction with bitter taste receptors, hence reducing the experience of bitterness. Disparities might explain perceptible variations in bitterness onset, duration, or aftertaste *in vivo*. In the current study, the distinction between the free and bound states of bitter compounds is overlooked. A classification of bitter compounds based on their free and bound states might be helpful to broaden the perspective of the binding behavior of bitter substances to bitter receptors. Further studies should integrate advanced separation techniques to distinguish between bound and free-state bitter compounds in the food matrix. UHPLC–MS/MS,⁴³ NMR,⁴⁴ and isothermal titration calorimetry⁴⁵ can help to elucidate molecular interactions, providing

insights into binding mechanisms. The application of molecular dynamics simulations may be useful in further analyzing the response of bitter compounds to bitter receptors after binding with MP.⁴⁶ Accordingly, we can develop strategies to mask bitterness in meat products, improve flavor design, and enhance consumer acceptance.

CONCLUSION

The present study elucidated the key taste substances imparted to SBS by *Z. bungeanum* and the behavior of flavonol glycoside–protein binding. According to random forest analysis, a total of 15 key taste substances were conferred by *Z. bungeanum* to SBS, including flavonoids, phenolic acids, and phenolic and amino acid compounds. Isoquercetin and astragalin, as flavonol glycosides, were chosen to identify the bitter masking ability. E-tongue results indicate MP has good activity in masking bitter taste. The two flavonol glycosides interacted with MP at low pH, protecting the secondary structure from damage and maintaining the stability of the hydrogen bonding structure, increasing the binding sites of proteins and flavor-presenting substances. Molecular docking results suggest that differences in the molecular geometries of isoquercetin and astragalin lead to differences in the binding with TAS2R14. The outcome provides a valuable view for investigating the interactions between taste compounds and food components.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jingfan Wang: Conceptualization, Methodology, Software. Jingfan Wang and Wei Jiang: Data curation, Writing – original draft preparation. Yu Qiang and Dong Han: Visualization, Investigation. Chunhui Zhang, Marie-Laure Fauconnier, and Giorgia Purcaro: Supervision, Writing – reviewing and editing. Feng Huang: Software, Validation. Prince Chisoro and Wei Jia: Formal analysis. Shuangmei Xia, Ping Yang, and Chenchen Qi: Resources, Funding acquisition.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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