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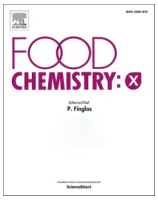

Glycation modification of protein hydrolysate from channel catfish (*Ictalurus Punetaus*) viscera to mitigate undesirable flavor: Unraveling structure and flavor characteristics

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ABSTRACT

To investigate the impacts of glycation modification on the structure and flavor of fish viscera-derived protein hydrolysates, channel catfish viscera was utilized and hydrolyzed by Flavourzyme, followed by glycation with glucosamine, xylose, ribose and glucose, respectively. The structural characteristics, taste and odor of glycated products were evaluated. The results revealed that glycation led to an increase in peptides (<1 kDa) and a decrease in peptide fraction (>5 kDa). UV and fluorescence spectra indicated a gradual rise in absorbance and fluorescence intensity. Glucosamine-induced glycation significantly mitigated bitterness, while enhancing saltiness and umami. GC–MS/MS analysis revealed that glycation led to reduced undesirable odor, and formation of aromatic compounds. LC-MS/MS identified Arg and Lys as the main modification sites. Interestingly, aldehydes were found to modify peptides via carbonyl-amine reaction, leading to decreased fishy odor. The present study contributes to flavor improvement of fish viscera-derived peptides through glycation and high-value utilization of fish viscera.

1. Introduction

The channel catfish (*Ictalurus punctatus*) is one of the most comprehensively farmed fish. Its global production has surged with the expansion of the aquaculture industry, exceeding 54 million tons in 2023 (Zhang et al., 2023). However, the farmed channel catfish has unpleasant off-flavors due to its diet habit and freshwater environment. During fish processing, a substantial amount of by-products, including skin, bones, and viscera, are generated in bulk. Notably, fish viscera of channel catfish contains 9.1 % protein and 44.5 % lipid (wet basis), presenting a significant opportunity for high-value application. But it is currently underutilized as animal feed, while separating various organs within fish viscera poses a hindrance (Zhang et al., 2023). The fishy odor in protein hydrolysates from freshwater fish viscera primarily originates from the fish itself. The fishy odorants mainly include geosmin (GSM) and 2-methylisoborneol (2-MIB) as well as volatile compounds such as aldehydes (e.g., hexanal, heptanal, nonanal) formed from lipid oxidation. Consequently, the efficient utilization of fish viscera produced during channel catfish processing, coupled with the mitigation of its unpleasant odor, has emerged as a critical concern in the industry.

Enzymatic hydrolysis is an important strategy for recovering protein-containing parts from fish viscera to generate high value-added protein hydrolysates. Fish viscera-derived protein hydrolysates have been reported to exhibit diverse bioactivities, such as antioxidant, antihypertensive, hypoglycemic, anticoagulant, and antibacterial activities, making it a promising source of functional food ingredients (Ramakrishnan, Jeong, Park, Cho, & Kim, 2023). However, protein hydrolysates from fish viscera tend to have undesirable flavors, mainly due to the presence of main volatile compounds (e.g. hexanal, heptanal, and (*E*)-2-octenal) formed by adsorption and lipid oxidation in aquaculture environments (Liu et al., 2021), which impedes their food application. Currently, several methods have been used to mitigate the undesirable flavor of protein hydrolysates including physical approaches (e.g. activated carbon adsorption), biological methods (e.g. microbial fermentation), and chemical techniques (e.g. Maillard reaction) (Y. J. Zhou et al., 2024). While activated carbon can partially

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reduce the unpleasant odor, it leads to significant protein loss during treatment. Microbial fermentation can reduce the odor to some extent but may introduce a distinct odor, while accurate control of fermentation conditions can be challenging. Hence, direct elimination of undesirable flavor during the preparation process of protein hydrolysates is of great significance. The Maillard reaction (glycation), also known as the carbonyl-amine reaction between food-derived proteins/peptides and reducing sugars, is capable of producing numerous aromatic compounds, which is promising for alleviating undesirable odors in fish viscera-derived protein hydrolysates. Furthermore, carbonyl-amine reaction involves the condensation of carbonyl groups (e.g. aldehydes and other fishy odorants) and amino group from protein/peptides, thereby contributing to the reduced fishy odor of protein hydrolysates (Cui et al., 2021). Additionally, the resulting aromatic compounds during the Maillard reaction can mask the undesirable odor (Y. Fu et al., 2020; Y. Fu, Zhang, Soladoye, & Aluko, 2020). However, the impact of glycation reaction time and types of reducing sugars on the flavor of the Maillard reaction products from fish viscera protein hydrolysates as well as the underlying structure-flavor relationship remain unclear. Normally, the Maillard reaction and inactivation of protease occur at high temperatures (Fu, Liu, et al., 2020). Consequently, simultaneous enzyme deactivation and the Maillard reaction can be achieved by the addition of reducing sugars.

In order to improve the utilization rate of fish viscera and effectively mitigate unpleasant flavor, Maillard reaction can be employed to improve the flavor of protein hydrolysates. Moreover, the effects of different reducing sugars and reaction times on the structural characteristics, flavor and volatile compounds of protein hydrolysates from fish viscera remain to be investigated. In this regard, channel catfish viscera was selected as raw material in this study. Flavourzyme was used to catalyze the enzymatic hydrolysis, followed by inducing the Maillard reaction of protein hydrolysates using four reducing sugars (glucosamine, xylose, ribose and glucose) concurrently with enzyme deactivation. The present study aimed to investigate the impacts of different reducing sugar types and reaction times on the structural characteristics, taste and volatile compounds of fish viscera-derived protein hydrolysate and to elucidate the underlying structure-flavor relationship.

2. Materials and methods

2.1. Materials and reagents

Channel catfish viscera was obtained from Chongqing Haohu Fishery Company (Chongqing, China), while swim bladder, gallbladder and impurities were removed. According to the method of ISO 1871:2009, the protein content of fish viscera was determined to be 9.03 % (wet basis). Glucosamine (purity >98 %), xylose (purity >98 %), ribose (purity >98 %), and glucose (purity >98 %) were purchased from Shenzhen Yinuo Food Ingredients Co., LTD. Flavourzyme was bought from Amano Company (Nagoya, Japan). *o*-Phthalaldehyde (OPA) and *N*-acetyl-L-cysteine were obtained from Shanghai Tengjun Biotechnology Co., LTD (Shanghai, China). Leu standard (purity, 99 %) was bought from Merck Chemical Technology Co., LTD (Shanghai, China). All other reagents employed in this study are of analytical grade.

2.2. Preparation of protein hydrolysates from fish viscera

The ultrasound-assisted defatting was performed with 0.06 % sucrose fatty acid ester solution (solid-liquid ratio 1:2.5, *w/v*) to remove lipids. The fish viscera mince is further ground in a grinder to obtain viscera mince at 4 °C. The resultant fish viscera was further mixed with deionized water in the weight ratio of 1:1. The pH of mixture was adjusted to 7.0 using NaOH (1 mol/L) followed by Flavourzyme (1 %)-catalyzed enzymatic hydrolysis at 50 °C for 1 to 7 h. Upon completion of enzymatic hydrolysis, the enzyme was inactivated at 100 °C. Meanwhile, four reducing sugars (glucosamine, glucose, xylose, and ribose) were introduced into the solution of protein hydrolysate at the mass ratio of 1:10 (sugar: protein) to initiate the Maillard reaction for 15, 30, 45, and 60 min to avoid the long-term high-temperature Maillard reaction to produce advanced glycation end-products and other harmful compounds. The resultant glycated hydrolysates were subsequently transferred to an ice bath for cooling, followed by centrifugation at 10000*g* for 15 min at 4 °C. The resulting Maillard reaction products were subjected to freeze-drying and stored at −18 °C for subsequent analysis. The native protein hydrolysates without Maillard reaction were selected as the control group.

2.3. Determination of degree of hydrolysis (DH)

The DH of protein hydrolysate was determined by OPA method (Y. Fu, Liu, et al., 2020). The OPA reagent consisted of 50 mM OPA (10 mL), 50 mM *N*-acetyl-L-cysteine (10 mL), 5 mL SDS (20 %, *w/v*) and 75 mL borate buffer (100 mM, pH 9.5). The prepared OPA reagent was stirred in the dark for 1 h before use. The absorption value was determined at 340 nm after sample (10 μL) was accurately mixed with 1.2 mL OPA reagent at room temperature for 10 min. The absorbance of the samples at different reaction times was determined respectively. The absorption of various concentrations of Leu standard solution was determined to plot the standard curve according to free amino group, and then used to calculate DH. The DH was calculated according to eq. 1 as follows:

$$\mathrm{DH}(\%) = \frac{h}{h_{tot}} = \frac{([NH_2] \times V_1)/m_1}{([NH_2]_{tot} \times V)/m} \quad (1)$$

where h is the number of peptide bonds partially hydrolyzed per gram of protein (mmol/g), h_{tot} is the total number of peptide bonds per gram of protein (mmol/g), $[NH_2]$ is the concentration of free amino group in the enzyme (mmol/mL), V_1 is the volume of enzyme (mL), and m_1 is the sample mass (g).

2.4. Molecular weight distribution analysis

The molecular weight distribution of the Maillard reaction products was determined by size exclusion chromatography (Yu et al., 2022). Ultimate 3000 HPLC (Thermo Scientific, Massachusetts, USA) equipped with Phenomenex BioSP SECS2000 column was employed. The eluent was acetonitrile aqueous solution (30 %) containing 0.1 % trifluoroacetic acid (*v*/v). The sample concentration was 1 mg/mL, the volume was 10 μL, the flow rate was 0.5 mL/min, and the detection wavelength was 214 nm. Chromeleon software was used to analyze the chromatographic data and calculate the size distribution of peptides by fitting standard samples and elution time eqs. MW calibration curves (LogMW = −0.6086 t + 6.9381) were obtained using the following standards, including Trp (204 Da), GLV (287 Da), SGNIGFPGK (1114 Da), insulin (5700 Da) and myoglobin (17,600 Da), where MW is molecular weight (Da) and t is elution time (min).

2.5. Color measurement

The concentration of each Maillard reaction sample was adjusted to 20 mg/mL, and the color was measured according to Yu et al. (2022). The CIELab scale was used to further evaluate the color change of Maillard reaction products. The CIELab parameters including L^* (brightness value), a^* (redness value) and b^* (yellowness value) of Maillard reaction products were determined by the NR10QC colorimeter (3NH Technology Ltd., Shenzhen, China). The total color difference (ΔE) was calculated according to the eq. 2 below.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

2.6. UV–vis scanning analysis

The reaction degree of Maillard reaction products was evaluated according to the method of Yu et al. (2022). A UV–vis spectrophotometer (UV-8000 A; Shanghai Yuanxi Instrument Co. LTD., Shanghai, China) was used to scan samples (5 mg/mL) in the range of 200–500 nm. The absorbance values of all the samples were meticulously measured and subsequently subjected to comparative analysis.

2.7. Fluorescence scanning analysis

A fluorescence spectrophotometer (version F93; Shanghai Jingke Instrument Co. LTD., Shanghai, China) was used to determine the fluorescence intensity of Maillard reaction products (5 mg/mL) to examine the alteration ins fluorescence intensity of each sample according to our previous method of Yu et al. (2022). The excitation wavelength was fixed at 347 nm, and the emission wavelength range was selected in the range of 400–600 nm.

2.8. Sensory evaluation

According to the method of ISO 3972, ten sensory panelists (five males and five females, with an average age of 22–25 years old) were recruited. The panelists were required to have no taste disorders and receive conventional sensory experiment training in the sensory laboratory for 3 weeks, three times a week, for 20 min each time. The five basic taste solutions at different concentrations were identified and recorded. During the training, the panelists were presented with five basic tastes to describe the taste characteristics of samples on the intensity scale of 0 to 15. Taste training was carried out using the following reference solutions, including salty sodium chloride solution (30 mmol/L), sour citric acid solution (20 mmol/L), sweet sucrose solution (50 mmol/L), bitter quinine sulfate solution (10 mmol/L) and umami sodium glutamate solution (30 mmol/L). Ethical approval for the involvement of human subjects in this study was granted by the Ethics Committee of Southwest University. Prior to sensory evaluation, the sensory panelists performed intensity-graded training on the 15-cm line scale, using the reference solutions. Quinine sulfate (0.1 g/L and 0.2 g/L for 5 and 10), sodium chloride (1 g/L and 2 g/L for 5 and 10), and sodium glutamate (2.5 g/L and 5.0 g/L for 5 and 10). At room temperature, water solution containing Maillard reaction products (2 %, *w*/w) was placed in a black cup with a plastic lid, coded with 3 digits, and presented in random order, which was assessed by sensory panelists.

2.9. GC–MS/MS analysis

GC–MS/MS analysis of volatile compounds in Maillard reaction products was performed an Agilent 7890 A-5975C GC–MS/MS (Agilent Technology Co. LTD, California, USA) according to Y. L. Li et al. (2021) with slight modifications. Briefly, each sample was prepared from samples (8 mL) and NaCl (1.0 g) in the 15 mL vial for headspace extraction. The sample vial was placed on the solid phase microextraction (SPME) device at the set temperature of 70 °C and rotating speed of 1000 rpm. The SPME extraction head was inserted into the headspace of the sample through the bottle cap, and the fiber head was pushed out. The extraction head was about 1.0 cm above the upper surface of the sample, and the headspace extraction was carried out for 40 min. The fiber head was retracted, and the extraction head was removed from the sample bottle. Then, the extraction head was inserted into the GC–MS inlet, the fiber head was pushed out, and the sample was analyzed at 250 °C for 3 min. The column was DB-WAX (30.0 m × 250 μm, 0.25 μm). The initial temperature was maintained at 40 °C for 5 min, increased to 120 °C at 5 °C/min, and increased to 230 °C at 10 °C/min for 5 min. Gasification chamber temperature: 250 °C; Transmission line temperature: 240 °C; Carrier gas: He; Carrier gas flow rate: 1.0 mL/min. Mass spectrum conditions: EI source; Electron energy 70 eV; Ion source temperature: 230 °C; Quadrupole: 150 °C; The scanning quality ranges from 20 to 500 Da. Qualitative analysis: NIST11 MS database. The retention time was used to identify the detected volatile compounds. Quantitative analysis: The area normalization method takes the percentage of the peak area of the identified compounds in the sum of the areas of all identified components as the quantitative result. The eq. 3 is indicated follows:

$$C_i = \frac{A_i}{A_1 + A_2 \cdots + A_i \cdots + A_n} \times 100\% \quad (3)$$

where C_i is the content of the identified compound (%); A_i is the peak area of the identified compound; n indicates the total number of identified compounds.

2.10. LC-MS/MS analysis

The peptide sequences and the modification sites were analyzed by LC-MS/MS. High-performance Liquid Chromatograph: Dionex U3000 (Thermo Scientific, Massachusetts, USA); Column: C18, 3 μm, 100 A, 75 μm × 15 cm; Mobile phase A: 0.1 % formic acid in water; Mobile phase B: 0.1 % formic acid in acetonitrile. The concentration of the injected sample was 1 mg/mL. The flow rate was fixed at 600 nL/min and eluted with 95 % phase A and 5 % phase B elution from 0 to 8 min, followed by 92 % phase A at 16 min, 87 % phase A during 16 to 39 min, 72 % phase A during 39 to 50 min, 5 % phase A and 95 % phase B elution at 55 min and then, 94 % phase A elution to 60 min. Mass spectrometer: Thermo Scientific Q Exactive; Spray voltage: 3.8 kV; Capillary temperature: 320 °C; Resolution Settings: Level 1 70,000@*m/z* 200, level 2 17,500@m/z 200; Parent ion scanning range: m/z 300–1400; Sub-ion scanning range: m/z 100; MS1 AGC: 3e6, ion implantation time: 60 ms; MS2 AGC: 5e4, ion implantation time: 80 ms; Ion screening window: 3.0 m /z; Fragmentation mode: HCD, energy NCE 27; Data-dependent MS/MS: Top 20; Dynamic exclusion time: 15 s. Software: PEAKS 8.5; Protein database: *Ictalurus punctatus* protein database, species No. 7998, downloaded from the NCBI database.

2.11. Statistical analysis

Each experiment was repeated in triplicate and the results were expressed as mean ± standard deviation. SPSS 22.0 was used for ANOVA, and Duncan was used for multiple post-hoc comparisons ($P < 0.05$ meant significant difference, $P > 0.05$ meant no significant difference). Principal component analysis (PCA) was performed using The Unscrambler X 10.4 and the results were further visualized.

3. Results and discussion

3.1. Physicochemical analysis of protein hydrolysates

3.1.1. DH analysis

DH is a crucial indicator for evaluating the extent of enzymatic hydrolysis by protease. Flavourzyme containing both *endo-* and *exo*-peptidase can degrade proteins into peptides and free amino acids, which contributes to the improved flavor of protein hydrolysates (Fu, Zhang, et al., 2020). As depicted in Fig. S1(a), the DH of fish viscera significantly increased with the progression of reaction times. Specifically, the DH of protein hydrolysates was increased to 9.17 % after hydrolysis for 7 h ($P < 0.05$), suggesting continuous degradation of fish viscera proteins by Flavourzyme at 50 °C. This led to a consistent generation of peptides and free amino acids in protein hydrolysate. Our current results were in agreement with several recently reported studies. Li et al. (2021) have optimized the enzymatic hydrolysis of grass carp fish bone catalyzed by Flavourzyme and found that the DH gradually increased until 8 h. Furthermore, Guo et al. (2023) have investigated the enzymatic hydrolysis of fish skin collagen at various reaction times and

indicated that during the initial 3 h, the DH rapidly increased to 9.05 % at 3 h, while the DH exhibited a gradual elevation from 3 to 6 h, culminating to 10.98 % (6 h).

3.1.2. Molecular weight distribution analysis

The molecular weight distribution of peptides serves as a crucial indicator of the extent of hydrolysis of fish viscera treated by Flavourzyme. The peptide size distribution of fish viscera-derived peptides at various reaction times was analyzed. As depicted in Fig. S1(b), the proportion of peptide fraction >5 kDa gradually decreased from 5.67 % to 3.49 %, whereas the peptide fraction with molecular weight below 1 kDa significantly increased from 77.81 % to 84.65 % ($P < 0.05$). Notably, after enzymatic hydrolysis for 7 h, the variation in peptide proportion across different ranges of molecular weight became more gradual, suggesting the extensive hydrolysis of fish viscera proteins into peptides and free amino acids. Recently, Guo et al. (2023) have reported that after enzymatic hydrolysis by Flavourzyme for 5 h, and fish skin collagen hydrolysate revealed a similar tendency. Specifically, the proportion of peptide fraction greater than 5 kDa decreased from 38.62 % to 8.16 %, while the proportion of peptides below 1 kDa rose from 1.31 % to 6.24 %. Our current results were in agreement with the reported study, demonstrating that with the extended hydrolysis time, the high-molecular weight peptides (> 5 kDa) gradually diminished, while low-molecular weight peptides (<1 kDa) accumulated. Based on the peptide size distribution and free amino group of protein hydrolysates at different reaction intervals, protein hydrolysate at 7 h was selected for further analysis.

3.2. Color changes of maillard reaction products induced by four reducing sugars

During the Maillard reaction, the color change of Maillard reaction products can reflect the degree of Maillard reaction (Shakoor, Zhang, Xie, & Yang, 2022). The color changes of Maillard reaction products at different reaction times are listed in Table 1. In general, the longer the reaction time, the more significant change in total chromatic aberration (ΔE^*). With the progression of Maillard reaction, compared with the control group, the a^* value of glucosamine-induced Maillard reaction products significantly decreased from 5.03 at 0 min to 3.31 at 60 min ($P < 0.05$). Meanwhile, with the extension of heating time, the b^* value remarkably increased from 0.27 to 1.72 ($P < 0.05$), indicating that the color of Maillard reaction products became more yellowish. Overall, the extent of Maillard reaction was relatively low in this study. In addition, the a^* value of xylose-induced Maillard reaction samples increased from 5.74 at 0 min to 5.85 at 60 min, the b^* value increased from −0.78 to 0.56 ($P < 0.05$), and the a^* and b^* values in ribose samples decreased from 4.54 to 3.38 ($P < 0.05$) and from −2.30 to −2.95, respectively. The a^* value of glucose-induced Maillard reaction products decreased from 4.54 at 0 min to 4.25 at 60 min, and the b^* value decreased from −2.30 to −2.49. Compared with glucosamine-induced Maillard reaction samples, the degree of color change of xylose-, ribose- and glucosamine-induced Maillard reaction was relatively low, suggesting that glucosamine-induced Maillard reaction was more efficient. The Maillard reaction products prepared from xylose and sesame meal protein hydrolysates (Hu et al., 2021), wheat gluten protein hydrolysates (A. Sun et al., 2023) and soybean meal hydrolysates (Huang et al., 2023; L. B. Sun et al., 2023) tended to become more yellowish and brownish in terms of color as the Maillard reaction progressed, confirming the formation and accumulation of Maillard reaction intermediates.

Table 1
Color changes of maillard reaction products at different reaction times.

Samples	Reaction time (min)	L^*	a^*	b^*	ΔE^*
Glucosamine	0	23.38 ± 0.03 d	5.03 ± 0.01 a	0.27 ± 0.03 e	–
	15	23.95 ± 0.02 c	4.57 ± 0.03 b	1.29 ± 0.06 b	1.26 ± 0.02 d
	30	24.81 ± 0.01 a	3.70 ± 0.05 c	0.75 ± 0.03 d	2.01 ± 0.03 c
	45	24.27 ± 0.10 b	3.28 ± 0.02 d	1.15 ± 0.02 c	2.15 ± 0.07 b
	60	24.84 ± 0.05 a	3.31 ± 0.03 d	1.72 ± 0.20 a	2.68 ± 0.01 a
Xylose	0	21.73 ± 0.02 b	5.74 ± 0.20 d	−0.78 ± 0.03 d	–
	15	20.13 ± 0.03 d	7.10 ± 0.05 a	0.42 ± 0.05 b	2.42 ± 0.06 b
	30	19.21 ± 0.06 e	5.24 ± 0.02 e	0.62 ± 0.03 a	2.93 ± 0.09 a
	45	20.85 ± 0.08 c	6.59 ± 0.03 b	0.24 ± 0.07 c	1.60 ± 0.12 c
	60	21.84 ± 0.03 a	5.85 ± 0.02 c	0.56 ± 0.02 a	1.35 ± 0.03 d
Ribose	0	22.90 ± 0.03 c	4.54 ± 0.01 a	−2.30 ± 0.03 a	–
	15	22.86 ± 0.04 c	4.32 ± 0.02 b	−2.78 ± 0.03 b	0.53 ± 0.01 c
	30	25.22 ± 0.04 a	2.71 ± 0.06 d	−3.80 ± 0.04 d	3.31 ± 0.06 a
	45	24.75 ± 0.06 b	3.41 ± 0.05 c	−3.05 ± 0.06 c	2.30 ± 0.03 b
	60	24.85 ± 0.03 b	3.38 ± 0.06 c	−2.95b ± 0.01 c	2.36 ± 0.03 b
Glucose	0	22.90 ± 0.04 b	4.54 ± 0.08 a	−2.30 ± 0.04 a	–
	15	22.89 ± 0.03 b	4.34 ± 0.06 b	−2.28 ± 0.10 a	0.20 ± 0.02 c
	30	22.88 ± 0.07 b	4.33 ± 0.04 b	−2.41 ± 0.03 b	0.24 ± 0.03 c
	45	22.84 ± 0.08 b	4.01 ± 0.09 c	−2.70 ± 0.03 c	0.66 ± 0.04 b
	60	23.70 ± 0.04 a	4.25 ± 0.01 b	−2.49 ± 0.01 b	0.87 ± 0.03 a

Note: Different letters within the same group indicate statistically different ($P < 0.05$).

3.3. The contents of free amino group in maillard reaction products induced by four reducing sugars

The content change in free amino group in Maillard reaction products can reflect the degree of Maillard reaction. As shown in Fig. 1, with the extension of reaction time, the content of free amino group in the glucosamine-induced Maillard reaction products first increased and then decreased due to the presence of amino group in glucosamine. The free amino group increased from 89.41 mmol/L to 97.37 mol/L at 15 min ($P < 0.05$), which was attributed to the introduction of glucosamine, and then the cross-linking reaction between glucosamine and protein hydrolysates significantly decreased the free amino group content to 65.67 mmol/L ($P < 0.05$). Xylose and ribose treatment decreased the free amino group from 89.48 mmol/L to 87.42 mmol/L ($P < 0.05$) and 80.43 mmol/L ($P < 0.05$) at 15 min, and then decreased rapidly until 30 min, and eventually decreased to 53.25 mmol/L ($P < 0.05$) and 59.68 mmol/L ($P < 0.05$) at 60 min, respectively. In addition, the glucose-induced Maillard reaction led to the remarkably decreased content of free amino group from 89.48 mmol/L to 59.78 mmol/L ($P < 0.05$), which was attributed to the cross-linking reaction between reducing sugar and protein hydrolysates, which consumed the free amino group in the protein hydrolysates. Overall, the concentration of free amino groups in the glucosamine-induced Maillard reaction samples exhibited the most significant variation, indicating the high reactivity of glucosamine to induce Maillard reaction. Our current results were consistent with several recently reported studies. Yan et al. (2021) have prepared Maillard reaction products from pea protein and reducing sugars (xylose, arabinose, ribose, glucose and galactose) at the mass ratio of 5:1 (*w*/w) and demonstrated that the content of free amino groups in Maillard reaction products significantly decreased after heat treatment, which suggested that consumption of free amino groups during Maillard

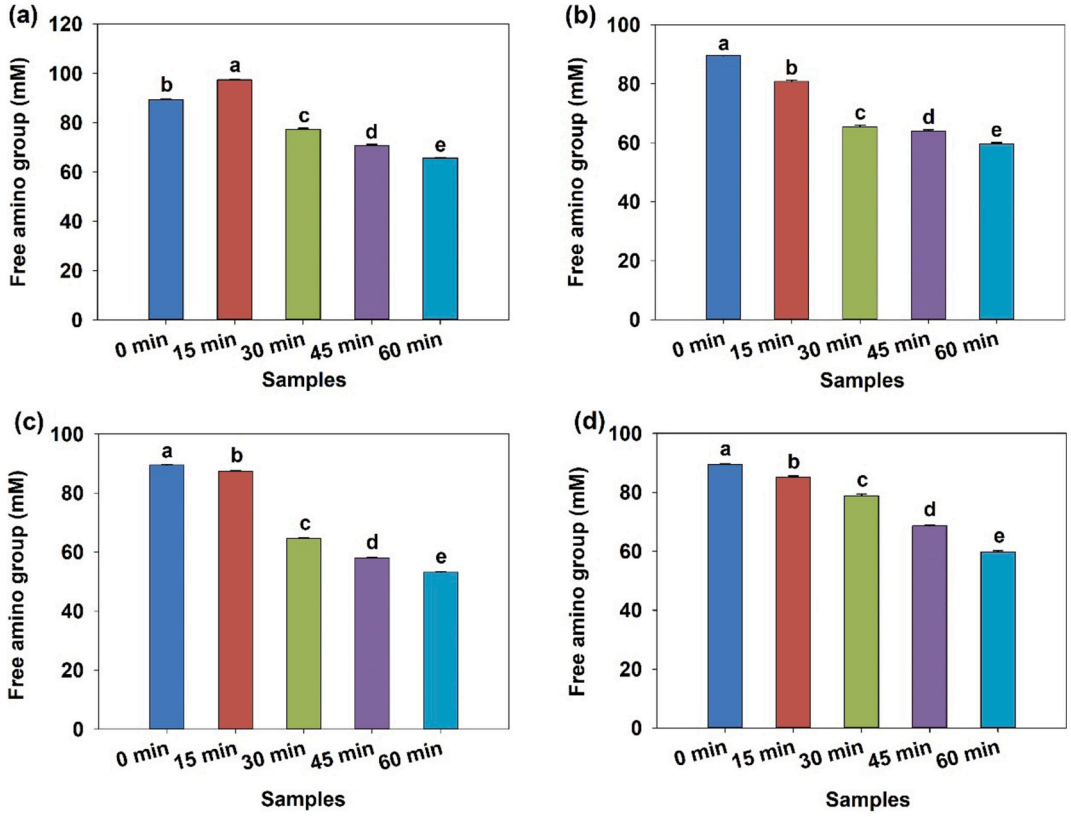

Fig. 1. Free amino group of maillard reaction products induced by glucosamine (a), xylose (b), ribose (c) and glucose (d) at different reaction times.

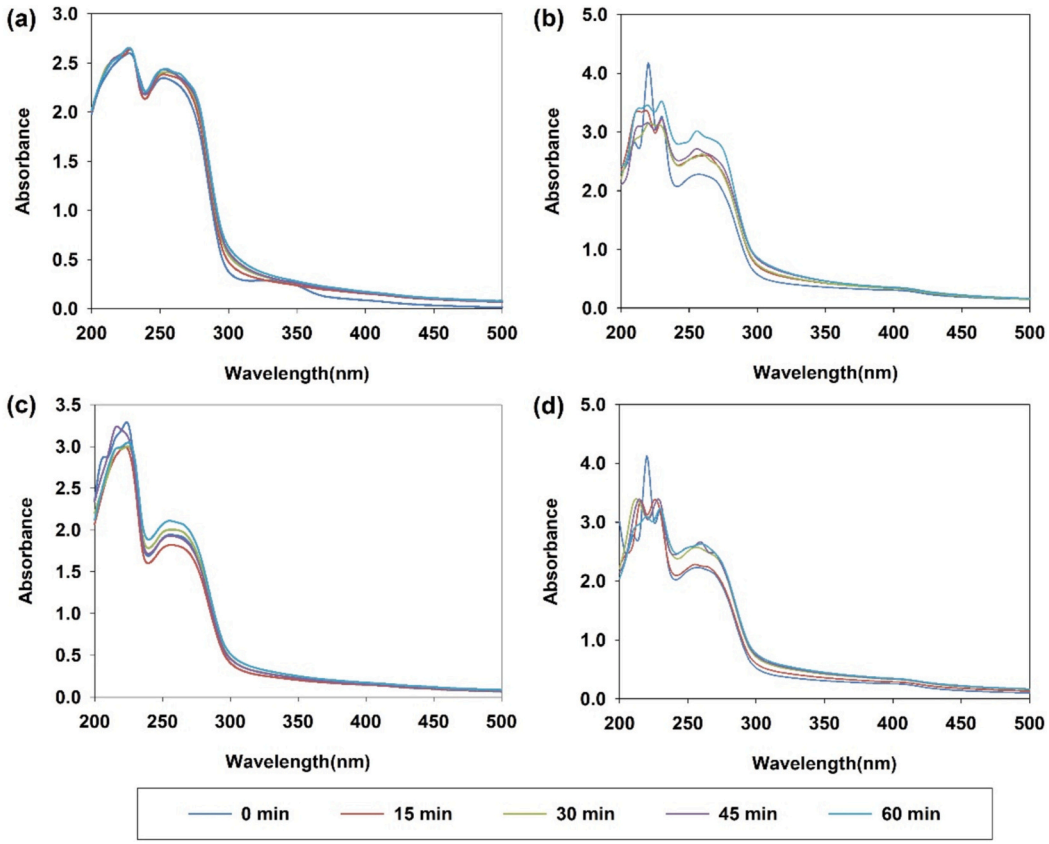

Fig. 2. UV absorption spectra of maillard reaction products induced by glucosamine (a), xylose (b), ribose (c) and glucose (d) at different reaction times.

reaction between sugars and proteins. Recently, Chen et al. (2023) have investigated the effects of different reducing sugars on the content of free amino group in Maillard reaction products of wolfberry seed meal, and the results showed that the content of free amino groups was significantly reduced after Maillard reaction ($P < 0.05$). Notably, the contents of aspartic acid and glutamic acid in xylose-induced Maillard reaction products were significantly higher, compared to those induced by fructose, arabinose, xylo-oligosaccharides, glucose, and galactose ($P < 0.05$).

3.4. Spectral analysis of maillard reaction products induced by four reducing sugars

3.4.1. UV scanning analysis

The Maillard reaction products at each stage exhibit distinct absorbance characteristics, so UV scanning can be used to monitor the degree of the Maillard reaction (Wei et al., 2019). The absorption value at 320 nm can be used to reveal the formation of Maillard reaction intermediates, while the absorption value at 420 nm is commonly used to monitor the formation of advanced glycation end-products (Yu et al., 2022). The UV scanning spectra results are shown in Fig. 2. At the wavelength of 320–420 nm, the absorbance of Maillard reaction products gradually increased with the extension of reaction time, indicating the formation of early and intermediate flavor compounds or precursors. Specifically, the absorbance of the Maillard reaction products was higher than that of the control group (native protein hydrolysates), and the absorbance of the Maillard reaction products followed the order: 60 min > 45 min > 30 min > 15 min. The present results showed the order of reactivity of four reducing sugars was glucosamine > xylose > ribose > glucose. Normally, pentose sugars have a higher reactivity than hexose sugars during the Maillard reaction, but glucosamine has an amino group, which endows it with a faster reaction rate and contributes to rapidly initiating the Maillard reaction in a short time (Fu, Liu, et al., 2020). The results indicated that with the extension of reaction time, more Maillard reaction intermediates were formed, while Maillard reaction intensified, with the glucosamine-induced Maillard reaction being the most reactive. The current trend of Maillard reaction was in agreement with the reported literature. Similarly, Dong et al. (2019) conducted UV scanning on Maillard reaction samples prepared from mussel meat, observing an absorption peak between 250 nm and 300 nm. Recently, the Maillard reaction between oyster hydrolysates and different reducing sugars showed that the absorption value at 420 nm gradually increased with the extension of reaction time. The absorption value at 420 nm represented the extent of browning, and 294 nm indicated the formation of early and intermediate flavor compounds or precursors (B. F. Fu et al., 2023).

3.4.2. Fluorescence scanning analysis

The formation of fluorescent compounds (Xiao, Huang, & Ho, 2024) in the early stage of the Maillard reaction can be used to evaluate the fluorescence products formed by the Maillard reaction (Liu et al., 2021; Luo, Li, & Ho, 2021). The fluorescence scanning spectra of Maillard reaction products at different times are illustrated in Fig. 3. In general, glucosamine-induced Maillard reaction products exhibited characteristic peaks adjacent to 550–600 nm. Xylose, ribose, and glucose-induced Maillard reaction samples displayed two characteristic peaks between 425 nm and 550 nm. Additionally, each Maillard reaction product demonstrated a higher fluorescence intensity in the range of 400–550 nm, compared to the control group (native protein hydrolysate). The results showed that fluorescent products were constantly formed during the Maillard reaction. The longer the reaction time, the higher the fluorescence intensity. The results showed that the fluorescence intensity of Maillard reaction products increased with reaction time, following the order: 60 min > 45 min > 30 min > 15 min > 0 min. In

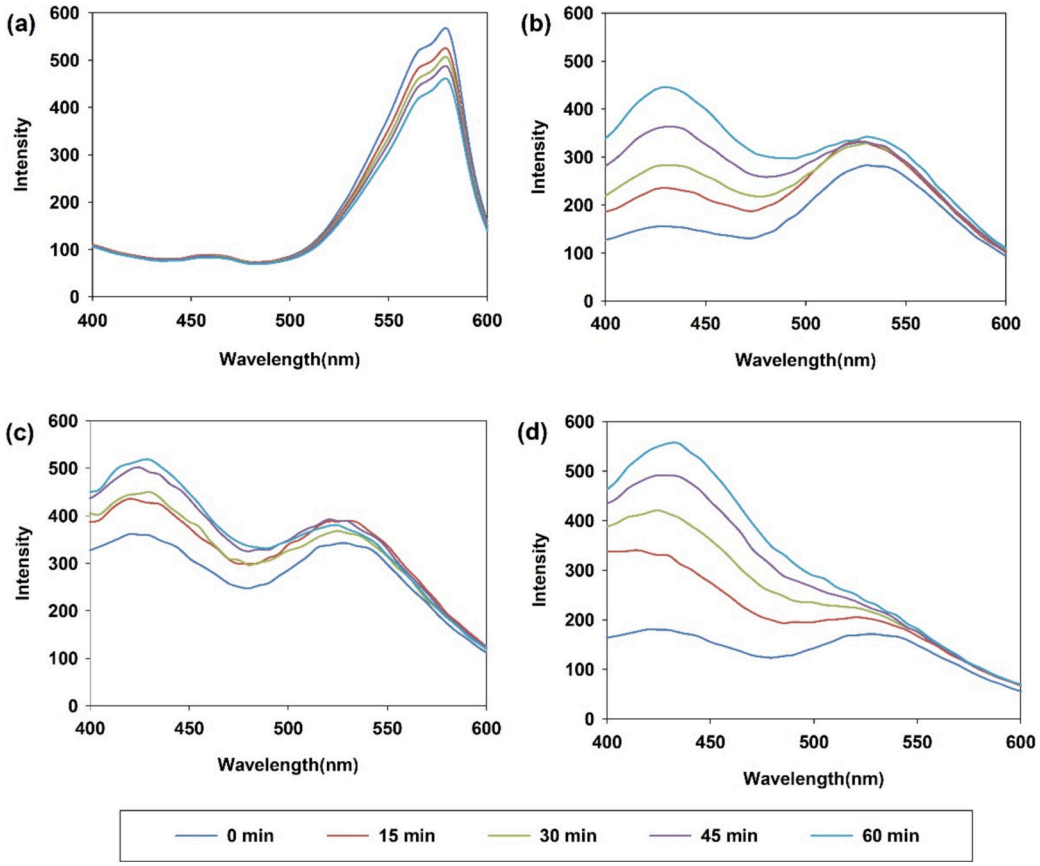

Fig. 3. Fluorescence spectra of maillard reaction products induced by glucosamine (a), xylose (b), ribose (c) and glucose (d) at different reaction times.

terms of four reducing sugars, the reactivity order was glucosamine > xylose > ribose > glucose. Hence, glucosamine-induced sample was selected for subsequent flavor analysis.

These present results indicated that the extent of the Maillard reaction deepened progressively with the extended reaction times. At the same time, in the Maillard reaction products induced by reducing sugars, the fluorescence intensity and the degree of change in the glucosamine group were found to be higher, compared to those in the other groups, suggesting that glucosamine can induce glycation more rapidly than other reducing sugars. Our findings were in agreement with previous studies and confirmed the fundamental principle of the Maillard reaction that as reaction time extends, Maillard reaction products progressively accumulate. It has been reported that Maillard reaction between collagen hydrolysates and reducing sugars revealed a rapid increase in the fluorescence intensity during the heating process. This augmentation was characterized by the accumulation of low molecular weight substances during the late stages of the Maillard reaction (Yu et al., 2022). Recently, X. J. Li et al. (2023) have prepared Maillard reaction products using xylose and corn zein, and demonstrated that the fluorescence intensity exhibited wide fluorescence spectral peaks in the range of 410–430 nm. At the temperature of 60 °C, the fluorescence intensity of Maillard reaction products increased over times. Similarly, the results indicated that the emission wavelength of the control sample was 384 nm, whereas that of the Maillard reaction products shifted to 405 nm, suggesting alterations in the protein structure due to the Maillard reaction. Furthermore, in the wavelength range of 450–550 nm, Maillard reaction products exhibited elevated fluorescence intensity (Qiu et al., 2024). Our results were consistent with the above-mentioned studies and confirmed that with the extension of reaction time, Maillard reaction products gradually were constantly formed.

3.5. Molecular weight distribution of maillard reaction products induced by four reducing sugars

The changes in the molecular weight distribution of Maillard reaction products can indirectly indicate the degree of Maillard reaction (Yu et al., 2022). As shown in Fig. S2, with the extension of reaction time, the proportion of peptides <1 kDa increased, while the proportion of peptides >5 kDa decreased. Meanwhile, the peptide content within each molecular weight range also exhibited gradual changes. The N-terminal of amino acids and peptides were quite reactive, which can easily cause crosslinking reactions with reducing sugars (Chen et al., 2023). In the glucosamine-induced Maillard reaction product, the proportion of peptide fraction <1 kDa significantly increased from 86.29 % to 91.34 % ($P < 0.05$). The content of peptide >5 kDa remarkably decreased from 4.79 % to 1.32 % ($P < 0.05$), while the proportion of each molecular weight fraction remained unchanged after 15 min, which may be due to the simultaneous occurrence of thermal degradation and crosslinking during the Maillard reaction. Despite the crosslinking between peptides and sugar during Maillard reaction, peptides may be subjected to degradation due to high temperature (Fu, Zhang, et al., 2020). Overall, during the Maillard reaction, the reactivity of glucosamine-induced Maillard reaction was the highest and the induction effect was the optimal. Therefore, glucosamine-induced Maillard reaction product was selected for subsequent analysis.

The molecular weight of peptides is a key parameter leading to the degradation and cross-linking properties of the peptides. A recent study has compared peptide size distribution of Maillard reaction products derived from porcine plasma (Fu, Liu, et al., 2020). It was found that the proportion of glycated peptides (< 1 kDa) increased, while the proportion of peptides >10 kDa decreased, suggesting that high molecular weight peptides (>10 kDa) were thermally degraded and cross-linked simultaneously during the Maillard reaction (Y. Fu, Liu, et al., 2020). Recently, Yan et al. (2021) have demonstrated the change in molecular weight distribution of Maillard reacted peptides from pea protein within 0–3 h, and found that the proportion of peptides <250 Da increased significantly from 20 % to about 40 % at 1 h. The proportion of peptides >1000 Da decreased from 10 % to 5 % from 0 to 1 h, and remained unchanged after 1 h, indicating that degradation and crosslinking occurred during the Maillard reaction. Taken together, our current results on the changes in the molecular of Maillard reaction products were in agreement with the above-mentioned studies.

3.6. Sensory evaluation of glucosamine-induced maillard reaction products

Quantitative descriptive analysis has been employed to evaluate the changes in the taste attributes of glucosamine-induced Maillard reaction products at different reaction times As illustrated in Fig. 4, with the extension of reaction time, the bitterness intensity of glucosamine-induced Maillard reaction products decreased from 7.5 to 5.0 ($P < 0.05$), saltiness intensity increased from 2.0 to 3.4 ($P < 0.05$), and umami intensity exhibited an insignificant increase ($P > 0.05$) from 3.6 to 4.5 ($P > 0.05$), indicating that the Maillard reaction could improve the taste profile of fish viscera-derived peptides, by reducing the bitterness intensity and enhancing the salt and umami taste.

The current results were consistent with a number of recently reported studies. It has been shown that the Maillard reaction products of oyster protein hydrolysate and xylose showed a remarkable saltiness-enhancing effect, and the glucose-induced Maillard reacted peptides displayed the highest saltiness (10.5), umami (3.0) and kokumi (2.5) intensities after reaction for 90 min, while the bitterness was at its lowest score (3.5) (B. F. Fu et al., 2023). Recently, fish skin-derived collagen glycopeptide has also been evaluated by descriptive sensory evaluation (Yu et al., 2022). It was observed that collagen glycopeptide had a significant saltiness and umami-enhancing effect. After the reaction for 4 h, the saltiness intensity of the sodium chloride solution containing collagen glycopeptide was the highest (9.33, saltiness intensity), and the saltiness-enhancing rate reached 71.51 % (Yu et al., 2022), suggesting its great potential as a food component for reducing NaCl content in formulated foods. Similarly, the glycated hydrolysates from porcine muscle and plasma had lower bitterness intensity and higher umami taste, indicating that glycation remarkably reduced the bitterness of peptides (Y. Fu, Liu, et al., 2020). Similarly, Liao, Wang, Chen, Zhang, and Liu (2020) demonstrated that the Maillard reaction products prepared with xylose and the heads and bones of *Takifugu obscurus* also showed a significant umami-enhancing effect, with the umami intensity increasing from 6.6 to 7.1 ($P < 0.05$).

3.7. GC–MS/MS analysis of glucosamine-induced maillard reaction products

The PCA bi-plot of volatile compounds derived from glucosamine-induced Maillard reaction products is illustrated in Fig. 5, describing 85 % and 10 % of the total variation in factor 1 (PC1) and factor 2 (PC2), respectively (Fig. 5). The present PCA results showed that 15-min sample and 0-min sample were in different quadrants. The obvious differences can be between them, while samples at 30 min, 45 min and 60 min were different from 0-min sample, suggesting that Maillard reaction could remarkably alter the flavor profile of protein hydrolysates. Notably, there are obvious differences between Maillard reaction products and native protein hydrolysates. In addition, a total of 9 volatile compounds were detected, mainly including aldehydes, alcohols, esters, acids, ketones, furans, alkanes, aromatics and pyrazines (Fig. S3). Overall, 30, 33, 38, 35, and 28 volatile compounds were identified in samples collected at 0, 15, 30, 45, and 60 min, respectively. Among them, 10 common compounds were alcohols, aldehydes and aromatic hydrocarbons. There were only 2 unique volatile components in 0-min sample, while 11, 13, 10 and 8 unique volatile compounds of samples at 15, 30, 45 and 60 min were found (Fig. S4), indicating that Maillard reaction at different reaction times generated distinctive volatile compounds, thereby resulting in the altered flavor profile.

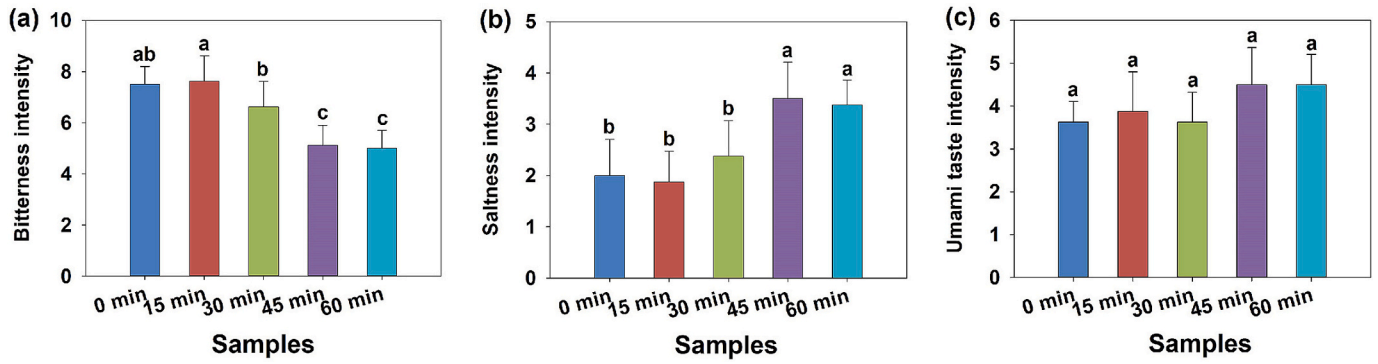

Fig. 4. Sensory analysis of bitterness (a), saltiness (b) and umami (c) intensity of maillard reaction products after different reaction times induced by glucosamine.

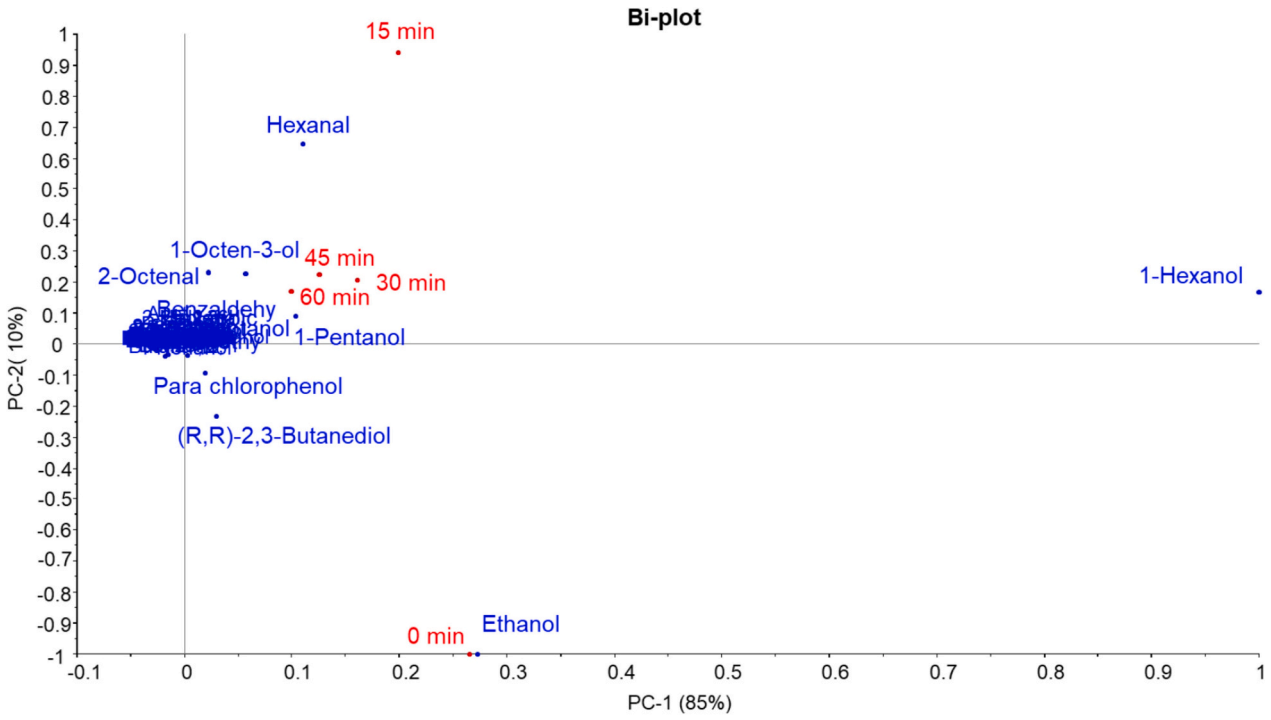

Fig. 5. PCA bi-plot analysis of maillard reaction products induced by glucosamine at different reaction times.

With the extension of reaction time, alcohols, ketones and esters first increased and then decreased, and aldehydes (as major fishy odorants) in general showed a decreasing trend. Among them, the native protein hydrolysates contain only three aldehydes, namely 2-methylbutanal, 2-heptenal and benzaldehyde. After reaction for 15 min, 6 types of aldehydes were detected, namely 2-heptenal, benzaldehyde, pentanal, hexanal, (E)-2-octenal and (E,E)-2, 4-decadienal. With the extension of reaction time, the contents of hexanal, 2-heptenal, 2, 4-decadienal, trans-2-octenal, 1-octen-3-ol and other fishy odorants first increased and then decreased, and no longer existed after 30 min. This fact suggested that the Maillard reaction led to the modified structure of fish viscera peptides, leading to the exposure and release of fishy odorants (Xu et al., 2024; Zhao et al., 2023). The longer the reaction time, the more pronounced the release of fishy odorants. Therefore, the number of aldehydes, including hexanal, 2-heptenal, 2,4-decadienal, and trans-2-octenal, exhibited an initial increase followed by a subsequent decrease. Moreover, the content of some fishy odorants also decreased after 30 min. As the reaction time prolonged, the content of various aromatic compounds such as benzaldehyde, 3-methylbutyric aldehyde, benzyl alcohol, phenylethanol, dimethyl phthalate, and heptyl formate gradually increased, indicating ongoing transformations during the Maillard reaction (Song, Du, Ding, Yu, & Wang, 2021; Xia et al., 2021). Overall, the odor compounds were gradually released and volatilized, leading to a gradual decrease in their contents. Concurrently, the Maillard reaction generated several aromatic compounds, while mitigating the undesirable odorants (especially aldehydes) (Table 3). Hence, the Maillard reaction contributed to the improved flavor profile of fish viscera-derived peptides.

Recently, Y. L. Li et al. (2021) have analyzed Maillard reaction products of grass carp bone peptides by SPME-GC–MS, and identified 62 volatile compounds, including 22 aldehydes, 10 alcohols, 4 ketones, 5 hydrocarbons, and 5 furans. There were 3 phenols, 3 esters, 5 pyrazines, and 5 other flavor compounds identified. The results showed that volatile aldehydes were the main volatile compounds in the Maillard reaction products, and their contents were relatively high, accounting for 26.04 %. Meanwhile, 2-methylpropanal elicited a pungent odor, and hexanal had a fishy and grassy odor. The content of hexanal decreased after Maillard reaction, indicating that Maillard reaction can enhance the flavor and aroma profile of grass carp protein hydrolysate, rendering it more refined and complete. More recently, X. H. Huang et al. (2023)

have prepared Maillard reaction products from xylose and mussel meat, and 37 volatile flavor compounds were identified in Maillard reaction products. Among them, sulfur compounds predominantly imparted a meaty flavor, characterized by a low threshold, yet they substantially contributed to the overall flavor profile. Pyrazine is mainly derived from the Strecker degradation in the Maillard reaction, and the α-amino ketone generated by the reaction of the intermediate with ammonia, indicating a toasty, nutty and caramel aroma. Furan compounds are formed by dewatering or cyclization of reducing sugars, which mainly provide a toasty flavor. Aldehydes primarily originate from the oxidative degradation of lipids and the thermal degradation of pentose and amino acids. Benzaldehyde, phenylacetaldehyde, 3-(methylthio) propionaldehyde, furfural, 3-methylbutyraldehyde, and 2-methylbutyraldehyde are associated with the aromas of nut/fruit, rose, meat, barbecue, and chocolate, respectively (Fu, Zhang, et al., 2020). Several other flavor compounds, including alcohols, esters, ketones, ethers, and hydrocarbons, typically possess higher odor thresholds and make minor contributions to the overall flavor of Maillard reaction products. However, they can contribute to modulating the overall flavor profile. Overall, the Maillard reaction can increase the contents of the aromatic compounds of samples, imparting an enriched flavor with reduced content of fishy odorants, thereby offering a viable approach for mitigating the fishy odor of protein hydrolysates from channel catfish viscera.

3.8. LC-MS/MS analysis

LC-MS/MS analysis can identify the peptide sequences and glycation sites of Maillard-reacted peptides. The main information on identified peptides is listed in Table 2, while the representative MS and MS/MS spectra are illustrated in Fig. S5 & S6, respectively. In general, Lys and Arg were identified as the primary modification sites, with Arg being predominating (Yu et al., 2022). This observation aligned with the previously reported finding that these two amino acids serve as potential glycation sites for food-derived peptides (Fu, Liu, et al., 2020; Zhou, Zhang, Dai, Zhang, & Fu, 2024). Interestingly, in addition to glucosamine-induced modifications, pentanal and hexanal-induced modifications were also found in the modified peptides, which may be due to the unique carbonyl structure characteristics of pentanal and hexanal with extremely high reactivity (Hseiky, Crespo, Kieffer-Jaquinod, Fenaille, & Pflieger, 2021; Huang et al., 2024). With the extension of reaction time, the number of identified peptide modifications gradually increased, and a total of 7 modified peptides were identified at 60 min. Some recent studies have shown that glycation modifications can exert a positive impact on the taste-active properties of protein hydrolysates from food sources. According to sensory evaluation, glycation modification plays a key role in the improvement of taste characteristics. Hence, the notable enhancement in salt and umami taste may be attributed to the Maillard reaction induced by glucosamine, resulting in the production of glycated proteins and peptides (Yu et al., 2022). At the same time, the carbonyl-amine reaction occurred between fishy odorants (especially hexanal and pentanal) and peptides, which can contribute to the mitigated undesirable flavor in protein hydrolysates. To the best of our knowledge, this is the first study that reported the reaction between the fishy odorants (especially hexanal and pentanal) and peptides, confirmed by LC-MS/MS analysis. Hence, the Maillard reaction not only generates desirable flavor compounds but also mitigates unpleasant fishy odors through carbonyl-amine reaction between fishy odorants and peptides. However, the underlying relationship between the structure of Maillard reacted peptides and the improved flavor necessitates further investigation.

4. Conclusion

The effects of glycation modification on the structure, taste and volatile compounds of fish visceral-derived peptides were investigated. The results of free amino group, UV, fluorescence spectra and peptide size distribution showed that glucosamine exhibited the highest reactivity in the glycation modification. Sensory evaluation results revealed that the bitterness intensity of glucosamine-induced glycated products decreased, while umami and salt taste increased. GC–MS/MS analysis showed that alcohols, ketones and esters first increased and then decreased during the Maillard reaction, while aldehydes generally decreased, suggesting the exposure and volatilization of the undesirable fishy odorants, while formation of more aromatic compounds. In addition, the main glycation sites were identified as Arg and Lys revealed by

Table 2
The modified peptides identified by LC-MS /MS and their modification sites.

Peptide sequence	Modification sites	Protein precursor	Sample
MVHWTDAER(+84.09)HIIADLWGK(+84.09) INHDEIGGQALARL	2 × Hexanal [R9; K18]	Hemoglobin subunit beta	15 min
QQRLIFAGK(+84.09)QLEDGR(+70.08) TLSDYNIQKESTLHLVLRL	1 × Pentanal [R17]; 1 × Hexanal [K11]	Ubiquitin C partial	15 min
QLEDGR(+70.08)TLSDYNIQK(+70.08)ESTLHLVLR	2 × Pentanal [R17; K26]	Ubiquitin C partial	15 min
SLSAKDKAVVKNLWAKIAPK(+84.09)VDD	1 × Hexanal [K21]	Hemoglobin subunit alpha	15 min
ADLWGKINHDEIGGQALAR(+162.05)L	1 × Glucosamine [R31]	Hemoglobin subunit beta	30 min
QQRLIFAGKQLEDGR(+162.05)TLSDYNIQKESTLHLVLRL	1 × Glucosamine [R17]	Ubiquitin C partial	30 min
QQR(+162.05)LIFAGKQLEDGRTLSDYNIQKESTLHLVLRL	1 × Glucosamine [R5]	Ubiquitin C partial	30 min; 60 min
PGPAGPSGGAGR(+162.05)T	1 × Glucosamine [R68]	Complement C1q tumor necrosis factor-related protein 2	30 min; 45 min; 60 min
SPGPAGPSGGAGR(+162.05)T	1 × Glucosamine [R68]	Complement C1q tumor necrosis factor-related protein 2	60 min
QQR(+70.08)LIFAGK(+84.09) QLEDGRTLSDYNIQKESTLHLVLRL	1 × Pentanal [R5]; 1 × Hexanal [K11]	Ubiquitin C partial	45 min
K(+70.08)R(+162.05)DDVVSFLKEQQDK	1 × Glucosamine [R590]; 1 × Pentanal [K589]	Transferrin	45 min
PGSPGPAGPSGGAGR(+162.05)T	1 × Glucosamine [R68]	Complement C1q tumor necrosis factor-related protein 2	45 min; 60 min
ADK(+162.05)IGAEALGR	1 × Glucosamine [K24]	Hemoglobin alpha 2	60 min
GPTGAPGPAGPK(+162.05)	1 × Glucosamine [K439]	Collagen alpha-1(XXV) chain isoform X1	60 min
QQR(+84.09)LIFAGKQLEDGR(+84.09)TLSDYNIQKESTLHLVLR	2 × Hexanal [R5; R17]	Ubiquitin C partial	60 min

Table 3
The volatile compounds in Maillard reaction products at different reaction times.

Category	Compounds	Relative content of volatile compounds in samples at different reaction times (%)				
		0 min	15 min	30 min	45 min	60 min
Aldehydes	2-Methylbutanal	0.06	–	0.15	–	–
	2-Heptenal	0.08	2.07	–	–	–
	Benzaldehyde	0.32	2.74	1.22	1.53	7.90
	Pentanal	–	1.39	–	–	–
	Hexanal	–	19.01	–	–	–
	(E)-2-Octenal	–	7.02	–	–	–
	(E,E)-2,4-Decadienal	–	0.99	–	–	–
	3,4-Dimethyl-benzaldehyde	–	–	1.28	–	–
	2,4-Dimethyl-benzaldehyde	–	–	–	1.67	–
	3-Methylbutanal	–	–	–	–	2.45
Alcohols	Ethanol	29.40	1.73	2.58	–	–
	1-Pentanol	4.03	4.50	9.56	7.43	9.38
	1-Hexanol	43.51	34.79	53.93	48.68	50.66
	1-Octen-3-ol	2.08	9.06	–	–	–
	1-Heptanol	2.27	2.52	4.88	4.10	–
	1-Octanol	2.04	1.42	3.13	2.80	3.13
	(*R*,*R*)-2,3-Butanediol	6.16	–	–	–	–
	1-Nonanol	0.87	–	0.95	–	–
	3-(methylthio)-1-Propanol	0.47	0.72	1.38	2.22	1.66
	Benzyl alcohol	0.15	0.37	0.35	0.59	0.68
	Phenylethyl alcohol	1.79	0.58	1.42	2.16	1.21
	2-Nonanol	–	0.82	–	–	–
	2-Decen-1-ol	–	0.36	–	–	–
	(5-Ethylcyclopent-1-enyl)methanol	–	0.81	0.83	–	1.70
	Cedrol	–	0.01	–	–	–
	trans-(2-Ethylcyclopentyl)methanol	–	–	1.02	0.82	–
	(E)-2-Octen-1-ol	–	–	1.10	–	–
	2,4-Decadien-1-ol	–	–	0.19	–	–
	4-Ethyl-Benzenemethanol	–	–	0.01	–	–
	4-Terpineol	–	–	–	0.89	–
	β-Methyl-benzene ethanol	–	–	–	0.11	–
	3,5-Dimethyl-benzenemethanol	–	–	–	0.05	–
Esters	Dihydro-5-pentyl-2(3H)-furanone	0.14	–	0.15	–	–
	Dimethyl phthalate	0.10	–	–	0.14	0.14
	Acetic acid, hexyl ester	–	2.39	–	–	–
	Decyl trifluoroacetate	–	–	0.06	–	–
	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	–	–	0.02	–	–
Acids	Formic acid	–	–	–	–	5.13
	Hexanoic acid	–	–	0.02	–	–
	Sorbic acid	–	–	9.59	15.19	–
	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	–	–	0.42	0.21	–
Hydrocarbons	Methylene chloride	0.04	–	0.13	0.15	0.43
	D-Limonene	0.11	0.53	–	–	0.57
	Dodecane	0.16	0.29	0.24	0.38	0.44
	Tridecane	0.12	0.35	0.17	0.29	0.39
	1-Ethyl-2,4-dimethyl-benzene	0.01	–	–	–	–
	1-Ethyl-2,3-dimethyl-benzene	–	–	–	–	0.09
	1,2,3,5-Tetramethyl-benzene	–	–	–	–	0.81
	2-Ethyl-1,3-dimethyl-benzene	–	–	–	–	1.66
Aromatic compounds	Isophorone	0.12	–	–	–	0.23
	Naphthalene	–	–	–	3.12	–
	Phenol	–	–	–	0.16	–
	1-Heptadecene	0.12	–	0.10	–	–
	Parachlorophenol	0.24	0.37	–	–	0.72
	2-Ethylfuran	–	–	0.24	–	–
	2-Heptanone	–	–	0.33	–	–
	1,3-Diazine	–	–	0.82	–	–
	3-Octanone	–	–	0.84	–	0.50
Ketones	2-Butanone	–	–	–	0.20	–
	Ethyl-1-propenyl ether	–	–	–	0.55	–
	Limonene	0.27	0.12	–	2.34	–
	Pyrazine	0.15	0.18	0.23	0.42	–
	3-Ethylcyclopentanone	0.18	0.09	0.10	0.16	0.19
	1-Ethyl-3,5-dimethyl-benzene	3.75	1.48	–	0.66	–
	o-Cymene	0.25	–	0.22	–	–
	1,2,4,5-tetramethyl-Benzene	–	1.18	–	–	–
	3-Chlorophenol	–	0.73	–	–	–
	2-Methylpyrazine	–	–	0.21	–	–
	2-Ethyl-1,4-dimethyl-benzene	–	–	0.96	–	2.48
	1-Methyl-3-(1-methylethyl)-benzene	–	–	0.35	–	–
Others	4-Isopropylidene-5,5-dimethyl-4,5-dihydro-1H-pyrazole	–	–	0.16	0.31	–
	2-[(*E*)-pent-2-enyl]furan	–	–	0.61	–	–
	(E)-2-Methoxy-4-(prop-1-enyl) phenol	–	0.79	–	–	–

(*continued on next page*)

Table 3 (*continued*)

Category	Compounds	Relative content of volatile compounds in samples at different reaction times (%)				
		0 min	15 min	30 min	45 min	60 min
	Benzyl nitrile	–	0.20	–	1.22	1.90
	2,4-Hexadiyne	–	–	–	0.80	1.30
	3-Methyl-2,4-hexadiene	–	–	–	0.65	–
	3-Ethyl-2-methyl-1,3-hexadiene	–	–	–	0.02	–
	2-Acetylthiazole	–	–	–	–	3.04
	Azulene	–	–	–	–	0.46
	Eugenol	–	–	–	–	0.01

LC-MS/MS. Meanwhile, pentanal and hexanal can cross-link with peptides by carbonyl-amine reaction, leading to the elimination of fishy odorants. Overall, glucosamine-induced glycation can mitigate the bitterness and undesirable odorants of fish viscera-derived protein hydrolysates, contributing to the improved flavor thereof.

Ethical statement

Those involved in the sensory evaluation were not forced to participate, fully disclosed the study requirements and risks, had written or verbal consent from the participants, did not release the participant data without their knowledge, and could withdraw from the study at any time.

CRediT authorship contribution statement

Binbin Yu: Writing – original draft, Investigation. **Xiaoli Gong:** Writing – review & editing. **Na Zhang:** Writing – review & editing. **Soottawat Benjakul:** Writing – review & editing. **Yuhao Zhang:** Writing – review & editing. **Yu Fu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.fochx.2024.101993.

Data availability

Data will be made available on request.

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