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Research Article

Antioxidative Activity and Volatile Profiles of Maillard Reaction Products between Giant Salamander (*Andrias davidianus*) Peptides and Glucose during the Heating Process

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To improve the antioxidant activity and flavor characteristics of giant salamander peptides, the changes in pH, browning extent, DPPH radical scavenging capacity, and reducing ability in Maillard reaction products (MRPs) between giant salamander peptides and glucose during the heating process (95°C and 0, 1, 2, 3, and 4 h) were investigated. The difference in volatile compounds of the MRPs was also analyzed by gas chromatography-ion mobility spectroscopy. The results indicated that the pH value of the MRPs shrank with the proceeding of heating time, while the browning extent and antioxidative capacity increased. 58 volatile compounds including 24 aldehydes, 12 alcohols, 8 esters, 5 ketones, 4 pyrazines, 2 furans, 1 pyridine, 1 ether, and 1 olefin were detected among the MRPs. At different stages of the reaction, the MRPs were dominated by aldehydes, and the relative amount of aldehydes first decreased and then increased with the intensification of the reaction time; the relative content of esters increased first and then decreased; the relative content of alcohols decreased gradually; and the relative amount of pyrazine and furan exhibited a significant increasing tendency. Principal component analysis (PCA) revealed that the cumulative contribution rate of the first two components reached 87.3%, indicating that the volatile compounds of MRPs at different reaction times were well differentiated. The correlation analysis demonstrated that the antioxidant activity was positively associated with the browning extent, ketones, furans, and pyrazines. These results might offer a certain reference for modifying the physicochemical traits of giant salamander peptides.

1. Introduction

Giant salamander (*Andrias davidianus*), also known as a "living fossil" and the largest amphibian species, has been listed as critically endangered since the 1980s [1]. This administration was mainly ascribed to habitat destroying and overcapturing because of their edible, economic, and medicinal values [1, 2]. In recent decades, artificial breeding and culture of giant salamanders have been successfully industrialized in many provinces of China, and it is legal to develop farmed giant salamander products, yet not wild populations [2, 3]. As their price dropped sharply with increasing quantities and yields annually, further development and exploitation of huge amounts of farmed giant salamanders are greatly in demand. Currently, nutrition analysis of giant salamanders [3–5], bioactive peptides [6–9], collagen/gelatin [10–12], segmentation processing, storage, preservation [13–15], and other aspects are frequently

reported. Along with the application and development trend of various food-derived bioactive peptides, functional peptides prepared from the meat of giant salamander by enzymatic hydrolysis have also been reported to have antioxidant, antibacterial, antidiabetes, and immune-enhancing effects [6–9]. However, there are still some technical problems to be solved, such as a strong fishy smell, difficulty in purification and enrichment of highly active components, and unclear activity mechanisms.

Maillard reaction is an important nonenzymatic browning reaction in food thermal treatment, mainly caused by the carbonyl ammonia reaction between reducing sugars and amino compounds [16–18]. The physicochemical and functional activities of Maillard reactions about sugars, proteins, and amino acids model systems were widely documented. At present, the research on the Maillard reaction of some reducing sugars with protein hydrolysates has also gradually become more and more. The previous research proved that the flavor, taste, and biological activity (antioxidant, antibacterial, etc.) of Maillard reaction products (MRPs) between protein hydrolysates/peptides and reducing sugars could be greatly improved [19–22], which is considered relatively safe and harmless in food systems. Therefore, the Maillard reaction has been deemed one of the potential alternative methods to modify the quality and function of protein hydrolysates/peptides.

Currently, the detection methods of volatile compounds in foods are diverse, such as GC-MS, gas chromatography-olfactometer, E-nose, and gas chromatography-ion mobility spectrometer (GC-IMS) [23, 24]. These technologies are widely employed in the detection of food-volatile compounds. Compared with common GC-MS technology, GC-IMS technology has the merits of easy sample preparation, rapidness, high susceptibility, high resolution, visualization, etc. [24, 25]. Chen et al. [18, 26] explored the cothermal Maillard reaction characteristics of fish scale gelatin hydrolysates and collagen peptide with reducing sugar, monitored the changes of polypeptide molecular weight, amino acid composition, and antioxidant activity before and after the reaction, and analyzed their volatile profiles by GC-IMS. Han et al. [17] and Cui et al. [22] explored the differences in antioxidant activities and volatile components before and after the Maillard reaction of scallop skirt protein hydrolysates. Zhao et al. [27] also studied the antioxidant activity and volatile compound characteristics of the Maillard reaction products between grass carp peptides and xylose. More and more research evidence indicates that the Maillard reaction has better modification and improvement impacts on the flavor and radical scavenging capacity of protein hydrolysates/peptides [28–30].

Previous studies have investigated the collagen and volatile compounds of giant salamander meat [10, 24, 31] and the purification and identification of bioactive peptides [8, 9, 32, 33]. Based on the abovementioned backgrounds about the improvement of flavor and antioxidative characteristics of food-derived peptides combined with the Maillard reaction, herein, the present work investigated the Maillard reaction characteristics of giant salamander peptides and glucose during the heating process, mainly monitoring the changes of the pH value, browning index, and antioxidant capacity of MRPs at different reaction stages. In addition, the fingerprints of volatile compounds of MRPs at different stages were visualized through GC-IMS technology to explore the correlation between the antioxidant activity and volatile compounds, with the hope of providing references for the modification and improvement of flavor quality and the antioxidant activity of giant salamander peptides.

2. Materials and Methods

2.1. Raw Materials and Chemicals. The freeze-dried powder of giant salamander peptides (GSPs, molecular weight less than 3000 Da, accounting for more than 93%) was provided by the Shaanxi Provincial Key Laboratory of Resource Biology (Hanzhong, China), and it was sealed and stored at −20°C before the experiment. Glucose was purchased from Tianjin Tongxin Chemical Co., Ltd. Analytical grade *n*-ketones (2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, and 2-nonanone, purities ≥99%) were bought from Guoyao Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of MRPs between Giant Salamander Peptides and Glucose. The MRPs between giant salamander peptides and glucose were manufactured according to a previous study by Han et al. [17], and the actual conditions were slightly modified. In brief, we prepared 30 mg/mL giant salamander peptide solution and 40 mg/mL glucose solution with deionized water, mixed them according to the volume ratio of 1 : 1, put them into 20 mL glass bottles, and then put them into a water bath for heating reaction (95°C and 0, 1, 2, 3, and 4 h). The corresponding MRPs of giant salamander peptide/glucose at different reaction times were cooled to room temperature for analysis.

2.3. Determination of pH. The pH value of the giant salamander peptide/glucose MRPs at different reaction times was measured by a precision digital pH meter. Final pH values were read and averaged at least 3 parallels.

2.4. Determination of the Browning Degree. According to the procedures of Zhao et al. [27], after diluting the MRPs of giant salamander peptide/glucose at different reaction times, the absorbance values at 294 nm (10-fold dilution) and 420 nm (20-fold dilution) were determined by a UV spectrophotometer (UV2100, Unico Instrument Co., Ltd., Shanghai, China), indicating the production of intermediate products and brown substances during the heating process, respectively. The average of three readings was taken for each absorbance.

2.5. Assay of Antioxidant Capacity. The antioxidant capacity of giant salamander peptide/glucose MRPs was evaluated by the DPPH free radical scavenging rate and reducing power, according to a previous report described by Jin et al. [34].

For the DPPH radical scavenging rate, in brief, the sample solution (1.0 mL) was blended in 0.1 M phosphate buffer (2.0 mL, pH 6.0) and 0.2 mM DPPH ethanol solution (2.0 mL). At ambient temperature, the dispersion was blended in the dark for 30 min and then centrifuged ($2000 \times g$, 10 min) in a centrifugator (TGL-16 MS, Xiangyi Centrifugal Instrument Co., Ltd., Shanghai, China). The absorbance of the upper solution was read at 517 nm using a spectrophotometer (UV2100, Unico Instrument Co., Ltd., Shanghai, China). The scavenging rate of the DPPH radical was calculated using the following equation:

$$\text{scavenging rate } (\%) = \left[1 - \frac{(A_s - A_0)}{A}\right] \times 100, \quad (1)$$

where A_s is the solution absorbance with the sample and A_0 is the solution absorbance with DPPH instead of the same volume of ethanol, while A is the absorbance of the solution with deionized water (1 mL) substituted for the sample.

For reducing power, the sample solution (1.0 mL) was blended with 0.2 M phosphate buffer (1.0 mL, pH 6.6) and 1% (w/v) potassium ferricyanide (2.0 mL). After keeping it at 50°C for 20 min, the fluid was added with 10% (w/v) trichloroacetic acid (1.0 mL). The mixture was centrifuged ($2000 \times g$, 10 min) in a centrifugator (TGL-16 MS, Xiangyi Centrifugal Instrument Co., Ltd., Shanghai, China) after the complete blend. The supernatants (2.0 mL) were drawn and blended with deionized water (2.5 mL) and 0.1% (w/v) $FeCl_3$ (0.3 mL). After keeping it at ambient temperature for 10 min, the absorbance of the solution was measured at 700 nm using a spectrophotometer (UV2100, Unico Instrument Co., Ltd., Shanghai, China). The higher for 700 nm absorbance, the greater the sample's reducing powder.

2.6. Detection of Volatile Flavor Compounds. The volatile flavor compounds of MRPs were determined on a GC-IMS device, according to a modified procedure [31]. Concisely, 2.0 mL of MRPs at different reaction times was transferred into a 20 mL headspace glass bottle. After incubation at 65°C for 10 min, the headspace gas (500 μL) was injected into the injector and examined by the GC-IMS instrument (FlavourSpec®, Germany). The program conditions were set as follows. The gas chromatographic prefractionation was done on an MXT-5 column ($15\,\text{m} \times 0.53\,\text{mm}$). The automatic injection conditions included incubation temperature of 60°C, 15 min incubation time, 500 rpm incubator speed, splitless mode, injection needle temperature of 85°C, and 500 μL injection volume. The 99.99% nitrogen was used as a vehicle air at a programmed speed as follows: 2 mL/min for 2 min, 30 mL/min for 8 min, 100 mL/min for 10 min, and 150 mL/min for 5 min. The IMS conditions were as follows: 45°C drift tube temperature and nitrogen (≥99.999%) as the drift gas at a flow rate of 150 mL/min. Several n-ketones were checked as immigrant markers for calculating the retention index (RI) of the individual organic components. By contrasting RI and the drift time (DT) via the segment libraries of the GC-IMS device, volatile flavor compounds were detected by matching DT and RI to those of the immigrant marker chemicals. The relative ratio of flavor compounds was correlated with the peak signal [23].

2.7. Statistical Procedure. The data were expressed as mean ± standard deviation ($n \geq 3$), and the t test was used for significance analysis ($p < 0.05$). The instrumental analysis included GC × IMS Library Search, LAV (Laboratory Analytical Viewer), and the gallery plot. All volatile compounds were identified through the retention index, GC-IMS NIST 2014 library search, and n-ketone standards. A plug-in PCA score and a Euclidean distance diagram by the headspace gas chromatography-ion mobility spectrometer (HS-GC-IMS) were also executed.

3. Results and Discussion

3.1. Changes in Physicochemical Indices and the Antioxidant Activity of MRPs at Different Reaction Times. In this study, giant salamander peptides and glucose solution were heated at different times to prepare MRPs (the appearance illustration shown in Figure 1). The pH value, browning degree, and antioxidant activity of the MRPs at different reaction times were monitored, and the results are shown in Figure 2. The pH value of the MRPs system showed a gradual decline after the heating reaction of the giant salamander peptide and glucose solution at different times (Figure 2(a)), which may be due to the continuous consumption of polypeptide amino acids during the Maillard reaction and the formation of acid compounds such as formic acid, acetic acid, methylglyoxal, and glyoxal [17, 35]. The browning degree of the Maillard reaction process is usually reflected by the absorbance at 294 nm (the production of intermediate products) and 420 nm (the production of brown substances at the advanced stage) [34]. Figure 2(a) also shows that the absorbance of MRPs at 294 nm and 420 nm after the heating reaction of giant salamander peptide and glucose solution at different times, after proper dilution, shows an upward trend. These data showed that the Maillard reaction of giant salamander peptide and glucose produced a large number of intermediate products, and these complex intermediate products were further polymerized/degraded into brown components [22, 27], which was consistent with the results of appearance of browning in Figure 1.

Many studies show that the Maillard reaction products of peptides and reducing sugar have a good antioxidant activity [26, 36–38]. Figure 2(a) shows the change rule of the DPPH free radical scavenging rate and reducing ability of MRPs after heating reaction of giant salamander peptide and glucose solution at different times. It can be seen that the DPPH free radical scavenging rate and reducing ability of giant salamander peptide and glucose MRPs show an increasing trend with the extension of reaction time. After 4 hours of reaction, their DPPH free radical scavenging rate and reducing ability are 6.9 times and 5.1 times of the initial value, indicating that the antioxidant activity has been greatly improved. The composition of the Maillard reaction products is rather complex. Due to different reaction conditions, some intermediate products of reducing ketone

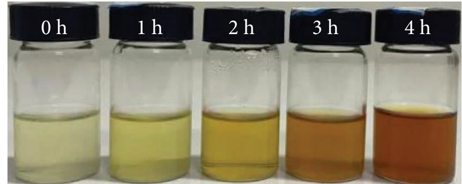

Figure 1: Appearance photo of giant salamander peptides/glucose MRPs at different reaction times.

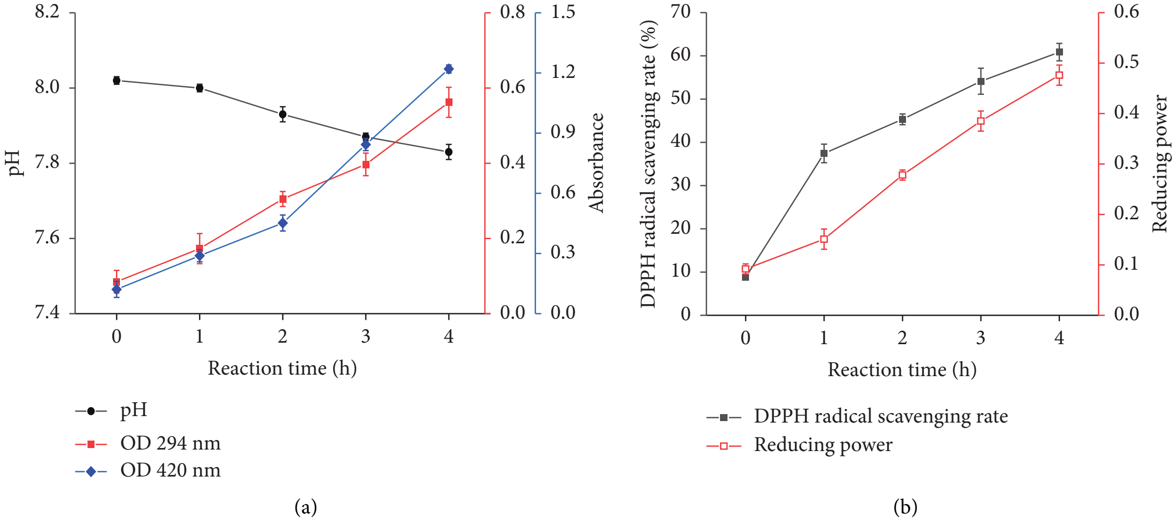

Figure 2: Physicochemical (a) and antioxidative activity (b) changes of Maillard reaction products derived from giant salamander peptides and glucose heated at different times.

structure, caramel products, and brown substances with furan ring and nitrogen (such as pyrazines and melanoids) are generally considered the physical basis of MRPs with high antioxidant activity [27, 39, 40].

3.2. GC-IMS Spectra of Volatile Compounds of MRPs at Different Reaction Times. GC-IMS was used to detect the volatile compounds of MRPs in the heating reaction of giant salamander peptide and glucose at different times. Figure 3(a) shows the 3D spectrum exported by the Reporter plugin in the LAV analysis software of the GC-IMS instrument. The *Y*-axis is the retention time, the *X*-axis is the migration time, the *Z*-axis is the signal peak intensity, each spot is a volatile compound, and the dark color represents the signal strength (the darker the color, the higher the relative content of volatile compounds). A volatile compound may contain monomers or dimers, which are subject to the content and nature of volatile compounds [23, 26]. From left to right, the 3D diagram shows the Maillard reaction products of giant salamander peptide and glucose of 0 h, 1 h, 2 h, 3 h, and 4 h, respectively. It is relatively difficult to visually identify the volatile compounds with the naked eye (Figure 3(a)), and further dimensionality reduction is required.

Figure 3(b) is a 2D plane top view obtained by projecting the 3D spectrogram, which is easier to compare the differences in volatile compounds of the MRPs at different reaction times. The volatile compounds of different MRPs samples were well separated by the gas phase ion migration spectrum, and the content of some compounds in different samples increased or decreased, reflecting slight differences [24, 26, 41] (yellow box area in Figure 3(b)). The researchers analyzed the differences in volatile compounds of fish scale gelatin hydrolytic peptides, collagen peptides, and reducing sugar coheating MRPs by the GC-IMS technology, indicating that there are certain differences between polypeptides and different MRPs volatile compounds [18, 26]. This study found that the GC-IMS characteristic spectrum of the volatile compounds of the MRPs in the heating reaction of giant salamander peptide and glucose at different times also showed relative differences, which might be related to the characteristics of raw materials, reaction conditions, and the Maillard reaction degree [27, 34, 36].

3.3. Identification and Qualitative Analysis of Volatile Compounds of MRPs at Different Reaction Times. GC-IMS qualitative volatile compounds mainly use normal ketone C4-C9 (2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, and 2-nonanone) as the external standards. By comparing the retention time and the migration time of various volatile compounds, the retention index of volatile compounds is obtained, and the qualitative analysis of volatile compounds is realized in accordance with the instrument database matching [23, 42]. The qualitative analysis results of volatile compounds in five MRPs at different reaction times are shown in Figure 4 (taking 0 h

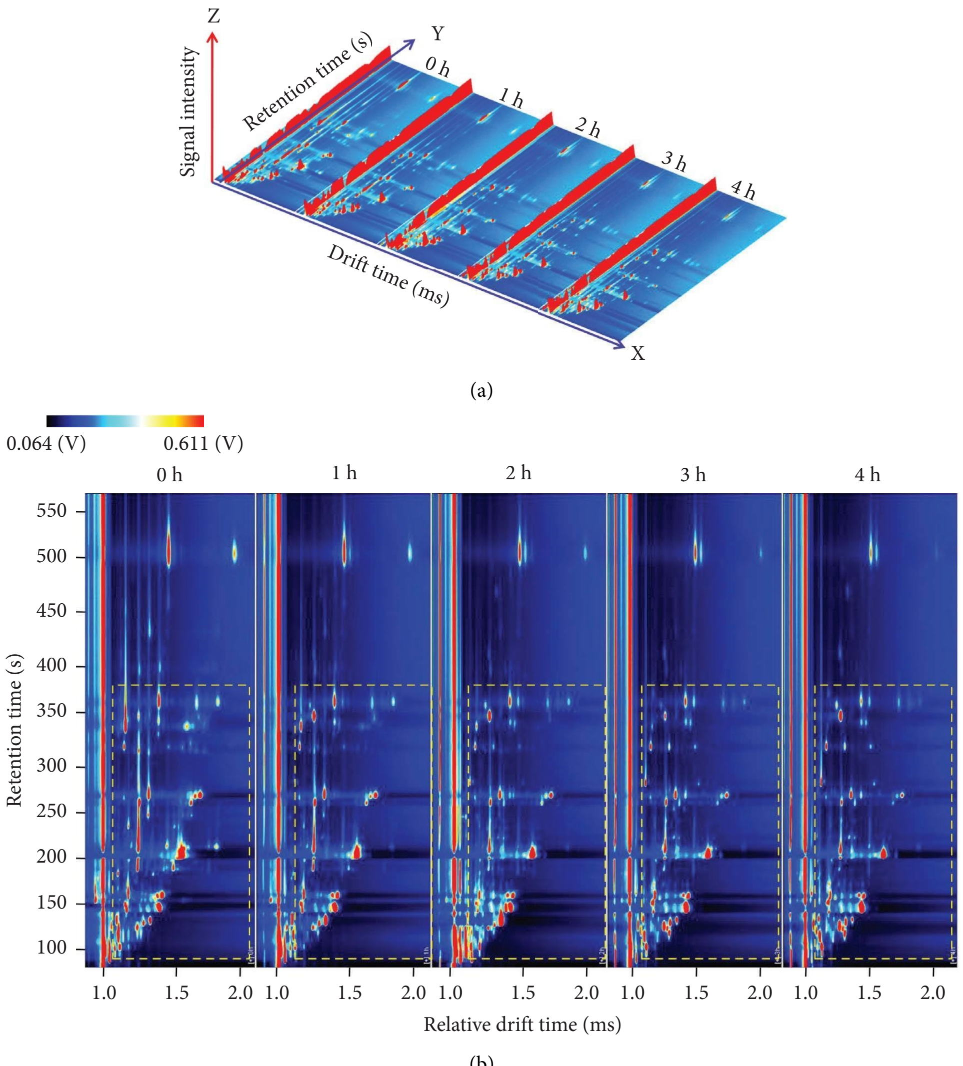

Figure 3: Three-dimensional (a) and two-dimensional (b) GC-IMS spectra of the Maillard reaction products derived from giant salamander peptides and glucose heated at different times.

sample as an example). 58 volatile compounds (monomers and dimers), including 24 aldehyde compounds, 12 alcohol compounds, 8 ester compounds, 5 ketone compounds, 4 pyrazine compounds, 2 furan compounds, 1 pyridine compound, 1 ether compound, and 1 olefin compound, have been identified. The names of various compounds are included in Table 1 for the retention index, retention time, migration time, and relative content information. It can be seen that before the Maillard reaction of giant salamander peptide with glucose (0 h), some characteristic compounds, such as hexanal, nonanal, and 1-octen-3-ol, were higher in content. With the progress of the Maillard reaction, some compounds changed significantly; especially, the content of 1-octen-3-ol decreased significantly (Table 1).

3.4. Volatile Compounds Fingerprint of Giant Salamander Peptides/Glucose MRPs. In order to better represent the difference in volatile compounds of the MRPs at different reaction times, the instrument's built-in plugin visualizes the fingerprint of volatile compounds of MRPs at different reaction times of giant salamander peptide and glucose (Figure 5). The figure shows the MRPs samples at different reaction times horizontally (0, 1, 2, 3, and 4 h from top to bottom and repeated for 3 times) and the same volatile substances in MRPs at different reaction times vertically (the darker the color, the higher the relative content) [24, 26]. It can be seen from the horizontal and vertical comparison in Figure 5 that the volatile compounds of giant salamander peptide and glucose MRPs at different reaction times are significantly different. It can be roughly seen from Figure 5 that the content of nonaldehyde, trans-2-octenal, 2-ethylhexanol, 1-octen-3-ol, 2-methyl-1-propanol butyrate, 1-pentanol, butyraldehyde, and other compounds in the 0 h MRPs sample is relatively higher (regions A and B). The content of 2-methylbutyraldehyde, glutaraldehyde, and ethyl acetoacetate in the 1 h MRPs sample is relatively higher (region C). The content of 2-pentylfuran, ethyl acetate,

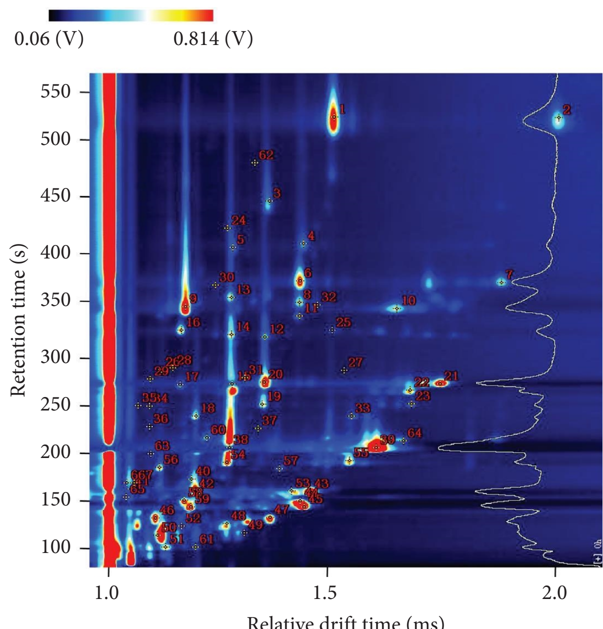

Figure 4: Volatile compounds searched through GC-IMS library from Maillard reaction products of giant salamander peptides and glucose heated at different times.

p-xylene, and other compounds in the 2 h MRPs sample is relatively higher (D region). The content of methyl caproate D, 3-methylbutyraldehyde, and other compounds in the 3 h sample is relatively higher (E region). The content of 2-hydroxy-4-methylvalerate, 2,6-dimethylpyridine, and other compounds in the 4-h sample is relatively higher (*F* region).

With the progress of the Maillard reaction, some volatile flavor compounds in the giant salamander peptide/glucose system have decreased significantly, such as 1-octen-3-ol with an earthy or fishy odor [31]. Several volatile compounds such as hexanal, heptanal, octanal, nonanal, (*E*)-hept-2-enal, and 1-octen-3-ol were reported in aquatic products as the typical fishy odor chemicals [23, 26, 27]. The present results found that the content of heptanal, octanal, nonanal, 1-octen-3-ol, and (*E*)-hept-2-enal in MRPs from 0 h to 4 h decreased significantly ($p < 0.05$) (Table 1 and Figure 5), indicating potential flavor improvement via the Maillard reaction. Meanwhile, some volatile organic compounds such as 2,5-dimethylpyrazine, 2-ethylfuran, and 2,6-dimethylpyrazine (Maillard reaction products with the potential antioxidant activity) increased significantly[27, 43]. Therefore, the Maillard reaction between giant salamander peptide and glucose could be used for enhancing flavor and the antioxidant activity. However, the combination of sensory evaluation and olfactory verification deserve further study.

To facilitate the comparison, the change rule of the relative content of various compounds with reaction times can be obtained from the normalization of the peak area in Figure 6. It can be seen that the MRPs at different reaction stages are dominated by aldehydes, and the relative content of aldehydes shows a trend of decreasing first and then increasing with the intensification of the reaction degree. The relative content of esters also increased first and then decreased. The relative content of alcohol showed a decreasing trend. The relative content of pyrazines and furans showed an obvious increasing trend. Chen et al. [26] identified 31 volatile compounds, including 11 aldehydes, 9 heterocyclic compounds (furans, pyrazines, alkenes, etc.), 8 ketones, 1 ester, 1 alcohol, and 1 acid, respectively, from fish scale gelatin hydrolytic peptides and xylose MRPs using the GC-IMS technology. Zhao et al. [27] identified 51 volatile compounds from grass carp peptides and xylose MRPs by headspace solid-phase microextraction combined with GC-MS, including 11 furans, 7 aldehydes, 5 ketones, 6 alcohols, 4 pyrazinepyrrole, and other hydrocarbons. In this study, 58 volatile compounds (Table 1), including aldehydes, furans, alcohols, esters, pyrazines, ethers, and olefins, were identified from the MRPs of giant salamander peptides and glucose at different reaction times by the GC-IMS. The types and quantities of these volatile compounds are quite different from those of the abovementioned studies, which may be related to the analytical methods, reaction conditions, raw material types, and statistical methods [34, 36]. In addition, the Maillard reaction components are very complex. The thermal degradation products of polypeptides, caramelization products and the decomposition, polymerization, and oxidation of precursors at different stages of the Maillard reaction may contribute greatly to the diversity of volatile components [36, 39]. In particular, with the progress of the Maillard reaction, the relative content of pyrazines and furans in the heating process between giant salamander peptide and glucose in this study increased sharply from 0.91% and 0.23% of the initial (0 h) to 3.58% and 5.66% of the 4 h, and these Maillard reaction products have a certain correlation with the antioxidant activity [27, 36, 43].

3.5. Similarity Analysis of Volatile Compounds of Giant Salamander Peptide/Glucose MRPs at Different Reaction Times. The volatile compound data identified by GC-IMS of giant salamander peptide/glucose MRPs at different reaction times were analyzed by principal component analysis, and the results are shown in Figure 7(a). It can be seen from Figure 7(a) that the contribution rates of the first two principal components are 64.9% and 22.4%, respectively, reaching 87.3% in total, which can represent and explain most of the information in the original data. The samples with the same reaction time are relatively clustered together, and the samples with different reaction times are thus distinguished, suggesting that the volatile compounds of the samples with different reaction times are well distinguished. Figure 7(b) shows the Euclidean distance map of giant salamander peptide/glucose MRPs at different reaction times. According to the distance reaction similarity between samples at different reaction times, the close distance between 0 h and 1 h samples and 2 h and 3 h samples indicates high similarity, while the distance between 1 h and 3 h samples and 2 h and 4 h samples indicates low similarity, and the Euclidean distance between samples at different reaction times is significantly greater than the average distance between parallel samples. The difference of MRPs samples at different reaction stages was indirectly realized through the Euclidean distance.

Table 1: Volatile compounds and their relative amounts identified from Maillard reaction products of giant salamander peptides and glucose heated at different times.

No.	Chemicals	Retention index	Retention time (s)	Drift time (ms)	Relative amount (%)				
					0 h	1 h	2 h	3 h	4 h
1	Nonanal-M	1111.9	511.849	1.47627	9.57 ± 0.18^{a}	8.45 ± 0.17^{b}	7.23 ± 0.16^{c}	6.56 ± 0.18^{d}	5.63 ± 0.24^{e}
2	Nonanal-D	1111.5	511.275	1.95095	2.43 ± 0.20^{a}	1.73 ± 0.11^{b}	1.21 ± 0.04^{c}	0.88 ± 0.05^{c}	0.64 ± 0.03^{d}
3	(*E*)-2-Octenal	1057.7	433.872	1.33978	1.11 ± 0.05^{a}	0.42 ± 0.06^{b}	0.28 ± 0.00^{c}	0.23 ± 0.01^{c}	0.20 ± 0.01^{d}
4	2-Ethyl-1-hexanol	1029.8	393.784	1.41183	0.55 ± 0.01^{a}	0.25 ± 0.01^{b}	0.16 ± 0.03^{c}	0.11 ± 0.01^{c}	0.11 ± 0.01^{d}
5	Octanal-M	1006.1	359.685	1.40436	3.79 ± 0.07^{a}	3.57 ± 0.04^{b}	3.02 ± 0.05^{c}	2.98 ± 0.04^{c}	2.34 ± 0.02^{d}
6	Octanal-D	1004.9	357.997	1.82992	1.14 ± 0.06^{a}	0.93 ± 0.05^{b}	0.73 ± 0.02^{c}	0.60 ± 0.01^{c}	0.37 ± 0.02^{e}
7	3-Octanol	988.9	339.428	1.40287	1.02 ± 0.04^{a}	0.79 ± 0.01^{c}	0.67 ± 0.03^{d}	0.87 ± 0.01^{d}	0.85 ± 0.03^{b}
8	Oct-1-en-3-ol-M	984.1	335.377	1.16247	8.83 ± 0.25^{a}	4.41 ± 0.22^{b}	2.35 ± 0.06^{c}	1.41 ± 0.06^{c}	1.22 ± 0.07^{d}
9	Oct-1-en-3-ol-D	981.8	333.351	1.60893	1.13 ± 0.06^{a}	0.37 ± 0.06^{b}	0.14 ± 0.01^{c}	0.11 ± 0.01^{c}	0.11 ± 0.01^{c}
10	2-Methylpropyl butanoate	951.1	307.203	1.33048	0.53 ± 0.03^{a}	0.29 ± 0.02^{b}	0.20 ± 0.02^{c}	0.19 ± 0.01^{c}	0.16 ± 0.01^{d}
11	2-Pentyl furan	994.3	344.031	1.2598	0.87 ± 0.03^{e}	4.06 ± 0.12^{c}	4.57 ± 0.14^{b}	1.59 ± 0.04^{b}	5.20 ± 0.08^{a}
12	(*E*)-Hept-2-enal	953.9	309.595	1.2598	1.51 ± 0.05^{a}	0.76 ± 0.01^{b}	0.54 ± 0.03^{c}	0.46 ± 0.01^{c}	0.39 ± 0.02^{d}
13	2-Heptanone-M	899.8	263.44	1.26121	4.53 ± 0.06^{a}	4.13 ± 0.04^{b}	3.12 ± 0.06^{c}	3.37 ± 0.02^{c}	2.82 ± 0.03^{d}
14	Ethyl acetoacetate	898.7	262.513	1.15135	0.13 ± 0.00^{d}	0.17 ± 0.01^{c}	0.21 ± 0.01^{ab}	0.22 ± 0.02^{ab}	0.19 ± 0.01^{b}
15	(*E*)-2-Hexen-1-ol-M	848.1	233.278	1.18567	0.95 ± 0.03^{a}	0.55 ± 0.02^{b}	0.43 ± 0.00^{c}	0.43 ± 0.01^{c}	0.44 ± 0.02^{c}
16	*n*-Hexanol-M	868.2	244.061	1.32411	0.88 ± 0.05^{a}	0.36 ± 0.03^{b}	0.16 ± 0.01^{d}	0.27 ± 0.02^{c}	0.11 ± 0.01^{e}
17	Heptanal-M	902	265.309	1.329	3.68 ± 0.10^{a}	3.46 ± 0.02^{b}	3.11 ± 0.03^{c}	3.37 ± 0.05^{c}	2.98 ± 0.01^{d}
18	Heptanal-D	899.9	263.499	1.69935	3.46 ± 0.15^{a}	2.97 ± 0.04^{b}	2.42 ± 0.08^{c}	2.16 ± 0.04^{c}	1.50 ± 0.02^{d}
19	2-Heptanone-D	892.7	257.348	1.63717	1.25 ± 0.11^{a}	1.07 ± 0.03^{b}	0.79 ± 0.03^{c}	0.52 ± 0.02^{c}	0.38 ± 0.01^{d}
20	*n*-Hexanol-D	870	245.044	1.63993	0.18 ± 0.02^{a}	0.04 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}
21	Benzene acetaldehyde	1040	408.532	1.25081	0.16 ± 0.01^{c}	0.13 ± 0.01^{d}	0.13 ± 0.01^{d}	0.18 ± 0.01^{d}	0.23 ± 0.01^{a}
22	Benzaldehyde-D	958.2	313.213	1.47163	0.18 ± 0.01^{c}	0.19 ± 0.02^{c}	0.19 ± 0.01^{c}	0.35 ± 0.01^{c}	0.43 ± 0.01^{a}
23	2,5-Dimethylpyrazine-M	915.3	276.611	1.11253	0.13 ± 0.01^{e}	0.27 ± 0.03^{d}	1.10 ± 0.07^{c}	2.44 ± 0.13^{c}	2.93 ± 0.03^{a}
24	2,5-Dimethylpyrazine-D	914.3	275.765	1.49731	0.03 ± 0.00^{d}	0.03 ± 0.00^{d}	0.07 ± 0.00^{c}	0.24 ± 0.01^{c}	0.31 ± 0.01^{a}
25	2,6-Dimethylpyrazine	917.5	278.512	1.13621	0.02 ± 0.00^{e}	0.07 ± 0.00^{d}	0.13 ± 0.01^{b}	0.11 ± 0.01^{b}	0.17 ± 0.01^{a}
26	3-Methylthiopropanal	905.3	268.151	1.08848	0.03 ± 0.00^{c}	0.04 ± 0.00^{c}	0.05 ± 0.01^{c}	0.08 ± 0.01^{b}	0.11 ± 0.00^{a}
27	Methyl hexanoate	906.5	269.157	1.28867	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}	0.14 ± 0.00^{a}	0.06 ± 0.00^{b}
28	(*E*)-2-Hexen-1-ol-D	848.1	233.277	1.51351	0.20 ± 0.02^{a}	0.05 ± 0.01^{b}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}
29	2,6-Dimethylpyridine	865.2	242.455	1.08649	0.02 ± 0.00^{c}	0.08 ± 0.01^{b}	0.32 ± 0.01^{a}	0.08 ± 0.01^{a}	0.08 ± 0.01^{b}
30	p-Xylene	866.4	243.092	1.063	0.01 ± 0.00^{c}	0.02 ± 0.00^{b}	0.08 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.00^{c}
31	Methylpyrazine	829.6	223.379	1.08649	0.05 ± 0.01^{c}	0.04 ± 0.00^{c}	0.14 ± 0.01^{b}	0.14 ± 0.01^{b}	0.17 ± 0.01^{a}
32	3-Methyl-1-pentanol	826.7	221.824	1.31655	0.03 ± 0.00^{e}	0.13 ± 0.00^{d}	0.19 ± 0.00^{c}	0.33 ± 0.00^{b}	0.37 ± 0.01^{a}
33	Hexanal-M	793.5	204.059	1.25499	4.12 ± 0.41^{c}	4.59 ± 0.03^{b}	3.97 ± 0.02^{c}	5.09 ± 0.05^{a}	4.84 ± 0.01^{ab}
34	Hexanal-D	793.5	204.059	1.56442	$13.99 \pm 0.55b^{c}$	14.81 ± 0.32^{b}	14.11 ± 0.08^{c}	15.3 ± 0.07^{a}	13.58 ± 0.1^{d}
35	Methyl isobutyl ketone	724.3	174.785	1.17502	0.43 ± 0.02^{b}	0.42 ± 0.01^{b}	0.26 ± 0.02^{c}	0.54 ± 0.01^{a}	0.55 ± 0.02^{a}
36	2-Ethyl furan	701.5	165.527	1.04763	0.05 ± 0.00^{e}	0.18 ± 0.01^{d}	0.33 ± 0.01^{c}	0.28 ± 0.01^{b}	0.46 ± 0.02^{a}
37	Pentanal-M	695	162.89	1.18284	1.77 ± 0.17^{c}	2.13 ± 0.06^{b}	1.47 ± 0.05^{d}	2.43 ± 0.03^{a}	2.17 ± 0.03^{b}
38	Pentanal-D	695.4	163.06	1.42513	4.39 ± 0.05^{a}	4.48 ± 0.11^{a}	3.49 ± 0.03^{c}	4.28 ± 0.05^{b}	2.99 ± 0.02^{d}
39	2-Methylbutanal-D	666.2	154.248	1.4043	3.72 ± 0.24^{e}	4.97 ± 0.19^{c}	4.45 ± 0.16^{d}	7.41 ± 0.07^{b}	8.02 ± 0.09^{a}
40	3-Methylbutanal-D	646.2	148.848	1.41364	3.42 ± 0.07^{e}	5.13 ± 0.10^{c}	4.33 ± 0.05^{d}	8.76 ± 0.03^{b}	8.89 ± 0.11^{a}
41	Ethyl acetate-M	608.2	138.614	1.09896	2.55 ± 0.07^{b}	3.22 ± 0.10^{a}	2.09 ± 0.01^{d}	2.33 ± 0.04^{c}	1.90 ± 0.04^{e}
42	Ethyl acetate-D	609.3	138.899	1.34179	2.59 ± 0.16^{c}	8.54 ± 0.12^{a}	2.19 ± 0.32^{d}	2.88 ± 0.03^{b}	2.13 ± 0.03^{d}

Table 1: Continued.

No.	Chemicals	Retention index	Retention time (s)	Drift time (ms)	Relative amount (%)				
					0 h	1 h	2 h	3 h	4 h
43	2-Butanone	588.2	133.214	1.25055	1.55 ± 0.19^{d}	1.68 ± 0.04^{c}	1.40 ± 0.02^{e}	5.28 ± 0.02^{b}	6.91 ± 0.07^{a}
44	Butanal-D	557.1	124.828	1.28647	0.40 ± 0.09^{d}	0.51 ± 0.03^{c}	0.50 ± 0.02^{c}	2.09 ± 0.02^{b}	2.68 ± 0.03^{a}
45	Butanal-M	547.6	122.27	1.10399	3.94 ± 0.15^{a}	1.97 ± 0.09^{c}	0.77 ± 0.02^{d}	3.42 ± 0.02^{b}	3.96 ± 0.02^{a}
46	Acetone	508.1	111.61	1.12051	1.03 ± 0.24^{d}	1.43 ± 0.08^{c}	1.49 ± 0.02^{c}	4.28 ± 0.10^{b}	5.23 ± 0.19^{a}
47	2-Butanol	580.3	131.082	1.15428	0.20 ± 0.04^{d}	0.21 ± 0.03^{d}	0.36 ± 0.05^{c}	0.63 ± 0.07^{b}	0.78 ± 0.02^{a}
48	Pentan-1-ol-M	762.7	190.349	1.24983	2.50 ± 0.12^{a}	1.70 ± 0.01^{b}	0.74 ± 0.01^{c}	0.63 ± 0.00^{d}	0.38 ± 0.02^{e}
49	(E)-2-Pentenal-M	751.7	185.889	1.10747	0.65 ± 0.02^{a}	0.28 ± 0.01^{b}	0.18 ± 0.01^{d}	0.24 ± 0.02^{c}	0.27 ± 0.01^{b}
50	(E)-2-Pentenal-D	748.7	184.671	1.36182	0.23 ± 0.03^{a}	0.05 ± 0.00^{b}	0.06 ± 0.00^{b}	0.04 ± 0.00^{b}	0.04 ± 0.01^{b}
51	2-Methylbutanal-M	668.9	154.97	1.1605	1.12 ± 0.09^{a}	1.03 ± 0.02^{b}	0.72 ± 0.01^{c}	1.09 ± 0.01^{c}	1.04 ± 0.05^{ab}
52	3-Methylbutanal-M	645.1	148.573	1.17425	1.79 ± 0.15^{a}	1.51 ± 0.05^{b}	0.85 ± 0.01^{d}	0.95 ± 0.01^{c}	0.85 ± 0.01^{d}
53	Ethyl butyrate	810.2	213.001	1.20764	0.23 ± 0.02^{a}	0.23 ± 0.01^{a}	0.18 ± 0.00^{b}	0.16 ± 0.01^{b}	0.12 ± 0.01^{c}
54	Ethyl 2-hydroxy-4-methylpentanoate	1082.3	469.36	1.30867	0.05 ± 0.01^{d}	0.13 ± 0.01^{c}	0.19 ± 0.01^{b}	0.10 ± 0.01^{b}	0.27 ± 0.01^{a}
55	3-Methyl-2-butenal	782.5	198.375	1.09033	0.05 ± 0.00^{e}	0.07 ± 0.00^{d}	0.09 ± 0.01^{c}	0.13 ± 0.01^{b}	0.17 ± 0.02^{a}
56	Butyl acetate	805.4	210.432	1.62335	0.21 ± 0.00^{a}	0.11 ± 0.00^{b}	0.08 ± 0.00^{c}	0.21 ± 0.01^{c}	0.05 ± 0.01^{d}
57	1-Propene-3-methylthio	715.1	171.02	1.03923	0.50 ± 0.02^{e}	0.70 ± 0.03^{d}	1.98 ± 0.03^{a}	0.91 ± 0.03^{c}	1.04 ± 0.03^{b}
58	3-Hydroxybutan-2-one	715.8	171.33	1.05409	0.05 ± 0.01^{b}	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}	0.06 ± 0.01^{a}	0.07 ± 0.00^{a}

-M and -D at the end of volatile chemicals denote monomer and dimer, respectively. Different lowercase letters in the same row denote significant difference ($p < 0.05$).

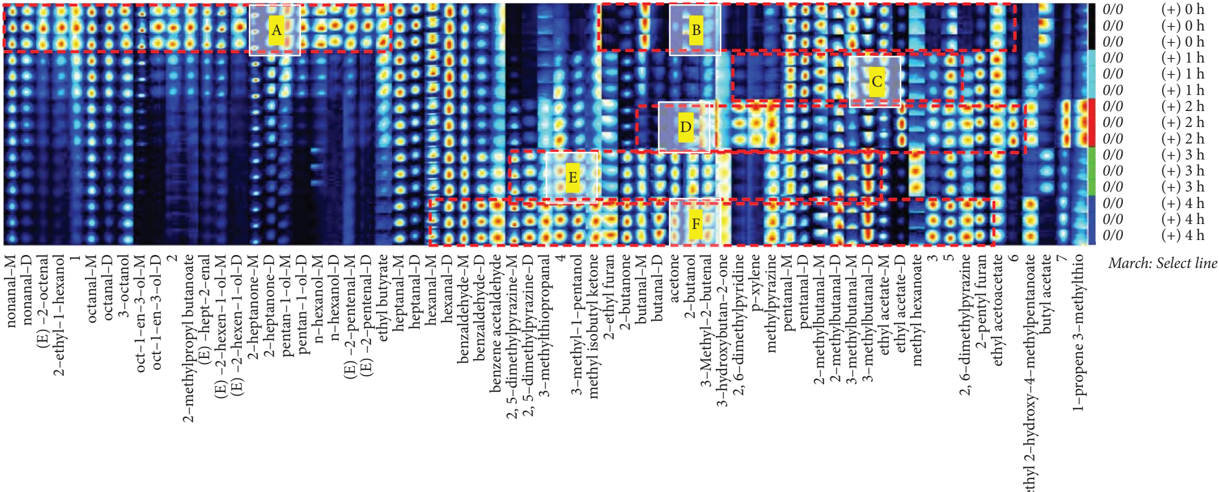

Figure 5: Gallery plot of volatile compounds in Maillard reaction products derived from giant salamander peptides and glucose heated at different times.

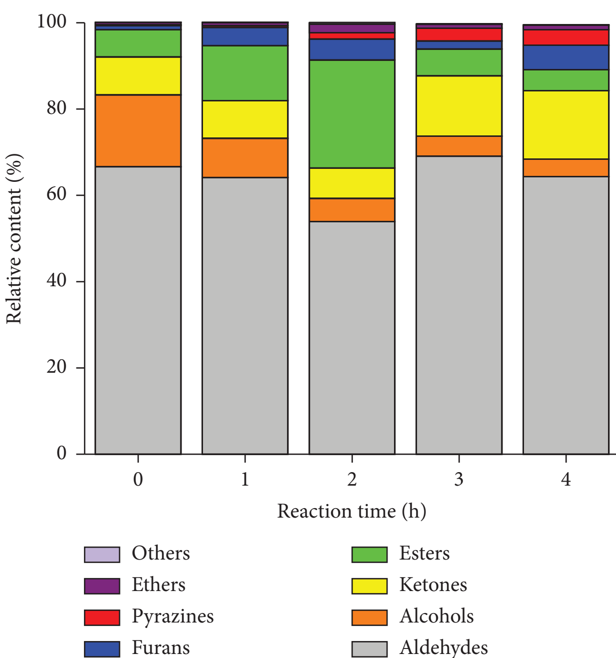

Figure 6: Relative content of volatile compound kinds in Maillard reaction products derived from giant salamander peptides and glucose heated at different times.

3.6. Correlation Analysis. Figure 8 shows the Pearson correlation analysis of the pH value, browning degree (absorbance at 294 nm and OD 420 nm), and antioxidant activity of giant salamander peptide/glucose MRPs at different reaction times, with various volatile compounds in Figure 6. Generally, the greater the Pearson correlation coefficient, the stronger the correlation. It can be seen from Figure 8 that pH has a significant negative correlation with the browning degree, DPPH free radical scavenging rate, reducing ability, and pyrazines ($p < 0.05$). The antioxidant activity was positively correlated with the degree of browning, ketones, furans, and pyrazines, but the reduction ability was only significantly positively correlated with pyrazines ($r = 0.99$, $p < 0.05$), but not with furans ($r = 0.66$, $p > 0.05$). The research showed that the antioxidative activity of grass carp peptide and xylose Maillard reaction products was significantly positively correlated with furans, while the difference in the positive correlation with pyrazines was not obvious. The analysis may be caused by the differences between GC-MS and GC-IMS in raw materials, reaction conditions, and volatile components analysis [26, 27, 36].

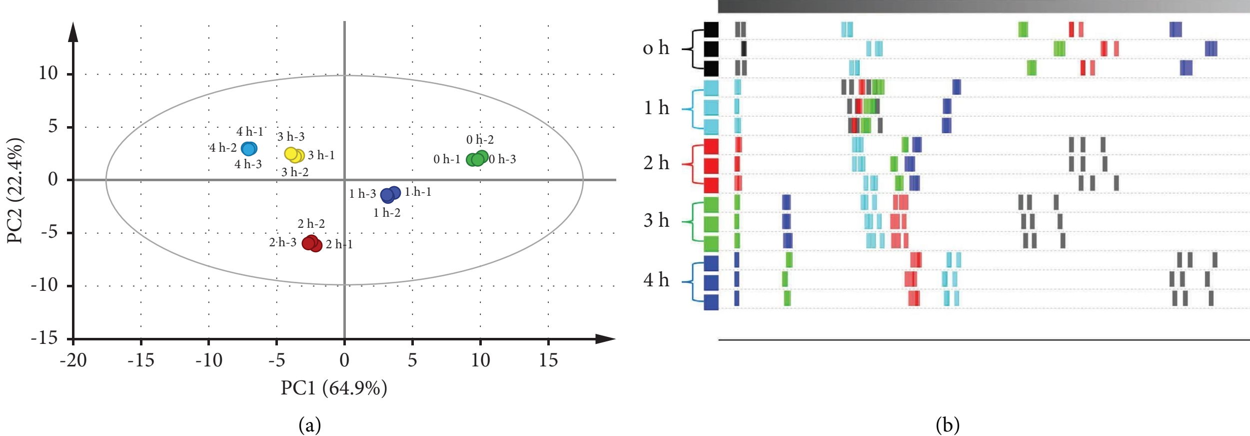

Figure 7: Similarity analysis of volatile compounds in Maillard reaction products derived from giant salamander peptides and glucose heated at different times: (a) PCA score plot and (b) Euclidean distance diagram.

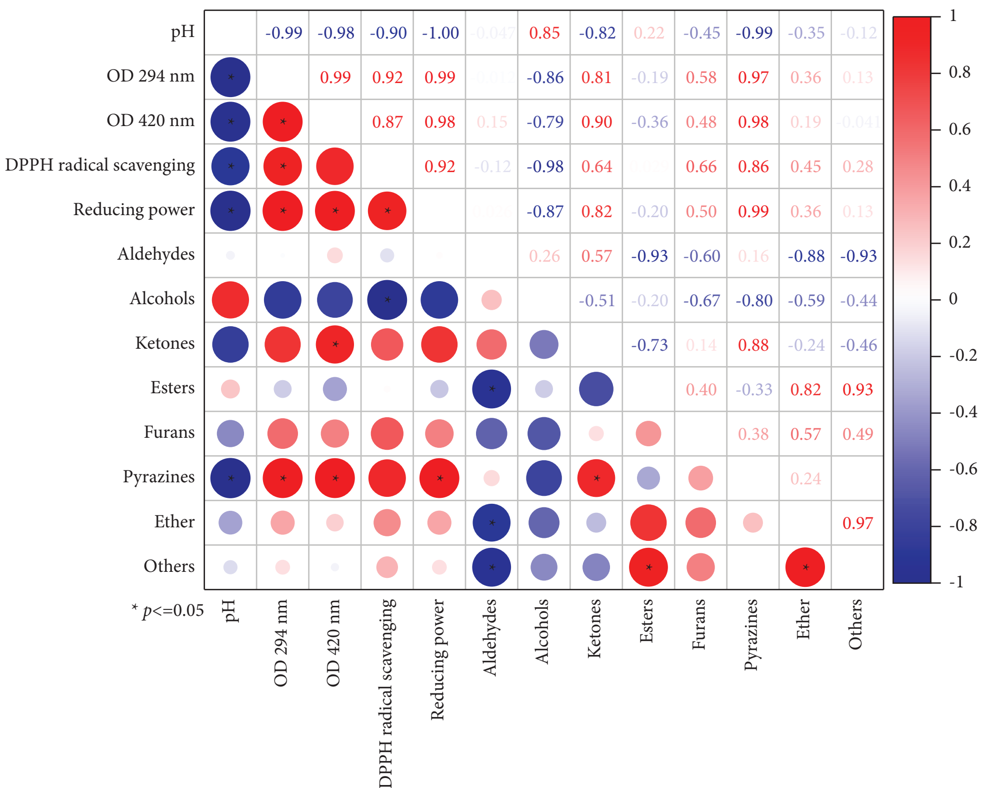

Figure 8: Correlation analysis between pH, browning extent, antioxidant activity, and volatile compounds.

4. Conclusion

In summary, with the prolongation of reaction time, the pH value of the MRPs between giant salamander peptide and glucose decreased, while the degree of browning, DPPH radical scavenging rate, and reducing power increased. A total of 58 flavor compounds were detected in the MRPs at different reaction stages, mainly dominated by aldehydes. The principal component and the Euclidean distance analysis showed that the volatile flavor compounds of MRPs at different reaction times could be well distinguished. During the heating process in the giant salamander peptide/glucose system, the relative content of the main fishy odor compounds decreased significantly, while pyrazines and furans increased, suggesting that the flavor and the antioxidant activity of giant salamander peptide have been favorably modified via the Maillard reaction. More work about verification through sensory evaluation and gas

chromatograph-olfactometry together with safety concerns of the MRPs deserve further studies and will be reported elsewhere.

Data Availability

All data generated or analyzed during this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Wenqian Wang conducted investigation, wrote the original draft, and performed plot analysis. Shibo Zhao and Jinjin Pei performed partial analysis, visualization, and language check. Pengfei Jiang reviewed and edited the manuscript. Ruichang Gao and Wengang Jin reviewed and edited the manuscript, supervised the work, and acquired funding.

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