Food Science of Animal Products

Research Article

Improvement on non-Na^+ saltiness via Maillard reaction between *Katsuwonus pelamis* hydrolysates and reducing sugar

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Abstract: Salt plays an important role in giving saltiness and adjusting taste in daily life. However, excessive intake of salt will cause certain harm to the body. Therefore, it has become an urgent need to develop new food processing strategies to reduce salt without reducing saltiness. In present study, Maillard reaction was employed to improve the flavor of *Katsuwonus pelamis* hydrolysates (KPSPs) with saltness. The change of flavor was measured by electronic tongue and sensory evaluation. After Maillard reaction of KPSPs and xylose, the bitter and other unpleasant flavor of the hydrolysates were reduced, whereas the saltiness and acceptability were enhanced significantly. The content of bitter amino acids of the Maillard reaction products of KPSPs (M-KPSPs) was reduced. Due to the covalent bonding between the peptide chain and the sugar molecules, the intrinsic fluorescence intensity was weakened with a redshift of the maximum emission wavelength, indicating that the tryptophan microenvironment became looser and more hydrophilic. The decrease in the composition of bitter amino acids and a change in the secondary structure of the peptides might lead to the change in the perception of flavor. The findings suggested that Maillard reaction affected the flavor characteristics, which provided a new idea for the improvement on non-Na^+ saltiness of healthy food additives via Maillard reaction between protein hydrolysate and reducing sugars.

Keywords: *Katsuwonus pelamis* hydrolysates; Maillard reaction; saltiness; structural change

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1 Introduction

With the improvement of living standards, people's eating habits gradually tend to be "healthier". To balance the relationship between "healthy" and "delicious", the seasoning process in food industry is crucial. Saltiness is one of the five basic tastes, which is mainly achieved by adding salt in people's daily diets. The main component of salt is sodium chloride, of which Na^+ is indispensable essential mineral elements for human body, which can maintain normal osmotic pressure and regulate the body's water and salt metabolism. However, long-term intake of high sodium salt will not only seriously affect the homeostasis of the internal environment, but also cause varying degrees of damage to the heart, liver, kidney and other organs[1]. The direct reduction of salt amount without any other strategies will bring about the deterioration of food salty perception, lack of flavor, poor product texture, shortened shelf life in food processing industry[2]. Therefore, reducing the amount of salt added without affecting the food quality and flavor has become a research hotspot. Current methods to reduce salt intake include optimizing salt structure and distribution, metal chloride substitution, and the addition of flavor enhancers[3–5]. As a kind of flavoring agent, salty peptides have shown great potential for development. At present, salty peptides and salty enhancing peptides have been prepared and identified from bovine bone, tilapia by-product, straw mushroom proteins hydrolysates, and so on[6–8].

Bonito (*Katsuwonus pelamis*) is a kind of economic marine fish widely distributed in tropical and subtropical waters. The traditional use of bonito is to make "Bonito Flakes", which is a kind of condiment with slight saltness, but the production process is complicated. A number of studies have shown that bonito is rich in protein with a variety of essential amino acids, which can ensure a good nutritional balance[9]. Our research group has prepared salty peptides from whole bonito fish by enzymatic hydrolysis. However, the bitter and fishy taste of the hydrolysate become obstacle to further applications. Therefore, in order to better use the hydrolysates of bonito, it is necessary to improve its flavor by structural modification.

Maillard reaction has been shown to have the ability to alter sensory characteristics, which may be an effective way to improve the acceptability of fish by-products[10–11]. Yan et al.[12] reported the salt-enhancing potential of Maillard reaction products (MRPs) generated from different reducing sugars and pea protein hydrolysates, and the MRPs with pentose possessed better salt-enhancing effect. At the same time, when 0.7% MRPs were mixed with 0.4% NaCl, the saltiness of the solution was significantly higher than that of the control group (0.5% NaCl), so even if the concentration of NaCl was reduced by 20%, the saltiness of the solution would not be reduced, indicating that MRPs are a good source of non-Na^+ saltiness enhancers. Katsumata et al.[13] prepared MRPs using soybean polypeptides and different carbonyl

compounds, and confirmed that MRPs showed a biphasic effect on people's perception of saltiness, that was, it enhanced NaCl response at low concentration and inhibited it at high concentration. At the same time, studies have shown that the formation of glycated peptides could mask bitter taste and show more ideal flavor and continuity[14–15]. Maillard reaction had promoted the development of flavor chemistry and had broken the scope of traditional flavor production technology in the condiment industry. The purpose of this study was to enhance the saltiness of bonito protein hydrolysates by Maillard reaction and to explore its possible mechanism. Results could provide reference for the development of economic fish as health food and non-Na^+ saltiness substitute in food engineering.

2 Materials and methods

2.1 Materials

Bonito was obtained from Zhangpu Fengziya Food Co., Ltd. (Zhangzhou, China). Xylose (Xyl), glucose (Glu), galactose (Gal), fructose (Fru), ribose (Rib), arabinose (Ara), *iso*-malto-oligosaccharide (IMO), xylo-oligo-saccharides (XOS), galactooligo-saccharides (GOS) and fructo-oligo-saccharide (FOS) were purchased from Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). NaCl (AR), and sodium hydroxide (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.2 Preparation of *Katsuwonus pelamis* hydrolysates (KPSPs) and the MRPs of KPSPs (M-KPSPs)

Katsuwonus pelamis was minced, added to distilled water at a ratio of 1:3.16 (g/mL) and then enzymatic hydrolyzed with alkaline protease (the weight ratio of enzyme to raw material was 5.25:100) at pH 7.0 and 50 °C for 185 min. After heating at 100 °C for 15 min to inactivate the enzyme, the mixture was centrifuged at 10 000 r/min at 4 °C for 20 min. The supernatant was collected and lyophilized, namely KPSPs.

KPSPs and different kinds of reducing sugars (Xyl, Glu, Gal, Fru, Rib, Ara, IMO, XOS, GOS and FOS) were dissolved in distilled water in a weight ratio of 1:1, the pH was adjusted to 7.0, and the reaction was heated in a water bath at 90 °C for 180 min. Separately heated KPSPs (50 mg/mL) solution was used as a control. Reducing sugar that could better enhance the saltiness of KPSPs was selected for the subsequent optimization of Maillard reaction conditions. In order to obtain MRPs with stronger saltiness, single factor experiment and response surface experiment were used to optimize the reaction conditions including the ratio of KPSPs to sugar, reaction time, pH and temperature. The browning degree (absorbance at 420 nm)[16] and saltiness were used as monitoring indexes.

2.3 Taste profile analysis

2.3.1 Sensory evaluation

The sensory assessment was conducted based on the method of Qiu et al.[17] with slight modification. Eight evaluators without taste and smell disorders were selected for this experiment with informed consent, all of whom had experience in sensory evaluation. To familiarize the evaluators with the evaluation criteria, they all performed sensory assessments of the edible NaCl with a series of standard concentrations firstly. The 5-point scoring method was adopted. The evaluator randomly tasted KPSPs and M-KPSPs of 30 to 100 mg/mL for 10 s, spat out the sample and recorded the equivalent concentration of NaCl solution as the saltiness of the samples. A 5-min interval after gargle with cold water was required before conducting the next determination.

2.3.2 Electronic tongue measurement

The taste profile of samples was tested by an electronic tongue (Smart tongue, Bosin, Shanghai, China), which was composed of 6 sensors (Platinum S1, Aurum S2, Palladium S3, Tungsten S4, Titanium S5, and Argentum S6). The signal amplification factor of the sensors was 100, and the detection lasted for 120 s. Each sample was repeated for 6 times. Different concentrations of NaCl solutions (10–60 mmol/L) were used as standards of salty taste to observe the changes in flavor.

2.4 Thermal stability analysis of KPSPs and M-KPSPs

The 20 mg/mL of KPSPs and M-KPSPs were heated in a constant bath shaker for 15 min at 40–100 °C, respectively, to explore the effect of heating temperature on the saltiness. Similarly, the samples were placed in a water bath at 100 °C for 10–40 min, and then the electronic tongue test was carried out after the samples were cooled down to room temperature.

2.5 Structural changes analysis

2.5.1 Determination of total amino acid composition

The concentration of amino acids was determined by amino acid analyzer (Aracus classic, MembraPure, Hennigsdorf, Germany). The pretreatment of samples was followed by Eric et al.[18]. The solid sample (KPSPs was 0.106 1 g and M-KPSPs was 0.098 8 g, respectively) was added to the tube with 10 mL 6 mol/L HCl. After being sealed by an alcohol spray lamp, the tube was placed to hydrolysis in an oven at 110 °C for 24 h. The waste residue was filtered from the hydrolysate and the filtrate was diluted to a 50 mL volumetric flask. Then 1 mL diluent was deacidified and dried at 60 °C using a vacuum deacidifier. After deacidification, 5 mL buffer was added to the dried powder and the mixture was filtered through a 0.45 μm filter before proceeding with machine analysis. The amino acid concentrations were determined by comparing the sample with a standard solution using the Clarity Chromatography Software (DataApex Company, Prague, Czech Republic).

2.5.2 Determination of Fourier transform infrared (FTIR) spectroscopy

KPSPs and M-KPSPs (1 mg) were mixed evenly with KBr (100 mg), and then ground and tableted before loaded to a FTIR spectrograph (360 Intelligent, Thermo Nicolet Co., Madison, WI, USA). The instrument parameters were set as scanning range of 400–4 000 cm^{-1}, instrument resolution 4 cm^{-1}, scanning times 64.

2.5.3 Determination of fluorescence spectra

The fluorescence spectra were analyzed according to the report of Chen et al.[19]. KPSPs and M-KPSPs were prepared to a concentration of 0.5 mg/mL with phosphate buffer (50 mmol/L, pH 7.0). The Maillard fluorescence spectra of the samples was obtained by fluorescence spectrophotometer (Agilent) at 347 nm excitation and 350–600 nm emission to analyze the formation of

MRPs. For the endogenous fluorescence analysis of peptides, the sample solution was scanned with an excitation wavelength of 290 nm and emission wavelength range of 300–540 nm.

2.5.4 Determination of circular dichroism spectra

According to previous reported method with some modification[20], the sample solution of 0.5 mg/mL was prepared with phosphate buffer (50 mmol/L, pH 7.0) and analyzed by circular dichroism at room temperature with the wavelength scanning range of 190–260 nm.

2.6 Statistical analysis

All measurements were performed with triplicate, and values are expressed as the mean ± standard deviation of triplicates from each of the two independent experiments. Data were statistically analyzed using IBM SPSS statistics 26.0 software. One-way analysis of variance was conducted, and significant differences were defined at $P < 0.05$ by Duncan's multiple comparison test.

3 Results and discussion

3.1 Optimization of M-KPSPs preparation conditions

The Maillard reaction plays a pivotal role in augmenting the flavor of protein hydrolysate. Previous reports showed that the type of reducing sugar affected the rate of Maillard reaction, and pentose could react more easily than hexose[21]. As shown in Figure 1, the browning degree of MRPs of pentose (Rib and Xyl) was higher than that of hexose. And the saltiness of KPSPs was enhanced to varying extents by different reducing sugars. Among the tested sugars, Xyl demonstrated the most significant impact, indicated by the highest sensory score of 1.73. Besides, according to the feedback of sensory evaluators, the Maillard reaction not only enhanced the perception of saltiness, but also decreased the perception of bitterness. The acceleration of the Maillard reaction rate contributed to the development of a more desirable flavor, thereby enhancing the sensory characteristics of the KPSPs. Consequently, Xyl was selected as the reducing sugar source for subsequent optimization.

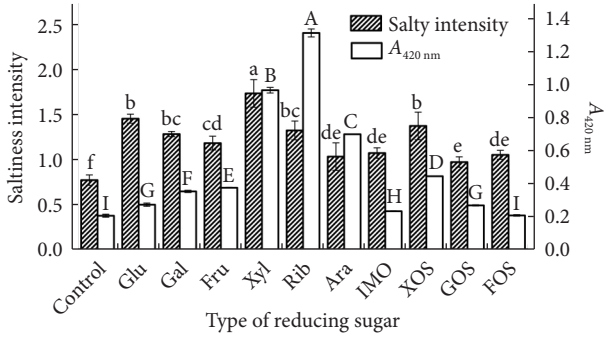

Figure 1 Effects of different reducing sugars on saltiness. Different letters indicate significant differences between groups ($P < 0.05$).

The influences of different reaction conditions on the saltiness of M-KPSPs were investigated. The initial aspect to be taken into consideration is the amount of Xyl introduced. As illustrated in Figure 2A, the saltiness intensity exhibited the maximum level when the mass ratio of KPSPs to Xyl was 4:1. Conversely, the lowest level of saltiness intensity was observed at a mass ratio of 1:4. This disparity can be ascribed to the elevated level of sweetness resulting from the inclusion of excess Xyl, which overshadowed the saltiness and consequently reduced its prominence. The influence of reaction time was shown in Figure 2B. At a reaction time of 150 min, the saltiness intensity reached the maximum point. With the elongation of the reaction time from 30 to 150 min, a progressive elevation in both the level of saltiness and the browning

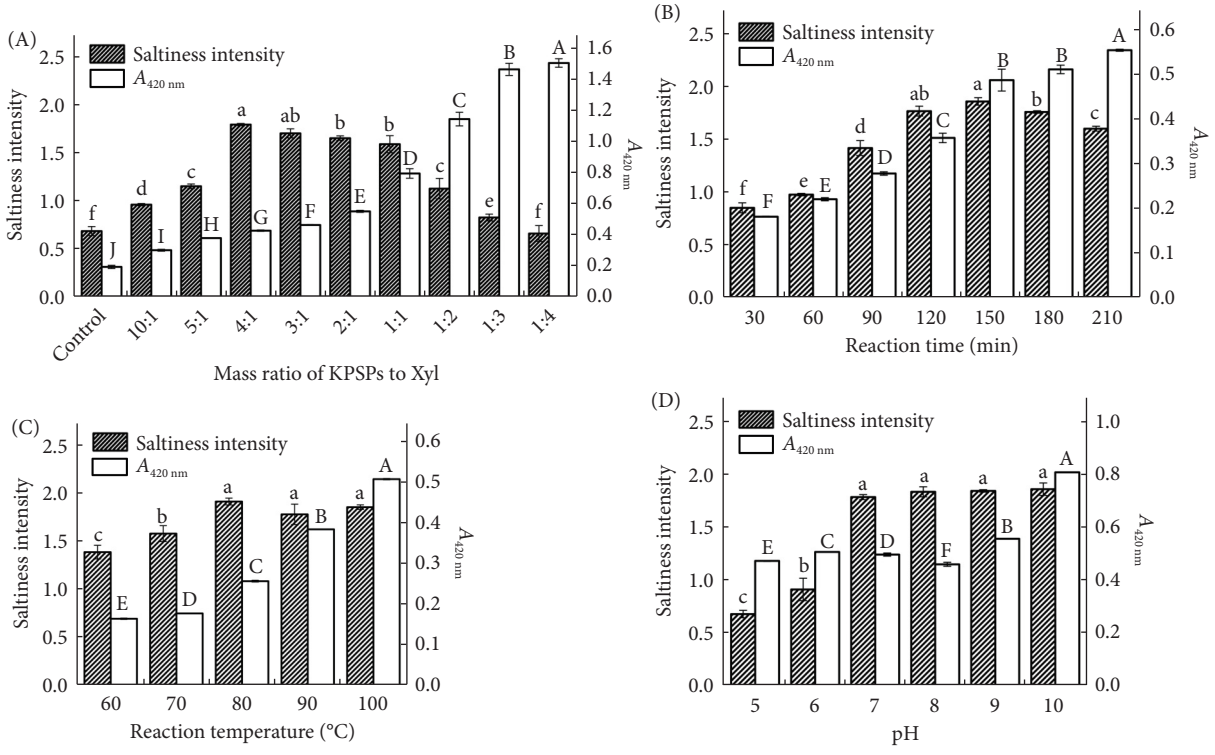

Figure 2 Effects of different reaction conditions on saltiness of M-KPSPs. (A) Mass ratio of KPSPs to Xyl, (B) reaction time, (C) reaction temperature, and (D) pH. Different letters indicate significant differences between groups ($P < 0.05$).

degree was observed. When the duration of the reaction surpassed 150 min, the browning degree exhibited continuous increase, while the perception of saltiness began to decline, which might be due to the production of undesirable flavors caused by the excessive caramelization reaction. Conversely, if the reaction time was too short, the full development of flavor and color would be prevented.

As depicted in Figure 2C, there was not statistically significant difference in saltiness when the temperature was set at 80, 90, and 100 °C. Therefore, temperature of 80 °C has been determined as the optimal condition. The impact of varying reaction pH on the intensity of saltiness and the level of browning was shown in Figure 2D. As the pH gradually increases from 5 to 7, the saltiness intensity also increases gradually. However, as the reaction pH continues to rise beyond 7, the saltiness intensity remains relatively stable without any significant differences. Several studies have indicated that optimal flavor can be achieved under weakly alkaline or neutral conditions. To prevent excessive introduction of Na^+ during pH adjustment, an initial reaction pH of 7 was selected as the most favorable condition.

On the basis of single factor experiments, Xyl additive amount (*A*), reaction time (*B*) and temperature (*C*) were selected as independent variables, and saltiness intensity was adopted as reaction parameters (Table 1). The three-factor and three-level experiments were conducted by Design-Expert 8.0.6 trial software, and the results were shown in Table 2. By regression analysis, the relationship between saltiness and each factor was obtained: Saltiness intensity = $1.800 + 0.092A + 0.130B + 0.160C + 0.095AB + 0.130AC + 0.340BC - 0.200A^2 - 0.260B^2 + 0.008C^2$.

Table 1 Factors and levels of response surface methodology.

Factor	Level		
	−1	0	1
Mass ratio of KPSPs to Xyl	5:1	4:1	3:1
Reaction time (min)	120	150	180
Reaction temperature (°C)	70	80	90

Regression analysis of variance (Table 3) showed that the model was extremely significant ($P < 0.01$). The R^2 of the regression model is 0.923 5, indicating that the model fits well and can be used to predict the preparation process parameters. According to the results of response surface, the optimal conditions were: the mass ratio of KPSPs to Xyl was 4.6:1, reaction time was 172 min, reaction temperature was 88.67 °C. The saltiness intensity was 2.20 under the optimal conditions. To facilitate the practical operation, the reaction temperature was adjusted to 89 °C and M-KPSPs were prepared with the saltiness intensity determined to be 2.18 ± 0.08.

Table 2 The experimental design and results of response surface.

Trial	*A* Mass ratio of KPSPs to Xyl	*B* Reation time (min)	*C* Reation temperature (°C)	Saltiness intensity
1	4:1	120	70	1.68
2	5:1	150	70	1.40
3	4:1	180	70	1.26
4	3:1	150	70	1.36
5	3:1	120	80	1.28
6	4:1	150	80	1.82
7	4:1	150	80	1.88
8	4:1	150	80	1.90
9	4:1	150	80	1.80
10	5:1	180	80	1.60
11	3:1	180	80	1.34
12	4:1	150	80	1.62
13	5:1	120	80	1.16
14	4:1	120	90	1.18
15	3:1	150	90	1.56
16	5:1	150	90	2.12
17	4:1	180	90	2.10

3.2 Saltiness enhancement of M-KPSPs

To characterize the change of saltiness before and after Maillard reaction, the electronic tongue analysis was conducted. The findings were presented in Figure 3. The combined contribution rate of PC1 and PC2 in terms of cumulative variance is 96.9%, indicating that these principal components effectively captured the overall taste characteristics of the sample. At equivalent concentrations of KPSPs and M-KPSPs, M-KPSPs exhibited higher saltiness than KPSPs. For instance, the saltiness of 50 mg/mL of M-KPSPs was equivalent to approximately 60 mmol/L NaCl, whereas 50 mg/mL of KPSPs was

Table 3 Variance analysis of the regression mode.

Source	Sum of square	Degree of freedom	Mean square	*F* value	*P* value	Significance level
Model	1.42	9	0.16	9.39	0.003 7	**
A Mass ratio of KPSPs to Xyl	0.068	1	0.068	4.07	0.083 3	
B Reaction time	0.13	1	0.13	7.44	0.029 4	
C Reaction temperature	0.20	1	0.20	11.81	0.010 9	*
AB	0.036	1	0.036	2.15	0.186 1	
AC	0.068	1	0.068	4.02	0.084 9	
BC	0.45	1	0.45	26.72	0.001 3	**
A^2	0.17	1	0.17	10.22	0.015 1	*
B^2	0.28	1	0.28	16.55	0.004 8	**
C^2	2.695×10^{-4}	1	2.695×10^{-4}	0.016	0.902 8	
Residual	0.12	7	0.017			
Lack of fit	0.068	3	0.023	1.86	0.277 2	
Pure error	0.049	4	0.012			
Total residual	1.54	16				
			$R^2 = 0.923\ 5$, $R^2_{Adj} = 0.825\ 2$			

Note: * Represents significant different with $P < 0.05$; ** represents extremely significant different with $P < 0.01$.

only equivalent to 40–50 mmol/L NaCl. Results of electronic tongue analysis indicated that the Maillard reaction effectively enhanced the perception of saltiness in KPSPs, which was consistent with the previous report of Sun et al.[22].

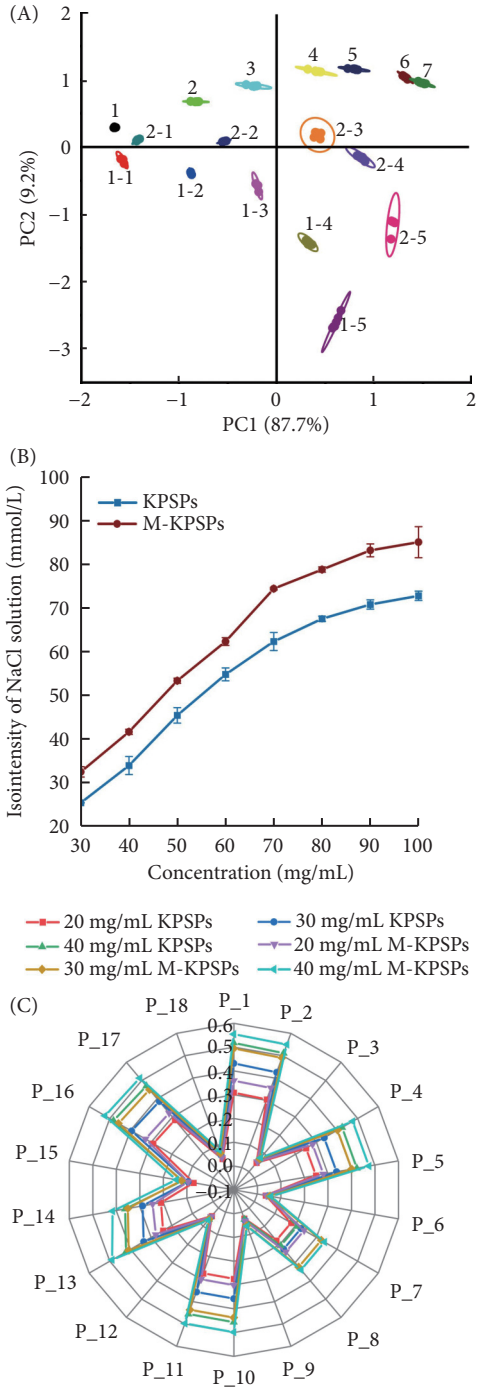

Figure 3 The change of saltiness (A, C) before and after Maillard reaction verified by electronic tongue and (B) artificial sensory evaluation. 1 to 7 represent NaCl solutions of 10, 20, 30, 40, 50, 60, 70 mmol/L; 1-1 to 1-5 represent KPSPs solutions of 10, 20, 30, 40, 50 mg/mL; 2-1 to 2-5 represent M-KPSPs solutions of 10, 20, 30, 40, 50 mg/mL. P_1 to P_18 represent the eighteen sensors in the electronic tongue.

Artificial sensory evaluation was conducted to assess the taste characteristics (Figure 3B). The results of the sensory evaluation indicated that the saltiness of the KPSPs and M-KPSPs exhibited a dose-dependent relationship. Furthermore, the equivalent NaCl concentration of M-KPSPs consistently exceeded that of KPSPs at the same concentration, which was in line with the findings of the electronic tongue analysis. The sensory evaluators reported a significantly higher acceptability of M-KPSPs compared to KPSPs. Additionally, our findings indicate that the trend of increased saltiness slowed down when the concentrations of KPSPs and M-KPSPs exceeded 70 mg/mL. According to the evaluators' feedback, the high concentration brought about distinct bitter taste, which might potentially mask its saltiness.

3.3 Thermal stability of KPSPs and M-KPSPs

To assess the thermal stability of the KPSPs and M-KPSPs (20 mg/mL), the variations of saltiness intensity under different temperature and time conditions were determined. The impact of varying heating temperatures on the flavor of KPSPs and M-KPSPs solutions was illustrated in Figure 4A. The contributions of variance contributed by PC1 and PC2 were 89.1% and 9.3%, respectively. The cumulative variance contribution rate of 98.4% indicates that the majority of the flavor information in the sample was captured. The saltiness of KPSPs remained relatively consistent after heating at 60 to 100 °C and the saltiness level was comparable to that of 20 mmol/L NaCl solution. M-KPSPs also exhibited good stability with saltiness comparable to 30 mmol/L NaCl solution. As shown in Figure 4B, when the heating time exceeds 20 min, the saltiness of both KPSPs and M-KPSPs showed an increasing trend with the extension of heating time, indicating that the samples were suitable for use in the process of food heat treatment and the saltiness would not decrease.

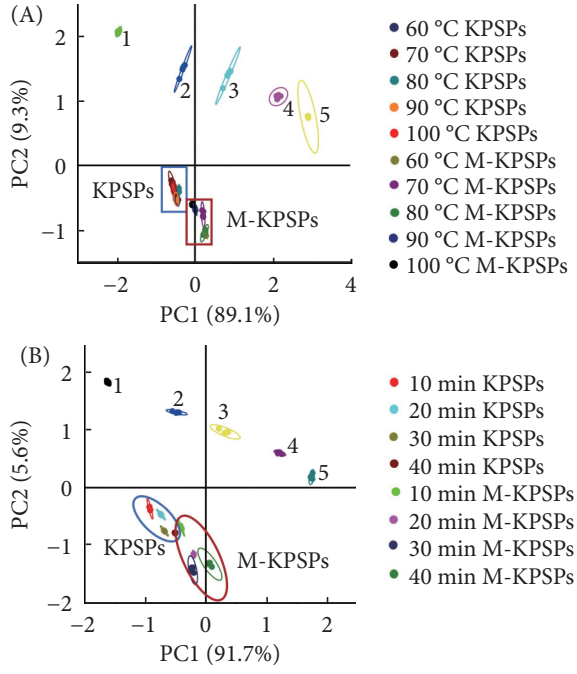

Figure 4 The thermal stability of KPSPs and M-KPSPs under different (A) heating temperature and (B) heating time. 1 to 5 represents NaCl solutions of 10, 20, 30, 40, 50 mmol/L.

3.4 Structural changes of KPSPs and M-KPSPs

3.4.1 *Total amino acid composition*

The composition of amino acids had important effects on the flavor of hydrolysate. As shown in Table 4, the Maillard reaction led to a

Table 4 Total amino acids composition of KPSPs and M-KPSPs.

Amino acid	Taste characteristic	Content (%)	
		KPSPs	M-KPSPs
Aspartic acid (Asp)	Sour, sweet, umami	6.992	6.568
Threonine (Thr)	Sweet, bitter	4.003	3.768
Serine (Ser)	Sweet, umami	3.245	3.075
Glutamic acid (Glu)	Umami	11.389	11.056
Glycine (Gly)	Sweet	4.086	3.604
Alanine (Ala)	Sweet	5.366	5.045
Valine (Val)	Bitter, sweet	0.931	0.853
Cysteine (Cys)		5.626	5.443
Methionine (Met)	Bitter, umami	1.580	1.453
Isoleucine (Ile)	Bitter	7.695	6.845
Leucine (Leu)	Bitter	2.248	2.134
Tyrosine (Tyr)	Bitter	4.435	4.013
Phenylalanine (Phe)	Bitter	1.742	1.773
Histidine (His)	Bitter	1.882	1.699
Lysine (Lys)	Bitter, sweet	7.782	6.922
Arginine (Arg)	Bitter	7.255	6.235
Proline (Pro)	Sweet, bitter	4.604	4.413
Total amino acids		80.860	74.900

reduction in the content of certain amino acids. Specifically, the content of Arg, Lys, Gly and Ile decreased the most. These findings highlighted the significance of these amino acids in the Maillard reaction. The content of total amino acids in KPSPs was measured to be 80.86%, while in M-KPSPs was 74.90%, which was aligned with the findings of Wen et al.[23] showing that the total amount of amino acids decreased following the Maillard reaction. The cross-linking of Xyl with the α-amino group at the terminal of the free amino acid or peptides was the underlying reason for this phenomenon[24]. Glu and Asp, classified as umami amino acids, constituted significant portion of the overall amino acid composition. These amino acids also play crucial roles in the perception of saltiness[25]. Several hydrophobic amino acids, including Phe, Tyr, Leu, Val, Pro and Ile, are known to elicit bitter taste sensation when subjected to heat[26]. As indicated in Table 5, the Maillard reaction led to a notable reduction in the hydrophobic amino acids of KPSPs, thereby inducing a decrease in bitterness and accentuating the perception of saltiness.

Table 5 Amino acid composition of KPSPs and M-KPSPs.

Amino acid type	Content (%)	
	KPSPs	M-KPSPs
Hydrophobic amino acid[a]	32.678	30.133
Acid amino acid[b]	18.381	17.624
Basic amino acid[c]	16.919	14.856
Human essential amino acid[d]	25.981	23.748

Note: [a] Includes Gly, Ala, Val, Leu, Ile, Pro, Tyr, Phe and Met; [b] includes Asp and Glu; [c] includes Lys, Arg and His; [d] includes Ile, Leu, Lys, Met, Phe, Thr and Val.

3.4.2 FTIR spectra

The Maillard reaction of KPSPs and Xyl was further verified by FTIR spectra. Figure 5A presented the FTIR spectra of KPSPs and M-KPSPs. KPSPs exhibit notable absorption peaks at 3 427, 1 641, 1 437, 1 120, and 618 cm^{-1}. The spectral features observed around 3 427 cm^{-1} were ascribed to the tensile vibration of the hydroxyl (–OH) functional group when subjected to tensile stress. And the peak became broader after Maillard reaction, which might be attributed to the introduction of –OH in the Xyl to peptides. Meanwhile, the absorptions of peaks located in the region 1 180–953 cm^{-1} were changed obviously in M-KPSPs, which was caused by the bending of C–H and stretching of C–C and C–O bonds of carbohydrates, indicating the conjugation of peptides and Xyl after heating[24,27].

3.4.3 Fluorescence spectra

Maillard fluorescence is ascribed to the formation of various new fluorophores, whose relative contribution may vary broadly depending on the site, extent of glycation and accompanying autoxidation[28]. Increase in Maillard fluorescence has been served as a characteristic index of peptides glycation. Figure 5B demonstrates the fluorescence intensity ranging from 400 to 450 nm of M-KPSPs was notably higher than that of the KPSPs, aligning with the typical characteristics of Maillard reaction in producing fluorescent substances. The phenomenon observed in this study may be attributed to the compounds generated through the Maillard reaction. This reaction involved a series of intricate reactions between reducing sugars and amino acids, leading to the formation of various molecules with unique structures, which exhibited distinct emission and absorption peaks on the fluorescence spectrum, ultimately resulting in Maillard fluorescence enhancement[29].

The endogenous fluorescence spectra of proteins/peptides of KPSPs and M-KPSPs were also characterized (Figure 5C). The fluorescence intensity of M-KPSPs was reduced, indicating that the covalent reaction with Xyl blocked the fluorescence signal of tryptophan residues, resulting in the quenching of peptide endogenous fluorescence. The maximum emission wavelength redshifted from 353 nm to around 365 nm under 290 nm excitation wavelength, indicating that the amino acids residues in peptide chain were covalently cross-linked with Xyl, and tryptophan residues were exposed to a more hydrophilic environment. Results suggested that the spatial structure of the peptides in KPSPs has been changed and become looser after Maillard reaction.

3.4.4 Circular dichroism spectra

Covalent interactions between peptides and reducing sugars via the Maillard reaction disrupt the organized secondary structure of peptides, such as α-helix and β-sheet, resulting in a more relaxed,

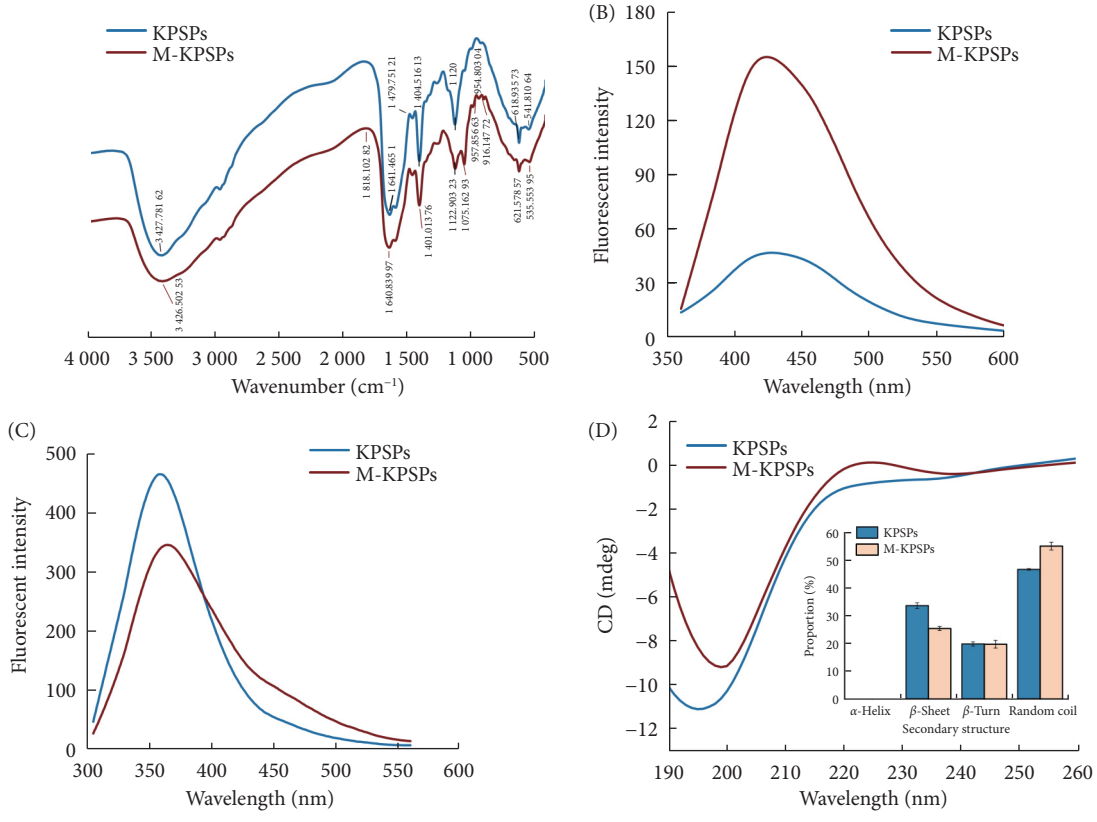

Figure 5 Structural changes of KPSPs and M-KPSPs analyzed by (A) FTIR spectra, (B) fluorescence spectra, (C) endogenous fluorescence spectra of peptides and (D) circular dichroism spectra.

partially permeable structure with an increase of random coil portion[30]. As shown in Figure 5D, α-helical structure was absent in KPSPs and M-KPSPs. After the Maillard reaction, the relative content of β-sheet decreased from 33.55% to 25.35%, and the random coil increased from 46.60% to 55.00%, indicating that the protein structure changed from order to disorder. Although there was no direct evidence of a link between increased random coil structure and flavor change, increased random coil might expose hydrophobic bitter amino acids within the peptide and promote their reaction with Xyl, resulting in decreased bitterness and increased saltiness, which was in accordance with the result of endogenous fluorescence spectra.

4 Conclusion

Maillard reaction was employed for the saltiness improvement of KPSPs. The MRPs of KPSPs and Xly exhibited strongest saltiness and the reaction conditions were optimized. Compared to KPSPs, the M-KPSPs exhibit enhanced saltiness, acceptability, and overall taste characteristics. After the occurrence of the Maillard reaction, there was a notable decrease in the concentration of hydrophobic amino acids, leading to a reduction in bitterness. Additionally, the high amount of Asp and Glu were recognized as significant contributors to the perception of saltiness in KPSPs and M-KPSPs. Results of FTIR spectra, fluorescence spectra and circular dichroism spectra confirmed the covalent binding of peptides and Xyl. The introduction of Xyl to KPSPs during the heat treatment increased the random coil structure potion of the peptides, loosened the spatial conformation, promoted the exposure of hydrophobic amino acids and the reaction with reducing sugars, thus alleviating the bitterness and improving the saltiness. The results of present study indicated that Maillard reaction played an important role in enhancing the saltiness perception of salty peptides. The synthetic use of enzymatic hydrolysis and Maillard reaction to prepare salty marine protein hydrolysates has the potential to partially replace NaCl in the development of novel healthy low sodium condiments.

Conflict of interest

The authors declare no conflict of interest.

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