Sensory characteristics of Maillard reaction products from chicken protein hydrolysates with different degrees of hydrolysis

Yongsheng Zhang, Yanping Wang, Fang Jiang and Huihui Jin

1. Introduction

Maillard reaction is a non-enzymatic reaction between the reducing sugars and amino groups of amino acids, peptides, or proteins (Danhegy, 1986). It has a great influence on some food properties such as color (Namiki, 1988) and flavor (Eric et al., 2013; Song et al., 2016). Maillard reaction products have been used as flavor enhancers. Some Maillard reaction products from protein hydrolyzates of animal (Li, Liu, He, Song, & Chen, 2015; Zhan, Tian, Zhang, & Wang, 2013) or vegetable (Su et al., 2011) origins were reported for their taste-enhancing properties. It has been reported that the Maillard reaction products from soybean protein hydrolyzates with 1000 to 5000 Da peptides, significantly increased the intensity of mouthfulness and continuity in umami solution and consommé soup (Ogasawara, Katsumata, & Egi, 2006). It was also reported (Lieske & Konrad, 1994) that using 2000–5000 Da chicken peptides as precursors of Maillard reaction, the flavor intensities were roughly 80–100 times greater than those produced by cooking an equivalent quantity of chicken by the conventional methods. Therefore, precursors play an important role in the Maillard reaction flavor.

Chicken is the cheapest commercially produced meat in the world. Chicken broth is highly appreciated as a flavoring for savory dishes because of its desirable taste characteristics (Dunkel & Hofmann, 2009). Although a number of studies have reported the volatile and non-volatile compounds of chicken flavor (Jayasena, Ahn, Nam, & Jo, 2013a, 2013b; Sun, Zhao, Cui, Zhao, & Yang, 2010), several important aspects of suitable precursors of chicken protein hydrolysates (CPHs) for the Maillard reaction flavor have not been fully understood. In the production of thermal reaction flavors, the type and content of reactants have a great influence on the taste characteristics of flavor, which includes amino acids, carbohydrates, and peptides (Liu et al., 2015; Yu et al., 2018). And amino acids and peptides are affected by the degree of hydrolysis (Wei, Thakur, Liu, Zhang, & Wei, 2018). To obtain desirable flavor precursors, especially free amino acids and appropriate peptides, methods for controlling the DH to acquire suitable chicken enzymatic hydrolysates require further investigations. Consequently, the effects of the compositions of amino acids and the difference in molecular weight (MW)
distribution of peptides, on the sensory characteristics of simulated chicken flavor, should be determined.

The objectives of the present study were to evaluate the sensory characteristics of Maillard reaction products prepared from chicken protein hydrolysates (MRP-CPHs) with different DH and to assess the potential of CPHs as flavor precursors by descriptive sensory analysis. In particular, the effect of DH and the molecular weight distribution of the hydrolysate on the stewed chicken flavor, mouthfulness, and umami taste. The changes in molecular weight distribution, pH values, free amino acid of CPHs, and MRP-CPHs were also examined.

2. Materials and methods

2.1. Materials and reagents

The chicken was purchased from a local market (Tianjin, China). Hens between the ages of 18 and 24 weeks were used. The breast meat was removed, cut into smaller pieces, and minced by a meat bowl cutter purchased from Guangdong Shaoquan Food Instrument Co., Ltd. Chicken breast meat had 21.53% crude protein, 2.83% crude fat and 72.69% moisture. The minced meat was vacuum packaged and then stored at −20°C for further use. Protease FH-G-NA-XII was obtained from Tianjin Nuooao Technology Development Co., Ltd, Trypsin was obtained from Nanning Pangbo Biological Engineering Co., Ltd. Amino acids were obtained from Tianjin Chunfa Bio-Technology Group. The other chemicals of analytical grade and HPLC grade were purchased from Tianjin No. 1 Chemical Reagent Factory.

2.2. Preparation of chicken protein hydrolysates

The minced chicken meat was thawed at room temperature and then mixed with distilled water at a ratio of 1:1 (w/v) in a flask. Then, the mixture was held at 55°C in a water bath, the protease FH-G-NA-XII and trypsin (each with 1500 U/g of chicken protein) were added simultaneously to the mixture and hydrolyzed at an initial pH of 6.5. The hydrolysis time was 1, 2, 3, 4, 5, 6, 7, and 8 h to prepare the chicken protein hydrolysates (CPHs) with different DH. After hydrolysis, the hydrolysate was heated in boiling water for 15 min to inactivate the enzymes. Then, the hydrolysates were cooled and centrifuged at 4000 rpm for 20 min. The supernatant was collected for further use.

2.3. Preparation of Maillard reaction products

CPHs solution (100 mL) was mixed with L-cysteine (0.5 g), thiamine (1 g), and xylose (1 g). The pH was adjusted to 6.5 using 6 M NaOH. The mixed solution was then transferred to a 250 mL, 3-neck, round bottom flask equipped with an electric mixer and a glass stopper. The reaction mixture was heated to 100°C with a SXKW electric jacket (Beijing, China) and the agitation speed was kept at 50 rpm. After 2 h of reaction, the flask was cooled in an ice-water bath immediately. All the reaction mixtures were named MRP-CPHs and transferred into a glass vial and, finally, stored at 4°C for further analysis and sensory evaluation. Meanwhile, the control sample was prepared by the same procedure except that the CPH was replaced by the same quantity of distilled water.

2.4. Determination of the degree of hydrolysis

The degree of hydrolysis was defined as the percentage of free amino groups cleaved from protein and calculated as the ratio between α-amino nitrogen and total nitrogen of CPH. The α-amino nitrogen was determined using formal titration method, by which a 5.0 mL of hydrolysate was added into 50 mL distilled water and the mixture was titrated with 0.1 M NaOH solution to pH 8.2 on a magnetically stirred device. After that, 10.0 mL of 38% (v/v) neutral formaldehyde solution was added and the titration was continued until the pH value reached 9.2. The consumed volume of NaOH from pH 8.2 to 9.2 was recorded as V₁. Meanwhile, the distilled water was used as a reagent blank, and the corresponding consumed volume of NaOH was recorded as Vₒ. The DH can be calculated by following Equation (1).

\[
\text{DH(\%)} = \frac{C \times (V₁ - Vₒ) \times V}{5 \times m \times p \times n} \times 100
\]

where C is the concentration of NaOH (mol/L), V is the total volume of hydrolysate, m is the mass of the chicken meat for hydrolysis, p is the percent of protein in the chicken, the protein content was determined by Kjeldahl method, and n represents the number of millimoles of the peptide bond in one gram of protein (mmol/g) (Adler-Nissen, 1979).

2.5. Free amino acid analysis

The Waters AccQ-Tag amino acid analysis Chemistry Package was used for free amino acid determination. Waters AccQ-Tag Fluorescence reagent 6-aminoquinolinyl-N-hydroxysuccinimidyl carbamate was used as a precolumn derivatizing reagent. The derivatized samples were analyzed by liquid chromatography (Waters 1525, Waters Co., Milford, MA, USA) equipped with 2996 Photodiode Array detector and Breeze 2 work station. The sample pretreatment, preparation, derivatization, and analytical procedure were followed according to the Waters AccQ-Tag Chemistry Package instruction manual.

The column was AccQ-Tag (3.9 mm×150 mm), while the mobile phase A (MP A) was Waters AccQ-Tag aqueous buffer, mobile phase B (MP B) consisted of acetonitrile/water (60/40, v/v). The elution conditions were 0 min (100% MP A), 0.5 min (98% MP A), 15 min (93% MP A), 19 min (90% MP A), 32 min (67% MP A), 33 min (67% MP A), 34-37 min (0% MP A), 38-64 min (100% MP A), and the flow rate was maintained at 1 mL/min. The column temperature was 37°C and 10 μL of sample was injected into the HPLC system. 2996 Photodiode Array detector was operated at a wavelength of 214 nm. For calculation, a calibration curve was obtained with a standard amino acid mixture (Waters Co., Milford, MA, USA), and quantification basis on retention time and peak area of samples.

2.6. Estimation of molecular weight distribution

The molecular weight distribution was determined according to the method reported by P. Liu et al., 2010. The liquid chromatography was equipped with 2487 UV detector and Breeze 2 work station. Chromatographic separation was performed with a TSK-G 2000 SWXL gel filtration column (7.8 mm × 300 mm × 10 μm, Tosoh Co., Tokyo, Japan). The mobile phase, consisted of acetonitrile/water/trifluoroacetic acid (45/55/0.1, v/v/v), was delivered at a flow rate of 0.5 mL/min. 0.2 mL of sample was
transferred to a 10 mL volumetric flask and diluted with mobile phase solution to volume, and mixed in an ultrasonic bath. Then the sample was filtered through a 0.22 μm membrane before injection.

The column temperature was 30°C and 10 μL of sample was injected into the HPLC system. 2489 UV detector was operated at a wavelength of 220 nm. The following standards were used to draw the molecular weight calibration curve: cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYY (451 Da), and tripeptide GGG (189 Da).

2.7. Sensory evaluation

Sensory evaluation was performed according to the method reported by different researchers (Eric et al., 2013; P. Liu et al., 2010; Ogasawara et al., 2006; Toelstede, Dunkel, & Hofmann, 2009) with some modifications.

Eight selected panelists (ages between 23 and 35, 3 females and 5 males) were selected from the College of Food Engineering and Biotechnology at Tianjin University of Science and Technology (Tianjin, China). All panelists had no history of known taste disorders and had attended a training session of descriptive sensory analysis with a duration of not less than 20 h. The following standard aqueous solutions were used as reference in the training: sodium chloride (1.5 g/L) for saltiness, sucrose (8 g/L) for sweetness, citric acid (0.25 g/L) for sourness, caffeine (0.05 g/L) for bitterness, monosodium glutamate (0.5 g/L) for umami taste, L-glutathione (1 g/L) for mouthfulness taste. Umami soup was introduced for sensory evaluation. The MRP-CPH solutions (1 g/L) were dissolved in umami soup individually. The umami soup consisted of monosodium glutamate (10 g/L) and sodium chloride (3 g/L). Chicken powder (5 g/L) was added to the umami soup as the control for sensory evaluation.

All test samples were adjusted to pH 7.0 by aqueous KOH (0.1 mmol/L) and held at 60°C in a water bath. Twenty-five-milliliter sample and 25 mL control solutions were served at the same time and evaluated in separated sensory booths. The sensory panel room was kept at 25 ± 1°C. The panelists were asked to taste the control sample before the sample evaluation and were asked to memorize the respective intensities. All samples were coded with three-digit numbers, served in a randomized order.

Quantitative descriptive analysis was applied to describe the sensory attributes of the samples. The test samples were scored in terms of sensory characteristics of boiled chicken flavor, umami, mouthfulness, continuity, and overall acceptance. Sensory evaluation was measured on a scale of 1 to 9. The average values were calculated by the scores given by the panelists. Based on the results of the pre-evaluation, the reference sample was assigned a score of 4 by the sensory evaluation team.

2.8. Statistical analysis

The statistical analysis was performed with the software SPSS 17.0 (SPSS Inc., Chicago, USA). The means of all the parameters were examined for significant difference by the one-way analysis of variance, and in case of significant difference (P < 0.05), the mean separation was performed by the Duncan’s multiple range tests. Data were expressed as means ± standard deviations of triplicate determinations.

3. Results and discussions

3.1. Changes in the pH and molecular weight distribution of CPHs and MRP-CPHs

Eight samples were hydrolyzed at different times ranging from 1 to 8 h, and the corresponding DH values after every hour were 12.15%, 13.35%, 15.80%, 17.85%, 18.01%, 18.64%, 18.96%, and 19.67%, respectively (Table 1). The changes in molecular weight distribution (MWD) and pH values of CPHs were determined (Table 1).

The pH variation indicates the intensity of the hydrolysis (Fernández & Kelly, 2016). The initial pH of the mixture was adjusted at 6.5, and the hydrolysis was carried out without external pH control. As shown in Table 1, the pH decreased significantly to 6.18 (p < 0.05) in the first 1 h. However, from 2 to 8 h, the pH changed very slowly from 6.13 to 6.08. The result indicated that in the initial stage of the hydrolysis, the chicken protein was decomposed to the peptides immediately (Mat, Cattenoz, Souchon, Michon, & Le Feunteun, 2018); and at the next stage, the strong buffer capacity of amino and carboxyl group of peptides and amino acids maintained the stability of the pH values. This is in accordance with our findings of the MWD determination of CPHs as shown in Table 1. The percentages of MWD in the range of 1500–6500 Da and >6500 Da in CPH1 were 4.66% and 4.82%, respectively. From 2 to 8 h, the hydrolysis was mainly in the range of 450–1500 Da and decreased by 41.38%; meanwhile, the percentage of MWD in the range of 200–450 Da was increased by 20.01%. However, the MWD <200 Da was relatively stable and was maintained at 10%. Overall, after the hydrolysis under various conditions, the pH and MWD showed a significant difference (p < 0.05) among the different CPHs. The results were similar with the previous studies (Huo & Zhao, 2009; Zhan et al., 2013).

The pH values and the changes of MWD of MRP-CPHs are shown in Table 1. After the Maillard reaction, there was a sharp drop in the pH of MRP-CPHs from 6.5 to about 5.4 (P < 0.05). And this result is in accordance with the previously reported findings (Brands & van Boekel, 2002), who determined that a pH variation of three or more units may occur during Maillard reaction due to the formation of organic acids. The pH change of the MRP-CPHs may indicate the degree of the reaction (Lan et al., 2010).

After the Maillard reaction, the MWD of the MRP-CPHs also changed significantly (P < 0.01), with a 175% average increase in the MWD <200 Da. This might be explained by the degradation of the large peptide molecules, and in our experiment, the MWD > 12,500 Da was disappeared after the Maillard reaction (data not shown). It might also be due to the addition of the amino acids. Correspondingly, the MWD of 200–450 Da, 450–1500 Da, 1500–6500 Da, and >6500 Da were decreased about by 17%, 29%, 19%, and 51% compared to their respective hydrolysates. The result was similar to previous research (Eric et al., 2013; Lan et al., 2010), who showed that the MWD of high molecular weight (>5000 Da) and low molecular weight (<1000 Da) of Maillard reaction products from the sunflower protein hydrolysate were significantly decreased. These scientists attributed this variation of MWD after the reaction to the small peptide cross-linking and the high molecular peptide decomposition.
**3.2. Changes in the free amino acid composition of CPHs and MRP-CPHs**

Free amino acid is an important flavor precursor in the Maillard reaction to form meat flavor (Mottram, 1998). Almost all free amino acids have some sweetness, bitterness, sourness, and umami, and therefore contribute to the characteristic tastes of foods (Kirimura, Shimizu, Mizikawa, Ninomiya, & Katsuya, 1969; Solms, 1969).

Changes in the free amino acid composition with different CPHs are shown in Table 2. The total free amino acid contents in CPHs were increased gradually from 14.99 g/L to 39.75 g/L with the increase in DH. Among these, the abundant free amino acids found in CPHs were leucine, valine, lysine, glycine, alanine, isoleucine, histidine, glutamic acid, and serine. In contrast, the contents of cysteine and proline were much lower, only reached 0.32 g/L and 0.81 g/L after 8 h of hydrolysis, respectively. These free amino acids may play an important role in the formation of meat flavor compounds during the thermal reaction. For example, sulfur-compounds which derived from cysteine seemed to be particularly important for the characteristic aroma of meat (Mottram, 1998). Other researchers (Jayasena et al., 2013b) revealed that the reaction of cysteine and pentose sugar could lead to characteristic meat flavor, especially for chicken and pork.

After the Maillard reaction, there were different degrees of decline in the free amino acid contents of all MRP-CPHs (Table 3). And for most amino acids, the contents of the free amino acid of the MRP-CPHs were highly correlated with the degree of hydrolysis of CPHs. The higher the degree of hydrolysis of CPHs, the more the free amino acids contents to reduce in MRP-CPHs. For example, compared to the CPH8, the contents of cysteine, valine, methionine and tryptophan in MRP-CPH8 were decreased by 81.3%, 74.8%, 74.3%, and 37.8%, respectively. Antonio Dario (Troise, Witalfsky, Fogliano, & Vitaglione, 2018) found that protein hydrolysis was the bottleneck step in the Maillard reaction. These results indicated that the Maillard reaction was positively correlated with the degree of hydrolysis of CPHs. This result is inconsistent with the results of the pH measurements (Table 1). On the contrary, the contents of tyrosine in MRP-CPHs were increased compared to the corresponding CPHs. This may be due to the decomposition of the peptide in the Maillard reaction (Karangwa et al., 2015; Yu et al., 2018). However, the proline contents had only a decrease of 3.8% after the Maillard reaction, this may indicate that low levels of proline participation in the thermal reaction.

### 3.3. Sensory evaluation

Sensory evaluation with umami solution was carried out in order to evaluate the flavor characteristics of the MRP-CPHs. The results of the sensory evaluation in the umami solution are shown in Figure 1. As shown in Figure 1, there was a significant difference (p < 0.05) in sensory attributes such as boiled chicken flavor, umami, mouthfulness, continuity, and overall acceptance between control and other MRP-CPHs. MRP-CPH1 showed a relatively weak boiled chicken flavor, mouthfulness, continuity, overall acceptance, and a very weak umami flavor attributes which were similar to the control. MRP-CPH2 and MRP-CPH3 showed a very strong boiled chicken flavor, mouthfulness, and overall acceptance attributes. MRP-CPH4, MRP-CPH5, and MRP-CPH6 had a good continuity and strong umami flavor, but they had a lower score in the mouthfulness and overall acceptance attributes. MRP-CPH7 and MRP-CPH8 showed relatively stronger boiled chicken and umami flavors and weaker mouthfulness, continuity, and overall acceptance attributes. Compared to all other sensory characteristics, the umami flavor of all samples was relatively weak. The sensory evaluation results were not in line with the previous results of the free amino acid analysis, indicating that the peptides may play a more important role than free amino acids in the sensory
Cambios en los contenidos de aminoácidos libres (g/L) en diferentes CPH. 

| Sample | CPH1 | CPH2 | CPH3 | CPH4 | CPH5 | CPH6 | CPH7 | CPH8 |
|--------|------|------|------|------|------|------|------|------|
| Aspartic acid | 0.43 ± 0.00 | 0.41 ± 0.00 | 0.35 ± 0.00 | 0.40 ± 0.01 | 0.57 ± 0.04 | 0.50 ± 0.05 | 0.68 ± 0.01 | 0.70 ± 0.01 |
| Glutamic acid | 0.69 ± 0.01 | 0.75 ± 0.01 | 0.82 ± 0.02 | 0.91 ± 0.02 | 1.03 ± 0.00 | 1.12 ± 0.00 | 1.15 ± 0.05 | 1.22 ± 0.02 |
| Serine | 0.36 ± 0.02 | 0.52 ± 0.02 | 0.64 ± 0.05 | 0.74 ± 0.05 | 0.88 ± 0.07 | 0.96 ± 0.08 | 1.02 ± 0.10 | 1.16 ± 0.13 |
| Glycine | 0.21 ± 0.00 | 0.16 ± 0.00 | 0.24 ± 0.02 | 0.45 ± 0.02 | 0.61 ± 0.03 | 0.81 ± 0.02 | 0.90 ± 0.02 | 0.98 ± 0.01 |
| Threonine | 0.48 ± 0.01 | 0.54 ± 0.06 | 0.66 ± 0.01 | 0.71 ± 0.04 | 0.80 ± 0.03 | 0.87 ± 0.01 | 0.93 ± 0.01 | 1.00 ± 0.04 |
| Proline | 0.28 ± 0.01 | 0.36 ± 0.07 | 0.58 ± 0.01 | 0.56 ± 0.04 | 0.58 ± 0.03 | 0.69 ± 0.03 | 0.76 ± 0.02 | 0.78 ± 0.02 |
| Alanine | 0.95 ± 0.01 | 0.99 ± 0.04 | 1.16 ± 0.06 | 1.26 ± 0.07 | 1.42 ± 0.00 | 1.53 ± 0.01 | 1.60 ± 0.01 | 1.66 ± 0.03 |
| Lysine | 1.27 ± 0.06 | 1.48 ± 0.15 | 1.81 ± 0.00 | 2.00 ± 0.08 | 2.22 ± 0.19 | 2.49 ± 0.05 | 2.57 ± 0.06 | 2.68 ± 0.00 |
| Methionine | 0.16 ± 0.00 | 0.18 ± 0.00 | 0.24 ± 0.02 | 0.25 ± 0.01 | 0.26 ± 0.01 | 0.26 ± 0.02 | 0.30 ± 0.02 | 0.38 ± 0.04 |
| Cystine | 0.15 ± 0.01 | 0.13 ± 0.01 | 0.12 ± 0.01 | 0.09 ± 0.01 | 0.10 ± 0.00 | 0.08 ± 0.00 | 0.07 ± 0.01 | 0.06 ± 0.00 |
| Histidine | 0.84 ± 0.01 | 0.88 ± 0.03 | 1.07 ± 0.09 | 1.23 ± 0.07 | 1.45 ± 0.01 | 1.62 ± 0.00 | 1.69 ± 0.03 | 1.75 ± 0.07 |
| Leucine | 1.71 ± 0.04 | 1.65 ± 0.07 | 1.47 ± 0.09 | 1.30 ± 0.04 | 1.01 ± 0.23 | 0.19 ± 0.01 | 0.75 ± 0.05 | 0.45 ± 0.06 |
| Tryptophan | 0.74 ± 0.01 | 0.77 ± 0.04 | 0.87 ± 0.01 | 0.90 ± 0.00 | 0.92 ± 0.01 | 0.93 ± 0.01 | 0.94 ± 0.01 | 0.97 ± 0.02 |
| Phenylalanine | 1.06 ± 0.00 | 1.30 ± 0.06 | 1.52 ± 0.04 | 1.59 ± 0.06 | 1.80 ± 0.01 | 1.91 ± 0.01 | 1.96 ± 0.07 | 1.95 ± 0.04 |
| Valine | 3.28 ± 0.09 | 2.66 ± 0.03 | 2.19 ± 0.05 | 2.06 ± 0.01 | 1.99 ± 0.13 | 1.77 ± 0.01 | 1.74 ± 0.05 | 1.64 ± 0.04 |
| Tyrosine | 1.35 ± 0.01 | 1.43 ± 0.05 | 1.54 ± 0.04 | 1.67 ± 0.09 | 1.84 ± 0.02 | 1.89 ± 0.02 | 1.98 ± 0.10 | 1.98 ± 0.09 |
| Total | 15.13 ± 0.21 | 15.94 ± 0.16 | 16.56 ± 0.06 | 17.49 ± 0.17 | 18.74 ± 0.29 | 19.06 ± 0.26 | 20.33 ± 0.28 | 20.65 ± 0.41 |

Las ocho muestras fueron denotadas por MRP-CPH seguidas de números arábigos de un dígito, donde "CPH" significa hidrolizado enzimático de proteína de pollo y los números arábigos 1–8 denotan el tiempo de hidrolisis enzimática de 1 a 8 horas, respectivamente.

* Ocho muestras fueron denotadas por MRP-CPH seguidas de números arábigos de un dígito, de tal forma que "CPH" significa hidrolizado enzimático de proteína de pollo y los números arábigos 1–8 denotan tiempo de hidrolisis enzimática de 1 a 8 horas, respectivamente.

4. Conclusiones

Los atributos sensoriales de la reacción de Maillard producidos fueron afectados por el DH, aminoácidos libres y los pesos moleculares de las proteínas hidrolizadas. En este estudio, todos los MRP-CPHs mejoraron las características de los disolventes, sabor de pollo de cocción, continuidad, y total aceptación del sabor de pollo. 

Características del MRP-CPHs. Este hallazgo fue en línea con los resultados de otros investigadores (Eric et al., 2013; Ogasawara et al., 2006; Zhan et al., 2013). Se demostró que el péptido de Maillard mejoraba la textura, el sabor y la continuidad del pollo. Además, los atributos evaluados fueron gradualmente mejorados con el DH de 12.15% a 15.8%, y el sabor y la continuidad de los atributos fueron mejorados con el DH de 12.15% a 18.01%. Estos resultados sugieren que el péptido de Maillard puede generar diferentes intensidades para atributos sensoriales diferentes (Karangwa et al., 2015).
that the CPHs with a DH range of 15.80% to 18.64% could be used as desirable precursors for chicken flavor.

Disclosure statement
No potential conflict of interest was reported by the authors.

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