



Antioxidant activity and sensory characteristics of Maillard reaction products derived from different peptide fractions of soybean meal hydrolysate



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ARTICLE INFO

Keywords:

Maillard reaction products
Peptide fractions
Soybean hydrolysate
Molecular weight distribution
Sensory characteristics
Antioxidant activity

ABSTRACT

Four peptide fractions PF1 (> 5 ;kDa), PF2 (3–5 ;kDa), PF3 (1–3 ;kDa), PF4 (< 1 ;kDa) were isolated from soybean hydrolysate using the ultrafiltration method. Then, D-xylose and L-cysteine were reacted with specific peptide solution at 120 ;°C for 2 ;h, and the molecular weight distribution (MWD), pH, colour, browning intensity, DPPH radical-scavenging activity, free amino acids and sensory characteristics of corresponding Maillard reaction products (MRPF1, MRPF2, MRPF3 and MRPF4) were evaluated, respectively. Peptides with low molecular weight showed higher contribution to the changes of pH, colour and browning intensity during Maillard reaction. The DPPH radical-scavenging activity of PF4 was significantly improved after Maillard reaction. Aroma volatiles and PLSR analysis suggested MRPF3 had the best sensory characteristics with higher contents of umami amino acids and lower of bitter amino acids, therefore it could be deduced that the umami and meaty characteristics were correlated with the peptides of 1–3 ;kDa.

1. Introduction

The Maillard reaction is a complex reaction between amine groups of free amino acids, peptides or proteins and carbonyl groups of reducing sugars (Hong, Jung, Kim, Lee, & Kim, 2010). Maillard reaction often occurs during food processing and storage, and it plays an important role in formation of flavour in foods. Therefore, the Maillard reaction products (MRPs) have been used as a variety of flavour enhancers in food which can improve the taste of mouthfulness, continuity and umami (Hofmann & Dunkel, 2012).

Peptides have been recognized as very pivotal taste-active components in foods. Four peptides Glu-Pro-Ser, Glu-Pro, Glu-Pro-Glu and Glu-Pro-Gln isolated from wheat gluten hydrolysates showed a strong umami taste (Schlichtherle-Cerny & Amadò, 2002). Peptides below 1200 ;Da from Spanish dry-cured ham were confirmed to be the main contributor to the flavour (Sentandreu et al., 2003). Peptides of molar weight (MW) between 1000 and 5000 ;Da isolated from MRPs were defined as “Maillard peptide”, as they could show strong taste-enhancing of umami, mouthfulness and continuity characteristics in umami solution (Ogasawara, Katsumata, & Egi, 2006), however, peptide fractions between 500 and 1000 ;Da from MRPs showed the strongest bitter taste characteristics (Deng, 2001). According to the sensory tastes in the

flavour industry, the distinct taste qualities of mouthfulness, continuity, richness and thickness were usually characterized as the “Kokumi”, and it was also reported that the cross-linking products in Maillard reaction with smaller molecular weight (< 1000 ;Da) could enhance the “Kokumi” taste rather than Maillard peptides (Karangwa et al., 2016). Nevertheless, owing to the complexity of the peptide composition and structure, only a few researches have been done to investigate the relationship among sensory characteristics of MRPs generated from different molecular weight peptides (Jisang & Youngsoon, 2009).

Soybean meal is the main by-product during soybean oil production, and it is a valuable protein source with a generally good nutritional balance for aqua feeds. Soybean peptide has been widely used in various industries because of its unique functional characteristics, such as water solubility, antiallergenic, antioxidant activity, higher digestibility and antimicrobial property. In recent years, previous research has been conducted about soybean protein or peptides in the generation of processed flavour enhancers, and soybean peptides were considered as an important flavour precursor and potentiator during the Maillard reaction (Liu et al., 2012; Song et al., 2013). A variety of MRPs rich in meaty, mouthfulness, Kokumi and umami could be formed by heating the mixture of soybean peptides and various sugars (Lan et al., 2010; Liu et al., 2012). Additionally, the MRPs were found to have equivalent

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antioxidant properties compared with those of conventional food antioxidants (Alfawaz, Smith, & Jeon, 1994).

In this study, four peptide fractions with different molecular weights were isolated from soybean meal protein hydrolysates by ultrafiltration. The antioxidant activity and free amino acids of peptide fractions were investigated. Furthermore, Maillard reaction products (MRPs) were prepared using xylose/cysteine/peptide fractions or hydrolysates models. The changes of molecular weight distribution, pH, colour, browning intensity, antioxidant activity, free amino acids and sensory characteristics of MRPs were investigated. The relationships between free amino acids, molecular weight distribution and the sensory characteristics of MRPs were also studied based on the partial least squares regression model. The results would provide a better understanding of the key factors that would influence antioxidant activity and sensory characteristics of MRPs. Furthermore, our investigation would lay the groundwork necessary for the design and development of dietary habits and functional foods.

2. Material and methods

2.1. Materials and chemicals

Soybean meal was provided by Qiangwang Food Co., Ltd. (Jieshou city, Anhui province, China). Alkaline protease (200,000 U/g) produced from *Bacillus licheniformis* was purchased from Beijing Aoboxing Bio-tech Co., Ltd. (Beijing, China). Flavourzyme 500 MG was purchased from Novo Co., Ltd. (Novozyme Nordisk, Bagsvaerd, Denmark). All the other chemicals and solvents were of analytical grade.

2.2. Preparation of soybean peptides

Soybean meal was crushed into powder (80 mesh sizes) by a high-speed grinding machine (Tianjin Instrument Co., Ltd., Tianjin, China). The main chemical compositions of the soybean meal powder were as follows: moisture 7.94%, proteins 45.82%, fat 3.56%, ash 6.41%.

Soybean meal powder was dissolved in distilled water with a final protein concentration of 6% (w/v), and pre-heated at 90 °C for 20 min. The mixture was adjusted to pH 10.0 with 6 M NaOH after cooling, and the mixture was hydrolyzed at 50 °C for 3.0 h using alkaline protease (5000 U/g, calculated as substrate protein content), and then the mixture was hydrolyzed at pH 6.5 50 °C for 4.0 h using Flavourzyme (500 U/g, calculated as substrate protein content). After hydrolysis, the hydrolysate was heated at 90 °C for 10 min to inactivate the enzymes, and the protein was precipitated in isoelectric point. The mixtures were centrifuged at 8000 r/min for 15 min at 4 °C, and the supernatants were stored at −20 °C for further use.

2.3. Preparation of peptide fractions with different molecular weight distribution and molecular weight distribution analysis

The supernatants of soybean peptides solution were fractionated through ultrafiltration membrane (Millipore Corporation Co., Ltd, USA) with a range of molecular weight cut-offs of 5, 3 and 1 kDa, respectively. Then the fractions with molecular weight distribution (MWD) of > 5 kDa, 3–5 kDa, 1–3 kDa and < 1 kDa were regarded as PF1, PF2, PF3, and PF4, respectively. Bicinchoninic acid (BCA) method was applied to determine the content of peptides. All the fractions were lyophilized and stored at −20 °C for further use. The MWD of the Maillard reaction product was determined by a Superdex Peptide 10/300GL column combined with an AKTA pure system (GE healthcare life Co., Uppsala, Sweden) at a flow rate of 0.5 ml/min monitored at 280 nm under isocratic condition (10 mM phosphate buffer, pH 7.0), and a calibration curve of molecular weight was used to calculate the MWD.

2.4. Preparation of Maillard reaction products

The mixture of peptides (1.5 g), D-xylose (0.6 g) and L-cysteine (0.3 g) were dissolved in 20 ml deionized water, and pH of the mixture was adjusted to 7.4 using 2 M NaOH. The solution was transferred into an oil bath and reacted at 120 °C for 120 min with magnetic stirring (800 r/min). Five Maillard reaction products (MRPs) derived from peptide fractions > 5 kDa, 3–5 kDa, 1–3 kDa, < 1 kDa and unfractionated soybean protein hydrolysates (PH) were named as MRPF1, MRPF2, MRPF3, MRPF4 and MRPH, respectively. The MRPs were cooled quickly with ice water and a portion of samples were stored at −20 °C for further chemical analysis, while the others were freeze-dried and stored at −20 °C for the functional evaluation. Furthermore, the native peptides (NPs) from unfractionated soybean protein hydrolysates and the corresponding peptide heating products (PHPs) without added D-xylose and L-cysteine in the Maillard reaction treatments were used as the controls in the whole investigation. All samples were prepared in duplicate.

2.5. Measurement of pH

The pH of MRPs and PHPs were measured by a pH meter (Model DELAT-320, METTLER TOLEDO, Inc., Shanghai, China) at room temperature.

2.6. Determination of browning intensity

The absorbance at 294 nm was used as an index of the uncoloured intermediate compounds which could be the important precursors of Maillard reaction (Barbanti, Mastrocola, & Lericci, 1990), and the brown polymers developed in the more advanced stages of the Maillard reaction could be measured at an absorbance of 420 nm (Morales & Jiménez-Pérez, 2001). The browning intensity of the samples was determined by a UV-Vis spectrophotometer (UV-2100, Unico Instrument Co., Ltd. USA) with a 50-fold dilution using distilled water for 294 nm and a 20-fold dilution measured at 420 nm.

2.7. Determination of colour

The colour of samples was determined by using a Chroma Meter NR-200 (Shenzhen 3NH Technology Co., Ltd. Shenzhen, China). The results were expressed as L^* , a^* and b^* . L^* was represented brightness or luminosity, a^* was used to indicate redness (+) greenness (−), and b^* was defined to show yellowness (+)/blueness (−).

2.8. Determination of DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined according to the method described by Gu et al. (2009) with some modifications. An equivalent volume of DPPH solution (0.1 mM in ethanol) was added to the MRPs solution (40, 8.0, 4.0, 2.0, 0.8, 0.4 mg/ml) defined as a sample, and an equivalent volume of ethanol was added to sample solution named as control, while the mixture of equivalent volume of ethanol and DPPH solution was regarded as blank. All the mixtures were then placed in the dark for 30 min at room temperature. The absorbance was determined at 517 nm using a UV-Vis spectrophotometer (UV-2100, Unico Instrument Co., Ltd. USA). Each sample was measured in triplicate. The DPPH radical-scavenging activity of samples was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = \left(1 - \frac{A_1 - A_s}{A_0}\right) \times 100$$

The A_1 represents the absorbance at 517 nm of sample, A_s indicates the absorbance at 517 nm of the control, and A_0 expresses the absorbance at 517 nm of the blank.

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