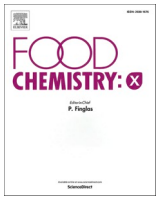
Food Chemistry: X 19 (2023) 100780

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Preparation of meaty flavor additive from soybean meal through the Maillard reaction

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ARTICLE INFO

Keywords:
Magnetic immobilized enzyme
Soybean meal
Enzymatic hydrolysate
Maillard reaction

ABSTRACT

Meaty flavor additive was prepared from soybean meal hydrolysate and xylose in the method of Maillard reaction. Under the conditions of reaction temperature 120 ℃, time 120 min and cysteine addition 10%, the Maillard products had strong flavor of meat. The content of free amino acids was 4.941 μ mol/mL in the products. There were 50 volatile flavor substances in Maillard reaction products according to GC–MS analysis. 4 mercaptans, 4 sulfur substituted furans, 3 thiophenes, 7 furans, 6 pyrazine, 3 pyrrole, 1 pyrimidine, 7 aldehydes, 4 ketones, 7 esters, 2 alcohols and 2 acids were included. The Maillard reaction products also have strong antioxidant activity. The scavenging ability of FRAP, DPPH radical, hydroxyl radical and $ABTS^{+}$ radical was 1.82%, 69.8%, 68.7% and 71.6% respectively. The products of Mailard reaction have potential to be used in food additives.

1. Introduction

Meaty flavor additive is a common and important food flavor in the field of food additives (Song et al., 2017). With the rapid development of economy and the improvement of living standard, human dietary constitution and living style have changed greatly, and there is more demand for food flavor. As a kind of food seasoning, meaty flavor additive plays an important role in improving food flavor and taste. It is widely used in the fields of seasoning bag, artificial vegetarian meat, puffed food and baked food (Li & Liu, 2021).

Meaty flavor additive can be divided into animal origin flavor, blended flavor and thermal reaction flavor according to the differents of production and processing methods. Meaty flavor additive from animals mainly comes from chicken, pork, beef, mutton and other animal meat, which has the disadvantages of high production cost and high content of saturated fatty acid (Kang, Alim, & Song, 2018). Blended meaty flavor additive is mainly prepared by mixing various flavor substances such as sulfur compounds, ketone compounds and hydroxyl compounds with a variety of spices to form a flavor base, and then mixing the flavor base with carriers such as salt or desiccant to form a product for application in food (Wei et al., 2019).

Although blended flavors account for a certain proportion of the meaty flavor additive market, most blended flavors are not enough in terms of characteristic flavor, aroma intensity and retention time, and heat resistance. In the process of production and processing, the selection of different spices and the proportion of raw materials added are not standardized, which restricts the development to a certain extent (Xia et al., 2021). Thermal reaction flavor is to use certain biological or chemical methods to hydrolyze protein in animals and plants, and the protein hydrolysate further undergoes Maillard or thermal degradation reaction to prepare substances without meaty flavor additive into substances with strong meaty flavor additive. Thermal reaction can form a stable and lasting flavor with full aroma, so it has become the mainstream method for preparing meaty flavor additive at present (Chen, Jiang, Xu, Geng, & Xu, 2020).

In this paper, non genetically modified soybean meal hydrolysate and reducing sugar were used as raw material in the Maillard reaction. The structure and flavor characteristics of Maillard reaction products

Abbreviations: SMH, Soybean meal hydrolysate; MRPs, Maillard reaction products; FRAP, ferric ion reducing antioxidant power; SMHM1, Maillard reaction product without cysteine1; SMHM2, Maillard reaction product without cysteine2; DPPH, 1,1-diphenyl-2-trinitrophenylhydrazine; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate.

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https://doi.org/10.1016/j.fochx.2023.100780
Received 27 December 2022; Received in revised form 25 May 2023; Accepted 2 July 2023
Available online 13 July 2023

(MRPs) were analyzed, and the meaty flavor additive from plants with antioxidant activity was prepared, so as to improve the utilization rate and added value of soybean meal.

2. Materials and methods

2.1. Materials

Soybean meal hydrolysate (SMH) was prepared in our laboratory, it was hydrolyzed by alkaline protease, and the degree of hydrolysis was 20.83%. Xylose, Alkaline protease (from Bacillus licheniformis, 200U/mg) and other reagents were purchased from Sigma-Aldrich company. Scanning electron microscope (S-3400N, Hitachi company, Japan), transmission electron microscope (H-7650, Hitachi company, Japan), ultraviolet visible spectrophotometer (UV-1800, Hitachi company, Japan), X-ray diffractometer (SmartLab-9kW, FEI, USA), laser particle size analyser (MS2000, Malvern, UK), vibrating sample magnetometer (VersaLab, Quantum Design, USA), Fluorescent Spectrophotometer (F-7100, Shanghai Precision Instrument, China), Fourier Transform Infrared Spectrometer (Spectrum Two, PE company, USA), Differential Scanning Calorimeter (SW 12.10, Mettler, Germanny), GC–MS (7890A-5975C, Agilent, USA) were used in the experiments.

2.2. Methods

2.2.1. Maillard reaction

1.0 g of SMH and 0.3 g of xylose were taken into a sealable spiral decomposition bottle, 20 mL of deionized water was added, the solution was mixed evenly, cysteine (3%, 7%, 10%, 20% and 30% of SMH mass) was added respectively. Maillard reaction experiments were did at temperature of 80 ℃, 100 ℃, 120 ℃, 140 ℃ and 160 ℃, and reaction time of 60 min, 90 min, 120 min, 150 min and 180 min. After reaction, the MRPs were cooled in ice water bath, and browning degree and sensory analysis were conducted respectively.

2.2.2. Flavor evaluation

15 members were selected to form a flavor evaluation team (10 women and 5 men, 22–30 years old). All team members had trained in descriptive flavor analysis and had experience in flavor characteristics of food samples. Before the flavor evaluation of the test sample, the evaluation personnel should use the control sample to evaluate the flavor. The control sample solution was a mixed solution of 0.5% salt and 1.0% sodium glutamate. The MRPs solution and the control solution were mixed at a ratio of 0.5:100. The evaluation indexes were delicate flavor, bitter taste, saline taste, meaty flavor, caramel flavor, mellow flavor, continuity, and overall acceptability. The score range was 1–10 points. Each sample was evaluated three times, and the average value of each index was taken as the final score.

2.2.3. Browning degree

MRPs were diluted to prepare a 2 mg/mL solution, deionized water was used as control, a spectrophotometer was used to measure the absorbance at 420 nm and 294 nm (Pirestani, Nasirpour, Keramat, Desobry, & Jasniewski, 2018).

2.2.4. Spectral analysis

10 mg dry solid sample of MRPs, SMH and MRPs without cysteine was taken respectively, the samples were pressed into tablets with KBr as the matrix. The infrared absorptions of the products were investigated under the conditions of 4 cm^{-1} resolution and the infrared spectrum wavelength of 4000–400 cm^{-1} (Yang, Oyeyinka, Xu, Ma, & Zhou, 2018).

MRPs, SMH and MRPs without cysteine were weighed and they were mixed with deionized water to prepare a 1 mg/mL solution, the ultraviolet spectrum of the sample was measure within the wavelength range of 190 nm to 500 nm respectively (Lertittikul, Benjakul, & Tanaka, 2007).

The fluorescence spectra of the samples were mesured under the conditions of excitation wavelength 347 nm, the emission wavelength 360 nm–550 nm, and the scanning speed was 1200 nm/min (Siewe, Kudre, Bettadaiah, & Bhaskar, 2020).

2.2.5. XRD

Target α diffraction method was used in the experiment, the phase purity and crystal structure of the dry solid sample were ananlyzed under the conditions of wavelength 0.1541 nm, voltage tube 40 kV, current tube 20 mA, Ni filter, scan step 0.02 °and 5°/min (Wang et al., 2022).

2.2.6. SEM

The dry solid samples were put on the carrier of the conductive adhesive, gold was sprayed under vacuum (90 s). Then the samples were put it into the SEM observation room to observe the sample state and take photos (Roth, Schwaminger, Peng, & Berensmeier, 2016).

2.2.7. Thermodynamic analysis

Differential Scanning Calorimeter (DSC) analysis was conducted at the nitrogen flow rate of 10 mL/min, the temperature rise rate of was 15 ℃/min, and the temperature measurement range of was 30–180 ℃ (Yang, Oyeyinka, & Ma, 2016).

2.2.8. Determination of free amino acids

Free amino acids were determined in HPLC method on a Nova Pak C18 column (3.9 × 150 mm, 5 μm). Column temperature 38 ℃, Mobile phase A: 50 mM K_2HPO_4, pH 6.5 (adjusted with 1:1 dilute phosphoric acid), Mobile phase B: acetonitrile/methanol (v/v, 50/50), Flow rate: 1 mL/min, Gradient elution (0–1.5 min, 0% B, 20.4 min, 40.7% B, 23.8–25.8 min, 70% B, 25.9–27.3 min, 0% B), UV-Detector wavelength 254 nm (Fickler, 2006).

2.2.9. GC–MS

The volatile compounds were analyzed by headspace solid phase microextraction (HS-SPME) combined with GC–MS. 2.0 g of solid sample was taken into a 20 mL headspace flask, 2.0 g of sodium chloride solution (30%, w/w) was added, and then 100 μL of 5 mg/mL 2-methyl-3-heptanone methanol water (50%, v/v) solution was added as the internal standard. SPME optical fiber was preheated at 270 °C for 30 min before analysis. The extraction of volatile matter was carried out at 60 °C using an automatic sampler (PALRSI120, Agilent, USA). Each sample was balanced for 15 min, and then the volatile compound was dissociated at 240 °C for 5 min (Richter, Eyres, Silcock, & Bremer, 2017).

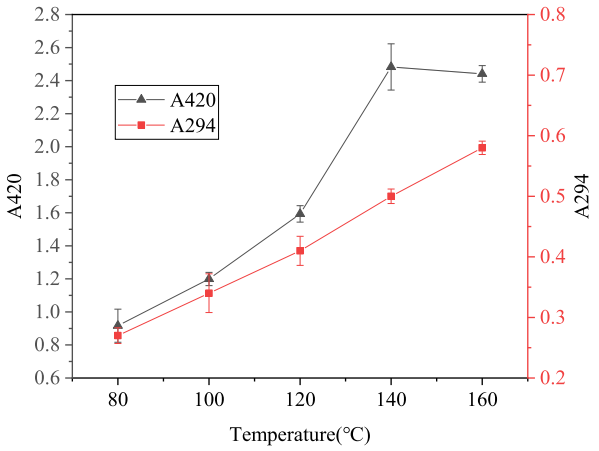
GC–MS column was ZB-WAXplus ™ Capillary column (60 m × 0.32 mm × 0.5 μm), helium flow was 1.0 mL/min. The temperature of the column oven was kept at 40 °C for 5 min, then the temperature was raised to 210 °C at the rate of 4 °C/min, and then the temperature was raised to 240 °C at the rate of 10 °C/min, and kept for 5 min. The mass spectrum recording range was 30–300 *m/z*.

2.2.10. Analysis of antioxidant activity

2.2.10.1. Radical scavenging ability of DPPH. 60 μL of anhydrous alcohol and 60 μL of the sample solution (0.5–3 mg/mL) was mixed evenly, 10 mL of 0.1 mmoL/L DPPH fresh solution was added, the reaction solution was shaken violently and left in the dark for 60 min (Yen & Hsieh, 2010). The mixture was centrifuged (3000 rmp) at 4 ℃ for 2 min. The absorbance of the supernatant was determined at 517 nm. Deionized water was used to prepare the control in the same way instead of the sample. The radical scavenging ability of DPPH was calculated as the following formula.

$$\text{DPPH radical scavenging ability } (\%) = \frac{A_0 - A_S}{A_0} \times 100\%$$

As represents the absorbance of the sample, and A_0 represents the



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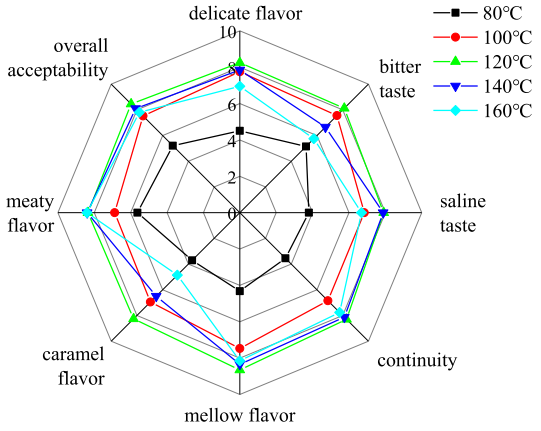

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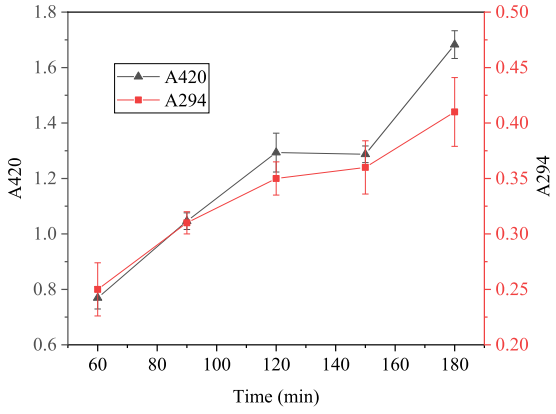

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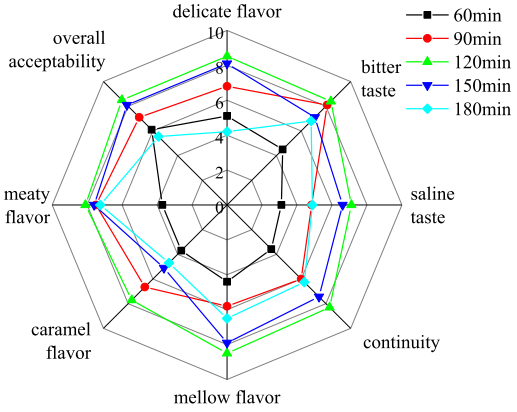

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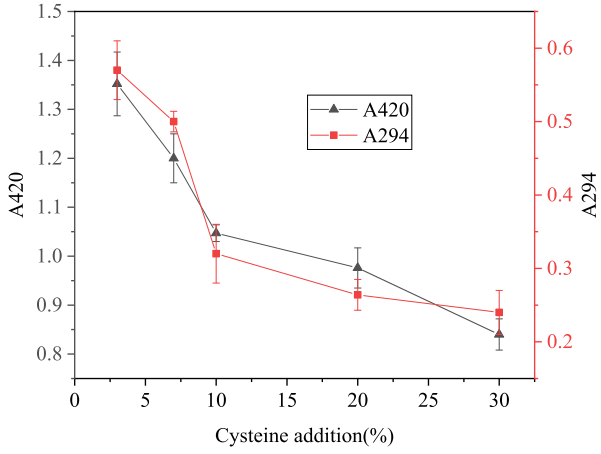

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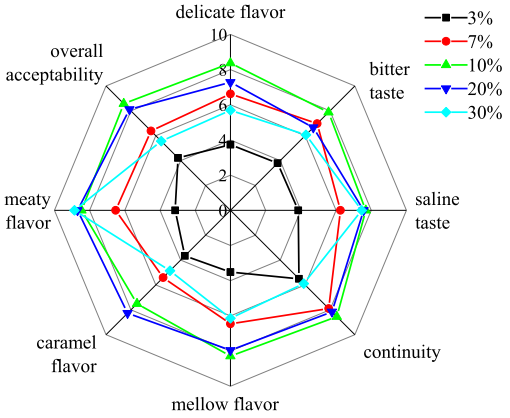

c

Fig. 3.1. . Effect of different temperatures, heating times and cysteine additions on browning strength (A, B, C) and flavor (a, b, c) of MRPs.

absorbance of deionized water.

2.2.10.2. Scavenging ability of hydroxyl radical. 100 μL of $FeSO_4$ solution (6 mmoL/L), 100 μL of H_2O_2 solution (6 mmoL/L), 100 μL of sodium salicylate solution (6 mmoL/L) and 100 μL of sample solution (0.5–3 mg/mL) were taken and fully mixed, left at room temperature for 20 min. The absorbance value of the solution was measured at 510 nm, and the following formula was used to calculate the scavenging ability of hydroxyl radical (Shao et al., 2017).

$$\text{hydroxyl radical scavenging ability } (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%$$

A_0 represents the absorbance of deionized water, A_1 represents the absorbance of the sample to be tested, A_2 represents the absorbance without sodium salicylate.

2.2.10.3. FRAP determination. Determination of ferric ion reducing antioxidant power (FRAP) is a method to measure the antioxidant capacity of samples by using the blue purple complex formed by ferrous ions and tripyridyltriazine under low pH conditions.

1.0 mL of sodium phosphate buffer solution (0.2 mol/L, pH6.6), 1.0 mL of potassium ferricyanide (1%, w/v) and 1.0 mL of sample solution (0.5–2 mg/mL) were taken and mixed well. The mixture was taken into a water bath of 50 ℃ constant temperature for 20 min, cooled to room temperature in the ice water bath. 1.0 mL of trichloroacetic acid (10%, w/v) was added into the mixture, and centrifugated at 4 ℃ 5000 r/min for 10 min after uniform mixing. 1.0 mL of supernatant and 200 μL $FeCl_3$ (0.1%, w/v) were taken and added it to 1.0 mL of distilled water, the ultraviolet absorbance was detected at 700 nm (Wu et al., 2014).

2.2.10.4. Scavenging ability of $ABTS^+$ radical. $ABTS^+$ radical was prepared by mixing 7 mmoL/L $ABTS^+$ solution and 2.45 mmoL/L potassium persulfate, and the mixed solution was kept in a dark place for 12–16 h before use (Rufián-Henares & Delgado-Andrade, 2009). $ABTS^+$ solution (kept for 2 days) was diluted with 5 mmoL/L phosphate buffer solution (pH 7.4) to the absorbance of 0.70 at 730 nm. 100 μL sample (0.5–3 mg/mL) was added into 1 mL of diluted $ABTS^+$ solution, 20 min later, the value of UV spectrophotometry was determined at 730 nm. The scavenging ability of $ABTS^+$ radical was calculated as following formula.

$$ABTS^+ \text{ radical scavenging ability } (\%) = \frac{A_0 - A_S}{A_0} \times 100\%$$

As represents the absorbance of the sample, and A_0 represents the absorbance of deionized water.

2.3. Statistical analysis

In this study, all test indexes were measured at least three times repeatedly. Origin 2018 software was used for drawing. SPSS 22.0 was used for analysis of variance, Duncan's test in one-way ANOVA was used to analyze the significance ($p < 0.05$), and Tukey's post-hoc for multiple comparisons ($p < 0.05$).

3. Results and discussion

3.1. Maillard reaction

3.1.1. Effect of reaction temperature on MRPs

1.0 g SMH, 0.1 g cysteine, and 0.3 g xylose were taken, they were reacted at the temperature of 80 ℃, 100 ℃, 120 ℃, 140 ℃, 160 ℃ for 120 min.

Temperature is the main factor which affects the reaction rate and products characteristics of Maillard reaction. With the increase of temperature, the absorbance values of Maillard products at A294 and A420 showed an upward trend (Fig. 3.1A). In general, the higher the heating temperature, the faster the reaction rate of the system was (Cheng, Mu, Jiao, Xu, & Chen, 2020). When the reaction temperature rised from 80 ℃ to 120 ℃, the absorbance of intermediate products and browning products rised slowly. When the temperature reached 140 ℃ and 160 ℃, the absorbance of intermediate products and browning products increased sharply, it indicated that the system reacted strongly at this time. When the temperature was too high, the reaction was too violent, which was not conducive to control the reaction rate.

When the reaction temperature was too high, the reaction system was forced to enter the final stage quickly, which resulted in accelerated browning. The temperature of the reaction system increased from 80 ℃ to 120 ℃, the caramel flavor, mellow flavor, meaty flavor and overall acceptability of Maillard reaction products increased (Fig. 3.1a). When the reaction temperature was 80 ℃, the reaction was insufficient due to the low temperature, the flavor concentration of Maillard products was insufficient, the bitterness was obvious, and the overall sensory score was low. When the reaction temperature was 100 ℃, each flavor index was better than 80 ℃, meaty flavor and caramel flavor gradually appeared. The overall flavor quality was not the best. When the reaction temperature was 120 ℃, the overall acceptability was good, the caramel flavor, meaty flavor and saline flavor were appropriate, the flavor was good. When the reaction temperature was higher than 120 ℃, the caramel flavor and bitter taste continued to increase due to the high temperature, which made people feel unhappy, so the score was low. The delicate flavor and mellow flavor also decreased gradually, and the reaction system might produce acrylamide, furan, hydroxymethyl furfural and other harmful substances for long time under the high temperature condition (Chen et al., 2019). In conclusion, the Maillard reaction temperature of 120 ℃ was the best.

3.1.2. Effect of reaction time on MRPs

1.0 g SMH, 0.1 g cysteine, and 0.3 g xylose were taken, they were reacted at the temperature of 120 ℃ for 60, 90, 120, 150, 180 min.

With the extension of heating time, the absorbance values of Maillard products at A294 and A420 showed an upward trend (Fig. 3.1B), which indicated that the browning intensity of Maillard products increased. During Maillard reaction, hydroxymethyl furfural and other substances would be formed, which would further react with amino compounds to form melanoidin substances (Bornhorst, Tang, Sablani, & Barbosa-Canovas, 2017). It was found that the absorbance of the intermediate products of Maillard reaction increased slowly when the reaction time was from 120 min to 150 min, which may be due to the fact that the rate of intermediate product formation was gradually lower than the rate of intermediate product converting to brown substance. When the heating time was 150 min, the absorbance of the final product did not change obviously, but when the heating time was 180 min, the absorbance was significantly larger. High temperature heating for a long time might promote the mutual polymerization of different substances, which resulted in the formation of toxic substances (Stanic-Vucinic, Prodic, Apostolovic, Nikolic, & Velickovic, 2013). Reaction time is one of the important factors, which affects the flavor of MRPs. The aroma and flavor of MRPs were different with the different length of reaction time.

When the reaction time increased from 60 min to 120 min, the caramel flavor, mellow flavor, meaty flavor and overall acceptability of MRPs increased correspondingly (Fig. 3.1b). When the reaction time was 60 min, because the time was too short, Maillard reaction might still be in the initial stage. The meaty flavor was weak, and bitter taste was very strong, so the sensory score was low (Cui, et al., 2017). At 90 min, some characteristic flavors began to appear, but the overall acceptability was not very good. When the reaction time was 120 min, the overall acceptability was good, caramel flavor, meaty flavor and saline flavor were appropriate, and all the scores were the best. When the reaction time was longer than 120 min, the caramel flavor was too strong, the delicate flavor and mellow flavor decreased gradually. It might be due to the heating time was too long, and the score was low. Long time of heating might produce more by-products and produce peculiar flavor in

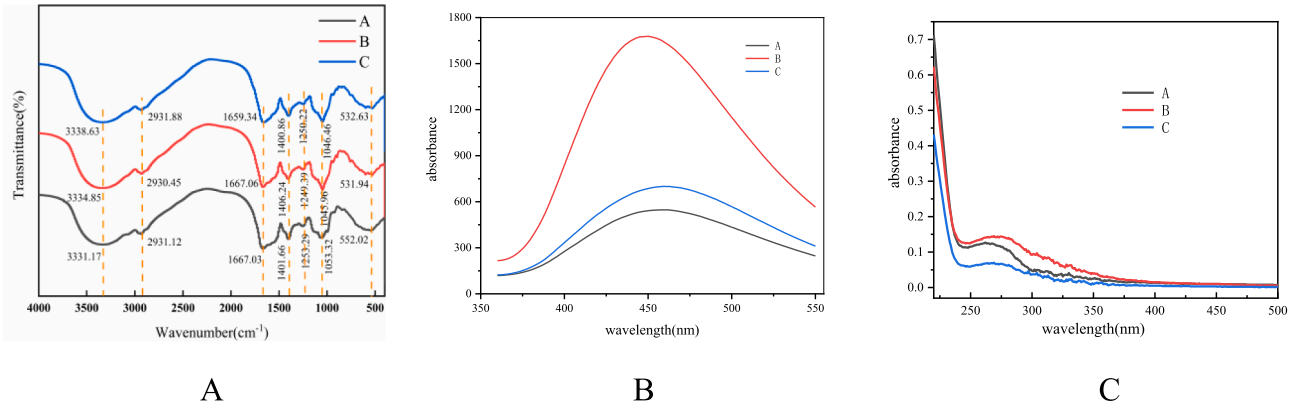

Fig. 3.2. FTIR, Fluorescence and UV spectra of MRPs. (A) FTIR spectra, (B) Fluorescence spectra, (C) UV spectra.

the product, the overall acceptability was poor (Aoki, Hiidome, & Kitahata, 1999). In conclusion, the Maillard reaction time of 120 min was the best.

3.1.3. *Effect of cysteine on MRPs*

1.0 g SMH and 0.3 g xylose were taken, 3%, 7%, 10%, 20% and 30% cysteine of SMH mass were added respectively, they were reacted at the temperature of 120 ℃ for 120 min.

With the increase of cysteine addition, the absorbance value of the reaction product gradually decreased, and the browning intensity of the product also gradually decreased (Fig. 3.1C). Cysteine is a kind of sulfur-containing amino acid, which can not only provide a unique flavor of the reaction system, but also can capture electrophilic compounds and prevent the formation of intermediate products in the reaction system due to its own nucleophilic group -SH, which will eventually further inhibit the formation of brown substances and prevent excessive browning of Maillard reaction products (Friedman & Molnar-Perl, 2012).

As a flavor precursor, cysteine can combine with dicarbonyl compounds to participate in pyrolysis or Strecker degradation to produce meaty flavor additive. The cysteine and xylose was the main factor to form meaty flavor in Maillard reaction (Zhao et al., 2019). With the gradual increase of cysteine addition, the meaty flavor, delicate flavor and overall acceptability of Maillard reaction products were generally improved, and the addition of cysteine significantly improved the delicate flavor (Fig. 3.1c). When the cysteine additon was small, the mellow flavor and meaty flavor were insufficient, and the reaction system had a bitter taste. At this time, the relative content of xylose in the system was more, so the caramel taste was slightly heavier. When the amount of cysteine addition was bigger than 10%, the peculiar flavor of Maillard products increased significantly. Due to the excessive amount of cysteine, the insufficient reaction in the system led to a more serious irritating smell of sulfur, and the sensory score decreased significantly. Therefore, the addition of cysteine was 10%.

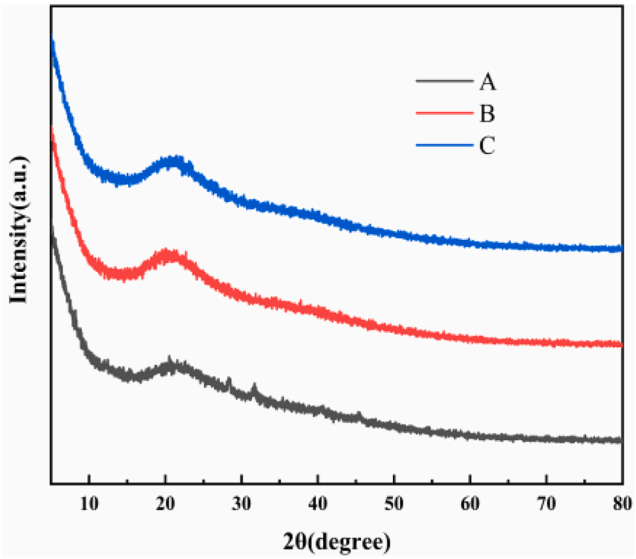

Fig. 3.3. XRD patterns of MRPs. (A) SMH, (B) SMHM1, (C) SMHM2.

3.2. *Characteristics of Maillard reaction products*

3.2.1. *Spectral analysis*

A large number of free amino acids would be converted to other groups in Maillard reaction. Three curves of soybean meal hydrolysate (SMH), Maillard reaction product without cysteine (SMHM1) and Maillard reaction product (SMHM2) all had absorption peaks near 3331 cm^{-1} and 2931 cm^{-1} (Fig. 3.2A), which were caused by the stretching vibration of O—H bond and C—H bond (Meng, Li, & Song, 2019). In the FTIR spectrum of SMH, it could be seen that there were characteristic absorption peaks near 1667 cm^{-1}, 1401 cm^{-1} and 1253 cm^{-1}, which were the main spectral absorption peaks of proteins, corresponding to the stretching vibration of C═O bond, N—H bond, C—N bond and N—H bond respectively. In the spectral diagram of SMHM2, the absorption peak at this position showed a blue shift. It indicated that the reduced sugar and soybean meal hydrolysate had successfully covalently combined in the Maillard reaction system, thus it led to the change of the absorption spectrum (Stanic-Vucinic, Prodic, Apostolovic, Nikolic, & Velickovic, 2013). In addition, the absorption peak near 1053 cm^{-1} was caused by the tensile vibration of C—O bond. The absorption peak here was slightly weakened and appeared blue shift after Maillard reaction. This indicated that the enzymatic hydrolysate of soybean meal formed a covalent bond with xylose and changed its structure through Maillard reaction.

Fluorescent compounds were formed at the early stage of Maillard reaction and could be used as precursors of browning compounds. The fluorescence spectrum of Maillard reaction products showed a single peak (Fig. 3.2B). SMHM1 had the highest absorption peak, which was because the presence of cysteine could significantly inhibit the formation and transformation of brown substances. When there was no cysteine in the reaction system, a large number of precursor substances of brown substances would produce, and the intensity of the absorption peak was large. The typical excitation wavelength of most fluorescent compounds produced in Maillard reaction is 340–370 nm, and the emission wavelength is 420–470 nm (Shrestha, Gemechu, & Meulenaer, 2013). The maximum emission intensity of SMH and SMHM2 was 455 nm and 460 nm respectively, and the fluorescence intensity of SMHM2 was higher than that of SMH, which indicated that the soybean meal

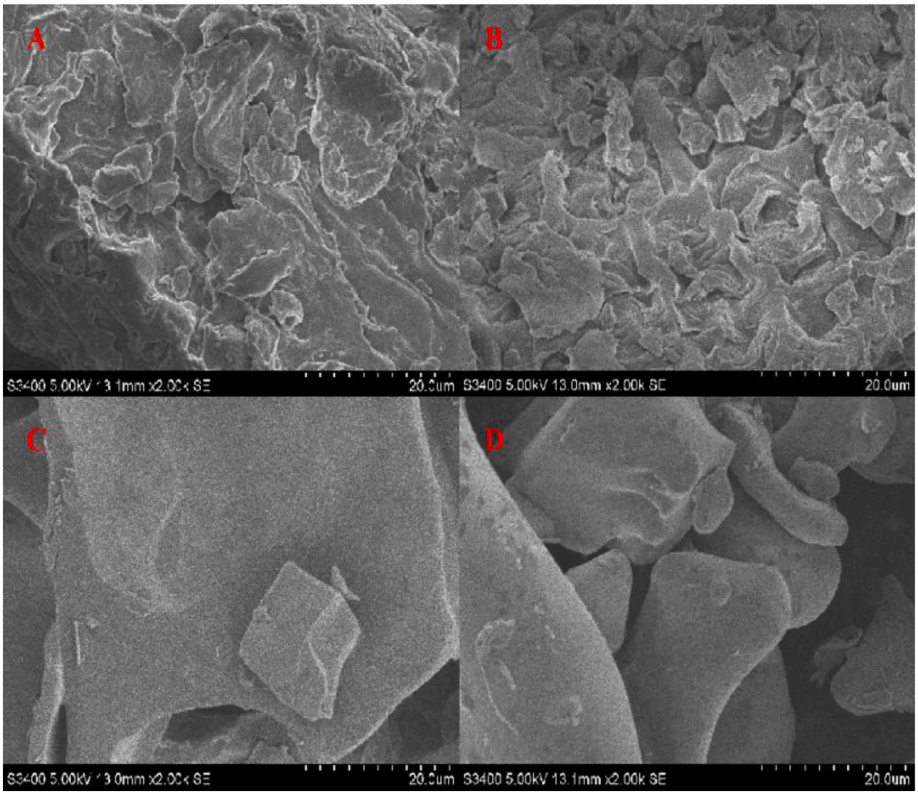

Fig. 3.4. SEM images of MRPs. soybean meal, (B) SMH, (C) SMHM1, (D) SMHM2.

hydrolysate conjugated with reducing sugar in the Maillard reaction. Without the effect of reducing sugar, no obvious absorption peak was detected in the range of 200 to 500 nm for SMH, the absorbance of SMHM1 and SMHM2 increased during the reaction, it indicated that new compounds were formed during the heating process (Fig. 3.2C). The absorption value at 294 nm can be used to reveal the formation of intermediate Maillard reaction products, the absorption value at 420 nm is usually used to detect the formation of advanced MRPs. In the primary stage of Maillard reaction, amino compounds and carbonyl compounds will generate Schiff bases, which will be converted into molecular rearrangement products again, thus it shows an absorption peak at 294 nm. SMHM2 and SMHM1 were observed characteristic absorption peaks near 275 nm, which might indicate the generation of new substances. The absorption value of SMHM1 at 294 nm and 420 nm was higher than that of SMHM2, which indicated that SMHM1 contains more brown substances, and the formation and transformation of brown substances could not be effectively inhibited without cysteine addition (Xu, Zhang, & Karangwa, 2016).

3.2.2. XRD

After the Maillard reaction, the products of SMHM1 and SMHM2 were amorphous (Fig. 3.3). In the figure, it could be seen that a broad peak appears near $2\theta = 20°$, which was attributed to highly disordered carbon atoms (Li et al., 2018). Two small peaks near 29.1° and 31.5° appeared in the diffraction figure of SMH, it might be due to the influence of inorganic impurities. After Maillard reaction, the diffraction peak angle of SMHM1 and SMHM2 shifted slightly to the left. This might be because xylose combined with soybean meal hydrolysate to promote the chain movement of the hydrolysate. The crystal structure was reduced by interfering with the peptide chain arrangement in the hydrolysate, and the crystallinity of the reactant was reduced after the reaction.

3.2.3. SEM

The morphology of different molecules before and after Maillard reaction was observed by SEM. The molecular surface of soybean meal powder remained rough, and there were some gullies with more irregular block distribution (Fig. 3.4A). The molecular surface of soybean meal powder became rougher, and the irregular block was more obvious after hydrolysis (Fig. 3.4B). The product surface was a rigid sheet structure after Maillard reaction, the surface became smooth and flat, and the rough, porous and irregular block structures disappeared (Fig. 3.4C, D). This change was due to the interaction between the molecules of glycosyl chains, polypeptide and amino acid residue, which resulted in changes in the spatial conformation of the peptide chain, thus formed a more compact plane structure (Fu, Xu, Luo, Xu, & Wan, 2021).

3.2.4. DSC

The denaturation temperature of Maillard product SMHM2 of soybean meal hydrolysate at high temperature was significantly higher than that of SMH. The denaturation initial temperature of SMH was 136.2 ℃, and the denaturation temperature was 157.8 ℃. Its denaturation temperature was the lowest, which indicated that more lysine residues might be exposed during heating (see Appendix Fig. 1.1). After glycosylation, the denaturation initial temperature of SMHM1 was 140.2 ℃, and the denaturation temperature was 161.1 ℃; the denaturation initial temperature of SMHM2 was 159.4 ℃, and the denaturation temperature was 169.2 ℃. There were two fluctuations of enthalpy values in the DSC curve of SMHM1, the amplitude of the fluctuations increased slightly. And there were many fluctuations of enthalpy values in the DSC curve of SMHM2, the amplitude of the fluctuations increased significantly (Appendix Fig. 1.1). It means that the components increased in both SMHM1 products and SMHM2 products, especially the components in SMHM2 products. This may be associated with the cysteine. No cysteine was added in SMHM1, and the components in the Maillard reaction products of SMHM1 were fewer. However, cysteine was added in SMHM2, and many new components were generated in the Maillard

Table 3.1
Free amino acid composition of different MRPs.

Amino acid	SMH		SMHM1		SMHM2	
	μmoL/mL	mg/g	μmoL/mL	mg/g	μmoL/mL	mg/g
Asp	0.020	0.093	0.204	0.944	0.202	0.933
Glu	0.393	2.009	0.216	1.108	0.385	1.841
Ser	0.148	0.542	0.253	0.923	0.186	0.681
Gly	0.082	0.216	0.088	0.231	0.099	0.258
His	0.220	1.185	1.003	5.407	0.047	0.254
Thr	0.836	3.459	0.281	1.160	0.148	0.613
Ala	0.202	0.625	0.212	0.655	0.343	1.062
Arg	0.464	2.811	0.424	2.569	0.160	0.971
Pro	0.268	1.072	0.223	0.891	0.037	0.149
Tyr	0.091	0.570	0.046	0.289	0.098	0.618
Val	0.116	0.475	0.062	0.253	0.103	0.423
Met	0.099	0.513	0.053	0.276	0.074	0.539
Cys	1.574	6.622	0.455	1.915	1.076	4.528
Ile	0.396	1.803	0.314	1.434	0.298	1.358
Leu	0.335	1.528	0.258	1.175	0.224	1.023
Phe	0.253	1.455	0.167	0.960	0.138	0.793
Lys	0.694	3.526	0.682	3.465	0.639	3.244
total amino acids	6.191	28.504	4.941	23.655	4.257	19.288
sweet amino acids	1.536	5.914	1.057	3.860	0.813	2.763
delicate amino acids	0.413	2.102	0.420	2.052	0.587	2.774
bitter amino acids	1.889	9.778	2.287	12.074	1.044	5.361
other amino acids	2.353	10.710	1.177	5.669	1.813	8.390

reaction products of SMHM2 (Zhang et al., 2018). Additionally, the thermal stability of SMHM2 was improved after Maillard reaction. It might be due to the enhancement of the spatial repulsion and electrostatic repulsion force of the coupling molecules caused by the glycosylation process, which prevented the aggregation of SMH at high temperatures (Liu, Zhao, Zhao, Ren, & Bao, 2012). In addition, Maillard reaction could enhance the thermal stability of protein through different interactions and the binding of exclusion volume. It had been reported that the covalent coupling of protein and polysaccharide through Maillard reaction could improve the thermal stability of protein and its mixture.

3.3. Content of free amino acid

The type and content of amino acids played an important role in the flavor of Maillard products. The composition and content of amino acids in the hydrolysate were determined by HPLC. The content of sweet taste amino acids (alanine, glycine, serine, threonine, proline) was 1.536 μmol/mL, the content of delicate amino acids (aspartic acid, glutamic acid) was 0.413 μmoL/mL, the content of bitter taste amino acids (arginine, histidine, isoleucine, leucine, methionine, phenylalanine, valine) was 1.889 μmol/mL, other amino acids content was 2.353 μmol/mL (Table 3.1). The products of soybean meal hydrolyzed by immobilized alkaline protease contained more hydrophobic amino acids, which might be because alkaline protease had the tendency to hydrolyze at the position of hydrophobic amino acid residues. It caused higher bitterness due to the hydrolysate of soybean meal contained hydrophobic amino acid residues at the C-terminal or *N*-terminal (Zhang, Su, & Zhao, 2022). The total free amino acid content of soybean meal hydrolysate was 6.191 μmol/mL. After Maillard reaction of soybean meal hydrolysate with xylose, the content of total free amino acids in Maillard reaction group and control group were 4.941 μmoL/mL, 4.257 μmol/mL, they were 31.24% and 20.19% lower than that in soybean meal hydrolysate, respectively. It indicated that some amino acids, as precursors, cross linked with carbonyl compounds in Maillard reaction, this resulted in the content reduction of amino acid. In addition, the content of free amino acids was also affected by Strecker degradation and peptide degradation. The content of delicate amino acid in Maillard reaction group was 0.587 μmoL/mL, it was 42.13% more than that in soybean meal. Delicate amino acid can not only improve the taste of products, but also can improve the mellow flavor and continuity of food (Guo & Xiong, 2013). Bitter taste amino acids would cause severe discomfort feeling, and the content of bitter taste amino acids in Maillard reaction group was 1.044 μmoL/mL. The content of bitter taste amino acids in soybean meal decreased by 44.73%, which indicated that the generation of bitter amino acids was less than the degradation.

3.4. GC–MS

The flavor composition and content of different volatile compounds were closely related to the flavor characteristics of samples. 50 kinds of volatile compounds were detected from Maillard reaction products, 4 mercaptans, 4 sulfur substituted furans, 3 thiophenes, 7 furans, 6 pyrazines, 3 pyrrole, 1 pyrimidine, 7 aldehydes, 4 ketones, 7 esters, 2 alcohols and 2 acids were included (see Appendix Table 3.2 for data).

Seven kinds of furans were detected in the samples. It was reported that furans could be formed by caramelization reaction and carbohydrate degradation reaction in previous studies. The furan content of SMHM2 and SMHM1 was 0.375 μg/g and 1.939 μg/g respectively, which indicated that the use of cysteine in Maillard reaction would inhibit the formation of sulfur free furan. Mercaptans, thiophenes and sulfur substituted furans were included in the sulfur compounds detected. Sulfur compounds were very important flavor substances in Maillard reaction process, which could provide saline taste, barbecue taste and meaty flavor. Furfuryl mercaptan and 2-methyl-3-furan mercaptan were the main sulfur substituted furans, and their contents in SMHM2 and SMHM1 were 1.422 μg/g and 0.265 μg/g. Some studies had shown that furfuryl mercaptan and 2-methyl-3-furan mercaptan were the main flavor components in cooked beef and chicken soup (Wanapat, Ngarmsang, Korkhuntot, Nontaso, & Rowlinson, 2000). Mercaptan compounds were not detected in SMHM1, but the content of mercaptan compounds in SMHM2 was 0.316 μg/g, which was more important in meaty flavor additive. Thiazole compounds were not detected in SMHM2 and SMHM1, and thiophene compounds were not detected in SMHM1. The content of thiophene compounds in SMHM2 was 0.197 μg/g. Previous studies had showed that 3-methylthiophene-2-formaldehyde and 3-methylthiophene in thiophene were the reasons for sulfur flavor in cooked meat products (Wang, Xie, Zhang, Xu, & Yang, 2022). Pyrazines were the main nitrogenous compounds. The pyrazines content of SMHM2 and SMHM1 were 0.078 μg/g and 0.720 μg/g respectively. A large number of pyrazines were considered to be the source of baking flavor and nut flavor. The content of pyrrole and pyrimidine was less in the reaction products. Aldehydes, acids, alcohols, esters and ketones were included in Oxygenated substances. These oxygenated compounds were usually produced by partial oxidation of fatty acids. Because they had high flavor thresholds, they generally did not cause too much impact on the sensory quality and flavor of food. The content of benzaldehyde in aldehydes was relatively high. SMHM2 and SMHM1 were 0.520 μg/g and 0.741 μg/g respectively. Benzaldehyde might cause peculiar smell.

3.5. Radical scavenging ability

DPPH scavenging ability of SMHM2 was significantly enhanced compared with SMH, and increased by 69.8% with the improvement of concentration (Fig. 3.5A). The hydroxyl radical scavenging ability of SMH did not change obvious, the hydroxyl radical scavenging ability of SMHM2 increased significantly with the improvement of concentration. When the concentration was 3 mg/mL, the scavenging ability was 68.7% (Fig. 3.5B). The antioxidant activity of Maillard reaction products was complex. The intermediate products generated during the reaction process contained reducing aldehydes, ketones, alcohols and other

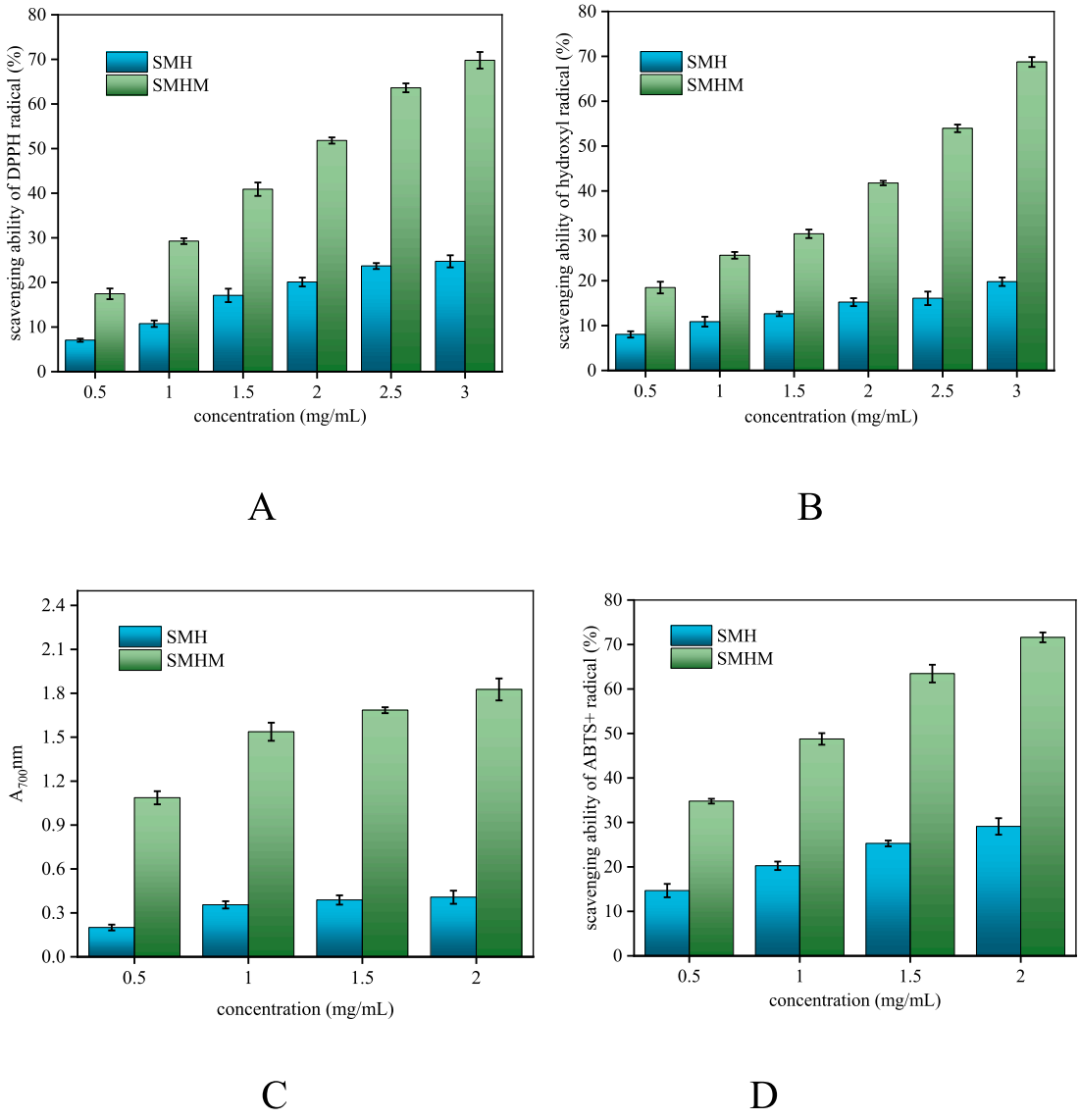

Fig. 3.5. Radical scavenging ability before and after Maillard reaction. DPPH scavenging ability, (B) hydroxyl radical scavenging ability, (C) FRAP scavenging ability, (D) $ABTS^{+}$ radical scavenging ability.

substances, which might lead to enhanced antioxidant activity (Jiang, Li, Zhao, Wang, & Hou, 2019). The reduction ability of SMH did not change significantly with the improvement of concentration, while the reduction ability of SMHM2 increased with the improvement of concentration. When the concentration was 2 mg/mL, the absorbance value was 1.82 (Fig. 3.5C). The $ABTS^{+}$ radical scavenging ability of SMH increased with concentration, but the radical scavenging ability of SMHM2 was significantly stronger than that of SMH after Maillard reaction (Fig. 3.5D). The reduction ability of Maillard reaction products might be due to the formation of a large number of brown substances and reducing ketones in the system, which could reduce Fe^{3+} to Fe^{2+}(Delgado-Andrade, Rufián-Henares, & Morales, 2005). Previous study reported that the reduction ability of α-lactalbumin was significantly enhanced after Maillard reaction with α-lactalbumin and glucose used as substrates. The scavenging ability of $ABTS^{+}$ radicals depended on the substances formed in Maillard reaction. The main compounds formed in Maillard reaction were melanoids, which were the main contributors to the scavenging ability of radicals. In addition, the reduction ability of the hydrolysate was improved by Maillard reaction. Zhang (Zhang et al., 2020) studied the Maillard reaction products of α-lactalbumin and galactose, they found that the Maillard reaction products had good antioxidation, in which the $ABTS^{+}$ radical scavenging ability was 69.81%. Wang studied that the macromolecular melanin of Maillard reaction products from xylose and glycine, the Maillard reaction products achieved higher browning and reducing capacity.

4. Conclusion

In conclusion, meaty flavor additive was prepared from soybean meal hydrolysate and xylose in the method of Maillard reaction. Under the conditions of reaction temperature 120 ℃, reaction time 120 min, and cysteine addition 10%, the Maillard product had strong flavor of meat, good continuity, suitable mellow and delicate flavor. The characteristics of Maillard reaction products were analyzed. The results showed that the Maillard reaction products were amorphous, and the content of free amino acids was 4.941 μ mol/mL, free amino acid content of delicate flavor 0.587 μ mol/mL. There were 50 volatile flavor substances in Maillard reaction products according to GC–MS analysis. 4 mercaptans, 4 sulfur substituted furans, 3 thiophenes, 7 furans, 6 pyrazine, 3 pyrrole, 1 pyrimidine, 7 aldehydes, 4 ketones, 7 esters, 2 alcohols and 2 acids were included in the products. The Maillard reaction

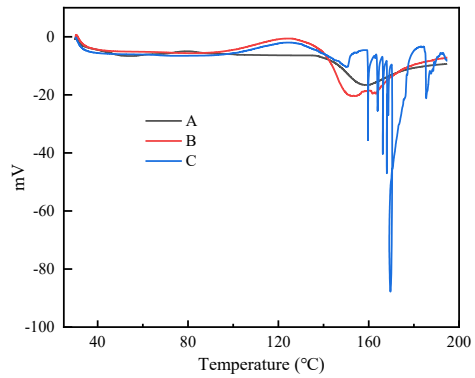

Fig. A1.1. DSC curve of MRPs. (A) SMH, (B) SMHM1, (C) SMHM2.

products also have strong antioxidant activity. The scavenging ability of FRAP, DPPH radical, hydroxyl radical and $ABTS^{+}$ radical was 1.82%, 69.8%, 68.7% and 71.6% respectively. The prepared products of Mailarrd reaction have the great potential application for the development of food additives.

CRediT authorship contribution statement

Xianhui Huang: Writing – original draft. **Peng Wang:** Software, Investigation. **Wenlin Xue:** Data curation, Methodology. **Jie Cheng:** Conceptualization. **Fuming Yang:** Writing – review & editing, Supervision. **Dianyu Yu:** Software, Validation. **Yongge Shi:** Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgements

This work was also financially supported by the Million Project of Heilongjiang Province (2020ZX08B01) and Academic backbone Project of Northeast Agricultural University (20XG015). The authors would like to thank the anonymous reviewers and the editor for their comments on this paper.

Appendix

Appendix Table 3.2. GC–MS analysis of Maillard reaction products.

Types	Compounds	SMHM2 (μg/g)	SMHM1 (μg/g)
mercaptan	Methyl mercaptan	0.044	ND
	3-Methoxyphenylmercaptan	0.052	ND
	1,1-Bis (ethylthio) ethane	0.023	ND
	2-Methyl-3-pentanethiol	0.197	ND
sulfur substituted furan	2-Methyl-3-furathiol	0.265	ND
	furfuryl mercaptan	1.422	ND
	Furfuryl methyl sulfide	0.021	ND
	Difurfuryl disulfide	0.019	ND
thiophenes	3-Methylthiophene	0.097	ND
	3-Methylthiophene-2-formaldehyde	0.061	ND
	2,5-Thiophenediol	0.039	ND
furan	furan	0.063	0.071
	2-Methylfuran	0.177	0.525
	furfural	0.094	1.157
	2-Amylfuran	ND	0.033
	2-Acetylfuran	ND	0.024
	Furfuryl alcohol	0.041	ND
	3-Furan methanol	ND	0.129
pyrazines	Pyrazine	0.021	0.018
	2-Methylpyrazine	0.041	0.125
	2,5-Dimethylpyrazine	0.016	0.440
	2,6-Dimethylpyrazine	ND	0.089
	trimethyl pyrazine	ND	0.028
	3-Ethyl-2,5-dimethylpyrazine	ND	0.020
pyrrole	indole	0.031	0.026
	1-furfurylpyrrole	ND	0.075
	2-Ethylpyrrole	0.015	ND
pyridine and pyrimidine	4,6-Dimethylpyrimidine	0.014	ND
aldehyde	Valeraldehyde	ND	0.034
	Hexanal	0.136	0.247
	Nonanal	0.023	0.071
	Benzaldehyde	0.520	0.741
	acetaldehyde	ND	0.015
	2-Methylbutyraldehyde	ND	0.039
	3-Methylbutyraldehyde	ND	0.158
Ketones	2-Acetone	0.039	0.173

(*continued on next page*)

(*continued*)

Types	Compounds	SMHM2 (μg/g)	SMHM1 (μg/g)
	2-butanone	0.076	0.069
	2-heptanone	0.055	0.041
	2-Pentanone	0.046	ND
esters	ethyl acetate	0.036	ND
	Benzyl acetate	ND	0.011
	Diethyl phthalate	0.019	0.024
	Butylated hydroxytoluene	ND	0.016
	Ethyl Hexadecanoate	0.036	ND
	Butyl acetate	0.047	ND
	3-Methylbutyl acetate	0.013	ND
alcohols	1-pentanol	0.032	0.021
	5-Methylbenzene-1,3-diol	ND	0.021
acids	Caproic acid	0.050	0.028
	Thioacetic acid	0.033	ND

ND means no detection.

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