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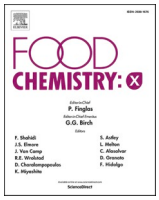

Prediction of flavor of Maillard reaction product of beef tallow residue based on artificial neural network

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ABSTRACT

The beef flavor of beef tallow residue was improved by enzymatic hydrolysis followed by the Maillard reaction, and the flavor could be predicted using an artificial neural network. Five beef tallow residue hydrolysates were prepared using different enzymes. The Flavourzyme and Papain (FP) hydrolysate had low molecular weight peptides and high degree of hydrolysis and free amino acid content. We identified 49 main compounds, including aldehydes, pyrazines, and furan.

Furan and pyrazine were the dominant volatile compounds in the five beef tallow residue-derived Maillard reaction products (MRPs), and their profiles and levels in the FP MRPs were high. The FP MRPs had the best sensory characteristics. The artificial neural network analysis revealed that the multiple input single output model had a better performance than the single input single output model, and the prediction accuracy was>90%, indicating that the MRPs sensory evaluation scores could be accurately predicted.

Introduction

Beef tallow is a kind of animal fat generated from the slaughter of cattle (Zhao, Xu, Yu, Yan, & Zhang, 2013). Compared with vegetable oils, the flavor of beef tallow is unique; therefore, it has been widely used in margarine, shortening, and other products (Moody, 1983). Suet, which is a layer of fat film on the beef abdomen rid, is an excellent raw material for preparing butter. The frying residue of suet is beef tallow residue, which contains a notable amount of protein. Most beef tallow residue is buried or used as chicken feed, causing resource waste and environmental pollution.

Beef flavor, which is popular among consumers, is affected by the beef grade, cutting method, cooking method, and temperature, and at least 38 aroma and flavor characteristics have been identified (Adhikari et al., 2011). The Maillard reaction and enzymatic protein hydrolysis are common techniques for improving the beef flavor. The degree of hydrolysis is regulated by the type of enzyme, reaction temperature, reaction time, pH, and other factors, among which the enzyme type is the most important (Hashemi, Aminlari, & Moosavinasab, 2014). Fu, Liu, Hansen, Bredie, and Lametsch (2018) hydrolyzed bovine muscle and porcine plasma using 10 different food-grade enzymes. The Maillard reaction was performed on the obtained hydrolysate to increase the content of small peptides (<0.5 kDa), which enhance umami taste and reduce bitterness. Chiang, Eyres, Silcock, Hardacre, and Parker (2019) obtained five beef bone hydrolysates with single and simultaneous enzymatic hydrolysis treatments. Compared with Bromelain® and Protamex®, the hydrophobic amino acid content in the hydrolysate obtained using Flavourzyme® decreased, and the Maillard peptide, pyrazine, and thioether contents increased, reducing the bitter taste and improving the meaty flavors. However, the ement of beef flavor via the

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hydrolysis of beef tallow residue-derived Maillard reaction products (MRPs) has not been reported.

Enzymatic hydrolysis and the Maillard reaction can increase the value of resources by masking the initial caramel-like flavor and improving the odor characteristics (Kouakou et al., 2014). However, applying this method is difficult due to the lack of quantitative research on the relationship between flavor characteristics and volatile components. The accuracies of conventional analysis methods, such as principal component analysis and linear regression analysis, are not sufficient for quantitative calculation because the features of the samples are reduced during the data simplification process. A quantitative analysis model is needed to address this limitation, and artificial neural networks (ANNs) are widely used in food sciences research. Huang et al. (2021) used multiple linear regression and an ANN to assess the ability of models to predict soluble solids, the acid content, and the ratio of soluble solids to titratable acid based on the mineral elements in fruits. Singh, Ruhil, Jain, Patel, and Patil (2009) used an ANN to model data on the deteriorative processes of ultra-high temperature-treated milk during storage. However, to the best of our knowledge, an ANN has not been built to predict the relationship between the beef flavor sensory evaluation score and the related components of beef tallow residue-derived Maillard reaction products.

Therefore, this work aimed to investigate how to predict the beef flavor of beef tallow residue-derived Maillard reaction products using an ANN. Qualitative and quantitative analyses of volatile components were performed by sensory evaluation and two-dimensional gas chromatography with mass spectrometry detection (GC × GC–MS). In addition, an ANN was used to examine the relationship between the sensory evaluation scores and the contents of related components.

Materials and methods

Chemicals and materials

Beef tallow residue (69 % protein, 17 % lipid, 3 % water) was obtained from Guanghanshi Maidele Food CO., ltd (Deyang, China). The enzymes Flavourzyme (50,000 U/mg) and Papain (2,000 U/mg) were obtained from Tanggui China Co., ltd. (Henan, China). Protamex® (31.4 U/mg) was purchased from Novo Co., ltd. (Novozyme Nordisk, Bagsvaerd, Denmark). Alcalase® (200 U/mg) was purchased from Wanbang China Co., ltd. (Henan, China). Other chemical reagents were obtained from National Chemical Reagent Co., ltd. (Shanghai, China).

Preparation of beef tallow residue-derived hydrolysates

Beef tallow residue was ground into powder and mixed with deionized water in a 1:4 ratio. The mixture was then hydrolyzed using Protamex (P), Alcalase (A), Flavourzyme (F), a combination of Flavourzyme and Alcalase (FA), and a combination of Flavourzyme and papain (FP). Table S1 lists the preparation methods for five beef tallow residue-derived hydrolysates under optimal hydrolysis conditions. The five hydrolysates (designated P, A, F, FA, FP) were centrifuged at 3,800 × g for 20 min after enzyme deactivation at 100 °C for 15 min. Supernatants were stored at 4 °C until use.

Determination of the degree of hydrolysis (DH)

The DH of the protein hydrolysates was calculated based on the method of Song et al. (2013), and α-amino nitrogen and total nitrogen were quantified using formaldehyde titration and the Kjeldahl method, respectively.

Free amino acid analysis

Peptides or proteins were precipitated by mixing 5 mL of the hydrolysate and 5 mL of 5 % trichloroacetic acid in a 20-mL volumetric flask (Song et al., 2013). After incubation at 25 °C for 2 h, the filtrate was obtained by filtering the mixture through filter paper (Whatman filter No.4). The filtrate was then centrifuged for 8 min at 13,000 × g and placed in a 4 °C constant temperature refrigerator.

Free amino acids in beef tallow residue-derived hydrolysates were analyzed according to (Liu et al., 2012). The sample (20 μL) was injected into an automated Agilent 1100 high-performance liquid chromatography (HPLC) system for analysis, with a flow rate of 1 mL/min, a column temperature of 40 °C, a Hypersil ODS column (4.6 mm × 250 mm × 5 μm), and a wavelength of 262 nm/338 nm. The mobile phase consisted of 0.15-mM acetonitrile/methanol/sodium acetate (2:2:1, v:v:v) and 0.6-mM sodium acetate. Amino acid standards were used to determine the calibration curve for calculation, and amino acids were identified and quantified based on the retention times and the peak area of standard compounds, respectively.

Molecular weight (Mw) distribution analysis

A Waters 600 liquid chromatography system (Waters Co., Milford, MA, USA) was used to analyze the Mw distribution of the beef tallow residue-derived hydrolysates. The system was equipped with an Empower workstation and Waters 2487 UV detector on a 2000 (300 mm × 7.8 mm) SWXL TSK gel filtration column. Acetonitrile/water/trifluoroacetic acid (40:60:0.1, v:v:v) constituted the mobile phase, and the flow rate was 0.5 mL/min. The sample (10 μL) was injected into the HPLC system at a column temperature of 30 °C. Five standards were used to obtain the Mw calibration curve: tripeptide GGG (189 Da), tetrapeptide GGYR (451 Da), bacitracin (1450 Da), aprotinin (6500 Da) and cytochrome *C* (12,500 Da). A UV detector at 220 nm was used to record the chromatogram, and the data were analyzed using gel permeation chromatography software.

Preparation of MRPs

The Maillard reaction products (designated P, A, F, FA, and FP MRPs, respectively) were obtained by mixing 10 mL of the hydrolysate and 3 % xylose in a beaker and reacting with magnetic agitation (130 RPM) for 60 min in a 100 °C oil bath. The five MRPs prepared using beef tallow residue-derived hydrolysates were immediately transferred to ice water for cooling and stored at 4 °C in a constant temperature refrigerator.

Sensory characteristics of MRPs

To determine the sensory characteristics of the five MRPs, sensory evaluation was performed according to the method of Schlichtherle-Cerny and Amado (2020) with some modifications. A team of four men and four women (23–45 years old) with experience in sensory evaluation from the School of Food Science and Engineering at Jiangnan University in China evaluated the samples. The eight panelists had previously received 3 h of training to define descriptive terms and determine the appropriate reference solutions for the samples. The reference solutions were prepared as follows: the caramel-like flavor was obtained by placing 1.0 g of Dove (Mars Food ltd., USA) syrup caramel in 20 mL of water, the umami flavor was 3.0 g monosodium glutamate (LIANHUA Food Co. ltd., Henan) in 20 mL of water, the sour flavor was 2.0 g lactic acid (Yakult ltd., Japan) in 20 mL of water, the meaty flavor was obtained by boiling 3.0 g of filet steak (Wanda supermarket ltd., Wuxi) in 800 mL of water for 1 h, the bitter flavor was obtained by adding 2.0 g Nestle (Nestle Co. ltd., Switzerland) coffe to 20 mL of water, the aromatic flavor was obtained by adding 2.0 g of Jinlongyu (Wilmar International Co. ltd., Shanghai) sesame oil to 20 mL of water. The evaluation was scored on a scale at 1 to 10 intervals (0 = tasteless, 10 = extremely intense), and the reference solutions were scored 5 points. The MRPs (60 mL) and 60 mL of each reference solution were simultaneously tasted in separate sensory booths at 22 ± 2 °C. The sensory evaluation was conducted in a Jiangnan University sensory laboratory,

which meets international standards.

GC × GC–MS analysis

GC × GC–MS analyses were performed using a Pegasus® 4D instrument (LECO Corp., St. Joseph, MI, USA). Volatile compounds were extracted via solid-phase microextraction with 75-μm Carboxen/Polydimethylsiloxane fiber (Supelco, Bellevue, PA, USA). Two grams of the MRP were placed in a glass bottle. The glass bottle was sealed with a lid and placed in a water bath at 60 °C for 25 min to allow the volatile compounds to reach equilibrium. The fiber was then desorbed at 250 °C for 5 min. The first-dimension separation was achieved on a polar Stabilwax® polyethylene glycol column (30 m × 0.25 mm inner diameter × 0.25 μm film thickness; Restek), and the second-dimension separation was achieved on a DB-17MS column (1.195 m × 0.25 mm × 0.15 μm). The initial temperature of the main oven was set to 40 °C for 3 min, increased to 230 °C at a rate of 10 °C/min, and then maintained at 230 °C for 6 min. The components were separated and identified by GC × GC–MS; total range MS with additional 2D selectivity and time-of-flight MS determination was used for those with low concentrations. Different modulation cycles (2, 4, 5, 6, and 8 s) were tested during the optimization stage to determine the optimal separation conditions. The chosen modulation cycle was 4 s, and the hot pulse duration was 0.8 s. The MS acquisition rate was 200 spectra/s. Solvent acquisition was delayed for 90 s to protect the MS analyzer from overexposure to the solvent. The ion source temperature was 210 °C, and the transfer line temperature was 250 °C. The data were acquired in scanning mode, and the electron impact voltage and the detector voltage were set to 70 eV and 1,430 V, respectively. The acquisition frequency was 100 spectra/s, and the mass sampling range was from *m/z* 35 to *m/z* 400. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min.

Volatile compounds were identified by comparing published literature, authentic standards, Wiley 07, and NIST 11 databases to the detector data (Kovats retention index, KI) of samples. The KI values were calculated with reference to the normal alkane series (C8-C23) under the same sample conditions. Assuming a relative response coefficient of 1 and a recovery ratio of 100 %, the approximate quantities of volatile compounds were estimated by comparing their peak areas to those of the internal standard obtained from the total ion chromatogram using the following formula:

$$W_i = f' * \frac{A_i * m_s}{A_s} \Big/ m$$

where A_i is the peak area of the sample i, A_s is the peak area of the internal standard, m_s is the mass of the internal standard, m is the mass of the sample, f' is a relative correction factor assumed to be 1, and W_i is the concentration (μg/g) of the compound i.

Mathematical modeling

There is a complex nonlinear relationship between the component contents and the sensory score. We used an ANN to build a sensory score prediction model, trained based on the experimental results and representative of the relationship. The model has three types of layers: the input layer, the hidden layer, and the multiple-model mechanism (Fig. 1).

The model input is the content of each compound, and the output is the sensory score. The input layer is used for processing input data. The activation function of the input layer is *relu*:

$$relu(x) = \begin{cases} x, x > 0 \\ 0, x \le 0 \end{cases}$$

The hidden layer is the main part of the neural network, consisting of hidden neurons to extract information from the input data. Considering the range of sensory scores from 0 to 10, the multiple-model mechanism limits the output in a reasonable range (Wang, Fan, Wang, & Wu, 2021). There are two regimes in the multiple-model layer: the maximum and the minimum reasonable value. The regime weights are calculated via the *softmax* activation function using the output of the hidden layer:

$$\boldsymbol{G} = [G_1, G_2]^T = softmax(w_h O_h + b_h)$$

where $\boldsymbol{G}$ is the weight vector of the regimes, G_j is the weight of the j^{th} regime, w_h is the weight matrix, O_h is the output of the hidden layer, and b_h is the bias vector.

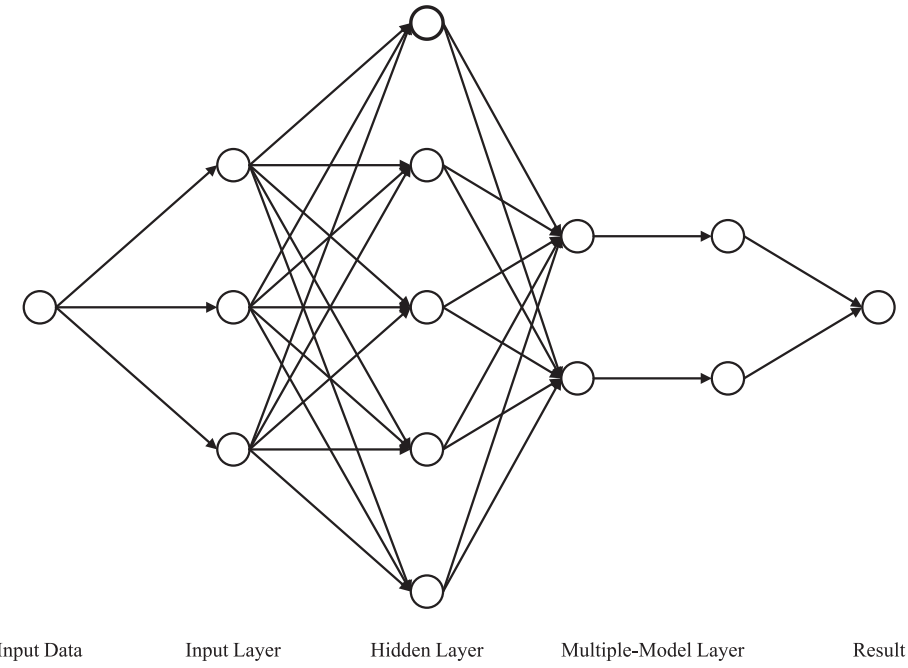

Fig. 1. The structure of analysis model. Input the different substances content, while the output is the sensory score. The input layer and the hidden layer consist of basic neuron, whose activation function is Rectified Linear Unit (ReLU). The multiple-model layer consists of two regimes: one regime represents the lowest sensory score, and the other represents the highest sensory score.

The value of each regime is not changed in training, but the weights of different regimes are regulated and determined by training. The result of the neural network is a weighted sum of multiple regimes:

$$O = \boldsymbol{R}^{\boldsymbol{T}}\boldsymbol{G} = \sum_{j=1}^{2} R_j G_j$$

The loss function of the model is the mean square error:

$$L = (\widehat{y} - y)^2$$

where $\widehat{y}$ is the prediction result of the model, and y is the label of the sample.

We divide the samples into the training dataset and the testing dataset, in which the ratio of training set to testing set is 4: 1. Each group of samples was used as a test set in turn, and the other four groups were used as a training set. The training dataset is used to train the model, and the testing dataset is used to test the prediction performance of the model. First, we conduct experiments and use the experimental data to train the ANN prediction model and obtain the complex nonlinear relationship between the content of each compound and sensory score.

Second, we conduct another experiment to obtain the contents of different components. We then process and input these data into the model to obtain the sensory prediction score. The difference between the prediction results and the true results can characterize the accuracy and generalization ability of the ANN model.

Statistical analysis

Analysis of variance was performed using SPSS version 13.0 (SPSS Inc., Chicago, Illinois). Significant differences between the beef tallow residue-derived MRPs were analyzed using a neural network based on a proxy model. $P \leq 0.05$ was considered significant. We used Python 3.7 analysis software; The model was built based on the TensorFlow-1.13.0. The Adam optimizer training method was used to train the model, with 1,000 training iterations. The learning rate was 0.002; the dropout rate was 0.95. We trained the model using the following datasets:

a) SISO: single input single output. Only the data related to the corresponding sensory score are used as the sample input.
b) MISO: multiple input single output. The data relating to the corresponding sensory score and other inputs are used as the sample input.

Results

DH of beef tallow residue-derived hydrolysates

The DH significantly differed ($P \leq 0.05$) between the beef tallow residue-derived hydrolysates produced using different enzymes (Table S1). The DH of the beef tallow residue-derived hydrolysates that were synergistically hydrolyzed using two enzymes was significantly higher than that of those hydrolyzed using one enzyme. Compared with single enzyme hydrolysis, the DH of the samples increased significantly after Flavourzyme pretreatment.

Free amino acids

The free amino acid contents of the beef tallow residue-derived hydrolysates are shown in Table 1. The FA and FP hydrolysates had significantly higher amino acid contents than the other hydrolysates. The amino acid content of the FA hydrolysate was the highest, and that of the P hydrolysate was the lowest. The amino acid contents of the five beef tallow residue-derived hydrolysates significantly differed ($P \leq 0.05$). Compared with other amino acids, the Asp, Glu, Ala, Lys, Leu, Phe, and Tyr contents were higher in the beef tallow residue-derived hydrolysates. The larger the degree of hydrolysis, the higher the free amino acid content.

Table 1
Free amino acid analysis of the five beef tallow residue hydrolysates.

Amino acid	P	A	F	FP	FA
	(mg/L)				
Asp	143.78^{e} ± 0.62	198.26^{b} ± 0.77	158.28^{d} ± 0.68	188.74^{c} ± 0.76	267.68^{a} ± 0.89
Thr	65.47^{e} ± 0.45	115.71^{d} ± 0.75	193.57^{c} ± 0.79	256.75^{b} ± 0.93	288.50^{a} ± 0.97
Ser	64.32^{d} ± 0.63	105.54^{c} ± 0.47	180.71^{b} ± 0.58	178.08^{b} ± 0.73	251.35^{a} ± 0.76
Glu	160.23^{e} ± 0.71	270.86^{b} ± 0.82	217.70^{d} ± 0.82	225.40^{c} ± 0.63	510.78^{a} ± 2.11
Pro	60.73^{b} ± 0.85	71.03^{a} ± 0.94	55.00^{c,d} ± 0.63	53.81^{c} ± 0.93	57.15^{d} ± 0.25
Gly	76.17^{d} ± 0.95	109.13^{c} ± 0.98	147.41^{b} ± 1.23	144.92^{b} ± 1.32	200.76^{a} ± 1.74
Ala	142.04^{e} ± 1.74	220.82^{d} ± 2.21	345.14^{b} ± 3.61	320.43^{c} ± 2.22	467.01^{a} ± 3.85
Cys	71.90^{c} ± 0.95	60.73^{a} ± 1.74	126.39^{d} ± 1.21	92.13^{c} ± 1.11	86.75^{b} ± 1.23
Val	58.46^{d} ± 1.08	53.09^{d} ± 1.01	88.96^{c} ± 0.94	144.00^{b} ± 1.32	219.54^{a} ± 1.84
Met	42.98^{e} ± 0.26	113.21^{c} ± 1.3	73.02^{d} ± 1.57	123.44b ± 1.98	160.16^{a} ± 1.31
lle	77.40^{e} ± 0.73	84.02^{d} ± 0.85	103.35^{c} ± 1.32	123.45^{b} ± 0.98	141.83^{a} ± 0.93
Leu	86.46^{e} ± 0.73	171.99^{d} ± 1.85	410.36^{c} ± 3.63	468.69^{b} ± 3.87	574.55^{a} ± 4.76
Tyr	471.33^{b} ± 4.78	477.97^{b} ± 4.74	391.74^{c} ± 4.74	463.46^{b} ± 4.93	527.33^{a} ± 4.36
Phe	200.18^{e} ± 2.76	453.54^{d} ± 3.99	585.49^{c} ± 4.83	602.21^{b} ± 5.54	898.46^{a} ± 7.53
Lys	123.12^{d} ± 1.32	231.82^{c} ± 3.16	280.29^{b} ± 2.76	284.85^{b} ± 2.93	409.04^{a} ± 3.84
His	150.61^{a} ± 2.02	82.31^{c} ± 0.83	42.11^{e} ± 0.42	53.34^{d} ± 0.47	97.67^{b} ± 0.74
Arg	40.86^{d} ± 0.33	41.15^{d} ± 0.32	393.50^{c} ± 2.99	427.56^{b} ± 3.75	480.04^{a} ± 3.81

Values bearing different lowercase letters (a, b, c, d and e) were significantly different ($p \leq 0.05$).

Mw distribution

The Mw distributions significantly differed ($P \leq 0.05$) between the five beef tallow residue-derived hydrolysates (Table 2). The samples were mainly composed of peptides and amino acids with a Mw < 2,000 Da. The proportion of low-Mw components gradually increased, and the proportion of high-Mw components gradually decreased with increasing DH. The low Mw (<1,000 Da) contents were highest in the FP

Table 2
Changes of molecular weight distribution (percent of total area) in five beef tallow residue hydrolysates.

MW (Da)	P	A	F	FP	FA
>10000	1.16^{b} ± 0.01	2.22 ^{a} ± 0.01	0.45^{c} ± 0.014	0.19 ^{d} ± 0.01	1.16^{b} ± 0.01
10000–5000	5.25^{b} ± 0.02	6.74 ^{a} ± 0.02	2.59^{c} ± 0.01	0.90 ^{d} ± 0.01	5.25^{b} ± 0.02
3000–5000	9.61 ^{a} ± 0.04	8.82^{b} ± 0.02	4.99^{c} ± 0.02	2.24 ^{d} ± 0.01	9.61 ^{a} ± 0.04
2000–3000	9.77 ^{a} ± 0.01	8.99^{b} ± 0.03	6.08^{c} ± 0.02	3.20 ^{d} ± 0.01	9.77 ^{a} ± 0.01
1000–2000	19.78 ^{a} ± 0.05	19.77^{b} ± 0.05	16.93^{c} ± 0.05	11.24 ^{d} ± 0.10	19.78 ^{a} ± 0.05
500–1000	23.14^{b} ± 0.10	21.03^{c} ± 0.16	28.36 ^{a} ± 0.22	24.77^{b} ± 0.07	23.14^{b} ± 0.10
180–500	24.15 ^{d} ± 0.08	24.34^{c} ± 0.02	33.83^{b} ± 0.03	46.82 ^{a} ± 0.01	24.15 ^{d} ± 0.08
<180	7.12^{c} ± 0.04	8.10^{b} ± 0.04	6.78 ^{d} ± 0.06	10.64 ^{a} ± 0.03	7.12^{c} ± 0.04

Values bearing different lowercase letters (a, b, c, d and e) were significantly different ($p \leq 0.05$).

hydrolysate and lowest in the A hydrolysate.

MRP sensory characteristics

The MRPs of beef tallow residue-derived hydrolysates were characterized by scoring their caramel-like, umami, sour, meaty, bitter, and aromatic flavors. Analysis of variance was conducted on the MRP sensory characteristic data, which were assessed in triplicate. Fig. 2 shows the average intensity values for the six attributes, which significantly differed ($P \leq 0.05$) between the MRPs. The FA MRPs had the strongest caramel-like flavor, and the F, FA, and FP MRPs had stronger bitter flavors than the other MRPs. The overall flavor of the P MRPs was not prominent. The FA MRPs also had the highest umami and sour flavor scores. The FP MRPs had significantly higher bitter, aromatic, and meaty flavor scores than the other MRPs.

MRP composition

The volatile compounds in the five MRPs are shown in Table 3. The volatile compound contents and profiles were affected by the enzyme pretreatment ($P \leq 0.05$). Forty-nine main compounds (relative content > 0.5 %), including aldehydes, pyrazines, and furans, were detected and identified. Furan and pyrazine were the dominant volatile compounds in all five MRPs. Pyrazine was the dominant component of the A MRPs, and the most abundant pyrazine was 2-ethyl-3,5-dimethyl-pyrazine. Furan was the most abundant component of the P MRPs, among which 2-pentyl-furan accounted for the largest proportion. FP MRPs were composed of mostly aldehydes, with nonanal accounting for the largest proportion of aldehydes. The five MRPs contained different amounts of acetic acid, and the acetic acid content was highest in the FA MRPs.

Relationships between MRP sensory characteristics and free amino acids, hydrolysate Mw

The SISO model predicted the corresponding flavor score according to the contents of the flavor components, examining only the relationship between the flavor components and the sensory evaluation score. The MISO model predicted the corresponding flavor score according to the contents of the flavor components and the interaction and masking effects between flavors. The prediction errors of the two datasets are shown in Fig. 3 and Table S2. Both prediction models perform well under most conditions. The maximum error between the prediction results and the true score was<1.5 and the minimum error was<0.01, demonstrating that the established model was sufficiently accurate to use in the prediction process. Except for acidic conditions, the MISO model performed significantly better than the SISO model.

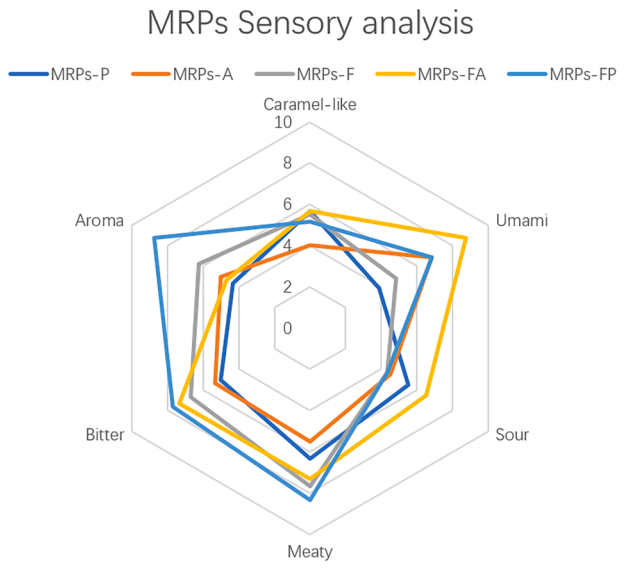

Fig. 2. The mean scores of the 5 attributes for the 5 MRPs in descriptive sensory evaluation. Mean scores for each attribute with different lowercase letters (a, b, c, d and e) were significantly different ($p \leq 0.05$). MRPs-P/MRPs-A/MRPs-F/MRPs-FA/MRPs-FP represented the Maillard reaction products from P/A/F/FA/FP, respectively.

Discussion

The DH, Mw distribution, amino acid composition, and volatile component composition indicated that the MRP characteristics were directly related to the selection of enzymes. Enzyme characteristics are principal contributors to the hydrolysis process. Protamex hydrolyzes proteins into small molecular peptides or amino acids with a wide range of hydrolysis. Flavourzyme is used to modify or hydrolyze small molecules or other macromolecular components to improve the unpleasant taste of peptides (Xiao-min et al., 2009). Alcalase can hydrolyze not only peptide bonds but also amide bonds, ester bonds, trans esters, and trans peptides. Papain is a Cys enzyme that exhibits extensive enzymatic activity in proteins, short peptide chains, amino acid esters, and amide links, preferentially cleaving essential amino acids, especially Arg, Lys, and Phe residues (Su, Li, & Wei, 2014). The synergistic combinations of Flavourzyme with Alcalase or papain greatly increased the DH. The single enzymes can only cleave peptide bonds of specific types, preventing deep hydrolysis of the beef tallow residue. However, pretreatment with Flavourzyme exposed more cleavage sites, resulting in more contact between enzymes and proteins and an increase in the DH.

Compared with the single enzyme treatments, combining Flavourzyme with Alcalase or papain generated hydrolysates with higher free amino acid contents. Pretreatment with Flavourzyme may have changed the secondary structure of the protein in the hydrolysate, increasing the contact opportunities between the second enzyme and the protein to produce more free amino acids. It is well known that the flavor characteristics of MRPs are affected by free amino acid content and composition (Lorenzen, Davuluri, Adhikari, & Grun, 2005). Yu, Tan, and Wang (2012) reported that Lys could form 2-acetyl-1-pyrroline and alkylpyrazines, and Cys could form potent flavor compounds via the Maillard reaction. The bitter amino acids are His, Arg, Ser, Phe, Met, Ile, Leu, and Val, of which Met, Arg, and His have the strongest bitter flavor (Lan et al., 2010). Specific hydrophilic amino acid residues and Glu provide the umami flavor (Arai, Yamashita, & Fujimaki, 1972, Arai, Yamashita, & Noguchi, 1973). Kirimura, Shimizu, Kimizuka, Ninomiya, and Katsuya (1969) attributed the sour flavor to Glu-Leu, Gly-Asp, and Ala-Glu, among other peptides.

The Mw distribution of hydrolysates also contributes to flavor profiles. Ogasawara, Yamada, and Egi (2006) reported that Maillard peptides with 1,000–5,000 Da Mw provide special flavor. In this study, the FA and FP hydrolysates contained more low-Mw peptides than the other three hydrolysates and had better flavor profiles. High-Mw peptides, especially the Maillard peptides (Lan et al., 2010), increase due to the cross-linking of low-Mw peptides during the thermal reaction (Zamora & Hidalgo, 2005, Dondero, Figueroa, MoraleS, & Curotto, 2006). The taste and mouthfeel of the product can be improved by increasing the content of Maillard peptides. Our results demonstrated that as the DH increased, more high-Mw peptides were hydrolyzed to low-Mw components. These low-Mw peptides form Maillard peptides through the subsequent Maillard reaction to improve the flavor and taste of the MRPs.

Comparing the five beef tallow residue-derived MRPs, the P, F, and FA MRPs had a stronger caramel-like flavor, probably because they were rich in furan (Van Boekel, 2006). Furan is an important volatile component contributing to beef flavor. The F hydrolysate had a high furan content, which is consistent with the research results of Chiang et al. (2019). The F, FA, and FP MRPs were more bitter than the P and A MRPs due to the reduction in bitter amino acids such as Arg, Met, and

Table 3
Volatile compounds of five MRPs analysed by GC*GC–MS.

NO.	Compounds	RI	P	A	F	FP	FA
			Peak area (%)				
Alcohols							
1	1-Octen-3-ol	1448	$3.31^{b} \pm 0.03$	$3.94^{a} \pm 0.04$	$3.94^{a} \pm 0.09$	$3.22^{b} \pm 0.07$	$3.20^{b} \pm 0.21$
2	(E)-2-Octen-1-ol	1544	$1.43^{b} \pm 0.02$	$2.45^{b} \pm 0.02$	$2.55^{a} \pm 0.02$	$2.47^{b} \pm 0.01$	$2.46^{b} \pm 0.03$
3	1-Octanol	1559	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.72^{b} \pm 0.05$	$0.50^{b} \pm 0.07$	$2.14^{c} \pm 0.05$
Esters							
4	Ethyl palmitate	1322	$11.07^{a} \pm 0.15$	$9.68^{b} \pm 0.24$	$10.68^{a} \pm 0.05$	$9.70^{b} \pm 0.13$	$9.60^{b} \pm 0.27$
Aldehydes							
5	Heptanal	1087	$1.45^{d} \pm 0.20$	$0.68^{e} \pm 0.07$	$0.94^{b} \pm 0.03$	$1.88^{c} \pm 0.05$	$2.83^{a} \pm 0.20$
6	2,4-Hexadienal	1096	$0.00^{b} \pm 0.00$	$1.64^{a} \pm 0.09$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
7	Heptanal	1182	$1.17^{d} \pm 0.12$	$1.65^{a} \pm 0.15$	$1.38^{b} \pm 0.04$	$1.37^{b} \pm 0.13$	$1.66^{a} \pm 0.19$
8	Octanal	1265	$0.82^{a} \pm 0.02$	$1.37^{b} \pm 0.01$	$1.76^{c} \pm 0.07$	$1.74^{c} \pm 0.07$	$2.34^{d} \pm 0.25$
9	Nonanal	1395	$5.73^{e} \pm 0.33$	$7.49^{c} \pm 0.05$	$8.37^{b} \pm 0.24$	$10.13^{a} \pm 0.28$	$9.91^{d} \pm 0.28$
10	(E)-2-Octenal	1426	$7.16^{c} \pm 0.05$	$5.93^{d} \pm 0.03$	$6.38^{b} \pm 0.05$	$7.77^{a} \pm 0.05$	$5.94^{d} \pm 0.04$
11	Decanal	1497	$0.00^{c} \pm 0.00$	$1.44^{c} \pm 0.05$	$1.50^{c} \pm 0.06$	$1.66^{b} \pm 0.05$	$2.31^{a} \pm 0.05$
12	Benzene acetaldehyde	1518	$2.21^{b} \pm 0.20$	$2.70^{b} \pm 0.07$	$3.94^{a} \pm 0.06$	$5.67^{a} \pm 0.18$	$4.72^{a} \pm 0.19$
13	(E)-2-Nonenal	1531	$1.12^{a} \pm 0.18$	$0.92^{b} \pm 0.04$	$1.31^{a} \pm 0.15$	$1.16^{a} \pm 0.15$	$1.30^{a} \pm 0.18$
14	(E)-2-Decenal	1654	$1.22^{b} \pm 0.03$	$0.92^{c} \pm 0.11$	$1.62^{a} \pm 0.19$	$1.09^{b} \pm 0.20$	$1.58^{a} \pm 0.016$
15	2,5-dimethyl-Benzaldehyde	1683	$1.28^{a} \pm 0.07$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.12^{b} \pm 0.01$
16	(E,E)-2,4-Nonadienal	1778	$0.38^{a} \pm 0.02$	$0.50^{b} \pm 0.01$	$0.51^{b} \pm 0.02$	$0.76^{c} \pm 0.06$	$0.71^{c} \pm 0.07$
17	(E)-2-Undecenal	1861	$0.69^{c} \pm 0.02$	$0.57^{d} \pm 0.02$	$1.29^{a} \pm 0.05$	$0.82^{b} \pm 0.02$	$1.25^{a} \pm 0.05$
18	(E,E)-2,4-Decadienal	2001	$1.74^{b} \pm 0.02$	$2.44^{d} \pm 0.01$	$2.95^{a} \pm 0.01$	$2.60^{c} \pm 0.01$	$2.78^{b} \pm 0.02$
Acids							
19	Acetic acid	1435	$0.17^{d} \pm 0.01$	$0.37^{b} \pm 0.02$	$0.33^{c} \pm 0.02$	$0.32^{c} \pm 0.02$	$0.55^{a} \pm 0.02$
20	Pentanoic acid	1803	$0.00^{c} \pm 0.01$	$3.02^{a} \pm 0.02$	$0.23^{c} \pm 0.01$	$0.07^{d} \pm 0.02$	$2.23^{b} \pm 0.02$
21	Hexanoic acid	2050	$1.30^{b} \pm 0.08$	$2.63^{a} \pm 0.05$	$2.24^{b} \pm 0.07$	$1.78^{d} \pm 0.05$	$2.08^{c} \pm 0.11$
22	Ethyl ester Tetradecanoic acid	2056	$0.20^{b} \pm 0.01$	$0.28^{c} \pm 0.07$	$0.98^{d} \pm 0.02$	$0.19^{b} \pm 0.01$	$0.12^{a} \pm 0.02$
23	Decanoic acid	2276	$0.50^{a} \pm 0.05$	$0.39^{b} \pm 0.01$	$0.30^{c} \pm 0.01$	$0.37^{b} \pm 0.01$	$0.44^{a} \pm 0.03$
24	Nonanoic acid	2370	$0.31^{b} \pm 0.01$	$0.16^{a} \pm 0.03$	$0.40^{c} \pm 0.07$	$0.39^{c} \pm 0.07$	$0.52^{d} \pm 0.02$
Ketones							
25	6-methyl-5-hepten-2-one	1239	$0.77^{a} \pm 0.05$	$0.54^{b} \pm 0.01$	$0.67^{a} \pm 0.02$	$0.47^{c} \pm 0.01$	$0.44^{c} \pm 0.03$
26	Butyrolactone	1312	$0.63^{a} \pm 0.03$	$0.68^{a} \pm 0.04$	$0.61^{a} \pm 0.02$	$0.54^{b} \pm 0.03$	$0.51^{b} \pm 0.03$
27	2-Octanone	1324	$0.48^{c} \pm 0.01$	$1.02^{a} \pm 0.05$	$0.00^{d} \pm 0.00$	$0.84^{b} \pm 0.03$	$0.95^{a} \pm 0.02$
28	2-Heptanone	1396	$0.00^{c} \pm 0.00$	$1.15^{a} \pm 0.04$	$0.00^{c} \pm 0.00$	$0.17^{b} \pm 0.01$	$0.00^{c} \pm 0.00$
29	2-Nonanone	1401	$1.11^{b} \pm 0.08$	$0.89^{c} \pm 0.09$	$0.56^{d} \pm 0.04$	$1.40^{a} \pm 0.204$	$1.43^{a} \pm 0.07$
30	2-Decanone	1472	$2.51^{b} \pm 0.20$	$2.45^{b} \pm 0.05$	$2.17^{c} \pm 0.04$	$2.78^{a} \pm 0.05$	$2.68^{a} \pm 0.05$
31	(E,E)-3,5-octadien-2-one	1490	$1.27^{b} \pm 0.12$	$1.83^{a} \pm 0.04$	$1.84^{a} \pm 0.13$	$1.15^{b} \pm 0.11$	$1.22^{b} \pm 0.12$
Phenols							
32	2,4-Di-*tert*-butylphenol	2223	$1.41^{a} \pm 0.13$	$0.59^{b} \pm 0.03$	$0.44^{c} \pm 0.03$	$0.41^{c} \pm 0.03$	$0.57^{b} \pm 0.11$
Furan							
33	2-pentyl-Furan	1235	$3.19^{a} \pm 0.04$	$2.71^{c} \pm 0.04$	$3.31^{b} \pm 0.04$	$2.45^{d} \pm 0.03$	$3.73^{a} \pm 0.05$
34	2-hexylfuran	1321	$0.43^{c} \pm 0.02$	$0.04^{d} \pm 0.02$	$0.62^{b} \pm 0.01$	$0.42^{c} \pm 0.02$	$0.73^{a} \pm 0.04$
Pyrazine							
35	2-ethyl-6-methyl-Pyrazine	1372	$0.57^{b} \pm 0.05$	$0.71^{c} \pm 0.07$	$0.94^{a} \pm 0.09$	$0.40^{c} \pm 0.24$	$0.74^{d} \pm 0.22$
36	trimethyl-Pyrazine	1402	$0.87^{d} \pm 0.02$	$0.52^{b} \pm 0.01$	$0.48^{b} \pm 0.03$	$0.32^{a} \pm 0.01$	$0.76^{c} \pm 0.03$
37	2-ethyl-3,5-dimethyl-Pyrazine	1436	$0.86^{b} \pm 0.02$	$2.93^{c} \pm 0.03$	$1.12^{a} \pm 0.03$	$0.84^{d} \pm 0.02$	$2.56^{c} \pm 0.03$
38	tetramethyl- Pyrazine	1466	$0.17^{c} \pm 0.01$	$0.82^{a} \pm 0.02$	$0.02^{d} \pm 0.01$	$0.02^{d} \pm 0.01$	$0.54^{b} \pm 0.01$
Alkane							
39	2,6,10-trimethyl-Dodecane	1354	$0.00^{d} \pm 0.00$	$0.43^{b} \pm 0.01$	$0.29^{c} \pm 0.01$	$0.87^{a} \pm 0.01$	$0.27^{c} \pm 0.01$
40	Tetradecane	1397	$3.66^{a} \pm 0.20$	$2.65^{c} \pm 0.04$	$1.15^{d} \pm 0.07$	$3.29^{b} \pm 0.09$	$1.21^{d} \pm 0.04$
41	Dodecane	1592	$1.21^{a} \pm 0.15$	$0.43^{c} \pm 0.03$	$1.01^{b} \pm 0.03$	$1.49^{a} \pm 0.23$	$1.15^{a} \pm 0.20$
42	Hexadecane	1600	$0.62^{b} \pm 0.02$	$0.68^{b} \pm 0.03$	$0.92^{a} \pm 0.03$	$0.43^{c} \pm 0.02$	$0.61^{b} \pm 0.02$
43	Tridecane	1768	$0.00^{d} \pm 0.00$	$1.17^{b} \pm 0.05$	$1.01^{c} \pm 0.06$	$0.00^{d} \pm 0.00$	$1.38^{a} \pm 0.08$
44	Nonadecane	1900	$1.93^{a} \pm 0.12$	$0.64^{d} \pm 0.03$	$0.78^{c} \pm 0.02$	$1.08^{b} \pm 0.11$	$1.01^{b} \pm 0.11$
Aromatic compound							
45	Naphthalene	1189	$1.32^{a} \pm 0.08$	$0.23^{c} \pm 0.01$	$0.03^{d} \pm 0.01$	$0.35^{b} \pm 0.03$	$0.25^{c} \pm 0.01$
46	1-chloro-4-(1-methylethenyl)-Benzene	1603	$0.70^{a} \pm 0.02$	$0.07^{b} \pm 0.01$	$0.06^{b} \pm 0.01$	$0.05^{b} \pm 0.01$	$0.00^{c} \pm 0.00$
47	methoxy-phenyl-Oxime	1846	$3.24^{a} \pm 0.09$	$0.59^{c} \pm 0.03$	$0.36^{d} \pm 0.01$	$0.47^{d} \pm 0.02$	$1.16^{b} \pm 0.06$

Values bearing different lowercase letters (a, b, c, d and e) were significantly different ($p \leq 0.05$).

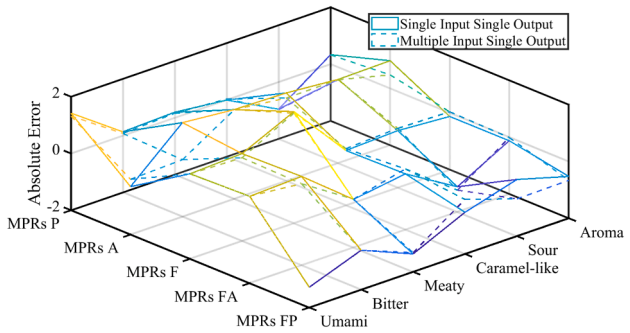

Fig. 3. The absolute error between the prediction sensory score of the model and the real score. The active line represents the error of SISO model, while the dotted line represents the MISO model.

His, and the cross-linking of peptides with Mw < 1,000 Da. The FP MRPs had the highest umami characteristics, which can be attributed to the high Glu content (Vinther Schmidt, Olsen, & Mouritsen, 2021). In the current study, the FA MRPs had the highest sour taste because they had the highest acetic acid content and a high acid peptide precursor content. The FP MRPs had the highest aromatic flavor score due to the high aldehyde content. Many aldehydes are produced by lipid oxidation; for example, unsaturated aldehydes degrade phenylalanine to the corresponding Strecker aldehyde (benzene acetaldehyde) via lipid oxidation, resulting in increased phenylacetaldehyde content (Zamora, Gallardo, & Hidalgo, 2007). Volatile components, such as 1-octanol, were only present in the samples hydrolyzed with Flavourzyme. Flavourzyme is a mixture of endoprotease and exoprotease, which can hydrolyze the peptide bonds within polypeptides and those from the *N*-terminal or C-terminal, resulting in the production of unique volatile compounds (Zhang et al., 2017). The sensory evaluation scores significantly differed between the five MRPs due to the differences in free amino acid composition, Mw distribution, and volatile component composition; a relationship between the sensory evaluation score and the related flavor substances was detected.

It has been demonstrated that the contents of heterocyclic compounds of sulfur, nitrogen, and their derivatives in food are related to meat flavor (Song et al., 2013). Sulfur compounds like Cys have been demonstrated to influence meaty flavor, but only a small amount of Cys was detected in these five MRPs, consistent with the research results of Song et al. (2016). These findings suggest that even a small amount of sulfur compounds can produce a sufficient meaty flavor. The F MRPs were rich in these essential meaty flavor compounds, but the FP MRPs had the highest meaty sensory score. The possible reason might be that the F MRPs were high in furans and pyrazines, which have strong caramel-like characteristics that could overpower the meaty flavor. Of the pyrazines detected, 2-ethyl-3,5-dimethylpyrazine was the most abundant. Ala is a significant precursor of aroma-related trialkyl pyrazines such as 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine (Cerny & Grosch, 1994). 2-Pentyl-furan is a typical furan compound produced during frying, which is degraded by hydroperoxide generated from the oxidation of linoleic acid (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003). Moreover, the five MRP samples all contained alkanes such as tetradecane and dodecane. Although alkanes did not provide the main flavor of beef tallow residue-derived MRPs, due to the high content, they were important components of the MRPs.

Yu, Low, and Zhou (2018) investigated the influence of the chemical composition of green tea beverages on consumer preferences. The results showed that the quality of the neural network model was higher than that of the linear partial least squares (PLS) regression model. This phenomenon can be explained by the improved nonlinear representation ability of the ANN; each neuron in the ANN can be seen as a PLS regression model. Additionally, the beef tallow residue MRPs sensory characteristic scores can be predicted using the ANN model built in this study, which could reduce the waste of human and financial resources brought by sensory evaluation. Similar research on ready-to-drink green tea beverages and titratable acid content has been conducted by Yu et al. (2018) and Huang et al. (2021), respectively. Compared with them, the model built in this study has higher accuracy. The model output lies within a reasonable range because an output processing method called the multiple-model mechanism was adopted when building the model. An ANN was also used to predict the sensory quality of Ultra High Temperature milk in Singh et al. (2009). The accuracy of the trained prediction model was higher than the model used in this study. This is because the problem in that studycan be seen as a simple linear model, while the problem in this study is much more complex.

In addition, research on the effects of volatile component contents on flavor has revealed a strong connection with the corresponding sensory evaluation (Shiqing et al., 2016). However, a compound that has a strong influence on one flavor may also react with other substances. That is, other volatile components in the food may also contribute to the sensory evaluation score. Moreover, sensory evaluation scores are objective, and one flavor may influence the score of another flavor, which is difficult to express using conventional prediction methods. We explored these contributions by building a SISO model and a MISO model. The SISO model only incorporates the relationship between flavor substances and sensory evaluation scores, whereas the MISO model also includes the interactions and masking effects among various flavors. The experimental results indicated that the MISO model had a better performance than the SISO model. These results suggest that the impact of the corresponding component on the score was the most important factor for predicting the score, and the other components were only had the modification function. Meanwhile, the accuracy of the sour flavor scores was lower in the MISO model than in the SISO model. The reason may be that the sour flavor was strong and not easily covered by other flavors. In addition to the sour flavor scores, the average values of the other flavors also slightly increasedcompared to SISO.

Conclusions

This study clearly showed that synergistic hydrolysis using Flavourzyme and papain could effectively increase the DH, free amino acid content, and low-Mw component contents of beef tallow residue-derived hydrolysates, and the subsequent Maillard reaction could improve the flavor. After the sugars and amino acids were cross-linked by the Maillard reaction, the beef tallow residue hydrolyzed with Flavourzyme and papain had the best meaty, aromatic, and bitter characteristics. The GC × GC–MS results revealed that compared with the MRPs of the hydrolysates prepared using a single enzyme, the MRPs of the hydrolysates prepared via synergistic hydrolysis using two enzymes had significantly increased volatile compound contents. Moreover, the ANN had high predictability for the flavor of the MRPs. Therefore, beef tallow residue-derived hydrolysates prepared with Flavourzyme and papain, followed by the Maillard reaction, can be used to produce beef flavor, and the ANN can be used to predict the flavor of the beef tallow residue-derived MRPs.

CRediT authorship contribution statement

Jingwei Cui: Conceptualization, Data curation, Methodology, Formal analysis, Visualization, Investigation, Software, Validation. **Yinhan Wang:** Data curation, Methodology, Formal analysis, Visualization, Investigation, Software, Validation. **Qiaojun Wang:** Funding acquisition, Project administration. **Lixue Yang:** Funding acquisition, Project administration. **Yiren Zhang:** Funding acquisition, Project administration. **Emad Karrar:** . **Hui Zhang:** Funding acquisition, Project administration. **Qingzhe Jin:** Funding acquisition, Project administration. **Gangcheng Wu:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Funding

acquisition, Project administration. **Xingguo Wang:** Funding acquisition, Project administration.

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

No data was used for the research described in the article.

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Appendix A. Supplementary data

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