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Contribution to aroma characteristics of mutton process flavor from the enzymatic hydrolysate of sheep bone protein assessed by descriptive sensory analysis and gas chromatography olfactometry

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ABSTRACT

Changes in the aroma characteristics of mutton process flavors (MPFs) prepared from sheep bone protein hydrolysates (SBPHs) with different degrees of hydrolysis (DH) were evaluated using gas chromatography–mass spectrometry (GC–MS), gas chromatography–olfactometry (GC–O), and descriptive sensory analysis (DSA). Five attributes (muttony, meaty, roasted, mouthful, and simulate) were selected to assess MPFs. The results of DSA showed a distinct difference among the control sample MPF0 and other MPF samples with added SBPHs for different DHs of almost all sensory attributes. MPF5 (DH 25.92%) was the strongest in the muttony, meaty, and roasted attributes, whereas MPF6 (DH 30.89%) was the strongest in the simulate and roasted attributes. Thirty-six compounds were identified as odor-active compounds for the evaluation of the sensory characteristics of MPFs via GC–MS–O analysis. The results of correlation analysis among odor-active compounds, molecular weight, and DSA further confirmed that the SBPH with a DH range of 25.92–30.89% may be a desirable precursor for the sensory characteristics of MPF.

1. Introduction

Mutton is widely consumed and increasingly popular in Western countries because of its nutritional and special flavor qualities [1]. Clearly, opportunities exist for the expansion of the mutton market. Meanwhile, much attention has been given to the development of simulated mutton flavor to meet different consumer demands (e.g., meatless products with special meat flavor) and different applications in food.

Abundant information on meat flavors from beef, chicken, pork, and other domestic red meat species is available. However, published data, particularly on mutton flavor, are limited. Although a few papers described the volatile compounds of cooked goat meat [2–4], the effect of precursors on mutton flavor formation remains unknown. No study that describes simulated mutton flavor has been found in the literature.

Meat flavor precursors can be divided into two categories: water-soluble components and lipids [5]. The former provides the "basic meat flavor" through Maillard reaction and, together with the latter, imparts the "characteristic flavor" through the coordination of Maillard reaction and lipid oxidation. In general, water-soluble precursors include free amino acids, peptides, and reducing sugars [6]. The different compositions of these precursors, which can be derived by controlling the degree of hydrolysis (DH), generate a significant difference in flavor because of the different levels of volatile formation under thermal reaction conditions [2]. Therefore, precursors play an important role in thermal reaction flavor. To obtain desirable flavor precursors, especially free amino acids and peptides, enzymatic proteolysis of the protein from various sources has been studied extensively. For many years, enzymatic proteolysis of hydrolyzed vegetable protein (HVP) and meat protein has been used to produce precursors of meat flavor [7–9]. Moon et al. [10] showed that simulated beef flavor could be obtained by adding soy protein isolates. These simulated meat flavors were favored by vegetarian and health-conscious consumers. Song et al. [11] reported that beef base prepared by beef hydrolysis with a suitable DH is useful in accentuating or extending the characteristic meat flavor. However, meat flavors based on HVP only partially simulate natural meat flavor and, from an economic point of view, meat flavor based on meat protein has negligible economic advantage. In contrast to the above two sources, enzymatic proteolysis of the protein of meat by-products does not only simulate realistic meat aroma but also reduce costs and increase their value.

As one of the by-products of the mutton processing industry, sheep bone is a rich resource of protein, amino acids, and other useful biological substances. In a previous study, sheep bone has

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a similar of protein and amino acid content to mutton. Collagen and chondroitin, which comprise approximately 90% of the protein in sheep bone, have higher physiological activities than common peptides. They could be converted into collagen peptides and amino acids by moderate enzymatic hydrolysis [12]. These moderate enzymatic hydrolysates can be used as the precursors for preparing mutton process flavors. Methods for controlling the DH to accomplish moderate enzymatic hydrolysis require further investigation. Therefore, the effects of the different compositions of amino acids and the different molecular weight (MW) distribution of peptides on the aroma characteristics of simulated mutton flavor should be determined. However, systematic studies on the effects of bone hydrolysate with different DHs on the aroma characteristics of simulated mutton flavor are lacking.

The primary objectives of this study are to evaluate the changes in the aroma characteristics of mutton process flavors (MPFs) prepared from sheep bone protein hydrolysates (SBPHs) with different DHs using descriptive sensory analysis (DSA) and to analyze the corresponding volatile odor-active compounds by gas chromatography–mass spectrometry–olfactometry (GC–MS–O). Through a correlation analysis of the DSA data, odor-active compounds, and MW of peptides, desirable SBPHs with the appropriate DH were recommended for mutton flavor, aiming to produce a desirable precursor for the sensory characteristics of mutton flavor. This study also aims to change waste products into useful materials and increase the utilization of sheep bone protein. The results of this study serve as a theoretical basis for the utilization of livestock bone.

2. Materials and methods

2.1. Materials

Lean mutton and sheep bone were purchased from Wal-Mart supermarket in Wuxi, China. HVP was provided by Tianning Flavor Fragrance Co., Ltd. (Shanghai, China). Serial n-alkanes (C_6–C_{26}), L-cysteine, alanine, glucose, thiamine, and taurine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). I + G (5′-IMP:5′-GMP = 50%:50%) was provided by Guangdong Dinghu Biotechnology Co. (Guangdong, China). Refined suet was purchased from Tianjin Tianyuan Oil and Fats Co., Ltd. (Tianjin, China). Alcalase and Flavorzyme were provided by Novozymes (Bagsvaerd, Denmark). Pure standards hexanol, heptanol, sulfurol, hexanal, heptanal, octanal, nonanal, benzaldehyde, acetic acid, pentanoic acid, hexylic acid, nonanoic acid and decanoic acid were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pure standards decanal, tetradecanal and 2-tridecanone were purchased from Tokyo Chemical Co. (Tokyo, Japan). Pure standards (E,E)-2,4-octadienal, (E,E)-2,4-dodecadienal, 12-methyltridecenal, 4-methylnonanoic acid, butyl 2-decenoate, 2,3-dimethyl pyrazine and benzyl methyl sulfide were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

2.2. Sample preparation

2.2.1. Preparation of SBPH

Fresh sheep bones were washed with tap water to clean the bloodstain and other impurities and autoclaved at 121 °C for approximately 4 h. The attached meat, fat, bone marrow, and fascia were removed, and the bones were washed repeatedly in hot water to eliminate residual fat. Subsequently, the bones were dried in an oven at 55 °C for 5 h and then ground into powder (80 orders) using a grinder. Bone powder (20 g) was mixed with 80.0 mL of deionized water. Afterward, the mixture dispersion was heated at 95 °C for 10 min to deactivate endogenous enzymes and denature bone protein. Enzymatic hydrolysis of bone powder was performed in two steps. Alcalase was utilized in the first step, and Flavorzyme was used in the second step. Hydrolysis was conducted using Alcalase at 55 °C, pH 8.5 (1.0 mol/L NaOH) with an E/S of 0.20 (LAPU/g based on protein content) for 2 h and then treated at 50 °C using Flavorzyme with an E/S of 0.20 (LAPU/g protein content). The pH was adjusted to 7.0 using HCl (1.0 mol/L) for 1, 2, 3, 4, 5, 6, and 7 h to prepare SBPHs with different DHs. After hydrolysis, the reactions were terminated by immersing the reaction vessel into a 95 °C water bath for 10 min with stirring to ensure the inactivation of the enzyme. The sample was then centrifuged at 4000 rpm for 20 min to remove the insoluble residue. The supernatant liquid was used for further analysis.

2.2.2. Preparation of MPF

A mixture of HVP (0.5 g), L-cysteine (1 g), glucose (0.5 g), thiamine (0.5 g), taurine (0.5 g), alanine (1 g), I + G (0.5 g), and oxidized suet (4 g) was dissolved in 81.5 g solution of SBPH. The solution was transferred into 50 mL screw-sealed tubes. The pH was adjusted to 6.5 with 6 mol/L NaOH, and the tubes were tightly capped and then heated in a thermostatic oil bath with magnetic stirring (150 rpm) at 120 °C for 120 min. After the reaction, the tubes were immediately cooled in ice-water, and the thermal reaction products named MPFs were obtained for further use.

2.3. Analysis of SBPH properties

2.3.1. MW distribution

To quantify the MW distribution of the hydrolysates, the SBPH samples were analyzed in triplicate using liquid chromatography. The SBPH samples were centrifuged at 3500 rpm for 30 min, and the supernatant liquid was stored at 4 °C prior to injection.

The MW distribution of the hydrolysate was determined using a Waters™ 600E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA) with a 2487 UV detector and Empower work station. A TSK gel, 2000 SWXL (300 mm × 7.8 mm) column (Tosoh Co., Tokyo, Japan) was used with 0.1% trifluoroacetic acid and acetonitrile (45:50) as the mobile phase. The flow rate was 0.5 mL/min. The column temperature was 30 °C, and 10 μL of sample was injected into the HPLC system. A MW calibration curve was obtained using 0.5 mg/mL each of the following standards from Sigma Chemical Co. (St. Louis, Mo, USA): cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYR (451 Da), and tripeptide GGG (189 Da). SBPH samples prepared according to the above-mentioned method were diluted 25 times with water and filtered by microporous filtering film before injection. Absorbance was monitored at 220 nm. The results were obtained and processed with the aid of Millennium 32 Version 3.05 software.

2.3.2. Amino acid analysis

An appropriate pretreatment of the SBPH samples was conducted before free amino acid analysis. An equivalent volume of trichloroacetic acid was added to the SBPH sample to precipitate the peptides and proteins. After incubation for 2 h at room temperature, the solution was filtered through Whatman filter paper No. 4. The filtrate was centrifuged at 7000 × *g* for 10 min, and the supernatant liquid was stored at 4 °C before injection.

The amino acid composition of the sheep bone protein was determined by RP-HPLC (Agilent 1100, Palo Alto, CA, USA) with a UV detector operated at 338 nm using a Hypersil ODS C18 column (4 mm × 125 mm, Thermo Co., NY, USA). The mobile phase consisting of 20 mM sodium acetate and 1:2 (v/v) methanol–acetonitrile was delivered at a flow rate of 1 mL/min. The column temperature was 40 °C, and 1 μL of sample was injected into the HPLC system. A calibration curve was obtained using 0.25–2 mM standard amino

acid mixture (Sigma Chemical Co., St. Louis, MO, USA). Quantification was performed based on the retention time and peak area of the standard compounds.

2.4. Analysis of MPF properties

2.4.1. Sensory analysis

DSA was conducted by adopting the method of Moon et al. [10] to obtain data describing the sensory attributes of MPF samples by eight well-trained panelists (four males/four females, aged 26 years to 48 years). The selection criteria for the panelists were availability and motivation to participate on all days of the experiment. All panelists passed the screening tests according to ISO standards [13]. Moreover, sensory analysis was conducted in panel booths at the university sensory laboratory that conform to international standards [14]. Four specific training sessions were performed. In the first session, the panelists discussed MPF aroma characteristics for sensory attributes. In the second and third sessions, the panelists were trained to reach consensus on the aroma descriptors and then to assess five selected aroma attributes (muttony, meaty, simulate, roasted, and mouthful) for further descriptive analysis. Finally, the samples were evaluated in triplicate using a 10-point interval scale (1 = none, 10 = extremely strong). The MPF samples were coded with random three-digit numbers and randomly presented for each panelist to avoid causing the so-called order effect. The reference materials [15] were as follows: pot roast (approximately 200 g mutton, wrapped in aluminum foil and baked for 1 h at 150 °C) was labeled "mutton-like" attribute; lean mutton (0.5 kg, 2.0 cm thick, purchased from Wal-Mart supermarket) prepared by removing all separable fat and then boiled in water for 2 h was labeled "meat-like" aroma; stewed mutton in Brown Sauce product (purchased from Wal-Mart supermarket) with a similar degree of aroma was labeled "simulate" attribute; 10 g mutton broth cube (Qiangwang Seasoning Food Co., Anhui, China, mutton flavor consisting of MSG, yeast extract, and mutton extract) dissolved in water was labeled "mouthfulness" attribute; ground roast coffee (Maxwell House Coffee Co., Kraft General Foods, Inc., White Plains, NY, USA) was labeled "roasted" attribute.

2.4.2. GC/MS analysis

To identify and quantify volatile odor-active compounds, the MPF samples were analyzed in triplicate using GC. Homogenized MPF sample (3 g) was distributed evenly in 15 mL headspace vials with a magnetic stirring bar. An internal standard, 1,2-dichlorobenzene (4 μL, 0.555 μg/μL in methanol), was added to each sample prior to trapping. The vial was sealed with a PTFE/BYTL septum and equilibrated at 55 °C for 30 min while exposed to SPME fiber (75 μm carboxen/polydimethylsiloxane) in the sample headspace. The fiber was transferred to the injection port of the gas chromatograph (Finnigan Trace GC–MS, Finnigan, USA), desorbed at 250 °C for 2 min, and then operated in splitless mode. The selection of desorption time (2 min) and desorption temperature (250 °C) was based on the principle of improving peak shape and sensitivity while reducing carryover from the previous analysis.

The compounds were separated on a DB-WAX column (J&W Scientific Inc., Folsom, CA, USA) 30 m in length, 0.25 mm in internal diameter, and 0.25 μm in film thickness. Helium (99.999%) was used as carrier gas at a constant velocity of 1.8 mL/min. The temperature program started at 40 °C for 3 min, increased at 6 °C/min to 80 °C, then at 10 °C/min to 230 °C with a final hold time of 10 min. Oven temperature was determined according to increasing reliability in terms of number of peaks and peak area. Peak width and initial threshold were set to 0.04 and 1, respectively. Peaks with peak areas below 10,000 were not considered reliable, and peaks with retention times of more than 35 min were deleted because they are high-boiling compounds with no aroma. The compounds were analyzed by MS. MS spectra were obtained in the electron impact mode with an energy voltage of 70 eV, an ion source temperature of 230 °C, and an emission current of 35 mA. The quadrupole mass filter was operated at 150 °C. The transfer line temperature was 250 °C. A mass range of 35 m/z to 450 m/z was recorded at 4.45 scan/s.

Volatile compounds were identified by comparing Kovats retention indices (KI) and MS fragmentation patterns with those of reference compounds or with mass spectra in the Wiley and NIST Library together with their retention indices. The KI values were calculated according to the method of Song et al. [15]. Additionally, the identities of several key active-odor components were confirmed through comparison with pure standards. The relative concentration of volatile compounds in the MPF samples was calculated based on the internal standard.

2.4.3. GC/O analysis

GC–O was performed using a Finnigan Trace GC equipped with a flame ionization detector (FID) and an OP275 sniffing port (GL Sciences Inc., Japan). The effluent from the capillary column was split 1:1 (v/v) between the FID and the sniffing port, using deactivated and uncoated fused silica capillaries as transfer lines. The sniffing cone was purged with humidified air to help maintain olfactory sensitivity by reducing the dehydration of the mucous membranes in the nasal cavity. A DB-wax column was used for analysis. Nitrogen was used as the carrier gas at a constant flow rate of 2.1 mL/min. The temperature program of the oven started at 40 °C for 3 min and then raised to 230 °C at a rate of 6 °C/min with a final hold time of 5 min.

To obtain the odor profile of the MPF samples, the eight panelists participated in a detection frequency method performed according to Niu et al. [16].

2.5. Statistical analysis

Sensory data from descriptive analysis were assessed by analysis of variance (ANOVA) using SPSS version 20.0. ANOVA with a Duncan's multiple comparison test was performed to determine the differences among individual MPF samples for each sensory attribute.

An overview of potential correlations among the samples, sensory attribute data, odor-active volatile compounds, and peptide MW was obtained through partial least square regression (PLSR) using Unscrambler X version 9.6 (CAMO ASA, Oslo, Norway). PLS1 and PLS2 models were calculated for comparison. PLS2 showed the relationship among the samples, sensory attributes, odor-active compound data, and peptide MW, and PLS1 showed the correlation between individual sensory attribute and GC/MS/O profiles or MW. All variables were centered and scaled to 1/Sedv to make each variable have a unit variance and zero mean before applying PLSR analyses and to obtain an unbiased contribution of each variable to the criterion. Regression coefficients were analyzed by modified jack-knifing [17], which allows the determination of uncertainty limits that correspond to ±2 standard uncertainties under ideal conditions. Based on the above information, the significance ($p < 0.05$) of the variable relationship in matrices X and Y were determined. All regression models were validated using full cross-validation.

3. Results and discussion

3.1. Analysis of the MW distribution and amino acid composition of SBPH

SBPHs were prepared according to the method described above. Seven samples were hydrolyzed under different conditions, and

Table 1
Changes of molecular weight (MW) distribution (percent of total area) in different SBPHs.

Sample	MW (Da)				
	>5000	1000–5000	500–1000	180–500	<180
SBPH1[a]	0.04 ± 0.01[b]	16.99 ± 0.02	27.36 ± 0.11	47.85 ± 0.31	7.76 ± 0.21
SBPH2	0.01 ± 0.00	10.60 ± 0.22	25.81 ± 1.24	56.44 ± 2.01	7.14 ± 0.18
SBPH3	–[c]	7.65 ± 0.05	21.74 ± 0.33	58.49 ± 0.09	12.12 ± 0.22
SBPH4	–	3.84 ± 0.12	15.86 ± 0.10	66.70 ± 0.18	13.60 ± 0.05
SBPH5	–	2.62 ± 0.18	11.17 ± 0.05	58.02 ± 0.07	28.19 ± 0.06
SBPH6	–	2.92 ± 0.09	11.32 ± 0.02	60.40 ± 0.23	25.35 ± 0.43
SBPH7	–	1.12 ± 0.05	7.54 ± 0.13	54.20 ± 3.08	37.15 ± 1.22

[a] Seven samples were denoted by SBPH followed by one-digit Arabic numbers, where "SBPH" represents sheep bone enzymatic hydrolysate and Arabic numbers 1–7 denote DH 10.06%, 14.09%, 18.15%, 22.18%, 25.92%, 30.29%, and 35.16%, respectively.
[b] Mean ± standard deviation (average of triplicate).
[c] None.

the corresponding DH values were 10.06%, 14.09%, 18.15%, 22.18%, 25.92%, 30.29%, and 35.16%, respectively. Each sample was further investigated.

The MW distribution of the hydrolysates, including peptides and free amino acids, can have a direct or indirect effect on the properties of MPFs, such as sensory perception and quality of the Maillard thermo reaction flavorings [11].

Peptides from hydrolysis could be used as a precursor [9] and also contribute directly to volatile formation under thermal reaction conditions. In addition, certain amino acids in the bound form of peptides could form Strecker aldehydes through Strecker degradation. The peptides in SBPHs also vary with the changes in the DH values (Table 1). Peptides lower than 180 Da (equal to dipeptides or free amino acids) in sheep bone protein gradually increased with increasing DH except for sample SBPH6. By contrast, the tendency of the peptides from 500 Da to 1000 Da and 1000 Da to 5000 Da was converse to that of the peptides lower than 180 Da, which showed a downward trend except for SBPH6. However, no apparent tendency was found in the peptides from 180 Da to 500 Da, equal to tetra-, tri-, or dipeptides, with the changes in DH values. In addition, peptides greater than 5000 Da decreased with increasing DH. When the DH reached 18.15%, no peptide greater than 5000 Da was found.

Meanwhile, free amino acids from hydrolysis also serve as important precursors in the thermal generation of an extensive range of characteristic meaty and roasty aroma compounds associated with cooked meat flavor [2,18]. The total free amino acid in SBPHs gradually increased from 3.7784 mg/g to 32.7527 mg/g dry basis with increasing DH (Table 2). In all samples except for SBPH1 (glycine, aspartic acid), arginine was the most abundant amino acid. This amino acid increased from 0.4396 mg/g to 6.1285 mg/g dry basis. The other abundant amino acids found in SBPHs are leucine, valine, glycine, lysine, alanine, and phenylalanine. Threonine, tyrosine, glutamic acid, and cysteine were also abundant in the samples. The concentrations of most of the free amino acids were similar to the values reported for goat meat [2]. These amino acids may play important roles in the aroma quality of MPFs because different contents of all individual free amino acids may generate different levels of volatile compounds through Strecker and Maillard reactions and through thermal decarboxylation and deamination reactions. For example, the potent odorants pyrazine and 2,3-dimethyl pyrazine, which are widely used in imitating cooked meat flavors to impart roasted and meaty attributes [19], can arise from the Strecker degradation of cysteine [20] and the thermal reactions of lysine [21] and glycine [22], and phenyl acetaldehyde and benzaldehyde of leucine, phenylalanine and methionine [23].

Table 2
Changes of amino acid composition (mg/g) in different SBPHs.

Amino acid[a]	Sample						
	SBPH1[b]	SBPH2	SBPH3	SBPH4	SBPH5	SBPH6	SBPH7
Asp	0.4527c	0.4439b	0.4605d	0.4278a	0.4828e	0.5582f	0.5979g
Glu	0.3524a	0.4631b	0.5376c	0.9546d	1.0916e	1.2177f	1.5928g
Ser	0.0205b	0.0107ab	0.0144ab	0.0002a	0.0223b	0.0585c	0.8144d
His	0.1320a	0.2139b	0.6324e	0.4996cd	0.4963c	0.5560d	0.7340f
Gly	0.6014a	0.7942a	1.0612b	1.7553c	2.0530d	2.3485e	2.5922e
Thr	0.3838ab	0.4953b	0.3641a	0.3437a	0.7972c	1.5382d	1.9389e
Arg	0.4396a	1.3880b	2.4070c	2.8069d	3.4092e	4.6897f	6.1285g
Ala	0.1360a	0.3437b	0.6761c	1.3774d	1.6031e	1.7479f	2.2169g
Tyr	0.1744a	0.3360b	0.5916c	0.9322d	1.2201f	1.1611e	1.6042g
Cys-s	0.1067a	0.2196b	0.3743c	0.5160d	0.5806e	0.6467f	1.0094g
Val	0.1974a	0.2991b	0.8089c	1.5186d	2.2006e	2.2355e	2.8846f
Met	0.1286a	0.1685b	0.2859c	0.4552d	0.4972e	0.5664f	0.7557g
Phe	0.2562a	0.4523b	0.6858c	1.2478d	1.3483e	1.5209f	2.0353g
Ile	0.1157a	0.1895b	0.2758c	0.4939d	0.6319e	0.7518f	0.9184g
Leu	0.0135a	0.4812c	1.7968f	0.9107e	0.9072d	0.0308b	4.2550g
Lys	0.2542a	0.4923b	0.8074c	1.1744d	1.5722e	1.9435f	2.5093g
Pro	0.0133a	0.4052f	0.5195g	0.0982b	0.3832e	0.1099c	0.1653d
Totals[c]	3.7784	7.1964	12.2993	15.5126	19.2959	24.7266	32.7527

Numbers with different letters (a–g) are significantly different from one another.
[a] Abbreviation of amino acid: Asp, aspartic acid; Glu, glutamic acid; Ser, serine; His, histidine; Gly, glycine; Thr, threonine; Cys-s, cysteine; Val, valine; Met, methionine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; Lys, lysine; Pro, proline.
[b] Six samples were denoted by the SBP followed by one-digit Arabic numbers, where "SBPH" represents for sheep bone enzymatic hydrolysate and Arabic numbers 1–7 denote DH 10.06%, 14.09%, 18.15%, 22.18%, 25.92%,30.29% and 35.16%, respectively.
[c] Total free amino acids of different SBPHs.

Table 3
Results of Duncan's multiple comparison test on mean sensory scores of eight MPF samples for the five attributes (mean scores).[a]

Sample[b]	Muttony	Meaty	Simulate	Roasted	Mouthful
MPF0	4.48a	2.28a	2.98a	5.70b	3.15a
MPF1	5.58b	3.65b	3.44b	6.67d	4.53b
MPF2	6.00c	5.70c	4.14c	7.49e	4.99c
MPF3	6.91d	6.60d	5.84d	6.57d	5.51d
MPF4	7.79f	7.17e	5.59d	5.72b	6.72e
MPF5	8.55g	8.51f	7.14e	8.46f	7.38f
MPF6	7.34e	5.78c	8.80f	6.23c	7.65g
MPF7	6.74d	6.45d	5.55d	5.29a	5.76d

[a] Mean scores (listed in ascending order) for each attribute within a column with different letters (a–g) are significantly different ($p < 0.05$) using Duncan's multiple comparison test ($n = 24$; eight panelists with three replications).
[b] Eight mutton-like process flavors were denoted by the MPF followed by one-digit Arabic numbers. 0–7, which represent samples prepared from without SBPH and with the DH 10.06%, 14.09%, 18.15%, 22.18%, 25.92%, 30.29%, and 35.16% of SBPH, respectively.

Additionally, Madruga et al. [2] revealed a subtle difference between the roasted aromas of two meats because of the relatively high amount of glycine in goat meat.

3.2. Sensory analysis of the MPF samples

The eight MPF samples were prepared without SBPH and with different DHs. Three replicates were applied to the sensory data to access the results. The mean scores of each sample for the five attributes and the results of Duncan's multiple comparison tests are shown in Table 3.

As shown in Table 3, the ranges of the eight MPF samples between the lowest and the highest mean scores for the muttony (4.07), meaty (6.23), simulate (5.82), and mouthful (4.50) attributes were relatively larger compared those for the roasted (3.17) attribute. These results may confirm a significant difference between the control sample MPF0 and other MPF samples for the muttony, meaty, simulate, and mouthful attributes, and less for the roasted attribute. MPF1 was strong in the roasted attribute but relatively weak in the muttony, meaty, simulate, and mouthful attributes. Similar results were found in MPF2, which was also strong in the roasted attribute. MPF3 and MPF4 were strong in all five sensory attributes. Compared with the other MPF samples, MPF5 showed very strong muttony, meaty, roasted attributes, whereas MPF6 showed very strong simulate and roasted attributes. In contrast to the MPF samples with SBPHs, MPF0 only showed relatively strong intensity in the roasted attribute and had very weak contribution to the other four sensory attributes. This finding is in agreement with the results of Simpson et al. [24], who presented the higher aroma quality of enzymatic samples against fresh sample extract. This finding may be attributed to the increase in free amino acid level from the enzymatic hydrolysis. Similarly, MPF samples with additional SBPHs would generate higher levels of volatile compounds from the free amino acids through Strecker and Maillard reactions and through thermal decarboxylation and deamination reactions. These results are again consistent with the fact that mutton flavor only based on MPF0 or pure amino acids and HVP could not simulate natural mutton aroma. Moreover, the five attributes gradually intensified as DH increased from 25.92% to 30.29%. These results indicated that MPFs with different DHs produced varied levels of volatile compounds and may generate different intensities for individual sensory attribute.

3.3. GC/MS/O analysis of the MPF samples

The volatile compounds of the MPF samples were separated and detected on a DB-WAX column. More than 100 peaks were observed in the GC–MS profiles, including 10 alcohols, 26 aldehydes, 8 ketones, 4 thiophenes, 3 thiazoles, 9 alkanes, 12 carboxylic acids, 3 furans, 4 esters, 5 pyrazines, 2 pyrroles, 2 alkenes, 2 phenols, 3 sulfur compounds, 1 pyran, and 7 unknown compounds. However, only 82 peaks were detected in at least two MPF samples except for MPF0.

Some of the compounds might have no contribution to the aroma characteristics of MPFs. Furthermore, no single character impact compound has been identified for either authentic meat or simulated meat flavor [10], and many volatiles from different components existing in specific quantitative proportions were found responsible for the meat flavors. Therefore, to evaluate the effects of SBPHs with different DHs on the formation of the flavor characteristics of MPFs, the selection of specific compounds from the 82 compounds in the MPFs to represent the mutton-like attributes in samples and qualification might be important. To qualify as a specific compound, the compounds should be positively correlated ($p \leq 0.05$) with sensory attributes in DSA. A correlation analysis was conducted between the DSA results and the 82 compounds. The results showed that only 58 compounds were positively correlated with certain sensory attributes ($p \leq 0.05$).

Meanwhile, the concentration of a compound does not necessarily reflect its perceived aroma intensity in a sample because of the different odor thresholds or different detector sensitivities for different compounds [10]. Based on GC–O assessment, a specific compound must also be odor-active, with detection frequency >50% (i.e., not less than half detection frequency out of all panelists). Based on the detection frequency method, the MPFs were subjected to GC–O analysis, and only 43 odor-active compounds possessed an odor activity in MPFs.

Combined with the above 58 compounds that were positively correlated with sensory attributes, only 36 can be regarded by GC–O as specific or odor active in MPFs with greater than 50% detection frequency. Among these 36 odor-active compounds, there were two compounds with structures unidentified by GC–MS because of their lower concentration, the high detection limit of MS, or their absence in the database. The 36 volatile compounds of MPFs measured by GC–MS–O analysis are shown in Table 4.

Among these 36 odor-active compounds, 26 were significantly influenced by SBPHs ($p \leq 0.001$, data not shown). In addition, 10 of these compounds were derived from the Maillard reaction, 14 were lipid degradation products, and 2 compounds were unidentified. Benzaldehyde and 12-methyltridecenal can be produced from the Strecker degradation of amino acids coupled with lipid oxidation in a classic Maillard reaction. Other aliphatic aldehydes with straight chains [hexanal, heptanal, nonanal, (E)-2-nonenal, (E,E)-2,4-octadienal, 2-undecenal, tetradecanal, and (E,E)-2,4-dodecadienal] were formed from lipid oxidation [25]. Lipid degradation also contributes to the formation of heptanol, 2-tridecanone, pentanoic acid, hexylic acid, 4-methylnonanoic acid, and 2-pentylfuran [11]. 4-Methylthiazole is the product of the reaction of carbonyls with the Strecker degradation products of cysteine, namely, ammonia and hydrogen sulfide [19]. Other heterocyclic compounds associated with typical flavors, such as 2-hexyl pyridine, pyrazine, and 2,3-dimethyl pyrazine, were also proposed to have been formed via Maillard reaction through the Strecker degradation of cysteine or the thermal reactions of lysine and glycine.

Among the above-mentioned compounds, some have already been reported in mutton aroma: hexanal (green, fruity, fatty), heptanal (fruity, nutty, oily), octanal (floral, fruity, fatty), nonanal (fatty, oily, nutty), decanal (rubber tubing, smokey, fatty), (E)-2-nonenal (green, fatty, tallowy), undecanal (fatty, oily, candle), dodecanal (fatty, wood, nutty), 2-undecenal (fatty, green, boiled meat), (E,E)-2,4-dodecadienal (oily, fatty, milk), 12-methyltridecenal (muttony, fatty), 2-undecanone (oily, fruity), hexylic acid (lamby, oily),

Table 4
Volatile flavor compounds of eight MPF samples measured by GC–O analysis.

No. [a]	KI [b]	Compound	Odor description [c]	Detection frequency								ID [d]
				MPF0	MPF1	MPF2	MPF3	MPF4	MPF5	MPF6	MPF7	
A1	1429	Hexanol	Green, fruity, oily	5	6	6	6	7	6	6	7	A,C
A2	1545	Heptanol	Floral	4	7	6	7	5	6	6	5	A,C
A3	2458	Sulfurol	Nutty, meaty, sulfury	–	5	6	7	7	8	8	6	A,C
A4	1097	Hexanal	Green, fruity, fatty	6	5	7	6	5	7	6	5	A,C
A5	1219	Heptanal	Fruity, nutty, oily	4	6	6	6	7	7	7	5	A,C
A6	1349	Octanal	Floral, fruity, fatty	4	5	5	4	5	5	5	5	A,C
A7	1473	Nonanal	Fatty, oily, nutty	5	5	6	5	6	7	8	4	A,C
A8	1591	Decanal	Rubber tubing, smokey, fatty	6	5	6	6	6	6	7	7	A,C
A9	1622	Benzaldehyde	Nutty	4	6	7	6	4	8	6	4	A,C
A10	1635	(E)-2-Nonenal	Green, fatty, tallowy	5	6	5	–	6	6	5	7	A
A11	1707	Undecanal	Fatty, oily, candle	5	4	5	4	4	4	5	4	A
A12	1783	(E,E)-2,4-Octadienal	Green, fruity, fatty	5	5	5	4	8	6	7	5	B,C
A13	1823	Dodecanal	Fatty, wood, nutty	6	6	5	6	6	7	6	6	A
A14	1826	2-Undecenal	Fatty, green, boiled meat	4	5	4	4	5	7	6	4	A
A15	1881	Tetradecanal	Fatty, milky, fruity	6	6	5	4	5	4	7	5	A,C
A16	1935	(E,E)-2,4-Dodecadienal	Oily, fatty, milk	4	4	5	5	6	5	5	–	A,C
A17	1993	12-Methyltridecenal	Muttony, fatty	–	4	4	6	7	8	7	5	A,C
A18	1702	2-Undecanone	Oily, fruity	5	5	6	6	6	6	6	6	A
A19	1766	Acetophenone	Nutty	7	7	6	8	6	7	6	5	A
A20	1929	2-Tridecanone	Buttery, nutty, oily	4	6	5	5	5	5	6	5	A,C
A21	1341	4-Methylthiazole	Nutty, green	–	6	5	–	5	5	7	5	A
A22	1538	Acetic acid	Pungency, sour	6	7	7	7	6	5	7	6	A,C
A23	1967	Pentanoic acid	Fatty, oily	5	5	6	–	5	5	6	–	A,C
A24	2012	Hexylic acid	Lamby, oily	6	5	–	6	–	6	7	5	A,C
A25	2278	4-Methylnonanoic acid	Roasted muttony, suet-like	4	4	6	7	8	8	7	5	A,C
A26	2306	Nonanoic acid	Cheese, fatty	5	6	5	4	5	6	6	4	A,C
A27	2413	Decanoic acid	Oily, rancid	6	6	6	6	5	6	5	6	A,C
A28	2870	2-Hexyl pyridine	Meaty, fatty, roasted	–	–	4	6	5	7	7	6	A
A29	1278	2-Pentylfuran	Metallic, earthy, meaty	–	–	4	4	7	8	6	7	A
A30	1631	Octyl 2-furancarboxylate	Oily, mushroom, metallic	6	6	–	4	6	5	7	–	B
A31	1678	Butyl 2-decenoate	Green, milk	4	5	4	4	6	5	4	5	B,C
A32	1254	Pyrazine	Nutty, green	4	–	4	6	5	7	6	–	A
A33	1391	2,3-Dimethyl pyrazine	Coffee, roasted, meaty, buttery	–	4	5	–	6	6	6	–	B,C
A34	2043	Benzyl methyl sulfide	Roasted, muttony, burning	–	4	4	5	6	6	7	4	B,C
A35	1692	Unknown	Suet-like	–	–	4	5	7	6	7	5	
A36	2138	Unknown	Bread	–	4	6	5	4	6	8	5	

–: Less than 50% detection frequency or not detected.
[a] Code representing the 36 odor-active compounds observed in GC–MS–O analysis.
[b] KI: Kovat's index in column DB-Wax.
[c] Odor description as perceived by panelists at a given retention index during GC–O.
[d] Identification method: A, identified by comparison with reference compounds based on MS spectra, RI, odor quality, and authentic compounds; B, identified tentatively by comparison with literature data based on RI and odor quality; C, identified by mass spectra and retention time consistent with pure standard.

4-methyl nonanoic acid (roasted, muttony, suet-like), nonanoic acid (cheese, fatty), decanoic acid (oily, rancid), and 2-pentylfuran (metallic, earthy, meaty) [4,26–30]. However, some of the detected odor zones might be described as "mutton" (such as 12-methyltridecenal, hexylic acid, 4-methylnonanoic acid, and benzyl methyl sulfide). 4-Methylocatanoic acid is related to the odor of mutton [31]. However, this acid was not detected in MPFs. Furthermore, sulfurol, 2-undecenal, 2-pentylfuran, and 2,3-dimethyl pyrazine were described as meaty odors, and other compounds possessed certain odor activity in MPFs (Table 4). The GC/MS/O profiles showed that some odor-active compounds were abundant in certain MPF samples. However, nine of these 36 compounds were absent or had low content in MPF0: sulfurol, 12-methyltridecenal, 4-methylthiazole, 2-hexyl pyridine, 2-pentylfuran, 2,3-dimethyl pyrazine, benzyl methyl sulfide, and two unknown compounds (A35 and A36). These compounds could contribute to the production of a different overall odor by adding their specific attributes to the aroma profile of MPFs. The absence of these odor-active compounds in MPF0 might be the cause of its weaker sensory characteristics compared with the other MPF samples. In contrast to the other MPFs, MPF5 and MPF6 showed significantly higher detection frequency scores for most odor-active compounds especially for sulfurol, benzaldehyde, 12-methyltridecenal, 4-methylnonanoic acid, 2-pentyfuran and nonanal, hexylic acid, benzyl methyl sulfide, and two unknown compounds (A35 and A36), respectively. These results indicated that MPF5 and MPF6 prepared with a DH range of 25.92–30.89% produced a wider range of odor-active compounds, whereas MPF0 seemed to lack these compounds. Moreover, the results confirmed the reliability of the panellists' MPF evaluation (Table 3).

3.4. Relationship among the MPF samples, sensory attributes, peptide MW, and GC/MS/O analysis

Fig. 1 shows the results of the PLS2 regression analysis, which describes the relationship among the GC/MS/O data (X-matrix), peptides with various MW distribution of SBPH samples (X-matrix) and sensory attributes (Y-matrix), and MPF samples (Y-matrix). The estimated regression coefficients from the jack-knife uncertainty test showed that the compounds heptanol, sulfurol, heptanal, octanal, nonanal, (E,E)-2,4-octadienal, 2-undecenal, tetradecanal, (E,E)-2,4-dodecadienal, 12-methyltridecenal, 4-methylthiazole, hexylic acid, 4-methylnonanoic acid, nonanoic acid, butyl 2-decenoate, benzyl methyl sulfide, and two unknown compounds were significant ($p \leq 0.05$) for one or more of the eight MPF samples and five significant sensory attributes. The calibrated explained variance for this model was PC1 = 51% and PC2 = 13%. Fig. 1 shows a correlation–loadings plot. The ellipses indicated 50% and 100% explained variance, respectively. A total of 41 Y variances (including 36 odor-active compounds and five peptides with various

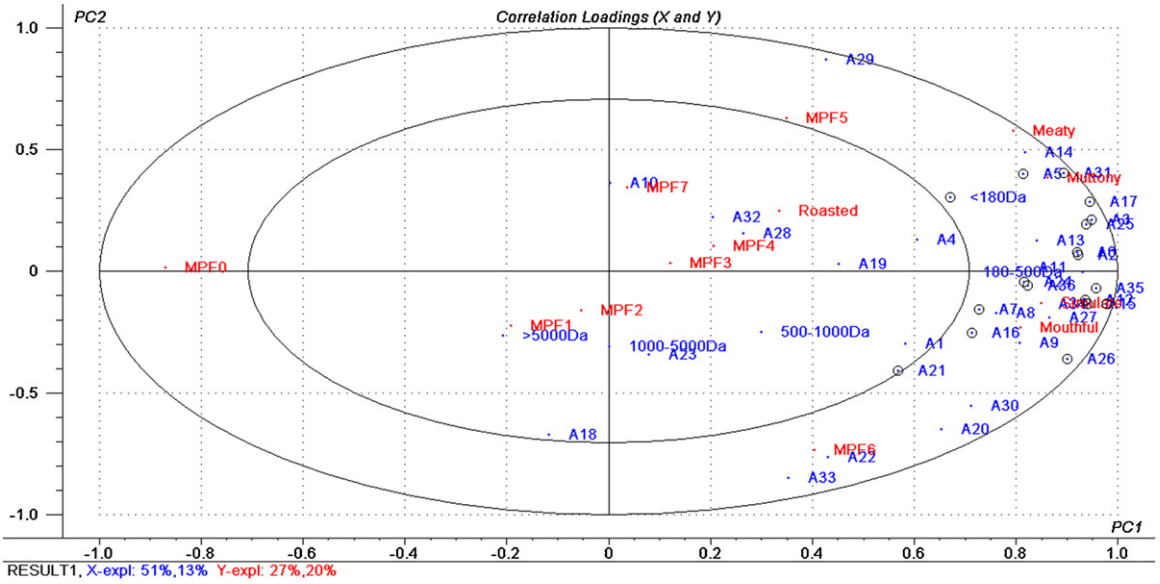

Fig. 1. PLSR correlation loadings plot of MW of peptides and GC–MS–O data in the X-matrix and MPF samples and sensory attributes in the Y-matrix. Ellipses represent r^2 = 0.5 and 1.0, respectively. GC–MS–O data of A1-36 correspond to the code compounds in Table 4.

MW) and 13 X variances (including eight MPF samples and five sensory attributes) were placed between the inner and outer ellipses, r^2 = 0.5 and 1.0, respectively, indicating that they were well explained by the APLSR model.

As indicated in Fig. 1, the MPF samples appeared to be separated along PC1 with MPF0 and MPF1 samples on the left side and samples prepared from higher DH on the right side of the plot. The area along PC2 was found to be explained by mouthful and simulate (in the lower part) as well as muttony, meaty and roasted (in the upper part). As shown in Fig. 1, the sample (MPF0) without added SBPH appeared to be strongly negatively correlated with all the sensory variables and all odor-active compounds (except 2-undecanone). This finding is in agreement with the sensory evaluation results (Table 3), where MPF0 had the lowest scores in nearly all sensory attributes except for the roasted attribute. This finding also agrees with the results of Song et al. [11], who explained that thermal reaction flavors can not be prepared only by HVP) but with addition of the SBPH to enhance the truly simulated natural meat aroma. The sample MPF1, located in the lower inner ellipses, seemed to have only covaried with peptides above 5000 Da. This finding might be attributed to the fact that MPF1 is the thermal reaction product from SBPH1, which had the lowest DH% and the highest peptide content (>5000 Da) compared with other SBPH samples. The samples MPF2, MPF3, MPF4, and MPF7 were near the origin of PLS2 loadings that showed weak correlation with the PLS2-factors. By contrast, the sample MPF5 covaried with the muttony, meaty, and roasted attributes and some odor-active compounds, including sulfurol, heptanal, 2-undecenal, 12-methyltridecenal, 4-methylnonanoic acid, 2-pentylfuran, butyl 2-decenoate, and peptides <180 Da. The sample MPF6 located in the lower righthand quadrant was correlated to the mouthful and simulate attributes and some compounds including nonanal, (E,E)-2,4-octadienal, tetradecanal, (E,E)-2,4-dodecadienal, 4-methylthiazole, nonanoic acid, unknown compounds, and peptides ranging from 180 Da to 1000 Da. These results are in agreement with the findings of Brennand [32], who showed that 4-methyloctanoic and 4-ethylocation acids, as important components with a strong "goat-like" odor and a low odor threshold value of 0.006 mg/kg in water, play important roles in sheep meat. The results are also in accordance with the research of Virginia et al. [27], who found that sulfur compounds might be the most closely associated with "cooked meat odors".

In order to support the results of PLS2 and to determine which compounds and peptides contribute greatly to each of the sensory attributes, PLS1 regression analysis was performed. In PLS1 regression modeling, the estimated regression coefficients were derived by the jack-knife uncertainty test. The significant compounds or peptides are shown in Table 5. As shown in Table 5, the muttony attribute was significantly correlated to sulfurol, tetradecanal, 12-methyltridecenal, 4-methylnonanoic acid, butyl 2-decenoate, and benzyl methyl sulfide, representing 66.99% of the variation in the muttony attribute. The compounds sulfurol, tetradecanal, (E,E)-2,4-dodecadienal, 12-methyltridecenal, 4-methyl nonanoic acid, and butyl 2-decenoate showed a positive effect but 2,3-dimethyl pyrazine showed a negative effect on the meaty attribute, representing 71.33% of the variation in the meaty attribute. Except for (E)-2-nonenal, 2-undecanone, pentanoic acid, and pyrazine, the other compounds showed positive correlation to the simulate attribute. Among these odor-active compounds, heptanol, octanal, (E,E)-2,4-octadienal, tetradecanal, and butyl 2-decenoate showed a significant influence. Moreover, all compounds except for pentanoic acid and 2-hexyl pyridine with 51.6% of the explained variation were positively associated to the mouthful attribute. These results are similar to those of other studies, in which the

Table 5
Standardized, estimated regression coefficients (significant) from PLS1 prediction models for the sensory attribute variables.

No[a]	Muttony[b]	Meaty	Simulate	Roasted	Mouthful
A2	–	–	0.03349	–	0.03916
A3	0.07197	0.0732	–	–	0.03840
A5	–	–	–	–	0.02655
A6	–	–	0.03595	–	–
A12	–	–	0.03816	–	0.04522
A15	0.02485	0.01831	0.03839	–	0.04475
A16	–	0.03069	–	–	–
A17	0.08632	0.09180	–	–	0.03703
A25	0.08176	0.08411	–	–	0.03316
A31	0.07747	0.08603	0.03583	–	0.03739
A33	–	−0.07380	–	–	–
A34	0.03904	–	–	–	–

–: Not significant.
[a] A1-36 correspond to the code compounds in Table 4.
[b] Regression coefficients derived by the jack-knife uncertainty test.

above compounds were found to be the key aroma compounds contributing to mutton aroma because of their low odor detection threshold value. As for the roasted attribute, all compounds had no significance and only explained 4.65% of the variation. This finding may be attributed to some of the key compounds that were undetected in this study or to evaluation errors of the panelists.

However, the relationship between peptides with different MWs and each sensory attribute was also determined by calculating the estimated regression coefficients using the jack-knife uncertainty test. Notably, the peptides ranging from 180 Da to 500 Da had a positive effect on the muttony, simulate, and mouthful attributes, and whereas those ranging from 180 Da to 1000 Da had a positive effect on the meaty and roasted attributes. However, none of them exhibited significant effects on any sensory attribute, indicating that the peptides might participate in the formation of aroma-active compounds. These findings may be attributed to evaluation errors of the panelists. Another reason might be the effect of the inclusion of free amino acid proportion in the peptide content that was calculated in this study.

4. Conclusions

DSA, GC–MS, and GC–O analyses were used in this study to describe changes in the aroma characteristics of MPFs prepared from different SBPHs. Experimental results suggested that DH was an important index in the preparation of meat flavors. DSA results demonstrated that MPFs prepared from SBPHs with different DHs showed superiority in one or more sensory attributes. The MPF with a DH of 25.92% was the strongest in the muttony, meaty, and roasted attributes, whereas that with a DH of 30.89% was the strongest in the simulate and roasted attributes. Further investigation was conducted on the eight MPFs based on volatile odor-active compounds evaluated by GC–MS–O. A total of 36 compounds were detected as key compounds representing the mutton flavor for the evaluation of the sensory characteristics of MPFs. Compared with the other samples, the SBPH with a DH range of 25.92–30.89% produced a wider range of odor-active compounds through thermal reaction. The results of correlation analysis among odor-active compounds, MW, and DSA confirmed that the SBPH with a DH range of 25.92–30.89% may be a desirable precursor for MPF.

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