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Contribution of beef base to aroma characteristics of beeflike process flavour assessed by descriptive sensory analysis and gas chromatography olfactometry and partial least squares regression

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ABSTRACT

Descriptive sensory analysis and gas chromatography–mass spectrometry (GC–MS) analysis were conducted to investigate changes in aroma characteristics of beeflike process flavours (BPFs) prepared from enzymatically hydrolyzed beef (beef base) of different DH (degree of hydrolysis) with other ingredients. Five attributes (beefy, meaty, simulate, mouthful and roasted) were selected to assess BPFs. The results of descriptive sensory analysis confirmed that BPF2 from beef base of moderate DH 29.13% was strongest in beefy, meaty and simulate characteristics; BPF4 and BPF5 from beef base of higher DH (40.43% and 44.22%, respectively) were superior in mouthful and roasted attributes respectively; while BPF0 without beef base gave weaker odour for all attributes. Twenty six compounds from GC–MS were selected as specific compounds to represent beef odour based on their odour-active properties assessed by a detection frequency method of GC–O and correlation of their contents with sensory attributes intensity. Correlation analysis of molecular weight (MW) of peptides, odour-active compounds and sensory attributes through partial least squares regression (PLSR) further explained that beef base with DH of 29.13% was a desirable precursor for imparting aroma characteristics of beeflike process flavour.

1. Introduction

Beef flavours have been increasingly found application in meat analogues and processed instant foods. Recently, there have been various types of simulated meat flavours such as simple blended spices [1], recombined flavour components isolated and identified from cooked or fried meat [2], prepared from hydrolyzed vegetable protein (HVP) or hydrolyzed yeast [3], however, the most common type is "thermal process flavour", which is a comparatively recent term given to a food flavour produced by heating a combination of two or more precursor materials under carefully controlled conditions [4]. The primary reaction occurring in this process is the Maillard reaction.

It is well-known that meat flavour is thermally derived and consists of "meaty flavour" and "species-specific flavour", which are imparted through coordination of Maillard reaction and lipid oxidation [5]. Therefore, precursors play an important role in the generation of process flavour. Generally, beef flavours are derived from the complex interactions of flavour precursors such as amino acids, peptides, sugars, thiamine, metabolites of nucleotides, and products of lipid oxidation. Considerable researches and patents have been done to develop beeflike flavour by Maillard reaction with various amino acids and sugars [6]. In contrast to pure amino acids, protein hydrolysates which contain free amino acids, peptides, nucleotides, reducing sugars, carbonyl compounds, and sulfur compounds, are inexpensive and have been used to produce beef flavours.

For many years, HVP like soybean protein has been selected as potential precursor for beeflike process flavour [3,7]. However, meat flavours based on HVP can only partially simulate natural meat aroma, therefore, the thermal reaction model system has been evaluated for other flavour precursors (e.g. enzymatically hydrolyzed animal proteins) [8]. Some early researches related to meat hydrolysates have been involved in the preparation of meat flavour [9]. The first attempt of heating enzymatically degraded meat to produce meat flavour was made by Chhuy and Day [10]. Similar processes starting with the proteolysis of meat and meat by-products have been described by others [11].

The degree of hydrolysis (DH) of meat protein is a very important index for preparing meat flavours. Barbel Lieske and Gerd Konrad [12] confirmed that strong meatlike flavour notes would be intensified by heating the partial hydrolysates of meat protein in the

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Table 1
Changes of molecular weight (MW) distribution in different HBPs.

MW (Da)	Samples				
	HBP1[a]	HBP2	HBP3	HBP4	HBP5
>5000[b]	0.25 ± 0.01[c]	0.00 ± 0.00	0.14 ± 0.01	0.07 ± 0.00	0.06 ± 0.01
1000–5000	6.75 ± 0.12	4.45 ± 0.18	5.00 ± 0.12	3.56 ± 0.01	3.13 ± 0.30
500–1000	19.05 ± 0.06	21.04 ± 0.10	18.11 ± 0.03	13.33 ± 0.09	12.69 ± 0.06
200–500	54.49 ± 0.05	59.31 ± 0.03	62.55 ± 0.12	69.44 ± 0.06	70.77 ± 0.01
>200	2.47 ± 0.02	1.21 ± 0.03	2.20 ± 0.06	4.59 ± 0.06	4.35 ± 0.01

[a] Five samples were denoted by the HBP followed by 1-digit Arabic numbers. Where "HBP" represents for beef enzymatic hydrolysate (beef base), the followed Arabic numbers 1–5 denote DH 25.35%, 29.13%, 35.40%, 40.43% and 44.22%, respectively.
[b] Peptides in HBP as mg/mL of beef base.
[c] Mean ± standard deviation (average of triplicate).

Table 2
Analyses of variance for the main effects and their interactions for each of the five attributes in descriptive analysis.

	F-values						Adjusted *F*-value	
	Sample (S) (df = 5)	Panelist (P) (df = 7)	Replication (R) (df = 2)	S × P (df = 35)	P × R (df = 14)	S × R (df = 10)	Sample[a] (S) (df = 35)	Sample[b] (S) (df = 10)
Beefy	213.41***	2.71*	3.18*	2.61***	0.33	2.32*	81.64***	66.76***
Meaty	416.65***	16.98***	2.96	11.48***	0.75	1.18	36.29***	33.60***
Simulate	401.53***	0.96	0.88	0.90	1.59	1.04	445.91***	441.35***
Roasted	67.36***	6.43***	2.58	2.24***	0.73	1.22	29.38***	22.58***
Mouthful	133.66***	1.11	3.17	2.03**	1.05	0.47	65.84***	71.21***

[a] Adjusted *F*-values of sample effect calculated using $MS_{sample \times panelist}$ instead of MSerror as described in the text.
[b] Adjusted F-values of sample effect calculated using $MS_{sample \times replication}$ instead of MSerror as described in the text.
* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.
*** Significant at $p \leq 0.001$.

presence of appropriate sulfur and carbohydrate sources compared with the total hydrolysates. However, there is still a lack of more systematic study for the impact of beef hydrolysate with different DH on the aroma characteristics of beeflike process flavour.

Even though a great number of volatile compounds (more than 1000) have been reported in cooked beef meat, only some of them are important in terms of the characteristic beef flavour. In recent researches, more great efforts have been made to find and identify key aroma compounds in beef via gas chromatography in combination with olfactometry (GC–O) [13,14]. However, a little was known about the aroma active components of beeflike process flavour prepared from enzymatically hydrolyzed beef, so called beef base.

The objectives of the present study are to (a) apply descriptive sensory analysis to describe and monitor the aroma attributes of beeflike process flavours (BPFs) derived from beef base with different DH, (b) analyze the volatile compounds released from BPFs by GC–MS and investigate the impact of beef base with different DH on their corresponding aroma-active compounds determined by GC–O, and (c) identify which aroma-active compounds and peptides of what MW have significant influence on individual sensory attributes through correlation analysis among molecular weight (MW) of peptides, aroma-active compounds and sensory attributes. Through the above analysis, the desirable beef base with suitable DH is then recommended for controlled proteolysis to prepare characteristic beef flavour precursors.

2. Materials and methods

2.1. Materials

Lean beef was purchased from Wal-Mart supermarket in Wuxi, China. Hydrolyzed vegetable protein (HVP) was provided by Tianning Flavour & Fragrance Co., Ltd. (Shanghai, China). Refined tallow was purchased from Anhui Muyang Oil and Fats Co., Ltd. (Anhui, China). DL-methionine, D-xylose, glucose, L-cysteine, L-glutamic acid, L-proline, thiamine and taurine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Alkaline protease, activity 2.4 AU/mL, and flavourzyme, activity 500 LAPU/g, were purchased from Novozymes (Bagsvaerd, Denmark). 1,2-Dichlorobenzene and methanol were of chromatography grade from TCI Development Co., Ltd. (Shanghai, China). Other authentic reference compounds were obtained from commercial sources and Sigma–Aldrich Co. Ltd. (Shanghai, China).

2.2. Sample preparation

2.2.1. Preparation of beef base

Lean beef (water content, 75.98%; protein content, 20.58%) was minced with a tissue-tearor and mixed with deionized water at a meat–water ratio of 7:3. The mixture dispersion was then heated at 95 °C for 10 min in order to make the endogenous enzyme

Table 3
The mean intensity values of the 5 attributes for the 6 BPF samples in descriptive sensory evaluation.[X]

Beefy		Meaty		Simulate		Roasted		Mouthful	
Sample[Y]	Mean score	Sample	Mean score	Sample	Mean score	Sample	Mean score	Sample	Mean score
BPF0	3.25[a]	BPF0	1.21[a]	BPF0	2.39[a]	BPF4	5.71[a]	BPF0	2.88[a]
BPF1	4.04[b]	BPF1	4.33[b]	BPF5	2.79[b]	BPF0	6.25[b]	BPF5	5.25[b]
BPF3	5.54[c]	BPF5	6.13[c]	BPF1	3.54[c]	BPF3	6.63[b]	BPF3	6.50[c]
BPF4	6.00[d]	BPF3	6.13[c]	BPF3	6.00[d]	BPF2	7.50[c]	BPF2	6.58[c]
BPF5	6.00[d]	BPF4	6.88[d]	BPF4	6.46[e]	BPF1	7.92[d]	BPF1	7.21[d]
BPF2	8.50[e]	BPF2	8.58[e]	BPF2	8.67[f]	BPF5	8.75[e]	BPF4	7.25[d]

[X] Mean scores (listed in ascending order) for each attribute within a column with different letters are significantly different ($p \leq 0.05$) using Duncan's multiple comparison test ($n = 24$; 8 panelists with 3 replications).
[Y] Six beaflike process flavours were denoted by the BPF0-5, which were prepared from without HBP and with HBP1, HBP2, HBP3, HBP4 and HBP5, respectively.

Table 4
Volatile flavour compounds of 6 BPF samples, determined by gas chromatography–olfactometry analysis, the compounds' detection frequencies (6 assessors, average of two sessions), statistical significance of beef base and odour descriptors.

Code[a]	Compound	Detection frequency						p[b]	Odour description[c]	ID[d]
		BPF0	BPF1	BPF2	BPF3	BPF4	BPF5			
A1	3-Methylbutanal	3	3	6	5	4	6	***	Chocolate, caramel, green, nutty	B
A2	Hexanal	3	4	6	3	4	5	***	Green, fruity	B
A3	Heptanal	3	5	5	4	4	4	***	Fruity, nutty	B
A4	(E,E)-2,4-Decadienal	5	5	5	3	4	5	***	Deep-fried, fatty, fried potato	B
A5	Nonanal	4	4	6	5	4	6	***	Grassy, green, beefy	B
A6	Decanal	5	4	5	5	5	6	NS	Rubber tubing, smokey	B
A7	2-Undecenal	4	4	3	5	4	3	***	Fatty, green, boiled meat	B
A8	Benzaldehyde	3	6	6	4	5	4	***	Pop corn, caramel, herby, metallic	B
A9	1-Octen-3-ol	5	5	5	6	5	6	NS	Mushroom	B
A10	2-Butanone	3	3	4	4	3	3	***	Sweet, buttery	C
A11	Unknown	–	–	4	–	–	–	***	Green, fatty	
A12	2-decanone	4	4	4	3	3	3	NS	Musty, fruity	C
A13	Hexanoic acid	3	3	3	3	4	5	***	Pungency, rancid	B
A14	Unknown	4	5	6	3	4	5	***	Tallow-like	
A15	2-Methyl-3-(methylthio) furan	3	3	6	3	3	6	***	Meaty, sulfurous, onion	B
A16	2-Methyl-3-furanthiol (2-MF)	–	3	6	4	–	–	***	Meaty, cooked rice	B
A17	Bis(2-methyl-3-furyl)disulfide	–	5	6	5	4	4	***	Meaty, coffee, metallic	B
A18	2-Pentylfuran	–	–	6	5	5	5	***	Metallic, earthy, green	B
A19	2-Acetyl-1-pyroline	4	5	3	3	3	6	***	Roasted, popcorn, coffee	B
A20	2-Ethyl-3,6-dimethylpyrazine	3	5	4	3	4	6	***	Nutty, roasted	B
A21	2,5-Dimethylthiophene	–	4	4	5	4	4	NS	Beef, sweet, ham, rancid	B
A22	3-Methyl-2-thiophenecarboxaldehyde	–	5	6	4	–	–	***	Sweet, beefy	C
A23	Sulfurol	–	3	6	6	–	–	***	Nutty	B
A24	2-Pentylpyridine	–	–	4	5	5	4	***	Nutty, beefy	C
A25	Unknown	–	–	4	4	3	4	***	Bread	
A26	δ-Nonalactone	3	5	5	4	5	5	***	Sweet, dairy, nutty	B

[a] Code representing the 26 odour-active compounds observed in GC–MS.
[b] $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; NS: not significant.
[c] Odour description as perceived by panelists at a given retention index during GC–O.
[d] Identification method: B, identified by comparing it with the reference compounds on the basis of MS spectra, RI, odour quality and authentic compounds; C, identified tentatively by comparing it with literature data on the basis of RI and odour quality.

deactivation and beef protein denaturation. Beef protein was consecutively hydrolyzed by alkaline protease and flavourzyme. It was first hydrolyzed at 60 °C for 3 h using alkaline protease with enzyme/substrate ratio (E/S) of 6.6×10^{-3} (AU/g minced meat) and pH of 8.0, and then treated at 50 °C using flavourzyme with E/S of 0.25 (LAPU/g minced meat) and pH 6.6 for 2, 4, 6, 8 and 10 h, respectively, to prepare the beef base with different DH. The resultant slurry was heated at 95 °C for 10 min to inactivate the enzyme and then centrifuged at 3500 rpm for 30 min to remove the insoluble residue. The supernatant was used for further analysis.

2.2.2. Preparation of beeflike process flavour (BPF)

Controlled oxidation of tallow: The oxidized tallow was prepared according to our early research [15]. The corresponding peroxide value (PV), acid value (AV) and p-anisidine value (p-AV) were 242.88 mequiv./kg tallow, 0.93 mg KOH/g tallow and 80.76, respectively.

A mixture of HVP (4 g), DL-methionine (0.2 g), D-xylose (1 g), glucose (1 g), L-cysteine (1 g), L-glutamic acid (0.25 g), L-proline (0.25 g), thiamine (0.6 g), taurine (0.5 g), oxidized tallow (10 g), was dissolved in 81.2 g solution of the beef base. 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 reaction, the tubes were immediately cooled in ice-water and the thermal reaction products named BPFs were sampled for further use.

2.3. Analysis methods

2.3.1. Determination of degree of hydrolysis (DH)

The degree of hydrolysis (DH) was defined as the ratio of free amino groups present in the hydrolysate to the total amount of amino groups in the protein. The free amino group was determined by a modified formol titration method [16]. The total nitrogen was determined by Kjeldahl method.

2.3.2. Determination of amino acids and molecular weight (MW) distribution

Amino acids and molecular weight (MW) distribution of beef base were determined according to the method reported by Lan et al. [17].

2.3.3. Sensory analysis

Quantitative descriptive sensory analysis was applied for evaluating BPFs by a well-trained panel consisting of 8 members at the age of 25–50, 5 females and 3 males. All panelists have passed screening tests according to ISO standards [18], and had previous experience with sensory evaluation. Sensory sessions took place in a sensory laboratory, which complied with international standards for test rooms [19]. Prior to the quantitative descriptive sensory analysis, the panelists had thoroughly discussed aroma properties of samples through three preliminary sessions, each spent 2 h, until all of them had agreed to use them as the attributes according to the objective of the present work. In total, 5 descriptors, including beefy, meaty, simulate, mouthful and roasted were used for the descriptive analysis. And the reference materials were as follows: pot roast (round bottom roast, approximately 200 g, wrapped with aluminum foil and baked for 1 h at 150 °C) was labeled "beef-like" note; defatted beef brisket (0.5 kg, 2.5 cm thick, purchased from Wal-Mart supermarket) boiled in water for 2 h was labeled "meat-like" aroma; stewed beef product, purchased from Wal-Mart supermarket, the similarity degree of aroma was labeled "simulate" note; 10 g bouillon cube (beef flavour consisting of MSG, yeast extract, and beef extract), dissolved in water was labeled "mouthfulness" attribute; ground roast coffee (Maxwell House Cof-

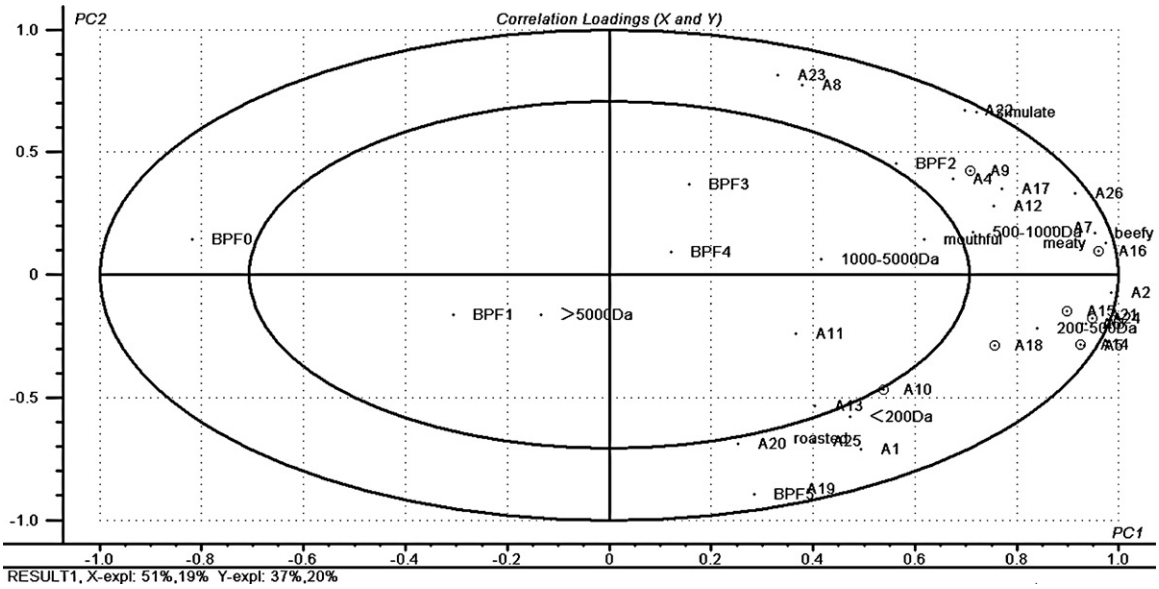

Fig. 1. An overview of the variation found in the mean data from the partial least squares regression (PLSR) correlation loadings plot for six samples. The model was derived from aroma-active compounds and molecular weight distribution of beef base as the *X*-matrix and BPF samples and sensory variables as the *Y*-matrix. The concentric circles represent 100 and 50% explained variance, respectively. Odour-active compounds of A1–26 correspond to the code compounds in Table 4.

fee Co., Kraft General Foods, Inc., White Plains, NY, USA) was labeled "roasted" note.

The sample solution (0.5%, w/w) was dissolved in umami solution. Umami solution consisted of 1.0% (w/v) monosodium glutamate (MSG) and 0.5% (w/v) sodium chloride (NaCl). About 30 mL of sample were served in opaque disposable plastic cups at the same time. To avoid temperature differences that could influence the assessment, the samples were kept at 45 °C in steel containers until evaluation, and the containers were completely sealed in order to avoid volatiles losses. Water and breads were available to the panelists throughout the analysis as this tended to stick to the palate. The samples were coded with random three-digit numbers and randomly presented for each panel to avoid causing a so-called order effect. The intensity of the descriptive terms was rated on a horizontal 10 cm continuous line scale, anchored "none" to the left and "extreme" to the right.

2.3.4. GC–MS analysis

The volatile compounds of BPFs were sampled with an SPME-fibre (75 μm, carboxen/polydimethylsiloxane), and assayed with a gas chromatograph–mass spectrometer (Finnigan Trace GC–MS, Finnigan, USA). The BPF (3 g) was weighed and placed in 15 mL vial. Immediately, 2 μL of 1,2-dichlorobenzene internal standard solution (0.555 μg/μL in methanol) was added to each sample prior to trap. The vial was sealed with PTFE/BYTL septum and equilibrated at 55 °C for 30 min exposed to SPME fibre in the sample headspace. After equilibrium, the SPME fiber was desorbed into the injector port at 250 °C for 2 min which was operated in a splitless mode. The volatile compounds were separated with a DB-WAX (30 m × 0.25 mm I.D., 0.25 μm film thickness; J & W Scientific Inc., Folsom, CA, USA). The temperature program employed was 3 min at 40 °C, a ramp of 6 °C/min to 80 °C, and then raised to 230 °C at the rate of 10 °C/min and held for 10 min. Helium was used as the carrier gas at a constant velocity of 1.8 mL/min. In order to get the linear retention index (LRI) values of the volatile compounds, a series of *n*-alkanes (C6–C26) were run under the same conditions. Mass spectra was obtained in the electron impact mode with an energy voltage of 70 eV and emission current of 35 Ua. The detector was set at a scanning range of 35 *m*/*z* to 450 *m*/*z* at a rate of 4.45 scans/s. Volatile compounds were either identified by comparison of ion spectra with authentic standards or tentatively identified using the NIST and WILEY library and Kovats retention index (KI). The KI values were calculated based on a series of *n*-alkanes (C6–C26). Where available, KI values of individual constituents were compared with KI values from authentic compounds run under the same GC–MS conditions. Approximate quantities of the volatile compounds were estimated by comparison of their peak areas with that of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms, using a response factor of 1.

2.3.5. GC–O analysis

The GC–O system consisted of a Finnigan trace GC (Finnigan, Perkin Elmer, USA) equipped with a flame ionization detector and a OP275 sniffing port (GL Sciences Inc., Japan). At the end of the capillary column the effluent was split 1:1 for FID and sniffing port, respectively, using deactivated and uncoated fused silica capillaries as transfer lines, and the sniffing cone was purged with humidified air to help in maintaining olfactory sensitivity by reducing dehydration of mucous membranes in the nasal cavity. The DB-wax column was also used for GC–FID and GC–O analyses. The initial oven temperature was maintained at 40 °C for 3 min, then increased to 230 °C at 6 °C/min, and maintained for 5 min. The carrier gas was helium supplied at a constant pressure of 122 kPa (2.1 mL/min).

Detection frequency method using a panel of six panelists was applied to obtain an odour profile for BPFs. There was no specific training session for the BPFs, but three of the six panelists had extensive experience with GC–O from other research. Each of the six assessors participated in perceiving the aroma compounds separated from BPFs at the sniffing port, and the number of panelists detecting odour components during GC–O was summed to acquire an aromagram for BPFs. The assessors were asked to state odour characteristics, if possible, whenever they detected an odour. The odour perception was recorded during the first 45 min, and at the sniffing port any odour that was reported by less than three of the six assessors was considered as noise.

2.4. Data analysis

Data from the descriptive analysis was evaluated by analysis of variance (ANOVA) using SPSS 13.0. ANOVA with Duncan's multiple comparison tests were performed to determine whether there were differences among individual samples for each sen-

sory attribute. When the interaction was found to be significant, an adjusted *F*-test was subsequently conducted based on using mean square of the interaction instead of mean square of error as the denominator for calculating *F*-value [20]. The correlations between MW of peptides, aroma-active compounds and sensory attributes were analyzed by partial least squares regression (PLSR) using the Unscrambler version 9.7 (CAMO ASA, Oslo, Norway). And the detailed PLSR analysis method has been previously described by Song et al. [21].

3. Results and discussion

3.1. Analysis of amino acid composition of beef base

The enzymatic hydrolysate of beef was prepared according to the method described above. Five samples were hydrolyzed for 2, 4, 6, 8 and 10 h and the corresponding DH values were 25.35%, 29.13%, 35.40%, 40.43%, and 44.22%, respectively. Each sample and related BPFs were used for further investigation.

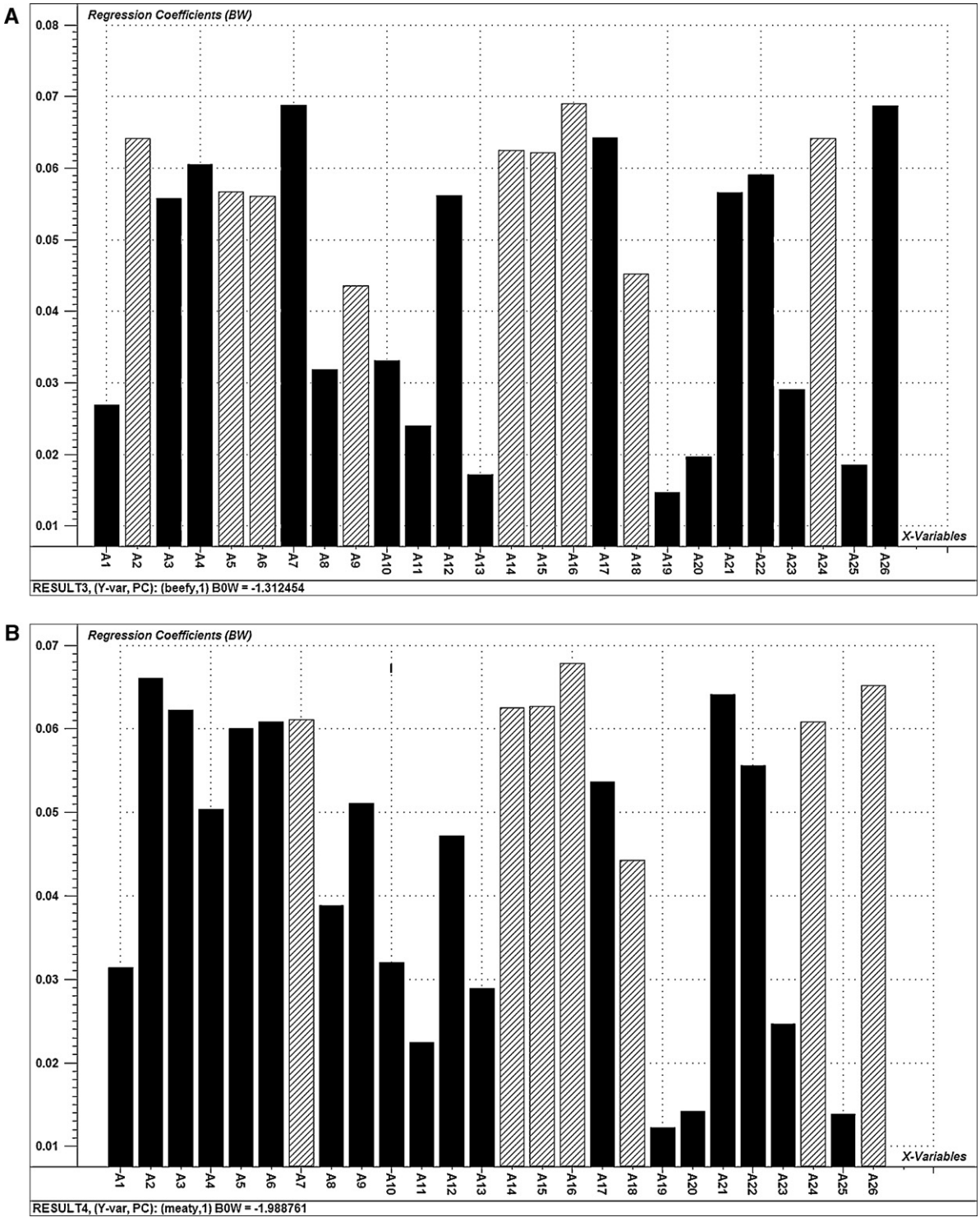

Fig. 2. Standardized, estimated regression coefficients and significance indications from PLS1 prediction models for the sensory attributes variables beefy (A), meaty (B), simulate (C), mouthful (D) and roasted (E) from aroma-active compounds. Odour-active compounds of A1–26 correspond to the code compounds in Table 4.

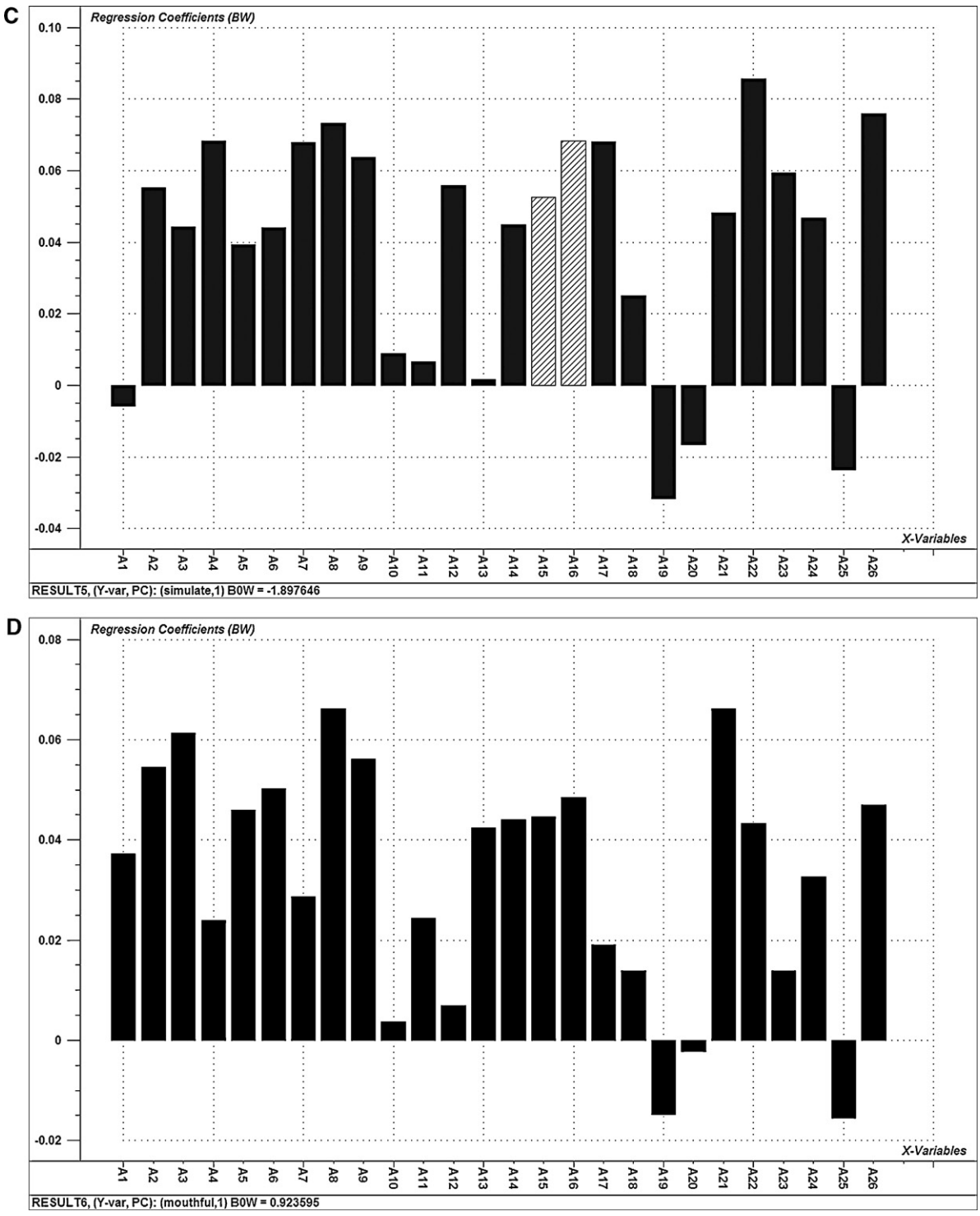

Fig. 2. (*Continued*)

The distribution of free amino acids (FAA) can directly or indirectly influence the sensory perception of BPFs. The total free amino acids gradually increased from 86.62 to 161.67 mg/g dry basis with an increase of DH. Levels of all individual amino acid were different in the experimental beef base irrespective of the conditions of hydrolysis used. Leucine was the most abundant free amino acid found in all samples, accounting for 19.26% to 20.68% of total free amino acids. The other abundant amino acid found in all samples was lysine, accounting for 16.94% to 17.99% of the total free amino acids. Others like arginine, alanine, phenylalanine and methionine were also high in free form. These amino acids may play important role in thermal process flavours. The potent odourants 3-methylbutanal, phenylacetaldehyde and numerous sulfur-containing compounds (e.g. dimethyltrisulfide, 3-(methylthio) propanal, and methanethiol) can arise from Strecker degradation of leucine, phenylalanine and methionine, respectively [22]. Lysine can undergo thermal reactions leading to formation of alkylpyrazines and 2-acetyl-1-pyrroline [23]. The remaining amino acids especially serine, cystine and proline were relatively low in quantity.

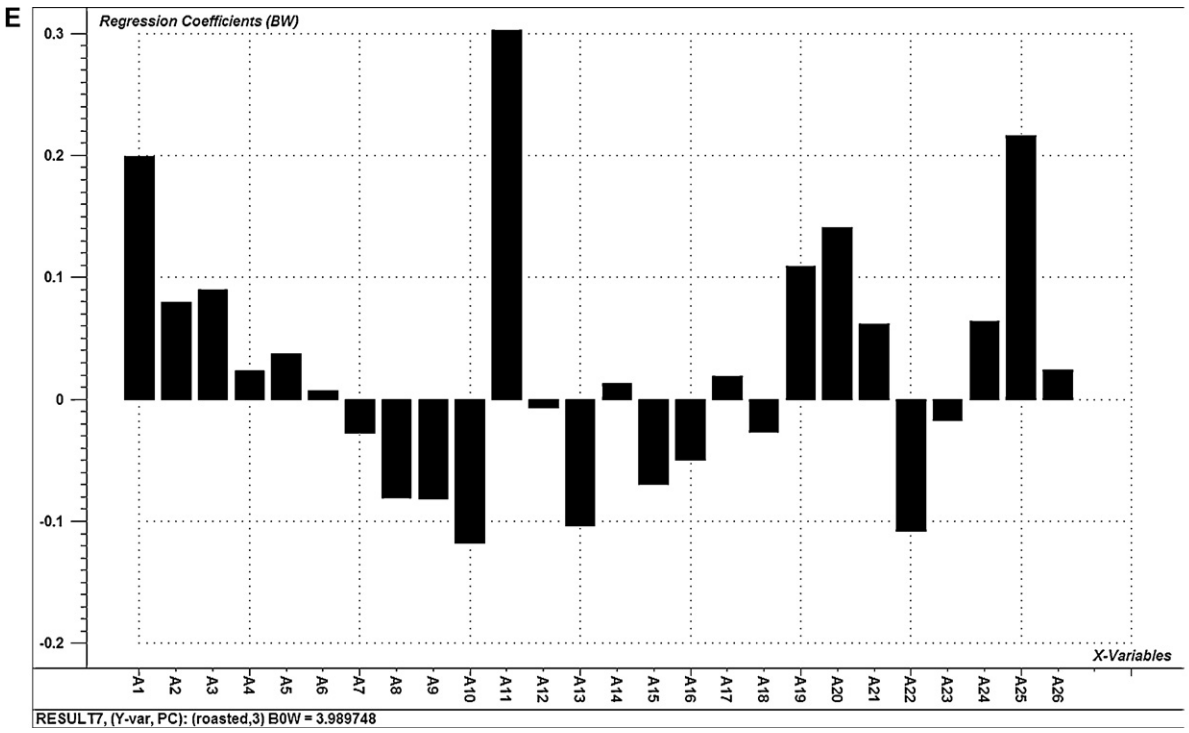

Fig. 2. (*Continued*).

Meanwhile, enzymatic protein hydrolysates contain a larger portion of peptides than that of free amino acids, owing to the higher selectivity and specificity of proteases. Despite information on the role of peptides in the formation of flavour compounds during thermal reaction is not as abundant as that on amino acids, it is advantageous when the partial hydrolysate containing peptides is used as a precursor for thermal process flavours [12]. Zhang et al. [24] also reported that peptides from casein hydrolysis contributed directly to volatile formation under thermal reaction conditions, and certain amino acids in the bound form of peptides underwent Strecker degradation to form Strecker aldehydes. The proportion of free amino acid to total amino acid (FAA:TAA) shows how much of each amino acid is still present as peptides. Results indicate that, due to the nature of enzymatic hydrolysis, almost 55% of the amino acid was still in bound peptides form in this study (data not shown).

The peptide content in hydrolyzed beef proteins (HBPs) would certainly vary with the DH changes. This was confirmed by the molecular weight distribution in all HBPs (Table 1). Results indicated that peptides above 500 Da in beef base as milligram per milliliter were gradually declined with increasing DH except for sample HBP2, whereas peptides from 200 Da to 500 Da, equal to tetra-, tri-, or dipeptides, showed an upward trend for all HBPs. However, there was no apparent tendency for peptides lower than 200 Da (equal to dipeptides or free amino acids) with change of DH.

3.2. Sensory characteristics of the BPF samples

An analysis of variance for BPFs was made, one sample without adding beef base was control and others were prepared from beef base with different DH. Three replicates were applied to sensory data to assess the results (Table 2). Significant differences among samples ($p \leq 0.001$) for all attributes indicated that the samples tested had different aroma intensities. Although, panelists were a significant source of variation in beefy, meaty and roasted attributes, this result is not uncommon in descriptive analysis, and suggests that the panelists used different scores scale to express their perceptions due to physiological differences in perceived intensity or differences in personal style of scoring, such as central or extreme raters. A significant replication effect ($p \leq 0.05$) was found for the beefy attribute, which might be partly influenced by the block effect of the replications in this study. Nevertheless, no significant interaction between panelist and replication was found, showing that all the panelists were reproducible in the triplicate tests for each attribute. In addition, there was no significant interaction between sample and replication in all attributes except "beefy"; this implies that intensities of beefy note in the samples were not rated similarly when they were replicated. However, a significant interaction between sample and panelist was observed for the "beefy" ($p \leq 0.001$), "meaty" ($p \leq 0.001$), "roasted" ($p \leq 0.001$) and "mouthful" ($p \leq 0.01$) attributes, indicating that the panelists were scoring samples not consistently for each attribute. And sensory data for the individual judges were examined to find the source of interaction. Sensory data revealed that two of the judges were using slightly different parts of the scale.

Because of the significant interaction effect between sample and panelist in all attributes except simulate, an adjusted *F*-test was performed using the mean square of sample × panelist interaction instead of mean square of error as a denominator, dealing with panelists as a random effect [20]. As shown in Table 2, the results of ANOVA after taking the variation of panelists into account showed that samples were significantly different ($p \leq 0.001$) in all attributes. Similarly, the adjusted F-test was carried out after taking the variation of replication into account. Also there was a noticeable difference ($p \leq 0.001$) between six BPFs in all attributes (Table 2).

ANOVA indicated significant differences ($p \leq 0.001$) among six HBPs in the intensity of the 5 attributes (Table 2). The mean intensity values of the 5 attributes and the results of Duncan's multiple comparison tests are shown in Table 3.

As shown in Table 3, BPF1 was very strong in roasted and mouthful notes but very weak in beefy, meaty and simulate notes. BPF2 was relatively even in all the 5 attributes and showed very strong beefy, meaty and simulate attributes compared to other samples. BPF3 was strong and even in all 5 attributes. BPF4 presented high-

est mouthful note. BPF5 was the strongest in roasted and with less extent in beefy, meaty and mouthful notes, while it was weak in simulate note. By contrast, the control sample BPF0 gave only strong roasted note and was very weak in the other 4 attributes, and this is consistent with the fact that meat flavours based on mixing of pure amino acids and hydrolyzed vegetable protein can only partially simulate natural meat aroma. Also, it is obvious that a significant increase in perceived beefy aroma intensity was observed when beef base with different DH were added to prepare BPF compared with control. In particular, BPF2 showed the strongest beefy, meaty and simulate characteristics compared to other samples, and each of other samples showed superiority in one or more attributes, respectively. This phenomenon implied that HBP with different DH produced varying quantities of volatile compounds; however, there was no apparent tendency for attributes changes with increasing DH.

Furthermore, the differences in flavour profile development amongst the BPFs are also supported by different contents of free amino acids, peptides and protein (Table 1). This may be explained by that certain peptides and free amino acids have an important effect on its unique flavour formation and perception. Peptides may directly or indirectly contribute to form aroma-active compounds instead of undergoing hydrolysis of peptide bonds to free amino acids. Some compounds formed from peptides might enhance the perception.

3.3. Effect of beef base with different DH on the volatile characteristics of BPFs

The volatile compounds of BPFs were separated and detected on a DB-WAX column: 19 alcohols, 16 aldehydes, 16 ketones, 13 thiophenes, 12 alkanes, 8 lactones, 8 furans, 7 carboxylic acids, 6 thiazoles, 4 pyridines, 3 sulfur compounds, 2 pyrazines, 2 pyrroles and 1 esters. They were either identified by comparison of ion spectra with authentic standards or tentatively identified using the NIST and WILEY library and further characterized by their retention indices.

To evaluate the influence of beef base with different DH on the formation of flavour characteristics of BPFs, the selection of specific compounds from GC analysis to represent the beeflike attributes in samples might be useful. Several researches [2,25] indicated that, in contrast to fruits or chocolates, no single character impact compound has been identified for either authentic meat or simulated meat flavours, and that a number of volatiles of different chemical classes existing in specific quantitative proportions were responsible for the meat flavours. In this study, multiple aroma compounds were also confirmed to be specific compounds responsible for the aroma characteristics of BPFs.

To qualify as specific compound, the compound detected by GC–MS should be positively correlated ($p \leq 0.05$) with attributes in descriptive sensory analysis. The correlation analysis was conducted between the descriptive analysis scores of the five sensory attributes and all identified compounds by means of GC–MS. Among 117 volatile compounds, 67 compounds were significantly correlated with the specific sensory attributes ($p \leq 0.05$) (data not shown).

It is well known that the concentration does not necessarily reflect the perceived aroma intensity of the compound in a sample due to the different odour threshold or differences in detector sensitivity for different compounds [26]. An additional criterion for a compound to be considered as specific compound is that it must be odour-active with greater than 50% detection frequency (i.e. half or more detection frequency out of all panelists) as assessed by GC–O. In this study, all samples were subjected to GC–O, based on the detection frequency method, to determine the odour-active compounds out of the range of volatiles. A total of 51 volatile compounds possessed an odour activity in BPFs. Meanwhile, based on the aforementioned correlation analysis and odour activity, among the 67 compounds that were positively correlated with sensory attributes, only 26 compounds were identified as odour-active in BPFs, with greater than 50% detection frequency by GC–O. Of these 26 compounds, 3 compounds were not able to be identified by GC–MS due to either small peak area or low quality of identification (Table 4).

Among the 26 aroma-active compounds, 16 compounds have already been demonstrated to be responsible for beef aroma: 3-methylbutanal (chocolate, pungent, sweet, roasted), hexanal (green), heptanal (green, fatty, oily), nonanal (soapy), (E,E)-2,4-decadienal (deep-fried, fat), decanal (sweet popcorn, fatty), 1-octen-3-ol (mushroom), 2-butanone (chemical, burnt), 2-decanone (musty, fruity), hexanoic acid (sweety), bis(2-methyl-3-furyl) disulfide (meaty), 2-methyl-3-(methylthio) furan (meaty, sweet, sulfurous), 2-methyl-3-furanthiol (2-MF) (meaty, cooked rice), 2-pentylfuran (metallic, earthy, green), 2-ethyl-3,6-dimethylpyrazine (nutty, roasted) and 2-acetyl-1-pyroline (roasted, popcorn, coffee) [13,17,27,28].

It is noted that 22 compounds were significantly influenced by beef base ($p \leq 0.001$). Eleven of them were Maillard reaction products, 8 derived from lipids and 3 compounds were not identified. 2-Methyl-3-furanthiol (2-MF), along with bis(2-methyl-3-furyl) disulfide which is a dimmer of 2-MF, is formed via thermal degradation of thiamin or as the thermal product of pentoses and cysteine [29]. 2-Methyl-3-(methylthio) furan was proposed to be formed via either Maillard reaction of ribose and cysteine, involving the reaction with methanethiol, or the reaction of 2-MF with methanethiol [30]. 2-Acetyl-1-pyroline and 2-ethyl-3,6-dimethylpyrazine can be produced from the Strecker degradation, which involves interaction of nitrogen containing molecules (e.g. α-amino acids) with dicarbonyls resulting from carbohydrate decomposition or lipid oxidation in a classic Maillard reaction. Other heterocyclic compounds associated with typical flavours, like 2,5-dimethylthiophene, 3-methyl-2-thiophenecarboxaldehyde and 2-pentylpyridine, were also the products of the reaction of carbonyls with Strecker degradation products, i.e. ammonia, hydrogen sulfide [31]. The autoxidation of saturated or unsaturated fatty acids from tallow produce hexanal, heptanal, nonanal, decanal, 2-undecenal and (E,E)-2,4-decadienal. Lipid degradation also contributes to the formation of 1-octen-3-ol, 2-butanone, 2-decanone, hexanoic acid and 2-pentylfuran. In addition, strecker degradation of leucine contributes to the formation of 3-methylbutanal.

Evidently, 9 compounds possessing an odour activity were not detected in BPF0 without beef base, including 2-MF, bis(2-methyl-3-furyl) disulfide, 2,5-dimethylthiophene, sulfurol, 2-pentylfuran, 3-methyl-2-thiophenecarboxaldehyde, 2-pentylpyridine, A11 and A25. Those compounds could contribute to the production of a different overall odour by adding their specific notes to the aroma profile of BPFs. For instance, 2-MF and bis(2-methyl-3-furyl) disulfide were reported as the most character impact compounds with high aroma values and were responsible for the meat-like odour in cooked beef [32]. The absence of these compounds for BPF0 might be the cause of its weaker beefy, meaty and simulate notes from sensory analysis compared with other BPFs (Table 3). Compound A11, which may contribute to characteristic of beef tallow flavour, was only detected in BPF2. 2-pentylfuran, A25 and 2-pentylpyridine were found in BPF2, BPF3, BPF4 and BPF5, while 2-MF, sulfurol and 3-methyl-2-thiophenecarboxaldehyde were only present in BPF1, BPF2 and BPF3. Among these, BPF2 showed significantly higher detection frequency scores for most aroma-active compounds than other samples especially for 2-MF, 2-pentylfuran, 3-methyl-2-thiophenecarboxaldehyde and sulfurol. Moreover, decanal, 1-octen-3-ol, 2-decanone and 2,5-

dimethylthiophene exhibited sweet, mushroom, alcoholic/milky, and rancid aroma respectively, and there were no different detection frequencies for these compounds in all BPFs.

Comparing this study with research reports [7], thiol, sulfide or disulfide group substituted furans at the 3-position have been regarded to be associated with typical meat-like aroma. In this study, 2-MF, bis(2-methyl-3-furyl) disulfide and 2-methyl-3-(methylthio) furan seemed to be major contributors to the aroma profile of BPF2, adding meaty, sulfury, onion and cooked rice notes. Meanwhile, 2-ethyl-3, 6-dimethylpyrazine and 2-acetyl-1-pyroline had an important impact on the roasted, popcorn and nutty notes of BPF1 and BPF5. Lipid-derived aroma-active aldehydes contributed to the fatty and meaty notes of BPFs. Compound A14 and sulfurol contributed, at different levels, to the tallow-like and beef-like notes of the BPFs especially for BPF2 while δ-nonalactone with sweet, dairy, nutty notes had a high impact on the profile of all BPFs. Based on those results, samples from BPF1 to BPF5 produced from beef base with different DH developed many

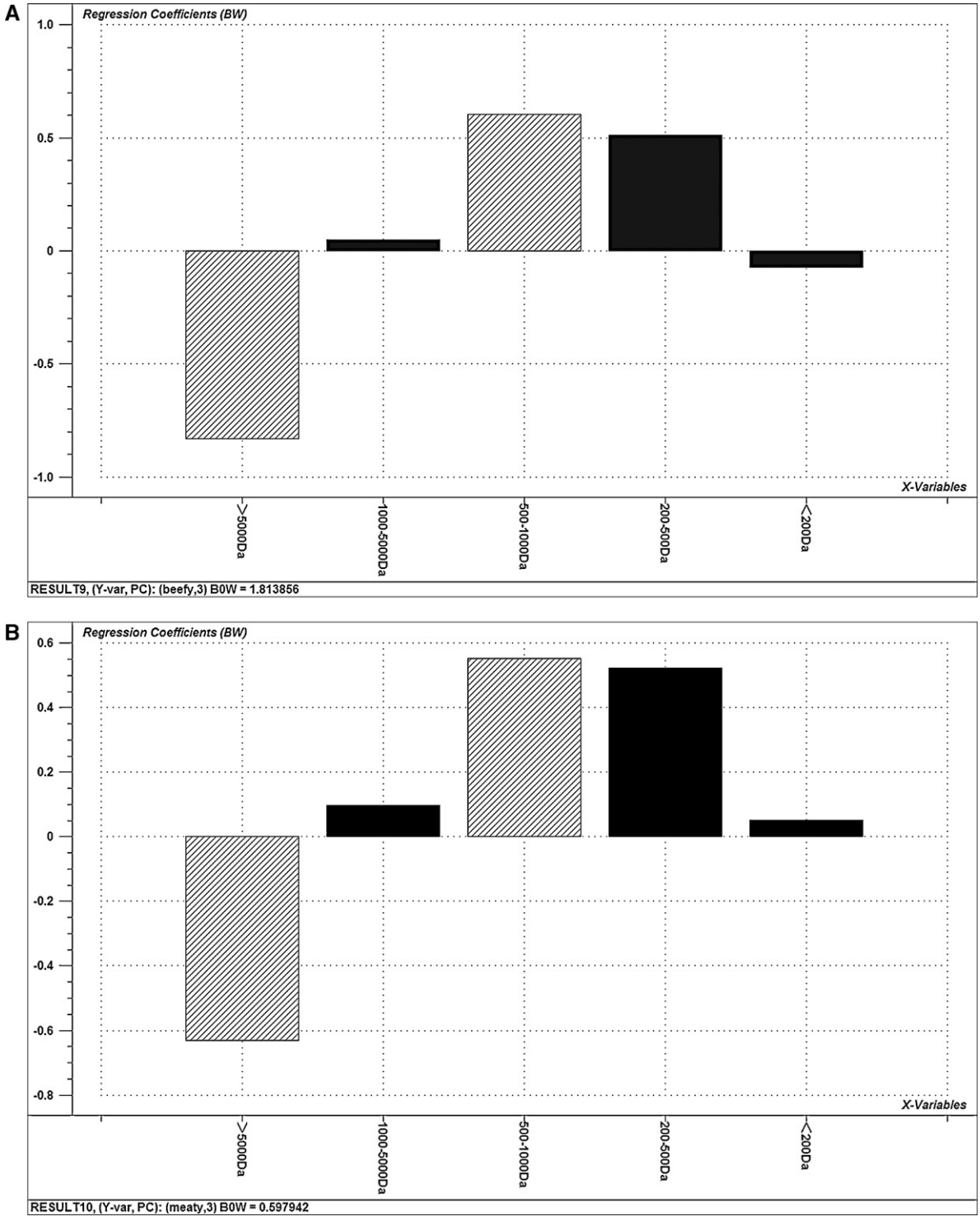

Fig. 3. Standardized, estimated regression coefficients and significance indications from PLS1 prediction models for the sensory attributes variables beefy (A), meaty (B), simulate (C), mouthful (D) and roasted (E) from MW of peptides.

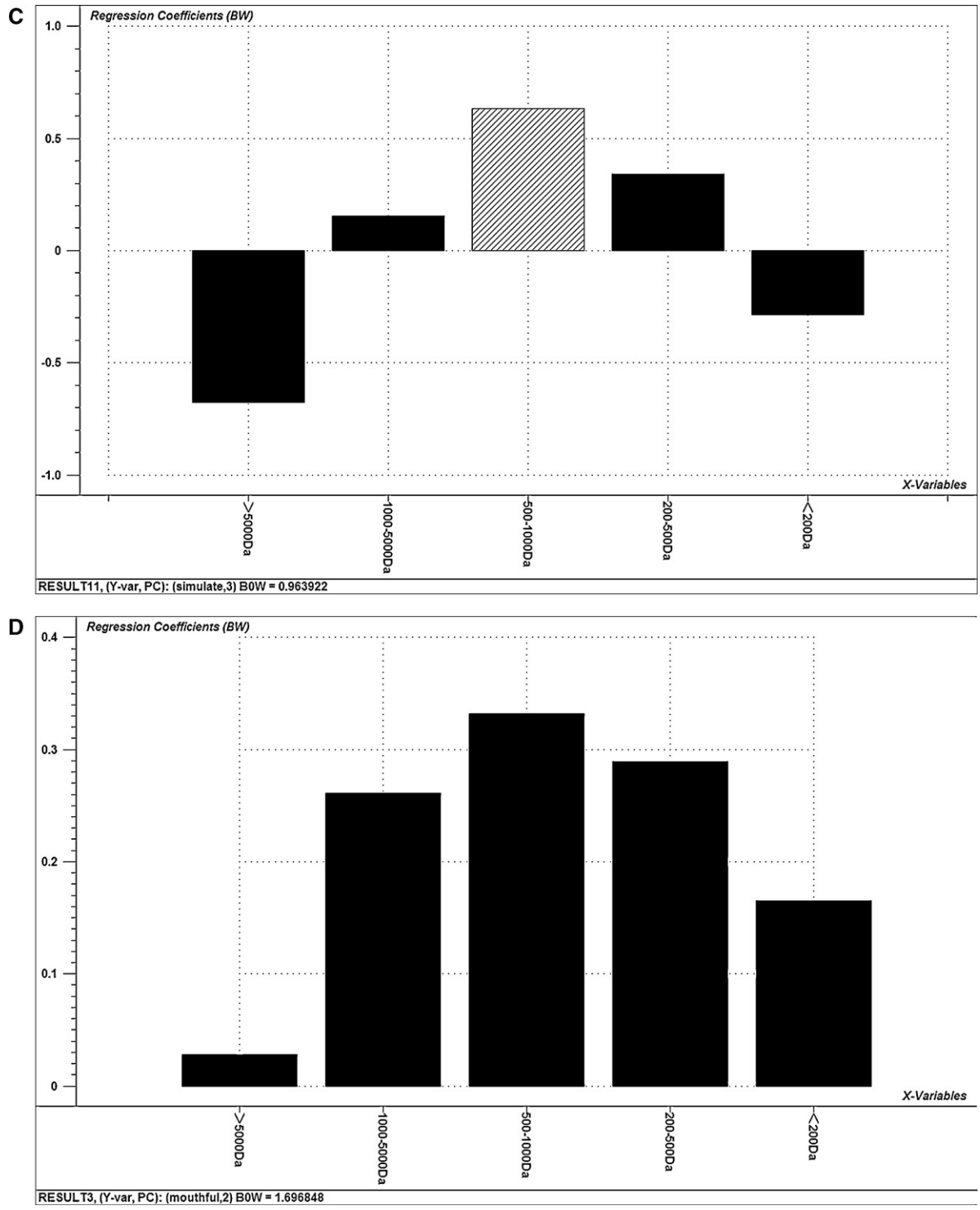

Fig. 3. (*Continued*)

more volatile compounds compared with BPF0 without beef base. And it seems that BPF2 produced from beef base of DH 29.13% developed a wider range of odour-active compounds while BPF0 seemed to miss most of them. From the point of aroma profile view, these results also confirmed the reliability of the panelists' sensory evaluation for BPFs (Table 3). As a consequence, it seems that partially hydrolyzed beef has an important influence on generation of odour-active compounds during beeflike process flavours. This different balance of volatiles through adding different beef base could lead to a diverse overall aroma.

3.4. Relationship between MW of peptides, odour-active compounds and sensory attributes of BPF samples

ANOVA-PLSR was used to process the mean data accumulated from sensory evaluation by the panelists and GC–O analysis. Table 4 corresponds to odour-active compounds identified by GC–O analysis of BPFs. The *X*-matrix was designated as GC–O measurements and peptides with various molecular weight distribution of beef base. The *Y*-matrix was designated as 0/1 design variables for BPF samples and sensory variables. The derived PLSR model included

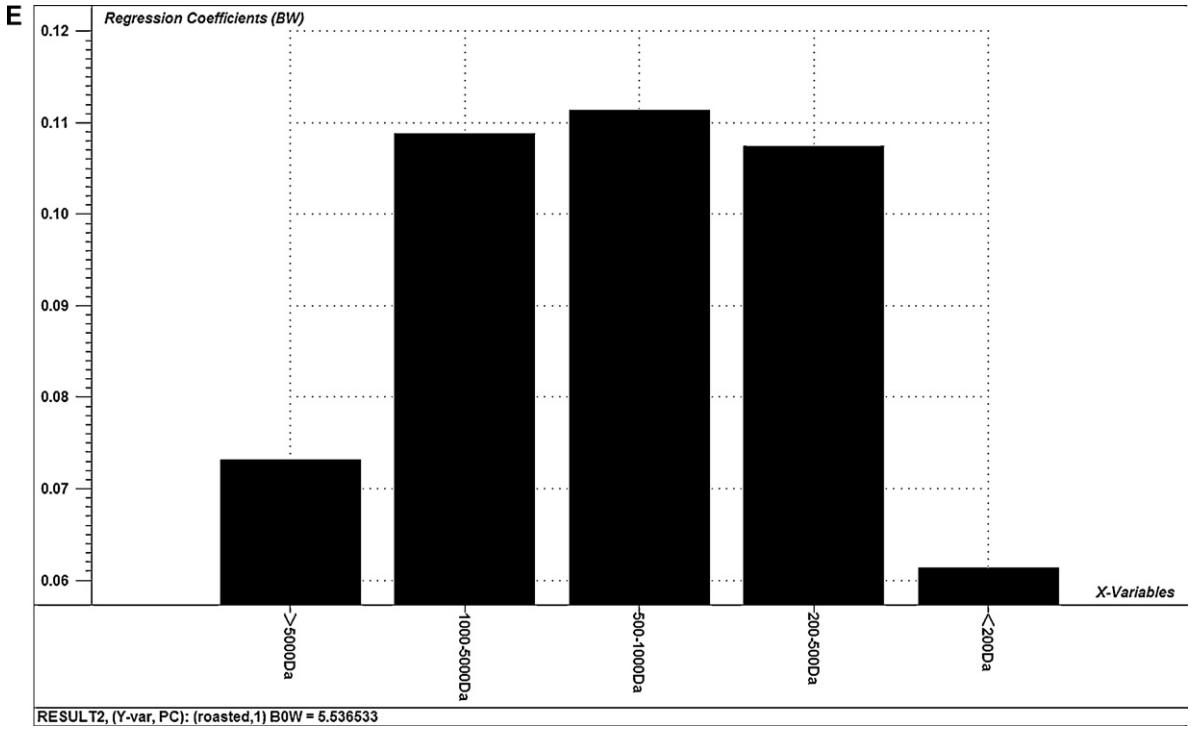

Fig. 3. (*Continued*).

three significant PCs explaining 84% of the cross-validated variance. Thus PC 1 versus 2 (Fig. 1) and PC 2 versus 3 were explored. PC 2 versus 3 was not presented here, as no additional information was gained through their examination. Further PCs did not provide any predictive improvement in the *Y*-matrix obtained. The estimated regression coefficients from the jack-knife uncertainty test show that the odour-active compounds 1-octen-3-ol, 2-MF, 2-methyl-3-(methylthio) furan, 2-pentylpidine, 2-pentylfuran, nonanal and butanone are significant ($p \leq 0.05$) for one or more of the six BPF samples and five significant sensory descriptors. The big circles indicate 50% and 100% explained variance, respectively. The resultant correlation loadings plot of PC1 and PC2 (Fig. 1) shows that BPF samples appears to be separated along PC1 with samples BPF0 and BPF1 on the left side and samples prepared from beef base of higher DH along the right side of the plot. The variation in PC2 was found to be explained by roasted (lower part) and the other sensory attributes (upper part). Six of the *Y*-matrix were placed between the inner and outer ellipses, $r^2 = 0.5$ and 1.0, respectively, indicating they were well explained by the PLSR model.

It is obvious that the control sample BPF0 without adding beef base was negatively correlated to all the sensory variables and all odour-active compounds even though it showed certain odour characteristics via panelists' evaluation. This phenomenon might be caused by sensory evaluated error, or it confirmed that process flavours only based on hydrolyzed vegetable protein cannot truly simulate natural meat aroma. The sample BPF1 seems only covaried with peptides over 5000 Da, and this is due to the fact that BPF1 was the thermal product from beef base HBP1, which showed the lowest DH and the highest content of peptides above 5000 Da (0.25 mg/mL) compared with others (Table 1). On the contrary, sample BPF2 covaried with beefy, meaty and simulate notes and some of the identified odour-active compounds and peptides ranging from 200 to 1000 Da. Sample BPF5 covaried with roasted note and the identified odour-active compounds 2-acetyl-1-pyroline, 2-ethyl-3,6-dimethylpyrazine and 3-methylbutanal and peptides lower 200 Da. The above presented results are in accordance with that of Mussinan et al. [33] who proposed that pyrazines contributed to characteristics of cooked foods due to the fundamental roasted aroma. These results are also similar with the findings of Specht et al. [34] who found that 3-methylbutanal was probably one of the compounds responsible for roast beef flavour.

To further investigate that which odour-active compounds have the significant contribution to sensory attributes of BPFs, PLS1 regression analysis was carried out. In addition, the significant variables for each sensory attributes were inspected by calculating estimated regression coefficients from the jack-knife uncertainty test (Fig. 2). All aroma-active compounds positively correlated to beefy and meaty notes (Fig. 2A and B). Beefy note was significantly correlated to hexanal, nonanal, decanal, 1-octen-3-ol, 2-methyl-3-(methylthio)furan, 2-MF, 2-pentylfuran, 2-pentylpyridine and unknown compound A14, and they explained 78.22% of the variation in beefy attribute. The compounds 2-undecenal, 2-methyl-3-(methylthio) furan, 2-MF, 2-pentylfuran, 2-pentylpyridine, δ-nonalactone and A14 showed a significant influence and also explained 72.61% of the variation in meaty attribute. Except for 3-methylbutanal, 2-acetyl-1-pyroline and 2-ethyl-3,6-dimethylpyrazine, the other aroma-active compounds showed positive correlation to simulate note, however, only 2-methyl-3-(methylthio) furan and 2-MF have significantly correlated to simulate note (Fig. 2C). These results are consistent with the findings of many researchers that the above compounds are the key aroma compounds in beef due to their low odour detection threshold value, e.g. 2-MF would display intensive aroma even present only in trace concentration due to the lower odour-perception threshold (0.005–0.01 μg/kg). In addition, all detected aroma-active compounds in this study showed non significance and some negative correlation with mouthful and roasted attributes (Fig. 2D and E) and only 2.27% and 16.26% of the variation were explained respectively. An explanation for the lacks of confidence of these models was that the mouthful perception was probably caused by non-volatile derivatives of nucleotides and peptides as well as minerals and roasted note imparted by other key aroma compounds which were not detected in this study. Another reason is probably caused by the error of panelists' evaluations.

Similarly, further studies on the relationships between peptides with different molecular weight distribution to the sensory attributes were carried out by calculating estimated regression coefficients from the jack-knife uncertainty test (Fig. 3). Peptides above 5000 Da had negative impact on beefy, meaty and simulate attributes (Fig. 3A–C), and showed to be significantly correlated to beefy and meaty notes. The above three attributes also showed statistically significant and positively related to peptides ranging from 500 to 1000 Da (Fig. 3A–C), while other peptides had no significant influences, indicating that peptides ranging from 500 to 1000 Da might participate in the formation of aroma-active compounds of beeflike flavour or intensify the perception of these compounds associated with beefy, meaty and roasted aroma. All peptides have been found positively associated with mouthful and roasted attributes; however, there was no significant influence for the two attributes (Fig. 3D and E). It further explained that mouthful note was indirectly imparted by peptides. Based on the correlation analysis, it can be noted that beef base with DH 29.13% which has the lowest contents of peptides above 5000 Da (not detected) and highest contents of peptides ranging from 500 to 1000 Da (21.04 mg/mL) (Table 1) is a desirable precursor for imparting aroma characteristics of beeflike process flavour, corresponding BPF2.

4. Conclusions

Beef base is proved to be useful in accentuating or extending the basic meat flavour character. Experimental results suggested that beef base with five different DH showed significant impact on aroma characteristics of BPF compared with control through descriptive sensory analysis. Beeflike process flavours prepared from different beef base showed superiority in one or more attributes, respectively. In particular, sample added beef base with DH 29.13% showed the strongest beefy, meaty and simulate characteristics. Further investigation for six BPFs based on aroma-active compounds assessed by GC–O. Twenty six compounds were selected as specific compounds to represent beef odour. Results indicated that beef base with DH 29.13% gives the development of a wider range of odour-active compounds compared with other beef base, while sample without beef base seemed to miss most of them. Meanwhile, PLSR analysis between MW of peptides, odour-active compounds and sensory attributes clearly showed that beef base with DH 29.13% was a desirable precursor for imparting aroma characteristics of BPF.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.10.046.

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