

Contribution of Chicken Base Addition to Aroma Characteristics of Maillard Reaction Products Based on Gas Chromatography-Mass Spectrometry, Electronic Nose, and Statistical Analysis

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Abstract Maillard reaction products containing 0-86.67% (w/w) enzymatically hydrolyzed chicken breast (chicken base) were analyzed. Descriptive sensory analysis was conducted and the 5 sensory attributes of meaty, caramel, umami, continuity, and off-flavor were used for evaluation. A sample group containing 65.00% chicken base had high scores for meaty note. A sample group containing 86.67% had a high score for caramel note. The electronic nose was used for measurement of flavor quality changes based on a metal oxide sensor (MOS) array. GC-MS was used for identification of volatile compounds. Heterocyclic sulfur and nitrogen oxygen compounds (especially 2-methyl-3-furanthiol, 2-methyl-furan, 2-pentyl-furan, 2-acetyl-furan, bis (2-methyl-3-furyl) disulfide, furfural, 2-acetyl-pyrrole, 1-methyl-pyrrole, thiazole, and 4-methyl-thiazole) were responsible for meaty and caramel attributes. Statistical analysis was used to interpret the distribution of samples and correlations among variations.

Keywords: chicken base, Maillard reaction product, sensory evaluation, electronic nose, gas chromatography-mass spectrometry

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Introduction

Thermal process flavors are produced by heating a combination of precursor materials under controlled conditions. The key reaction occurring in this process is the Maillard reaction (1). Precursor materials mainly include amino acids, peptides, sugars, and thiamine. Desirable meaty flavors were attributed to the water-soluble protein fraction (1,2). Hydrolyzed vegetable protein (HVP), like soybean protein, is a source of amino compounds that participate in the Maillard reaction (3-5). HVP based-flavor can only partially simulate the natural meaty aroma. Therefore, hydrolyzed animal proteins as flavor precursors are used in thermal reaction model systems for preparation of meat flavors (6,7). Chhuy and Day (8) attempted to produce a meat flavor by heating enzymatically degraded meat hydrolysates. Consequently, studies of meat proteolysis and meat by-products have been done (9,10). Modeling of amino acid-reducing sugar mixtures is being used to study the pathway of Maillard reactions and the properties of products derived from the reactions (11-13). Although modeling studies provide valuable thermodynamic information, obtained knowledge may not be totally and directly applicable to real meat systems. Due to meat protein hydrolysates used as flavor precursors, it is imperative to elucidate the effects of meat protein hydrolysates on flavor changes in order to obtain a savory, meaty flavor. Previous studies concentrated on the degree of hydrolysis and on reaction factors of temperature, pH, time, and amounts of reducing sugar. This study focused on the effects of hydrolyzed protein proportions on flavor attributes.

Solid phase micro extraction (SPME)-GC-MS and human sensory evaluations are common methods used for odor analysis in the food industry (14-16). Recently, use of an

electronic nose has been popularized due to rapid detection of volatiles (17,18). Although electronic nose analysis can discriminate between samples, a new way to combine electronic nose and sensory evaluation for simultaneous use is needed. Partial least squares regression (PLSR) can be used to combine GC-MS analysis with sensory evaluation, and even to incorporate the electronic nose response. Score plots can be used to interpret differences and similarities among samples while corresponding loading plots can be used to explain what factors are responsible for differences among samples. Based on correlations between these analyzes, a suitable amount of meat protein hydrolysate to be used can be identified. Principal Component Analysis (PCA) is frequently used for multivariate data analysis. No report exists regarding correlations between meat protein hydrolysates (meat base) and the complexity of aroma. Therefore, this should be a novel perspective for study of aromas produced by Maillard reactions.

The objectives of this study were (a) to apply descriptive sensory analysis to assess the aroma attributes of Maillard reaction products (MRPs) stemming from chicken base with use of additives, (b) to detect key volatile compounds using GC-MS and to discriminate MRP samples rapidly using an electronic nose, (c) to simplify multidimensional data generated using PCA and to classify samples based on agglomerative hierarchical clustering (AHC), and (d) to identify correlations between sensory attributes and major volatile compounds based on an electronic nose response with PLSR.

Materials and Methods

Materials Chicken breast was purchased from a local TESCO supermarket in Shanghai, China in November of 2013. Protamex (activity 1.5 AU/g) (AU/g, anson units per gram is measured with the Novozymes analysis method, which is based on denatured hemoglobin in 0.02 mol/L Ca^{2+} buffer solution) was purchased from Novozymes (Bagsvaerd, Denmark). D-glucose, D-xylose, L-cysteine, hydrochloride, glycine, β -alanine, thiamine hydrochloride, 2-octanol (chromatography grade), ethanol (chromatography grade), and other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of enzymatic hydrolyzed chicken breast (chicken base) Chicken breast (protein content of 12.83% measured using the Kjeldahl method) (15) was minced using a tissue-tearor and mixed with deionized water at a meat-water ratio of 1:3 in an enzyme reactor with mechanical stirring at 500 rpm. The pH was adjusted to 6.5 with 1 mol/L NaOH, then chicken breast was hydrolyzed in a double deck glass reactor equipped with circulating

water bath at a constant temperature of 50°C for 3 h using protamex with a 0.3% enzyme/substrate ratio (E/S). The resultant slurry was heated in boiling water bath equipped with magnetic stirrer at 98°C for 15 min to inactivate the enzyme, then partly centrifuged with equipment of CT15RT versatile refrigerated centrifuge (Techcomp Ltd., Shanghai, China) at $4,360\times g$ for 20 min for removal of insoluble residue. The supernatant was used for analysis of the degree of hydrolysis (DH).

Preparation of Maillard reaction products (MRPs)

Mixtures of D-glucose (1.0 g), D-xylose (0.5 g), L-cysteine hydrochloride (1.0 g), glycine (0.5 g), β -alanine (0.5 g), thiamine hydrochloride (1.0 g), sodium chloride (NaCl) (2.5 g), and monosodium glutamate (0.5 g) were prepared. Mixtures were blended with 52, 39, 26, 13, and 0 g of chicken base in triplicate, then the total weight was adjusted to 60 g using deionized water and the pH was adjusted to 8.0 using 4 mol/L NaOH. Then, solutions were transferred to 90 mL screw-sealed tubes that were tightly capped, then heated in a thermostatic oil bath with magnetic stirring (150 rpm) at 105°C for 60 min. After reaction, tubes were immediately cooled in ice water and products were named A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, E1, E2, and E3 for subsequent analysis. Samples labeled as “A” were prepared using 52 g of chicken base. “B” samples were prepared using 39 g of chicken base, “C” samples were prepared using 26 g of chicken base, “D” samples were prepared using 13 g of chicken base, and “E” samples were prepared without chicken base.

Determination of the degree of hydrolysis (DH) The degree of hydrolysis was defined as the ratio of free amino groups present in hydrolysates to the total amount of amino groups in protein. The number of free amino groups was determined using a modified formol titration method (19).

Sensory analysis Quantitative descriptive sensory analysis using a 10 point interval scale (0=none, 9=extremely strong) was applied for evaluation of MRPs. Evaluation was performed by a well-trained panel of 6 women and 4 men of 22–45 years of age. All panelists had experience with sensory evaluation and had been trained according to ISO 8586-1:1993(E) (15) and American Society for Testing and Materials (ASTM STP 758, 1981) (20) standards. Evaluations took place in a standardized sensory environment following ISO 8589:2007(E) (15) standards and followed acceptable sensory practice (20). Panelists stated and discussed characteristic sample aromas during 3 preliminary sessions until agreement was reached. The 5 sensory attributions of meaty, caramel, umami, continuity, and off-flavor were used for the descriptive analysis. Samples were maintained in a 50°C water bath in order to avoid a temperature

influence and coded with a 3 digit number in a random order to avoid the order effect (15). Samples were evaluated using a 10 point interval scale in triplicate. Results were recorded as average scores of 3 sets of parallel testing. During the process of sensory evaluation, panelists were allowed to breathe fresh air for 10 min after 3 samples were evaluated in order to relieve sensory fatigue.

Electronic nose analysis A FOX 4000 nose from Alpha-MOS (Toulouse, France) was used equipped with 18 metal oxide sensors and a headspace auto sampler HS100. All sensors were divided into 3 clusters (T, P, and LY) based on different oxides. For analysis, 0.5 g of a sample was transferred to a 10 mL glass vial and capped with a Teflon/silicon rubber cap. Samples were temperature conditioned at 22°C before placement into the auto sampler. Parameters of the analysis were 1) an auto sampler incubation temperature of 60°C and an incubation time of 10 min, 2) an analyzer acquisition duration of 20 s at a flow-rate of 150 mL/min, and 3) a syringe flushing time of 120 s with reference air. Analysis was performed for 5 samples in triplicate.

SPME-GC-MS analysis Seven g of sample and 1.0 g of NaCl were weighted by analytical balance (Mettler Toledo international Co., Ltd., Shanghai, China) and placed in a 20 mL headspace transparent glass vial. Then, 10 µL of 2-octanol (411 mg/L in ethanol) as an internal standard was added to samples using a pipette to facilitate quantitative analysis. Vials were sealed using a PTFE/BYTL septum to avoid the loss of volatile compounds. To maintain a constant temperature of 60°C, vials were placed in a water bath. After 10 min of equilibration, extraction was performed using SPME-fiber (75 µm carboxen/polydimethylsiloxane) conditioned 30 min at 230°C. After 1 h of adsorption, the fiber was thermally desorbed in a GC (Agilent Technologies 7890A) injector at 250°C for 5 min. Chromatographic separations were done using a HP-INNOWAX capillary column (60 m×0.25 mm×0.25 µm; J&W Scientific Inc., Folsom, CA, USA). The oven temperature was programmed

at 50°C for 3 min, ramped to 230°C at a rate of 4°C/min, and held for 10 min. The flow of carrier gas (helium) was 1 mL/min in a split ratio of 2:1. The MS apparatus worked as a detector and operated with an ionization voltage 70 eV and an emission current of 35 Ua. The detector was set at a scanning range of $m/z=35-450$ at a rate of 4.45 scans/s.

Identification of volatile compounds was carried out either by comparison of ion test spectra with authentic standard spectra, or by matching with mass spectral libraries (WILEY and NIST database) (15) and reported retention index (RI) values. RI values were calculated based on a series of n-alkanes (C7-C30) (Sigma-Aldrich, St. Louis, MO, USA) that were injected under the same chromatographic conditions as for test samples. Relative percentage amounts of volatile compounds were estimated based on comparison of peak areas with an internal standard compound (10 µL of 411 mg/L 2-octanol in ethanol). Analyses were performed in triplicate.

Data analysis Descriptive analytical data were evaluated using the analysis of variance (ANOVA) function of SAS 8.2. An ANOVA with Duncan's multiple range test (MRT) identified significant differences at ($p<0.05$) among samples for each attribute. Data acquired using the electronic nose were analyzed using PCA. GC-MS data were processed using the PCA and hierarchical cluster analysis (AHC) functions of XLSTAT 2010. Partial least squares regression analysis (PLSR) was performed using Unscrambler 9.7 (CAMO ASA, Oslo, Norway).

Results and Discussion

Sensory evaluation analysis Results of Duncan's MRT using mean scores is shown in Table 1 and Fig. 1 using the 5 sensory attributes of meaty, caramel, umami, continuity, and off-flavor. Scores of each attribute among the 5 groups were significantly ($p<0.001$) different, which indicated that the samples had significantly different aroma intensities for all sensory attributes. Group B for meaty presented the

Table 1. Mean scores of 5 MRP groups for each attribute in descriptive sensory evaluation

Sample	Mean score ¹⁾				
	Meaty (***) ²⁾	Caramel (***)	Umami (***)	Continuity (***)	Off-flavor (***)
A	5.125 ^c	8.625 ^a	5.125 ^c	6.958 ^b	1.750 ^c
B	8.375 ^a	6.167 ^b	6.542 ^b	7.875 ^a	1.125 ^d
C	6.417 ^b	5.792 ^b	6.958 ^b	4.750 ^{cd}	3.875 ^b
D	5.125 ^c	4.250 ^c	7.875 ^a	5.250 ^c	4.292 ^b
E	4.583 ^c	5.708 ^b	4.792 ^c	4.167 ^d	6.542 ^a

¹⁾Mean attribute scores within a column with different letters are significantly different ($p<0.001$) using Duncan's multiple range test ($n=30$, 10 panelists with 3 replications).

²⁾*** indicate significance at $p<0.001$, which indicated that the samples had significantly different aroma intensities for all sensory attributes.

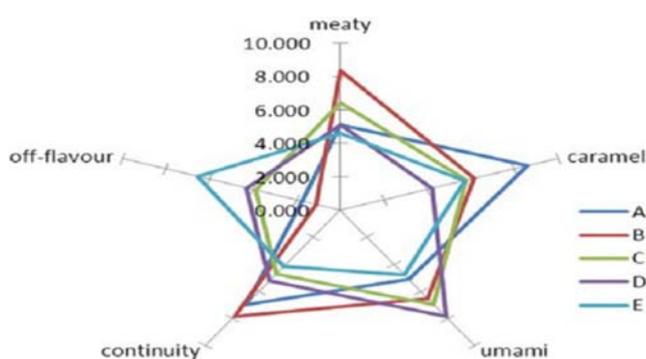


Fig. 1. Mean sensory scores for the 5 groups of MRP samples.

strongest scores, and followed by group C. Group A showed strong and group D showed weak caramel scores. Group D had the highest sensory scores for umami, and groups B and A showed stronger scores for continuity than other groups due to interactions between a meaty aroma and a caramel note. In contrast, group E, the control group, showed a significant ($p < 0.05$) difference from other groups for the five sensory attributes. Group A received the highest score of 8.625 points for the caramel note (Fig. 1), perhaps due to an excessive amount of peptides in chicken base. Group B exhibited the highest scores for meaty and continuity at 8.375 and 7.835 points, respectively. Group C exhibited high scores of 6.417 points for meaty and 6.958 points for umami, but a low score of 4.750 points for continuity. Group D received the highest marks of 7.875 points for umami. Group E exhibited highest marks of 6.542 points for off-flavor, but lower points for other attributes, which is caused of no chicken base. The characteristic aroma of MRPs was influenced by chicken base addition. Group B, rather than A, had pleasant scores for the meaty attribute. Therefore, a 65.00% chicken base content was suitable for use in the reaction system.

Results of electronic nose analysis Response values for 18 sensors are shown in Fig. 2 for the electronic nose. Group A (strong caramel) had a high positive response on sensors P, T and LY2/LG, while group B (strong meaty) had a high negative response on other LY sensors. The response value of group E (without chicken base addition) was lower than for other groups with chicken base addition. Response values varied with the molecular functionality of odorants, which derived from Maillard reaction products.

PCA is a commonly used method for multivariate data analysis based on limitation of the number of variables (21). The goal of PCA is to distinguish groups using principal components. A score plot for the first 2 factors (F1 and F2) explained the aroma differences of MRP samples with different chicken base addition (Fig. 3). In addition, factor F1 explained 95.02% of the total variation, while 3.46% of the total variance was explained by F2. Group E, without chicken base, was distributed in the left half of Fig. 3. Other groups with chicken base addition were scattered in the right half and distributed from left to right. The distribution of samples explained the aroma differences of MRPs with different amounts of chicken base addition. PCA results confirmed that the electronic nose could be regarded as a valid method for identification and classification of MRPs between different formulas of chicken base.

Analysis of volatile aroma compounds Volatile compounds analysis was performed using GC-MS. More than 100 volatile compounds were detected and 34 volatile compounds were used for further analysis (Table 2), based on frequent detection in samples, odors presenting meaty, caramel, umami, or other odors, and direct or indirect relationships with the Maillard reaction (1,6,11,14,15,17,25,26). The volatile compounds included 7 carboxylic

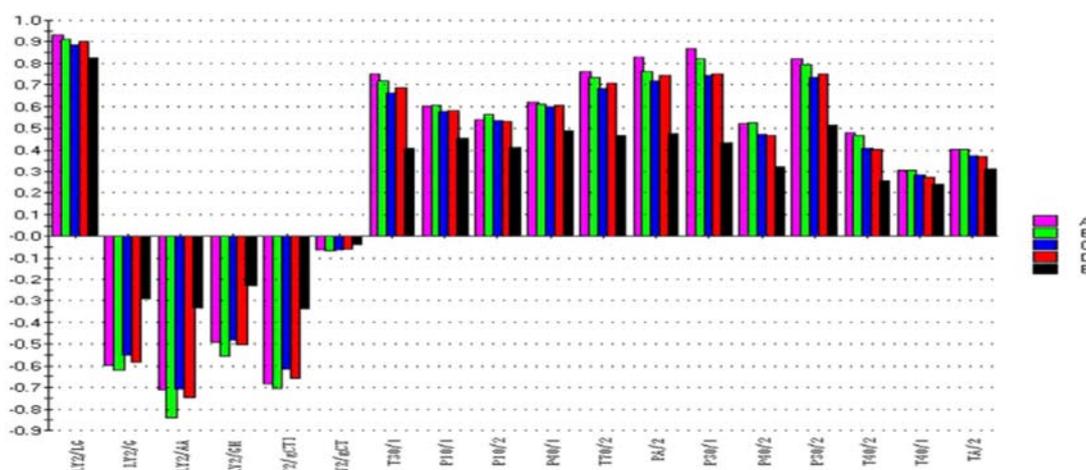


Fig. 2. The 5 sample group based on response values of electronic nose sensors.

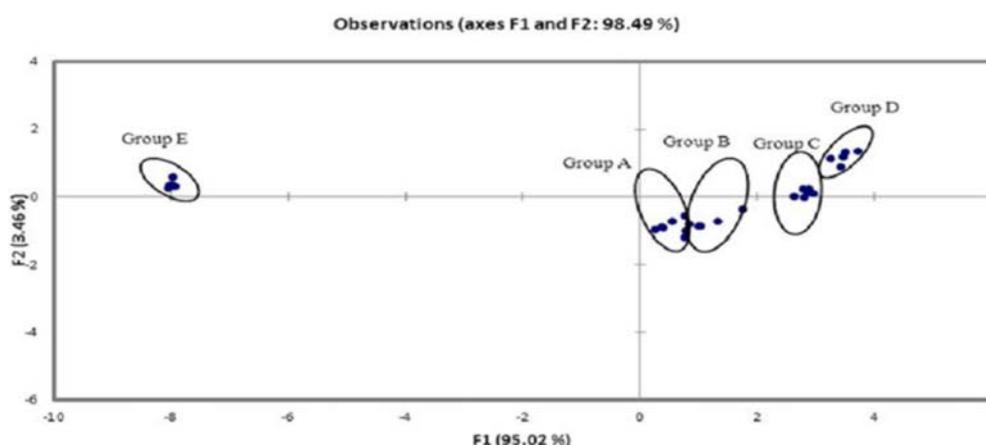


Fig. 3. A PCA plot based on electronic nose data.

acids, 6 aldehydes, 5 furans, 4 thiazoles, 4 ketones, 2 pyrroles, and other compounds. All 34 volatile compounds made a contribution to the 5 sensory attributes. Half of the aroma compounds containing heterocyclic sulfur and nitrogen oxygen compounds were formed via the Maillard reaction, including pyrroles, thiazoles, thiophene, pyrazines, furans, and their derivatives. Certain aldehydes were derived from Strecker degradation, such as 3-methyl-butanal (27,28). The internal fat in chicken breast could have been responsible for ketones and acids.

PCA analysis To achieve an even distribution of variances and mean values, chromatography data are usually normalized before analysis (Unscrambler Tutorials) (29). PCA results for GC-MS data are shown in Fig. 4 after data normalization. The first two factors F1 and F2 explained 39.69 and 31.28% of the total variance, respectively (Fig. 4A). Therefore, the first two factors could be used for interpretation of observations. Groups A, B, and E were clearly separated from groups C and D. The F1 score separated groups A and B from other groups as the scores were oppositely distributed on the horizontal axis (Fig. 4B). Group E was clearly separated from other groups by the F2 score. Aroma compounds with high positive loadings on F1 were mainly 2-furanmethanethiol, 2-methylthiophene, and 2-acetylthiazole. Negative loading compounds included 2-acetylfuran, furfural, and some aldehydes. Most compounds contributed to meaty, caramel and umami attributes. Positive loadings on F2 included octanoic acid and some ketones, and negative loadings included 1-methyl-pyrrol, 2-acetyl-pyrrole, 2-methyl-furan, 2-pentyl-furan, 2-methyl-3-furanthiol, pyrazine, and thiazole. Ketones and acids were considered to be the cause of an off-flavor.

AHC analysis AHC analysis was performed to assess the typology of odors. Two main clusters of group A (the first cluster) and all other groups (the second cluster) were

revealed (Fig. 5). The second cluster was distinguished by 3 layers of subclusters. Groups C and D were discriminated in the third layer subcluster, and group E was discriminated from other groups in the second layer. Group B was distinguished from other groups in the first layer subcluster. AHC results were consistent PCA results for chemical compounds. Both analyses elucidated characteristic aromas produced by Maillard reactions attributed to addition of chicken base.

PLSR analysis PLS2 was carried out to gain an overview of relationships between aroma compounds, electronic nose sensors, and sensory features (Fig. 6). All variables were standardized before regression (1/SDev) to obtain unbiased contributions for each variable. Full cross-validation was used to validate the model. A correlation loadings plot of PC1 versus PC2 is shown in Fig. 6A. Other APCs are not present in Fig. 6 as no correlation information was obtained. Explained model variance was PC1=28% and PC2=26%. Large circles in Fig. 6 indicate 50 and 100% explained variance, respectively. Twenty-two Y variables (caramel, umami, continuity, off-flavor, and 18 electronic sensors) and 17 characteristic compounds were located between inner and outer ellipses ($r^2=0.5$ and 1.0, which indicate that the variance is explained 50 and 100%, respectively), indicating explanation by the PLSR model.

Distribution of MRPs in a score plot showed agreement with aroma compounds and sensory attribute analyses (Fig. 6B). For PC1, group E without addition of chicken base was located on the left side with all other groups on the other side. For PC2, group C, and D were located on the bottom, group B in the middle, and group A on the top. Group A was mainly responsible for the caramel attribute, while group B had a good correlation with the meaty attribute (Fig. 6), in agreement with sensory evaluation analytical results. Both the caramel and meaty attributes made contributions to the continuity attribute. However,

Table 2. Volatile compounds identified in MRPs using SPME-GC-MS

Code	Compounds	RI ¹⁾	ID ²⁾	Odor description	Concentration ($\mu\text{g/g}$)				
					A	B	C	D	E
1	2-Furanmethanethiol	1469	MS,RI	coffee, roast	ND ³⁾	0.018 \pm 0.004 ⁴⁾	0.015 \pm 0.002	0.010 \pm 0.003	0.003 \pm 0.002
2	1-Methyl-pyrrole	1135	MS,RI	roast, coffee	ND	0.005 \pm 0.002	ND	ND	ND
3	2-Acetyl pyrrole	2027	MS,RI	nut, walnut, bread	0.012 \pm 0.002	0.002 \pm 0.002	ND	ND	ND
4	Maltol	2001	MS,RI	caramel, sweet	ND	0.007 \pm 0.004	ND	ND	ND
5	2-Methylfuran	878	MS,RI	chocolate	0.615 \pm 0.003	0.234 \pm 0.006	0.139 \pm 0.000	0.061 \pm 0.003	0.021 \pm 0.003
6	2-Pentylfuran	1237	MS,RI	green bean, butter	0.028 \pm 0.001	0.003 \pm 0.000	0.003 \pm 0.001	0.002 \pm 0.000	ND
7	2-Methyl-3-furanthiol	1339	MS,RI	meat	0.885 \pm 0.008	0.234 \pm 0.004	0.169 \pm 0.005	0.103 \pm 0.003	0.035 \pm 0.003
8	2-Acetylfuran	1549	MS,RI	balsamic, gasoline	0.037 \pm 0.004	ND	ND	ND	ND
9	Bis (2-methyl-3-furyl) disulfide	2216	MS,RI	meaty, coffee, metallic	0.118 \pm 0.004	ND	ND	0.018 \pm 0.002	ND
10	3-Methyl butanal	923	MS,RI	chocolate, caramel	ND	0.011 \pm 0.003	ND	ND	ND
11	Hexanal	1101	MS,RI	grass, tallow, fat	0.05 \pm 0.008	ND	ND	0.005 \pm 0.003	ND
12	Nonanal	1416	MS,RI	fat, citrus, green	0.36 \pm 0.005	0.029 \pm 0.002	0.051 \pm 0.003	0.020 \pm 0.003	0.008 \pm 0.005
13	2-Octenal	1453	MS,RI	green, nut, fat	0.04 \pm 0.004	ND	ND	0.003 \pm 0.001	ND
14	Furfural	1504	MS,RI	bread, almond, sweet	1.205 \pm 0.009	ND	ND	0.003 \pm 0.003	0.002 \pm 0.002
15	Benzaldehyde	1567	MS,RI	caramel, popcorn	0.124 \pm 0.003	0.017 \pm 0.002	0.021 \pm 0.003	0.010 \pm 0.002	0.005 \pm 0.001
16	2-Methylthiophene	1078	MS,RI	meat, sulfur	0.121 \pm 0.002	0.071 \pm 0.005	0.055 \pm 0.004	0.038 \pm 0.004	0.007 \pm 0.001
17	1-Octanol	1585	MS,RI	chemical, metal, burned	ND	0.011 \pm 0.004	0.018 \pm 0.004	0.009 \pm 0.002	0.004 \pm 0.001
18	Pyrazine	1254	MS,RI	roast, fruit, nut	ND	0.005 \pm 0.002	ND	0.002 \pm 0.002	ND
19	Thiazole	1286	MS,RI	nut, sulfur	ND	0.002 \pm 0.000	ND	0.001 \pm 0.002	ND
20	4-Methylthiazole	1315	MS,RI	roasted meat	0.043 \pm 0.002	0.009 \pm 0.002	0.012 \pm 0.002	0.009 \pm 0.003	0.007 \pm 0.000
21	2-Acetylthiazole	1692	MS,RI	roast, nut, sulfur	ND	0.005 \pm 0.002	ND	0.002 \pm 0.002	0.002 \pm 0.002
22	5-(2-Hydroxyethyl)-4-methylthiazole	2371	MS,RI	bean, meat	1.886 \pm 0.005	0.537 \pm 0.004	0.455 \pm 0.005	0.255 \pm 0.002	0.183 \pm 0.006
23	2-Methyltetrahydrofuran-3-one	1299	MS,RI	sweet, nut, butter	ND	0.005 \pm 0.003	ND	0.003 \pm 0.003	ND
24	2-Octanone	1305	MS,RI	milk, mushrooms	0.065 \pm 0.003	0.029 \pm 0.003	0.025 \pm 0.003	0.029 \pm 0.002	0.026 \pm 0.001
25	3-Mercapto-2-pentanone	1393	MS,RI	meat, milk	0.062 \pm 0.004	0.029 \pm 0.006	ND	0.007 \pm 0.003	0.007 \pm 0.003
26	2(3)-Heptanone	1202	MS,RI	fruity, flowery	0.040 \pm 0.007	0.019 \pm 0.004	ND	0.011 \pm 0.002	0.002 \pm 0.002
27	Benzyl benzoate	2706	MS,RI	balsamic, oil, herb	0.065 \pm 0.008	0.019 \pm 0.001	0.049 \pm 0.002	0.019 \pm 0.003	0.025 \pm 0.003
28	Formic acid	1558	MS,RI	sour	ND	0.109 \pm 0.004	0.121 \pm 0.003	0.056 \pm 0.003	0.031 \pm 0.002
29	Acetic acid	1493	MS,RI	sour, viniegra	0.140 \pm 0.004	0.026 \pm 0.003	0.025 \pm 0.003	0.010 \pm 0.003	0.008 \pm 0.001
30	Hexanoic acid	1888	MS,RI	sweet, pungent	ND	ND	0.018 \pm 0.008	ND	ND
31	Octanoic acid	2105	MS,RI	sweet, cheese	0.028 \pm 0.006	ND	0.010 \pm 0.003	ND	0.034 \pm 0.007
32	Decanoic acid	2323	MS,RI	rancid, fat	0.565 \pm 0.006	ND	0.097 \pm 0.007	0.012 \pm 0.004	0.027 \pm 0.006
33	Tetradecanoic acid	2672	MS,RI	rancid, fermented	0.304 \pm 0.003	ND	0.097 \pm 0.001	0.040 \pm 0.003	0.010 \pm 0.003
34	n-Hexadecanoic acid	2955	MS,RI	Mushroom, sour	1.066 \pm 0.010	0.317 \pm 0.002	0.761 \pm 0.003	0.415 \pm 0.010	0.017 \pm 0.004

¹⁾Linear retention index calculated using an HP-INNOWAX capillary column (60 m \times 0.25 mm \times 0.25 μm) using a homologous series of n-alkanes (C₇-C₃₀).

²⁾ID, Identification proposal is indicated by the following; MS, identification by comparison of EI mass spectrum with Wiley and Nist mass spectral database; RI, identification by comparing retent indexes with literature data (5,17,22-24) and flavornet database (<http://www.flavornet.org>, accessed June 2007), Acree, 2004 (on C20M stationary phase) and the LRI Database on the Web (<http://www.odour.org.uk/lriindex.html>) in the literature.

³⁾ND, not detected

⁴⁾Mean \pm SD of triplicates

group C and D exhibited good correlation with umami. In contrast, group E exhibited poor correlation with caramel and meaty attributes, but strong correlation with off-flavor. Thus, enzymatic protein hydrolysates, as flavor precursors, played an important role in formation of the overall flavors of MRPs. Peptides and free amino acids that take part in

Strecker degradation and amino-carbonyl reactions influence MRPs flavor. Both high and low percentages (86.67, 43.33, or 21.67%) of chicken protein hydrolysates produced a negative influence on overall acceptance. Group A, with 86.67% chicken base, showed a stronger caramel attribute, while group B prepared with 65.00% chicken base showed

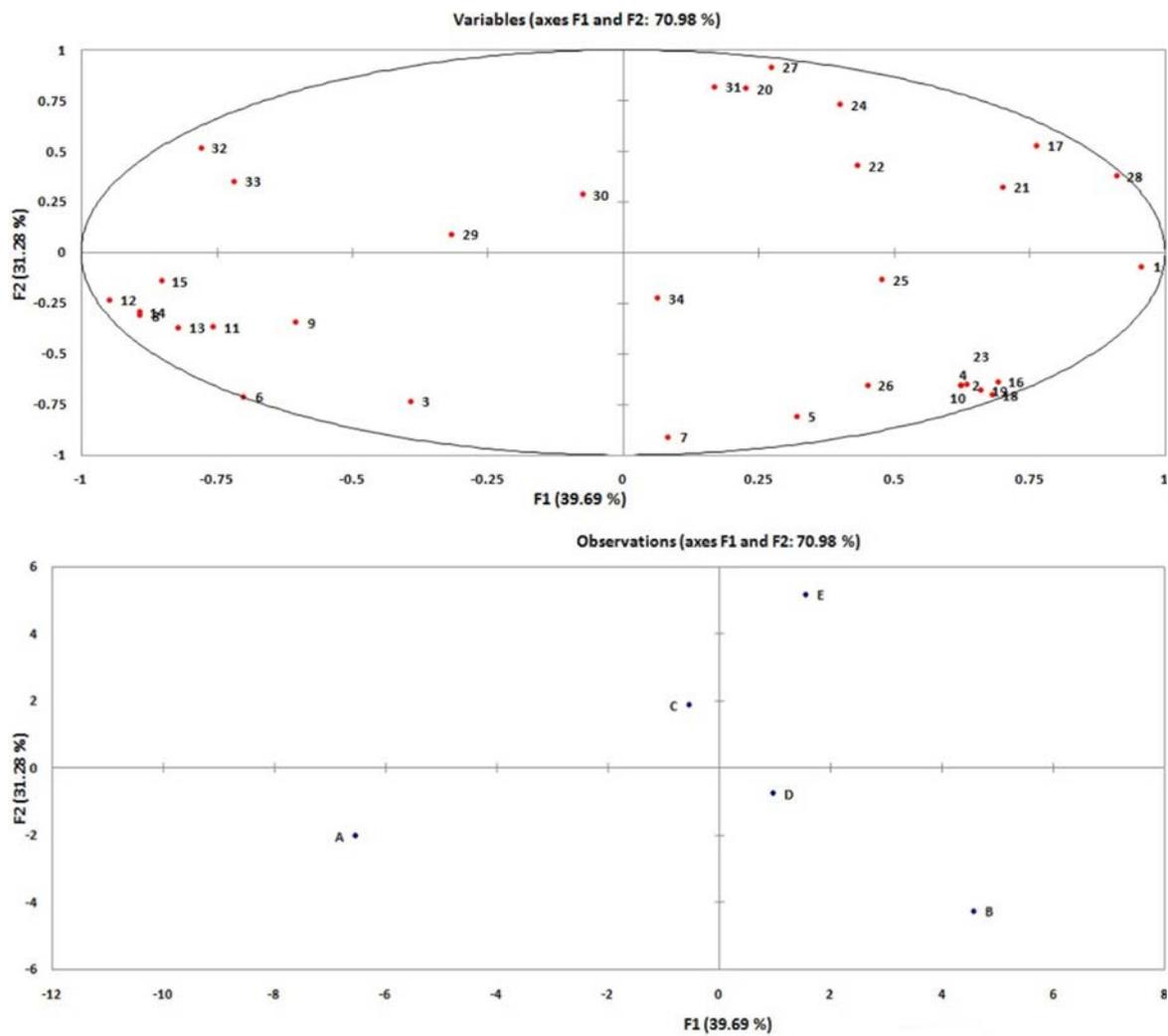


Fig. 4. Correlations between variables and factors (A) and factor scores (B) for PCA based on normalized GC-MS data of 5 MRPs groups.

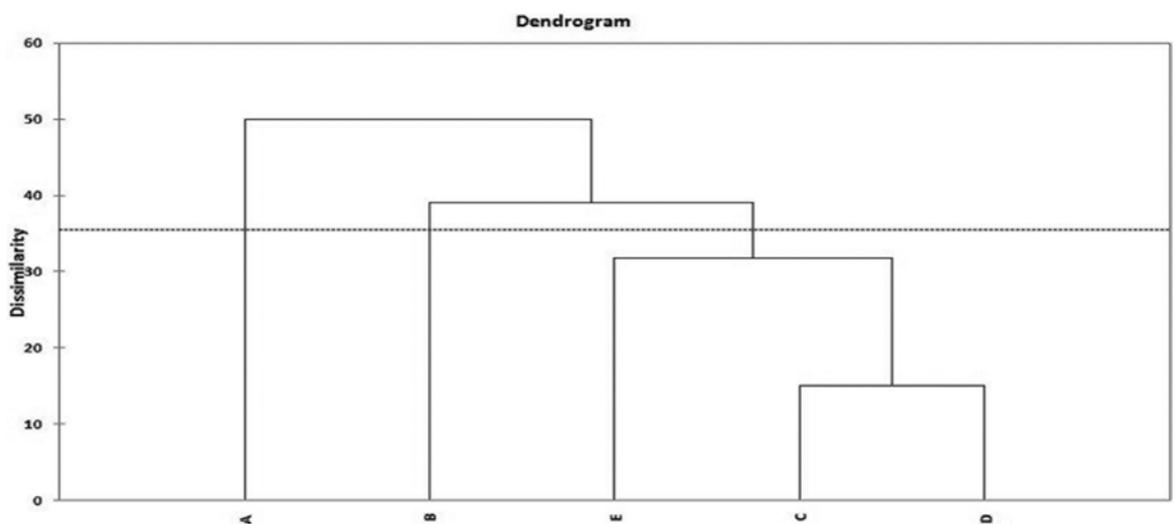


Fig. 5. Agglomerative Hierarchical Clustering (AHC) analysis using normalized GC-MS data of 5 MRPs groups.

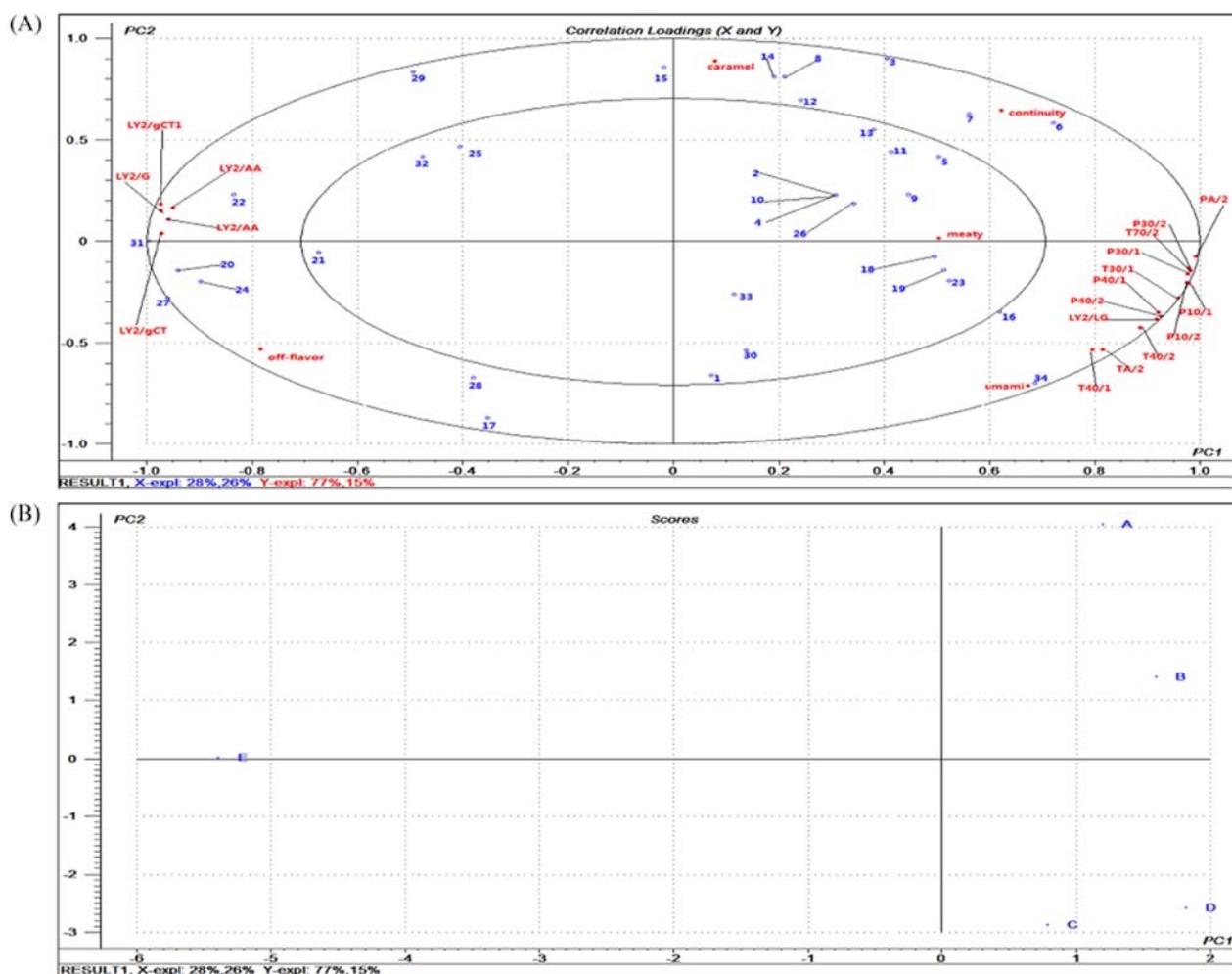


Fig. 6. Variations in mean values based on partial least squares regression (PLSR) correlation loadings (A) and scores (B).

a stronger meaty attribute. Other groups showed poor overall acceptance. Based on the electronic nose response, groups were categorized into 2 clusters comprised of LY2/G, LY2/AA, LY2/GH, LY2/gCT1 and LY2/gCT, and another cluster comprised of P10/1, P10/2, P40/1, PA/2, P40/2, P30/1, P30/2, T30/1, T70/2, T40/2, T40/1, TA/2 and LY/LG. The 2 clusters were distributed on the opposite sides along PC1. Distribution of metal oxide sensors depended on the type of sensors and the molecular functionality of odorants. Types T and P were based on tin dioxide (SnO_2) and LY sensors were chromium-titanium oxides ($\text{Cr}_{2x}\text{Ti}_x\text{O}_{3y}$) and tungsten oxide (WO_3) sensors.

A correlation loadings plot (A) showed correlations between sensory characteristics and chemical compounds (Fig. 6). Group E (samples without chicken base) was correlated with off-flavor. The off-flavor attribute was correlated with formic acid, octanoic acid, 2-octanone, 1-octanol, and benzyl benzoate. These chemicals may be caused by lipid auto oxidation (6,30). Groups C and D were mainly correlated with umami because of hexadecanoic acid and 2-methyl-thiazole. Group B was more associated

with a meaty attribute than the other groups. Related compounds mainly constituted 1-methyl-pyrrole, 2-methyl-furan, bis (2-methyl-3-furyl) disulfide, thiazole, pyrazine, 3-methylbutanal, 2-octenal, and 2-methyltetrahydrofuran-3-one, most of which are heterocyclic compounds containing nitrogen or sulfur produced during the thermal process (11,15,17). Group A had a strong link with caramel terms. The components 2-acetyl-pyrrole, 2-acetyl-furan, nonanal, furfural, and benzaldehyde made a contribution to the caramel term. The correlations loading plot showed agreement with results of odor description. Distribution of electronic nose sensors proved to be valid for discrimination of samples. PLS2 results showed correlations between chemical compounds, electronic nose sensors, and sensory evaluation.

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