Flavor Analysis of Mrps Made from Different Enzymolysis Products Based on Electronic Nose and Electronic Tongue

Xiaopan Fan\textsuperscript{1,a}, Li Ding\textsuperscript{2,b}, Hua Liu\textsuperscript{1,c}, Yiming Liu\textsuperscript{1,d}, Jianbo Liu\textsuperscript{1,e} and Lizhen Ma\textsuperscript{3,*}

\textsuperscript{1}College of Engineering and Technology, Tianjin Agriculture University, Tianjin, China
\textsuperscript{2}Coco Yixiang (Jiangsu) flavor industry Co., Ltd, Jiangsu, China
\textsuperscript{3}College of Food Engineering and Biotechnology, Tianjin Agriculture University, Tianjin, China

*Corresponding author e-mail: 391794215@qq.com, a308925471@qq.com, b36028808@qq.com, c41599386@qq.com, d421334362@qq.com, e764282636@qq.com

Abstract. The effects of different enzymatic hydrolysis methods on the odor and taste of the Maillard reaction products of minced beef-bone protein were studied. Electronic nose and electronic tongue were applied to analyze the volatile substances and taste components. The results indicated that the Maillard reaction products (MRPs) had improved organoleptic and meaty characteristics, especially that of Pf and DE groups. Moreover, DE is the most potential treatment for producing meat flavor as it consumes less energy source and improves production efficiency.

1. Introduction

Maillard reaction plays an important role in the formation of meat flavor compounds. Amino acids (cysteine, cystines) and sugars are used as substrates to reduce the reaction to produce meat flavor substances through Maillard and Strecker. Enzymolysis technology has been widely used in protein deep processing and active ingredient extraction because of its advantages of mild, high efficiency, easy control, safety, product functional diversity, and so on. Yu et al [1] used neutral protease and papain to optimize the enzymolysis conditions of eel head, and then developed a high-grade seafood flavor seasoning after Debittering and decolorizing treatment. Song et al [2]. Found that the content of low molecular weight peptide and amino acid in the hydrolysate of bovine bone protein pretreated by lipase increased significantly, and the product had rich and full beef aroma after Maillard reaction.

Electronic nose and electronic tongue are a new intelligent sensory instrument for complex volatile components, which can quickly reflect the overall quality information of samples, and has the characteristics of simplicity by simulating human olfaction and taste to achieve the detection of food sensory and quality. Now, they have been widely used in the field of food. [3-5] Panigrahi S. [6] used electronic nose system to evaluate the freshness of beef. Ramajaki T. [7] based on electronic nose technology to distinguish different kinds of ice cream, detect the taste of cheese and carry out pattern recognition of cheese aroma mixture. Yan Simin et al. used electronic tongue technology to distinguish the true and false of Anji white tea by PCA and plsda. The results showed that plsda was better than
PCA in classification. Li et al. used electronic tongue to distinguish Tieguanyin from four different places of origin. The results showed that compared with BP neural network, Levenberg Marquardt training algorithm model had better discrimination for tea. Palit et al. compared the effects of different pretreatment on the classification of black tea by electronic tongue. The results showed that electronic tongue had good recognition ability for black tea.

In this paper, the MRPs made from six kinds of enzymatic hydrolysates were used as the research object. The electronic nose and electronic tongue were used to analyze the flavor of MRPs. Combined with the sensor response value and principal component analysis, the flavor of MRPs were comprehensively evaluated from the two aspects of smell and taste, providing reference for its use as spice matrix.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Minced beef-bone (MBB) was obtained from Guayue Food Co., Ltd (Tianjin, China), which contains a notable amount of muscle, connective tissue, bone and fat, and therefore represents a valuable source of proteins, containing approximately 13.7%-32.9% moisture, 16.7%-24.9% protein (collagen), 8.2%-26.8% fat, and 35.8%-48.1% ash. The raw materials were stored at -18°C till required. Food-grade Flavourzyme (500 LAPU/g) and Protamex (1.5AU/g) were purchased from Novozymes Biological Technology Co., Ltd in China (Beijing, China). NaOH, formaldehyde and other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). All chemicals used were of analytical grade. L-cysteine, glycine, alanine and VB1 were purchased from Jizhou Biotechnology Co., Ltd (Hebei Province, China). Xylose and glucose were purchased from Shandong Xiwang Sugar Co., Ltd (Shandong Province, China).

2.2. Preparation of Maillard Reaction Products (MRPs)

Frozen MBB was thawed at 4 °C for 8 h. Then, it was mixed thoroughly with distilled water at a ratio of 1:4 and extracted at 120 °C for 4 h and 0.1 MPa in a autoclave sterilizer (TOMY Co., Ltd., Tokyo, Japan), which was named as minced beef-bone protein extract (MBBE). MBBE was was further enzymatically hydrolyzed into six enzymatic products, including control (C), Flavourzyme (F), Protamex (P), Flavourzyme followed by Protamex (Fp), Protamex followed by Flavourzyme (Pf) and combination of Flavourzyme and Protamex (DE). The xylose (1.2%), glucose (1.2%), L-cysteine (0.9%), glucine (0.45%), alanine (0.45%) and VB1 (1.8%) were added into the six enzymolysis products, and the mixtures were transferred to conical flasks (250 mL) and adjusted to pH 5.5 with 1 M acetic acid. Then, they were placed in an autoclave sterilizer (TOMY Co., Ltd., Tokyo, Japan) at 110 °C for 60 min. These six Maillard reaction products (MRPs C, MRPs F, MRPs P, MRPs Fp, MRPs Pf and MRPs DE) were immediately cooled in ice water (0°C) and filtered through qualitative filter paper, and the supernatants were stored at -18 °C for further analyses.

2.3. Electronic nose (E-nose) analysis of MRPs

E-nose was used to identify the flavor quickly and succinctly. The MRPs were analyzed using an electronic nose system (PEN3, Germany), consisting of an array of 10 metal oxide gas sensors (W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W and W3S). 10.0 ± 0.1 g of each sample was placed in a glass vial (15 mL) and equilibrated at room temperature (25 ± 2°C) for 20 min. The hermetic vial was pierced by a Luer lock needle connected to a 3 mm Teflon tubing by using headspace air intake [8]. The headspace gas was pumped into the sensor chamber at a constant rate of 300 mL/min. The preparation time was 5 s before the E-nose measurement began, and the measurement process lasted for 60 s, during which the absorbed gases were tested each second. The sensor automatic cleaning time was 60 s and was automatically zero cleared for 10 s. Each sample was analyzed in triplicate, and the average values were used for the subsequent study.
2.4. Electronic tongue (E-tongue) analysis of MRPs

E-tongue containing 7 sensors (SRS, SWS, BRS, STS, UMS, SPS and GPS) with a standard Ag/AgCl reference electrode was used in this study. The first 5 sensors were sensitive to acidity, sweetness, bitterness, saltiness and umami, which can reflect the relative intensity of the five flavors of different samples. Each sample (20 mL) in the glass was measured for 120 s, and then distilled water was used to clean the sensors to ensure that stable potentials were obtained. Each sample was analyzed eight times.

2.5. Statistical analysis

Principal component analysis (PCA) was used to describe and process the multivariate data acquired by the E-nose and electronic tongue sensors.

3. RESULTS AND DISCUSSION

3.1. E-nose analysis of MRPs

Table 1. Composition of sensors in the electronic nose and their performances

| Sensor name | Performance description | Representative compounds | Response threshold (mL/m³) |
|-------------|-------------------------|--------------------------|---------------------------|
| W1C         | Aroma components        | toluene                  | 10                        |
| W5S         | Sensitive to nitrogen oxides | NO₂                  | 1                          |
| W3C         | Ammonia, Aroma components | benzene                  | 10                        |
| W6S         | Mainly hydrogen selective | H₂                      | 100                       |
| W5C         | Aromatic hydrocarbons   | propane                  | 1                         |
| W1S         | Sensitive to methane    | CH₄                      | 100                       |
| W1 W        | Sensitive to sulfides   | H₂S                      | 1                         |
| W2S         | Sensitive to ethanol    | CO                       | 100                       |
| W2W         | Aroma components, Sensitive to organic sulfides | H₂S                  | 1                         |
| W3S         | Sensitive to alkanes    | CH₄                      | 10                        |

Figure 1. Effect of 10 sensors of E-nose on the response value of MRPs

The electronic nose used for analysis is equipped with 10 highly sensitive metal sensors with different properties and is capable of representing different types of volatile compounds (Table 1).

The typical E-nose response to MRPs samples prepared by different enzymatic hydrolysis methods (C, F, P, Fp, Pf, and DE) is presented in Fig. 1a. The W1W sensor had the highest response value to the samples, followed by W2W, showing that the majority of the flavor components of MRPs were sulfides.
These results are consistent with the study of Gong, Cao & Xie, et al. [9], who reported that sulfur compounds played an important role in the aroma composition of beef. In fact, these compounds are an important source of essential meat flavor, including H2S, methyl mercaptan, 2, 2'-methyl propyl mercaptan and 3-methyl-2-butyl mercaptan, etc. Except for group P, the response values of W1W for MRPs after enzymatic hydrolysis were significantly improved in contrast to C. This is possibly due to that sulfur compounds are made of sulfur-containing amino acids (methionine, cysteine and cystine). As shown in Fig. 1a, the sulfur-containing amino acids content of group P hydrolyzed by Protamex was the lowest (142 µg/mL). The results indicated that during the process of enzymatic hydrolysis, MBBE was hydrolyzed and degraded into peptides and free amino acids, which provided sufficient precursor for the Maillard reaction and then promoted the flavor formation of MRPs. Meanwhile, simultaneous hydrolysis using two enzymes (DE) could promote the release of sulfur compounds in MRPs, adequately. In addition, compared with the other treatments, DE is energy-saving, and is able to enhance the production efficiency and reduce the production cost.

The principal component analysis (PCA) is a linear transformation of the original data vector by dimension reduction, and the whole process includes no sample information. The most important factor and the highest contribution rate were obtained by this method to find an access to manage the differences between samples. The results of the E-nose data for MRPs are presented in Fig. 1b. As shown in Fig. 1b, in the correlation matrix model, the first 2 principal components (PC1 and PC2) accounted for 97.01% and 2.44% of the total variance, respectively, and the total contribution rate of 99.45%, which was higher than 90%, indicated that these two principal components can be used to reflect the actual situation of the sample. The variance contribution rate of PC1 was far greater than that of PC2 in the PCA rate. It shows that when the different samples are farther away in the X-axis, the samples have a greater difference, while the difference of the samples in the Y-axis has no significant effect. It can be shown that the MRPs DE and Pf had a far Euclidean distance. Meanwhile from Fig. 2b, P, F and C had a close distance.

3.2. E-tongue analysis of MRPs

![E-tongue response to MRPs samples](image)

**Figure 2.** Effect of 5 sensors of E-tongue on the response value of MRPs

Typical E-tongue response to the MRPs samples prepared by different enzymatic hydrolysis methods (C, F, P, Fp, Pf, and DE) is presented in Fig. 2a. The electronic tongue can be described as an analytical tool with 7 sensors, including SRS, GPS, STS, UMS, SPS, SWS and BRS, and SRS, SWS, BRS, STS and UMS are sensitive sensors for acidity, sweet, bitterness, saltiness and umami, which can give the relative intensity of the five tastes of different samples. The response value of the six MRPs on the
sensor was expressed from 0 to 12. As shown in Fig. 2a, the MRPs prepared using Flavourzyme or Protamex alone (F and P) were significantly different from the others (C, Fp, Pf and DE). Fp and Pf had stronger umami and bitter taste than DE and C.

PCA based on E-tongue is shown in Fig. 2b. The characteristic areas of the 6 groups of samples detected by the electronic tongue are not overlapped, indicating that the E-tongue can separate the MRPs prepared by different enzymatic solutions. As seen from the diagram, the first principal component contribution rate was 85.85%, which was significantly higher than PC2 (12.49%). The result demonstrated that the farther away the center of gravity of the sample on the X axis, the greater the difference between the samples. MRPs DE and MRPs C had the farthest distance compared with other groups, which showed that the MRPs prepared by simultaneous hydrolysis of two enzymes had the highest overall taste difference with the MRPs without enzymatic hydrolysis, followed by MRPs F. MRPs P, Fp and Pf had little difference with MRPs C.

4. Conclusion

MRPs were prepared from five enzymatic hydrolysates, and their flavor differences were evaluated comprehensively from the perspectives of odor and taste. E-nose test results shown that the MRPs DE and Pf had a far Euclidean distance. MRPs P, F and C had a close distance. MRPs prepared by simultaneous hydrolysis of two enzymes had the highest overall taste difference with the MRPs without enzymatic hydrolysis. The combination of E-nose and E-tongue can evaluate the flavor of products more comprehensively.

Acknowledgments

This study was financially supported by the Project of science research and development fund of Tianjin Agricultural University (20190112) and the Innovation and Entrepreneurship Training Program for College Students (201910061024).

References

[1] Yu J, Chen Meizhen. Preparation and application of hydrolyzed eel head protein by enzymatic method [J]. Food industry technology, 2001, 22 (1): 45-46.
[2] Song S, Li S, Fan L, et al. A novel method for beef bone protein extraction by lipase-pretreatment and its application in the Maillard reaction [J]. Food Chemistry, 2016, 208:81-88.
[3] Netale C, Macagnano. Electronic nose and sensorial analysis: comparison of performances in selected case [J]. Sensor and Actuators B, 1998, 50 (2): 246-252.
[4] Zhao Jianxin, Dai Xiaojun, Liu Xiaoming, et al. Comparison of aroma compounds in naturally fermented and inoculated Chinese soybean pastes by GC-MS and GC-O factor metry analysis [J]. Food Control, 2011, 22 (6): 1008-1013.
[5] HIROYUKIA F, TOMOHODEA Y, KAZUNORIB O. Efficacy and safety of Touchi extract, an alpha-glucosidase inhibitor derived from fermented soybeans, in non-insulin-dependent diabetic mellitus [J]. J Nutrition Biochemistry, 2001, 12 (6): 351-356.
[6] Panigrahi S., S. Balasubramanian, H. Gu, et al. Neural-network-integrated electronic nose system for identification of spoiled beef [J]. LWT, 2006, (39): 135-145.
[7] Ramajaki T., H. Alakomi, L. Ritvanen, et al. Application of an electronic nose for quality assessment of modified atmosphere pack aged poultry meat[J].Food Control, 2006,(17): 5-13.
[8] Kiani, S., Minaei, S., & Ghasemi-Varnamkhasti, M. Application of electronic nose systems for assessing quality of medicinal and aromatic plant products: a review [J]. Journal of Applied Research on Medicinal & Aromatic Plants, 2016, 3 (1), 1-9.
[9] Gong, J., Cao, C., Li, H., Du, R., Ling, Z., & Xie, J., et al. The initial-maillard pathway to meat flavor during the preparation of thermal reaction meat flavorings [J]. Journal of Chinese Institute of Food Science & Technology, 2016.