Assessment the flavor of soybean meal hydrolyzed with Alcalase enzyme under different hydrolysis conditions by E-nose, E-tongue and HS-SPME-GC–MS

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ABSTRACT

In the present study, E-nose, E-tongue, and headspace-solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) technology combined with Principal Component Analysis (PCA) were employed to evaluate the flavor characteristics of the volatile and the non-volatile substances generated during the enzymatic hydrolysis of the soybean meal by Alcalase. The results showed that the enzymatic hydrolysis effectively reduced the content of soybean odorous substance 1-octene-3-ol and led to better flavor. However, the excessive enzymatic hydrolysis resulted in the deterioration of the enzymatic hydrolysates flavor. In addition, the study on the flavor of enzyme-treated soybean meal was still deficient. Therefore, it would be interesting to study the difference in the flavor of soybean meal after enzymatic hydrolysis under different conditions. In addition, compared to other enzymes, Alcalase had high rate efficiency. Therefore, it would be interesting to study the difference in the flavor of soybean meal under enzymatic hydrolysis under different conditions. However, current researches on the enzymatic hydrolysis of soybean meal were mostly focused on the process of enzymatic hydrolysis (Sun, Chen, & Liu, 2005; Liu, Zhang, Feng, & Xue, 2010; Chen et al., 2019), improvement of protein solubility (Neto et al., 2017), fermentation (Wang et al., 2017), and the production stage. The study on the flavor of enzyme-treated soybean meal was still deficient. Therefore, it would be interesting to study the difference in the flavor of soybean meal after enzymatic hydrolysis under different conditions. In addition, compared to other enzymes, Alcalase had high rate of recovery protein and high degree of hydrolysis (DH) under similar condition, and more low molecular weight polypeptides were generated from the crude protein via gradual hydrolysis, leading to a remarkable improvement in protein solubility and other functional properties (Yasemi, Ghomi, Darnahal, Mohammadzadeh, & Amini, 2013; Meinschmidt, Sussmann, Schweiggert-Weisz, & Eisner, 2016).

Flavor is the distinctive sensory character of the food and is important for evaluating the nutritional value (Sivakumar & Bautista-Baños, 2014). Solid-phase microextraction (SPME) is a sample pretreatment prospecs and research significance. However, current researches on the enzymatic hydrolysis of soybean meal were mostly focused on the process of enzymatic hydrolysis (Sun, Chen, & Liu, 2005; Liu, Zhang, Feng, & Xue, 2010; Chen et al., 2019), improvement of protein solubility (Neto et al., 2017), fermentation (Wang et al., 2017), and the production stage. The study on the flavor of enzyme-treated soybean meal was still deficient. Therefore, it would be interesting to study the difference in the flavor of soybean meal after enzymatic hydrolysis under different conditions. In addition, compared to other enzymes, Alcalase had high rate of recovery protein and high degree of hydrolysis (DH) under similar condition, and more low molecular weight polypeptides were generated from the crude protein via gradual hydrolysis, leading to a remarkable improvement in protein solubility and other functional properties (Yasemi, Ghomi, Darnahal, Mohammadzadeh, & Amini, 2013; Meinschmidt, Sussmann, Schweiggert-Weisz, & Eisner, 2016).

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technology for the analysis of the components in a sample. This pre-treatment process is fast and safe as it integrates extraction, concentration, and injection leading to an improved speed of analysis. Recently, it has been widely used in the identification of volatile components (Ruo-Nong & Chemistry, 2015; Cao et al. 2020). Gas chromatography-mass spectrometry (GC–MS) can detect and compare the specific types of volatile substances in the samples but cannot analyze the contribution of these volatile substances to the flavor characteristics. Considering that the electronic nose (E-nose) can obtain the overall flavor information of a sample, the combination of GC–MS and E-nose can be beneficial for the study of sample flavor at both macroscopic and microscopic levels (Cheng, Qin, Guo, Hu & Wu, 2013). E-nose technology is been widely used in food testing and environmental monitoring. It has also been able to preliminarily diagnose lung cancer (Shlomi et al., 2017) as well as achieve noninvasive diagnosis of diabetes in clinic (Seesaard, Sriprapradang, Kittiyakara, & Kerdcharoen, 2016). The electronic tongue (E-tongue) and the E-nose are powerful tools for distinguishing flavor characteristics, which characterize small differences in taste and odor of foods from various sources without considering subjective factors (Zaragozá et al., 2013).

In the current study, we combined the HS-SPME-GC–MS, E-nose, and E-tongue technology with principal component analysis to investigate the effects of different conditions of enzymatic hydrolysis on the flavor of soybean meal hydrolysates. The results provided a theoretical basis for improving the flavor of the soybean protein and related products.

Materials and methods

2.1. Sample preparation and processing

The raw soybean “Northeast Soybean” was purchased from Suguo Supermarket (Nanjing, China), and the soybean was subjected to low temperature pressing at 60 °C using an oil press to obtain the soybean meal. The Alcalase protease used in the enzymatic hydrolysis was purchased from Nanjing Vazyme Biotech Co., Ltd. Based on methods described previously (Monge Neto et al., 2017) with minor modifications. The soybean meal and water were homogenized at a ratio of 1:10 (m / v) and inactivated the enzyme at 90 °C for 15 min. Based on previous experiments (Chen, Lin, Gao, & Shen, 2017; Zhang, Olsen, Grossi, & Otte, 2013), the optimum temperature for the enzymatic hydrolysis was set at 55 °C and the optimal pH was 8.0. There are many factors that affect the hydrolysis of the soy protein by proteases, such as pH, temperature, enzyme dosage, time, etc. The optimum temperature and pH are specific for different enzymes and substrates. Therefore, the amount of enzyme and the time of enzymatic hydrolysis were set as variables to group the samples (Table 1).

2.2. Determination of the DH

The process of enzymatic hydrolysis of a protein is accompanied by the release of carboxyl groups or amino groups, and the number of the two groups being released could affect the solution pH. According to Fernández’s pH-stat method (Fernández, Ayoo, & Kelly, 2016), the degree of proteolysis can be calculated based on the consumption of NaOH during the process of hydrolysis using the following equation:

\[
DH = B \times N_a \times \frac{1}{\alpha} \times \frac{1}{M_p} \times \frac{1}{n_{\text{hot}}} \times 100\%
\]

where B is the volume (mL) of NaOH consumed during the hydrolysis; \(N_a\) represents the concentration of NaOH (mol/L); \(\alpha\) represents the dissociation constant of a specific amino acid (Adler, 1986); \(M_p\) represents the total amount of the substrate protein (g); \(n_{\text{hot}}\) represents the number of moles of peptide bonds in 1 g of raw protein, and the soy protein isolated is 8.38 mmol/g.

2.3. E-nose analysis

The E-nose analysis of odor was performed according to the method of Yang (Yang et al., 2016) with minor modifications. There were ten samples in total and each sample measurement was taken in triplicates. One mL of the enzymatic hydrolysates was placed in a 10 mL glass vial, heated to 60 °C for 10 min in the headspace, and 0.5 mL of the solution was aspirated for further analysis with an acquisition time of 120 s. The volatile odor of the enzymatic hydrolysates was characterized using an E-nose system (FOX 3000, Alpha MOS, Toulouse, France) based on 12 metal oxide gas sensors (MOS sensors) made of different materials. The sample gas from the headspace of the vial was pumped into the sensor chamber at a constant rate of 100 mL/min using clean air as a carrier gas through a Teflon tube attached to the needle.

2.4. E-tongue analysis

Based on a protocol described previously, (Fang, Yang, Kimatu, Zhao, An, & Hu, 2017), the analysis was carried out using an ASTREE e-tongue-taste fingerprint analyzer (Alpha MOS, France). The detection sensors used in this analysis included CTS (for salty taste), NMS (for umami), AHS (for sourness), SCS (for bitterness), as well as PKS and CPS (both for general purpose).

2.5. Analysis of free amino acids

The free amino acids from the enzymatic hydrolysates under different conditions were extracted according to the method described previously (Zhang, Qiu, Lu, & Chen 2017). The free amino acids in the enzymatic hydrolysates were determined using an automatic amino acid analyzer (L-8900, Hitachi Ltd, Japan).

2.6. HS-SPME-GC–MS analysis

The analysis method was performed as described previously (Zhang, He, Gao, Ma & Li 2017) with minor modifications. Three mL of soybean meal hydrolysate was accurately measured and placed in a 20 mL pre-balanced headspace bottle at 60 °C for 30 min. To extract the volatile compounds from samples, a 50/30 μm aged diethylbenzene/carbon molecular sieve/polydimethylsiloxane fiber was used for extraction. The fiber was inserted into the sample vial and exposed to the HS at 60 °C for 30 min to collect the analytes. Finally, the fiber was removed and inserted into the injection port of GC-MS apparatus to identify the volatile compounds.

The analysis was conducted using GC–MS apparatus (7890A/5975C, Agilent Technologies, Santa Clara, CA, USA). Volatiles were separated
by using a HP-5MS type column (30 m × 0.250 mm × 0.25 μm). The inlet temperature was 240°C with a flow of 1.0 mL/min and the injection volume was 1 μL. Temperature variations in the program were as follow: initial temperature was set at 40 °C for 3 min, increased to 120 °C at the speed of 5 °C/min, held constant for 5 min, then increased to 200 °C at the rate of 8 °C/min, held constant for 8 min, then rise to 240 °C at 10 °C/min and held constant for 10 min.

The temperature of electron ionization source and transmission line were 230°C and 240°C, respectively. The mass spectra were obtained by electronic impact at 70 eV, and the data was collected at a rate of 1/scan over the range of 35–500.

2.7. Data analysis

AlphaSoft V9.1 was employed to organize the PCA data and radar fingerprints. The least significant difference (LSD) multiple comparison test was performed using SAS (V9.2, SAS Institute, USA) within 95% confidence level. All data were collected in triplicates.

Results and discussion

3.1. The changes of hydrolysis degree under different enzymatic hydrolysis conditions

DH represents the cleavage of peptide bonds and also affects protein recovery, functional properties and sensory qualities of the hydrolysates (e.g., bitterness) (Himonides, Taylor, & Morris, 2011). The changes in the DH under different conditions of enzymatic hydrolysis were shown in Table 1. The DH value was increased with the prolonging of the time under different enzymatic dosage. This result was consistent with the previous reports on protein hydrolysis, where DH increased as the time of enzyme increased (Wei, Thakur, Liu, Zhang, & Wei, 2018). However, the absolute increase of DH at 6000 u/g enzyme dosage was significantly (P < 0.05) lower than that of 2000 or 4000 u/g. This might be due to the fact that when the amount of the enzyme reached a threshold, the enzymatic hydrolysis of enzyme reached a plateau or there might be competitive inhibition in the enzymatic hydrolysis of Alcalases. This result was in accordance with the enzymatic reaction kinetics of alkaline protease. (Elçin, Dilek & Belma, 2011).

3.1.1. E-nose

E-nose is sensitive to the odor of the samples, and slight changes in the composition of the volatile compounds may result in a different
sensor response. Therefore, this technology has been used in various analytical fields such as food, beverages, cosmetics, medicine, and agriculture (Loutfi, Coradeschi, Mani, Shankar & Rayappan, 2015). In this study, the E-nose equipped with 12 sensors was used to analyze the integrated flavor profile of the enzymatically hydrolyzed soybean meal. The intensity of the sensor curve depended not only on the composition of the odor molecules, but also on their concentrations. The representative E-nose intensity curves were reported in Fig. 1. The signal intensity values that change appearance of 12 sensors during enzymatic hydrolysis stage was consistent. Fig. 1A showed the E-nose intensity curve of the soybean meal before enzymatic hydrolysis, and Fig. 1B exhibited the E-nose intensity curve of the soybean meal after enzymatic hydrolysis. The conditions of enzymatic hydrolysis were as follows: the amount of enzyme added was 6000 u/g, and the time of enzymatic hydrolysis was 5 h. Significant differences in sensor signal intensity were observed between non-enzymatic sample and enzymatic hydrolysis sample, indicating the significant changes in the composition of the volatile compounds in the enzymatic hydrolysates. The differences might be due to the formation of new volatile compounds during the enzymatic hydrolysis of soybean meal under these conditions.

To clear demonstrate these data, a radar fingerprint of the volatile components in the soybean meal hydrolysates under different conditions of enzymatic hydrolysis was organized (Fig. 2A). Significant differences were observed in the signal intensity of the same soybean meal hydrolysates by different sensors, and the sensor signal intensity of the soybean meal hydrolysates under different conditions of enzymatic hydrolysis by the same sensor also presented differences. Previous studies showed that LY2/G was sensitive to amines, alcohols, ammonia and ketones; LY2/GH was sensitive to amines and ammonia; LY2/gCTL was sensitive to hydrogen sulphide; PA/2 was sensitive to nitrogen compounds; T70/2 was sensitive to alcoholic vapours; T30/1 was sensitive to organic solvents and light polar molecules (Zhu, Chen, Wang, Niu, & Xiao, 2017; Michishita et al., 2010; Su et al., 2013; Yao et al., 2015). Radar fingerprint chart of three different hydrolysis time pointed at 2000 u/g of enzyme were almost completely overlapped. Whereas, the signal intensity of PA/2, T70/2, T30/1 at 4000, 6000 u/g were significantly (P < 0.05) higher than the untreated or 2000 u/g treated samples, and they were increased with the rise of the enzyme dosage or the prolonging enzymatic hydrolysis time, indicating that the aromatic compounds were continuously generated during these processes. The signal intensity of LY2/G, LY2/AA, LY2/GH, LY2/gCTL at 6000 u/g enzyme dosage with three different time points were significantly (P < 0.05) lower than other conditions. The results showed that amines, ammonia, hydrogen sulphide and some other compounds leading to unpleasant odour were accumulated when 6000 u/g enzyme dosage was used. The development of unpleasant odor at 6000 u/g may be related to excessive enzymatic hydrolysis of Alcalase. A previous study showed that, Alcalase has a special catalytic effect on hydrophobic amino acids,
which optimizes the flavor of the hydrolyzed products. However, unpleasant smell was produced when it was excessively enzymatically produced. While the continuous generation of aromatic compounds may be due to new volatile compounds, such as alcohols and esters, formed during enzymatic hydrolysis (Synowiecki, Jagietka, & Shahidi, 1996).

### 3.1.2. PCA analysis of the volatile components from E-nose

PCA is a statistical program that utilizes orthogonal transformation to convert one set of observation values of potentially related variables into another set of values of linearly uncorrelated variables (Rattray, Hamrang, Trivedi, Goodacre, & Fowler, 2014). Statistical analysis of the results based on PCA highlighted the different volatile components in the profiles under different conditions of enzymatic hydrolysis. The total contribution rate above 85% indicated the good feasibility of the method (Liu, Wang, Li, & Fowler, 2014). Statistical analysis of the results based on PCA highlighted the different volatile components in the profiles under different conditions of enzymatic hydrolysis. The total contribution rate above 85% indicated the good feasibility of the method (Liu, Wang, Li, & Wang, 2012). The PCA of volatile compounds in the soybean meal hydrolysates under different conditions of enzymatic hydrolysis were demonstrated in Fig. 2B. The contribution rates of the volatile compounds in the different soybean meal hydrolysates. Sample 7 showed a clear overlap with sample 3, 4, and 6 indicating that these two main components provided the maximum information on the volatile compounds.

### 3.1.3. HS-SPME-GC-MS analysis

In order to further evaluate the volatile components that were present in the soybean meal hydrolysates under different conditions of enzymatic hydrolysis, the volatile compounds were monitored by HS-SPME-GC-MS. Table 2 and table S1 reported the relative contents of the volatile compounds under different conditions. Sixty four volatile compounds were identified in ten samples, including 8 hydrocarbons, 16 alcohols, 11 aldehydes, 6 carboxylic acids, 11 esters, 9 ketones, 1 olefinic terpene and 2 heterocyclic compounds.

#### Table 2

The relative contents of the main volatile constituents in soybean meal under different enzymatic hydrolysis conditions.

| NO. | Compounds name       | RT       | Relative content (%) |
|-----|----------------------|----------|----------------------|
|     |                      | 0 h      | 1 h      | 3 h      | 5 h      |
|     |                      | u/g      | 2000     | 4000     | 6000     | u/g      | 2000     | 4000     | 6000     | u/g      | 2000     | 4000     | 6000     |
|     |                      | 0        | 1        | 3        | 5        | 0        | 1        | 3        | 5        | 0        | 1        | 3        | 5        |
|     | Alcohols             |          |          |          |          |          |          |          |          |          |          |          |          |
| 1   | 3-hexanol            | 1.99     | 0.7      | 0.44    |          |          |          |          |          |          |          |          |          |
| 2   | 3-methyl-1-butanol   | 4.08     | 13.25    | 14.57   | 14.22    | 14.57    | 17.1     | 18.56    | 13.66    | 14.95    | 15.05    |          |          |
| 3   | 3-hexanol            | 4.22     | 1.29     |          |          |          |          |          |          |          |          |          |          |
| 4   | 1-pentanol           | 9.82     | 1.53     |          |          |          |          |          |          |          |          |          |          |
| 5   | 1-hexanol            | 14.71    | 13.66    | 14.95   | 15.05    |          |          |          |          |          |          |          |          |
| 6   | 3,5-octadien-2-ol    | 16.55    | 1.34     | 0.34    | 0.29     | 0.46     |          |          |          |          |          |          |          |
| 7   | 1-octanol            | 18.37    | 1.07     | 1.57    | 0.98     |          |          | 0.98     | 1.60     | 1.56     | 1.77     |          |          |
| 8   | 1-octene-3-ol        | 20.67    | 15.59    | 15.26   | 14.77    | 13.78    | 13.10    | 13.19    | 15.98    | 15.07    | 15.38    |          |          |
| 9   | 1-heptanol           | 24.43    | 1.10     | 0.54    |          |          |          |          |          |          |          |          |          |
| 10  | 2-ethylhexanol       | 27.29    | 0.64     |          |          |          |          |          |          |          |          |          |          |
| 11  | 1-decan-4-ol         | 31.43    | 2.67     | 1.59    | 0.85     | 0.95     | 0.95     |          |          |          |          |          |          |
| 12  | Phenylethanol        | 33.24    | 0.64     | 7.9      | 8.47     | 9.38     | 7.48     | 7.9      |          |          |          |          |          |
|     | Aldehydes            |          |          |          |          |          |          |          |          |          |          |          |          |
| 13  | Hexanal              | 5.54     |          | 3.09    | 1.54     | 1.58     | 2.07     | 2.73     | 3.57     | 4.24     |          |          |          |
| 14  | 2-hexenal            | 6.38     |          | 6.39    |          |          |          | 1.03     | 1.34     | 1.4    |          |          |          |
| 15  | Furfural             | 10.74    |          | 0.9     | 1.45     | 0.92     | 1.29     | 1.26     |          |          |          |          |          |
| 16  | (E)-2-octenal        | 14.54    |          | 0.9     |          |          |          |          |          |          |          |          |          |
| 17  | (E)-2-nonenal        | 26.64    |          | 0.82    | 0.73     | 3.44     | 3.57     | 4.21     |          |          |          |          |          |
|     | Esters               |          |          |          |          |          |          |          |          |          |          |          |          |
| 18  | 3-methylbutyl acetate| 5.42     |          | 2.50    | 2.47     | 2.3     | 2.33     | 2.48     | 2.66     | 2.98     | 2.5    |          |          |
| 19  | Ethyl heptanoate     | 8.48     |          | 0.96    | 1.5      |          |          | 1.04     | 1.2      |          |          |          |          |
| 20  | γ-decalactone        | 8.88     | 0.98     | 0.68    | 7.49     | 8.5      | 7.46     | 7.9      | 8.70     | 7.4      | 7.9     | 7.43     |          |
| 21  | Butyro lactone       | 10.55    | 4.59     | 4.60    | 3.95     | 5.30     | 4.59     | 5.37     | 4.2      | 5.01     | 4.2    |          |          |
| 22  | Ethyl citrate        | 13.69    | 3.29     | 3.10    | 3.51     | 2.85     | 3.2      | 3.99     | 2.9      | 3.18     | 2.84    |          |          |
| 23  | Butyl butyrate       | 17.27    | 1.35     | 1.46    |          | 2.37     |          |          |          |          |          |          |          |
| 24  | Ethyl myristate      | 17.30    |          | 0.44    | 0.71     |          |          | 0.59     |          |          |          |          |          |
| 25  | γ-decalactone        | 22.86    | 4.13     | 4.21    |          |          |          | 4.76     |          |          |          |          |          |
| 26  | Methyl palmitate     | 24.82    |          |          |          |          |          | 2.16     |          |          | 0.89    |          |          |
| 27  | Triacetin            | 28.99    | 0.88     | 3.68    |          |          |          | 2.91     |          |          |          |          |          |
| 28  | Ethyl palmitate      | 32.95    | 0.93     | 3.03    |          |          |          |          |          |          |          |          |          |
in the undegested soybean meal, however, the large quantities of these two components were detected after enzymatic hydrolysis. The relative content of 3-methyl-1-butanol in the 9 enzymolysis groups was more than 13%, thus making it one of the main flavor substances in the enzymolysate.

Among the three different concentrations of the enzyme, the relative content of aldehydes at all three time points from 6000 u/g treatment group were higher than that of 2000, 4000 u/g groups. This result may be due to the production of new aldehydes including 2-hexenal with pungent odor and (E)-2-nonenal with oily smell at all three time points from 6000 u/g treatment group were significantly (P < 0.05) increased, compared to other treatment groups. These resulted in an unpleasant odor of the sample at 6000 u/g enzyme dosage at all three time points, which was consistent with the E-nose results.

In addition, the ester types were increased from 4 types to 11 types after enzymatic hydrolysis. Among the esters, 3-methylbutyl acetate, relative content of hexanal with pungent odor and (E)-2-nonenal with grassy smell and furfural which was similar to benzaldehyde odor. The relative content of hexanal with pungent odor and (E)-2-nonenal with oily smell at all three time points from 6000 u/g treatment group were significantly (P < 0.05) increased, compared to other treatment groups. These resulted in an unpleasant odor of the sample at 6000 u/g enzyme dosage at all three time points, which was consistent with the E-nose results.

3.2. Changes in non-volatile components

3.2.1. Free amino acids

The flavor amino acids including leucine, threonine, proline, lysine, tryptophan, and methionine in the enzymatic hydrolysate provide a variety of flavors. They are also considered as good sources of essential amino acids. The smooth flavor and rich taste of the enzymatic hydrolysates were attributed to the interaction among the amino acids. The free amino acids mainly involved umami amino acids (e.g., Glu, Asp), sweet amino acids (e.g., Thr, Ala, Gly, etc.) and bitter amino acids (e.g., Val, Leu, Phe, Arg, etc.). The contents and proportion of these amino acids were the important factors affecting the flavor of the enzymatic hydrolysates.

As listed in Table 3, the essential amino acid contents were increased after enzymatic hydrolysis, indicating that the soybean meal had a higher nutritional value after enzymatic hydrolysis and were good raw materials for preparing nutrient-rich seasonings and flavored mellow feed. The content of bitter amino acid, sweet amino acid, and umami amino acid in the enzymatic hydrolysate were gradually increased with increasing the time of enzymatic hydrolysis. Furthermore, the free amino acid contents were increased along with the increase of the enzyme amount at the same hydrolysis time. Although the relative contents of Asp and Glu were lower than bitter amino acids, the taste threshold of Asp and Glu was lower, leading to an improved flavor of the enzymatic hydrolysates at 4000 u/g with 3 h treatment. The increase of Val, Leu, Phe, Arg after 5 h enzymatic hydrolysis were greater than other 2 time points at 4000 u/g. Therefore, the flavor of hydrolysates at 4000 u/g with 5 h was inferior to 4000 u/g with 3 h.

3.2.2. E-tongue analysis

Based on the analysis performed by E-tongue on the enzyme-treated samples, as shown in Fig. 3A, significant changes in the bitterness and the umami taste of the enzymatic hydrolysate were observed. With an increase in the enzymatic hydrolysis time, the bitterness and the umami taste increased. At all three time points from 6000 u/g, the enzymatic hydrolysate showed a strong bitter taste (high SCS values), this may be due to the excessive enzymatic hydrolysis, the hydrophobic amino acids in the peptide gradually exposed to the outside of the side chains,

Table 3

| FAA     | 2000 u/g | 4000 u/g | 6000 u/g |
|---------|----------|----------|----------|
|         | 1 h      | 3 h      | 5 h      | 1 h      | 3 h      | 5 h      | 1 h      | 3 h      | 5 h      |
| Asp     | 0.006 ±  | 0.012 ±  | 0.017 ±  | 0.014 ±  | 0.025 ±  | 0.029 ±  | 0.023 ±  | 0.021 ±  | 0.028 ±  |
| Threonine | 0.014 ±  | 0.004 ±  | 0.009 ±  | 0.009 ±  | 0.004 ±  | 0.002 ±  | 0.002 ±  | 0.001 ±  | 0.003 ±  |
| Ser     | 0.002 ±  | 0.001 ±  | 0.002 ±  | 0.002 ±  | 0.005 ±  | 0.007 ±  | 0.115 ±  | 0.115 ±  | 0.115 ±  |
| Glu     | 0.001 ±  | 0.003 ±  | 0.002 ±  | 0.017 ±  | 0.019 ±  | 0.024 ±  | 0.019 ±  | 0.019 ±  | 0.024 ±  |
| Ala     | 0.003 ±  | 0.004 ±  | 0.002 ±  | 0.004 ±  | 0.012 ±  | 0.017 ±  | 0.017 ±  | 0.017 ±  | 0.017 ±  |
| Cys     | 0.005 ±  | 0.007 ±  | 0.007 ±  | 0.007 ±  | 0.012 ±  | 0.016 ±  | 0.012 ±  | 0.012 ±  | 0.016 ±  |
| Val     | 0.002 ±  | 0.002 ±  | 0.003 ±  | 0.001 ±  | 0.007 ±  | 0.011 ±  | 0.007 ±  | 0.007 ±  | 0.011 ±  |
| Met     | 0.001 ±  | 0.004 ±  | 0.003 ±  | 0.003 ±  | 0.007 ±  | 0.012 ±  | 0.007 ±  | 0.007 ±  | 0.012 ±  |
| Ile     | 0.001 ±  | 0.002 ±  | 0.004 ±  | 0.002 ±  | 0.007 ±  | 0.011 ±  | 0.007 ±  | 0.007 ±  | 0.011 ±  |
| Leu     | 0.002 ±  | 0.002 ±  | 0.004 ±  | 0.003 ±  | 0.007 ±  | 0.012 ±  | 0.007 ±  | 0.007 ±  | 0.012 ±  |
| Tyr     | 0.004 ±  | 0.020 ±  | 0.023 ±  | 0.027 ±  | 0.031 ±  | 0.034 ±  | 0.013 ±  | 0.016 ±  | 0.019 ±  |
| Phe     | 0.006 ±  | 0.034 ±  | 0.037 ±  | 0.041 ±  | 0.045 ±  | 0.046 ±  | 0.009 ±  | 0.012 ±  | 0.015 ±  |
| Lys     | 0.003 ±  | 0.006 ±  | 0.007 ±  | 0.051 ±  | 0.053 ±  | 0.055 ±  | 0.06 ±  | 0.06 ±  | 0.06 ±  |
| His     | 0.004 ±  | 0.026 ±  | 0.038 ±  | 0.040 ±  | 0.045 ±  | 0.046 ±  | 0.04 ±  | 0.04 ±  | 0.04 ±  |
| Arg     | 0.002 ±  | 0.025 ±  | 0.038 ±  | 0.043 ±  | 0.047 ±  | 0.05 ±  | 0.09 ±  | 0.09 ±  | 0.09 ±  |
| Ile     | 0.001 ±  | 0.008 ±  | 0.012 ±  | 0.014 ±  | 0.017 ±  | 0.021 ±  | 0.09 ±  | 0.09 ±  | 0.09 ±  |
contacted the taste buds and produced bitter taste. As the enzymatic hydrolysis progressed, more and more side chains were exposed, resulting in the increase in bitterness (Lovsin-Kukman, Zelenik-Blatnik, & Abram, 1996). As the enzymatic hydrolysis time and enzyme dose were increased, the umami taste of the enzymatic hydrolysate continued to increase (high NMS values). This may be due to the increase in the contents of Asp and Glu as the enzymatic hydrolysis progresses (Table 3). Aspartic and glutamic acid are sodium glutamate (MSG) ingredients (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). The e-tongue is able to distinguish the taste of different foods, identify the different fermented products, and describe the taste of different processed foods. In addition, the CPS values were increased with the increase of enzyme dose and duration of hydrolysis at the same dosage of enzyme (Fig. 3A), indicating general acceptance of soybean meal was improved with middle or high dose of enzyme. The results based on the e-tongue measurement were significantly correlated to the human sensory evaluation scores, indicating that the e-tongue can be utilized to characterize the flavor of the soybean meal hydrolysates or other food types.

To further understand e-tongue data, these results were statistically analyzed via PCA to highlight the differences in the distribution of nonvolatile compounds. A total contribution rate of over 85% suggested the feasibility of the method. The PCA analysis on the nonvolatile compounds under different conditions of enzymatic hydrolysis was shown in Fig. 3B. The variance contribution rates of PC1 and PC2 were 80.80% and 14.93%, respectively, with the cumulative variance contribution rate of 95.73% (more than 85%), indicating that the two principal components provided maximum information on the flavor of non-volatile compounds. According to the PCA chart, with the same amount of enzyme, as the time of hydrolysis was increased, PC1 presented a rising trend, while PC2 showed a decreasing trend. The results from the e-tongue was able to completely distinguish the flavor of the enzymatic liquids with different times of enzymatic hydrolysis at the same enzyme dosage. Meanwhile, the figure showed that under the same time for enzymatic hydrolysis, PC1 rose, while PC2 decreased along with an increase in the enzyme amount, demonstrating that the results of the E-tongue could completely distinguish the flavor of the enzymatic hydrolysates with different enzyme amounts under the same enzymatic hydrolysis time.

Conclusion

In this study, GC-MS, E-nose and E-tongue technology were employed to analyze the effects of different enzymatic conditions on the flavor of soybean meal hydrolysates. It revealed that different flavor was observed before and after enzymatic hydrolysis, a total of 64 volatile compounds were identified in all samples and the content of main soybean odor substance (1-octene-3-ol) was effectively reduced after
enzymatic hydrolysis. The flavor after enzymatic hydrolysis was improved, but undesirable flavor was produced in the case of excessive enzymatic hydrolysis, such as 6000 u/g at all three time treatments. Appropriate enzymatic hydrolysis with Alcalase improved the umami taste of the enzymatic hydrolysates. In addition, both radar graph and PCA of E-tongue distinguished the samples of the soybean meal hydrolysates from different conditions. In summary of all parameters, at 4000 u/g with 3 h treatment, the odor components of the soybean meal were decreased from 30.17% to a minimum of 13.10% while the Alcalase enzymatic hydrolysis of soybean meal effectively optimized its flavor. The results of this study provide a theoretical basis for the development of soybean meal and the improvement of its flavors and related products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfochx.2021.100141.

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