

Research Article

Analysis of VOCs in Lueyang Black Chicken Breast Meat during the Steaming Process with GC-IMS and Stoichiometry

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Steamed chicken breast meat attracts people for its unique flavor and nutritional benefits. In this study, the sensory evaluation of Lueyang black chicken breast meat during steaming was first performed, and their volatile organic compounds (VOCs) were further analyzed by gas chromatography-ion mobility spectroscopy (GC-IMS) combined with stoichiometry. The sensory results demonstrated that the Lueyang black chicken breast meat steamed for 15–30 min was more acceptable in taste, flavor, and chewiness. A total of 60 volatile flavor signal peaks were obtained, and 46 VOCs were recognized from qualitative analysis, containing 24 aldehydes (51.19–72.57%), 8 ketones (10.15–16.97%), 9 alcohols (7.98–13.16%), 2 furans (2.24–10.85%), 2 esters (0.54–1.56%), and 1 ether (0.05–2.47%). A stable and reliable prediction model was set up by orthogonal partial least squares-discriminant analysis (OPLS-DA), and 18 characteristic VOCs (including 10 aldehydes, 3 alcohols, 3 ketones, 1 furan, and 1 ether) were picked out through variable importance in the projection (VIP>1.0 and $p < 0.05$). Principal component analysis (PCA) results indicated that the cumulative contribution ratio was 92% with PC1 68.7% and PC2 23.3%, respectively, indicating that these characteristic VOCs could well discriminate the steaming time of Lueyang black chicken breast meat. Heatmap clustering analysis also demonstrated a similar distinguishing effect. These results could provide references for the research, development, and quality control of ready-to-eat steamed products for Lueyang black chicken breast meat in the future.

1. Introduction

Chicken is the second favorite meat item after pork in the human diet. Among chicken varieties, black chickens are highly sought after due to their nutritional and medicinal value [1–3]. Lueyang black chicken is an ancient and excellent chicken breed in Lueyang County, Shaanxi Province of China, and has been certified as a geographical indication of agricultural products by the Ministry of Agriculture of China since 2017. Given its increasing quantity and scale, efforts have been made in the processing of Lueyang black chickens to increase their added value. To date, the genetics

[4, 5], mitochondrial whole genome and molecular phylogeny [6], meat nutrition and transcriptome [7, 8], breeding [9–13], eggshell color [14, 15], and inosine acid content determination [16] of Lueyang black chicken have been studied. For a long time, slaughtered Lueyang black chicken has often been sold on the market, and there are fewer processed products. Meanwhile, there are several studies on the processed products of Lueyang black chicken, such as chicken sausage [17], chicken and *lentinus edodes* sauce [18], and chicken jerky [19].

Steaming, boiling, frying, and roasting are common cooking methods for chicken in daily life. Different cooking

methods can lead to different texture and flavor profiles of the final products. Chicken breast meat is the tender part of chicken with high protein and low fat, and humans can easily cook, digest, and absorb it. Meanwhile, volatile flavor profiles of the processed chicken products are also very important quality parameters. There have been already several studies on the volatile organic compounds (VOCs) in raw chicken breast [20] and processed chicken breast (roasted, steamed, fried, etc.) based on gas chromatography-mass spectrometry (GC-MS) and gas chromatography-ion mobility spectrometry (GC-IMS) [21–24].

Compared to GC-MS, GC-IMS is a novel emerging tool used for separating and detecting VOCs in food with low cost, rapid response, visualization, and high sensitivity [25, 26]. GC-IMS technology has been broadly utilized for the diagnosis and analysis of VOCs in restewed chicken breast [24], red-cooked chicken [27], braised chicken [28], and soft-boiled and boiled chicken [29, 30]. A large number of studies have proven that the GC-IMS is more effective than the common GC-MS, particularly for detecting trace volatile substances [21–24, 27–31], so the two complementary technologies can work together to provide the whole flavor profiles in foods.

Our previous studies analyzed the nutritional content and antioxidant effects of the soup made from Lueyang black chicken on aging mice induced by D-galactose [32] and optimized processing conditions of Lueyang black chicken sausage by single factor and orthogonal test [17]. However, there is still a lack of research on VOCs in the cooking process of Lueyang black chicken. This study aims to evaluate the sensory quality of Lueyang black chicken breast meat during the steaming process and further detect their VOCs by GC-IMS combined with stoichiometry, which would shed light on the research, development, and quality control of ready-to-eat steamed products of Lueyang black chicken in the near future.

2. Materials and Methods

2.1. Materials and Chemicals. Three 8-month-old fresh Lueyang black chicken cocks, with a body weight of 2.42 ± 0.43 kg, were purchased from Black Phoenix Black Chicken Breeding Co., Ltd. located in Lueyang County (Hanzhong, China). After killing the chickens, the breast meat was taken out and delivered to the laboratory on ice. Analytical grade n-ketones (2-pentanone, 2-butanone, 2-heptanone, 2-hexanone, 2-octanone, and 2-nonanone, purities $\geq 99\%$) were provided by Guoyao Reagent Co., Ltd. (Beijing, China).

2.2. Preparation of Steamed Breast Meat of Lueyang Black Chickens. The steamed chicken breast meat was referenced according to Fan et al. [21], with slight adjustments. The breast meat of Lueyang black chicken was first washed clean, cut into approximately $7 \text{ cm} \times 2.5 \text{ cm} \times 2 \text{ cm}$ in size, and put in the steamer after steaming for 5, 15, and 30 min (labeled as Z5, Z15, and Z30, respectively). The raw breast meat (unsteamed, labeled as Z0) was used as control. It was

removed and cooled, and then the steamed and unsteamed meat was crushed with a tissue masher homogenizer (JJ-2B, Changzhou, China) for analysis of VOCs.

2.3. Sensory Evaluation. Based on the Chinese national standard for sensory evaluation of meat products (20142773-T-601), 10 evaluators (including 5 men and 5 women) with experience in meat evaluation were invited to score three steamed chicken breast meat samples in four aspects, including taste, aroma, chewiness, and tissue state, with a total score of 100. The final sensory score = taste $\times 0.3$ + aroma $\times 0.3$ + chewiness $\times 0.3$ + tissue status $\times 0.1$, and the specific sensory assessment standards are listed in Table 1 [33].

2.4. GC-IMS Assay of Breast Meat VOCs. GC-IMS was utilized to analyze the volatile flavor substances in breast meat samples of Lueyang black chicken at different steaming times. Each kind of meat sample was measured 3 times. Concisely, 2.0 g of breast meat at different steaming times was put in a 20.0 mL headspace vial and 500.0 μL headspace gas nitrogen (purity $\geq 99.99\%$) was injected into the injector after incubation at 60°C for 15 min and then detected by GC-IMS instrument (FlavourSpec[®], Germany) [31]. The gas chromatographic separation was performed on the MXT-5 column ($15 \text{ m} \times 0.53 \text{ mm}$) at 60°C with nitrogen (purity $\geq 99.99\%$) as a carrier gas for 20 min. The start-up gas flow rate was 2.0 mL/min, maintained for 2 min, linearly enlarged to 10 mL/min within 10 min, and then linearly expanded to 100 mL/min within 20 min. The IMS was performed with 45°C IMS detector temperature and 150 mL/min nitrogen (purity $\geq 99.99\%$) flow rate and analyzed for 30 min. The n-ketones mentioned above were used as migrant markers to obtain the relative ratio of VOCs in the breast meat according to the retention index (RI) and the drift time (DT) provided by the fragment library of the instrument.

2.5. Statistical Analysis. The VOCs were qualitatively identified using the built-in NIST 2014 and self-built IMS database software. The Reporter plug-in allows for direct comparison of spectral differences between samples. The Gallery Plot plugin can be used to compare the fingerprint spectrum, allowing for intuitive and quantitative comparison of VOCs' differences between different samples. Excel 2010 was used to draw the bar graph of relative content changes of different components. PCA and the establishment and validation of OPLS-DA modeling were carried out by SIMCA-P 14.1 software. The cluster heatmap was drawn using the BioDeep tool assistant (<https://www.biodeep.cn/home/tool>).

3. Results and Discussion

3.1. Sensory Scores of Lueyang Black Chicken Breast Meat at Different Steaming Times. According to the sensory evaluation standards shown in Table 1, evaluators conducted

TABLE 1: The sensory assessment standards of chicken breast meat.

| Index | Scoring criteria | Score |
|---------------|---------------------|-------|
| Taste | Fresh and delicious | 20~30 |
| | Not outstanding | 10~19 |
| | No taste | 0~9 |
| Aroma | Strong fragrance | 20~30 |
| | Light fragrance | 10~19 |
| | No fragrance | 0~9 |
| Chewiness | Easy to chew | 20~30 |
| | Easier to chew | 10~19 |
| | Hard to chew | 0~9 |
| Tissue status | Tight | 8~10 |
| | Slightly fluffy | 4~7 |
| | Fluffy | 0~3 |

a sensory evaluation on the steamed chicken breast meat samples of Z5, Z15, and Z30 (Figure 1(a)), and the sensory scores bar graph is shown in Figure 1(b). As can be seen from the results of the sensory evaluation (Figure 1), as the steaming time extended, the volume of breast meat continued to increase, the tissue was more fluffy, and the taste, aroma, and chewiness were better. The total sensory score of sample Z30 was the highest, followed by samples Z15 and Z5. Due to the short steaming time, the taste, aroma, and chewiness of sample Z5 were poor, and there were significant differences compared with samples Z15 and Z30, respectively ($p < 0.01$). The taste and aroma of sample Z30 were better than those of sample Z15, while there was no significant difference between them, and sample Z30 is easier to chew and more fluffy than sample Z15. Therefore, based on different eating habits, consumers who prefer chewy meat can choose the breast meat steamed for 15 min, while consumers who prefer meat that is easy to chew can choose the breast meat steamed for 30 min. The Lueyang black chicken breast meat steamed for 15–30 min was more acceptable in taste, flavor, and chewiness.

3.2. GC-IMS 3D and 2D Spectrum of VOCs in Four Chicken Breast Meat Samples. The VOCs of the breast meat samples Z0, Z5, Z15, and Z30 were measured by GC-IMS. The GC-IMS 3D spectra of four breast meat samples were obtained (Figure 2(a)). The DT, ion relative retention time (RT), and signal intensity were the X, Y, and Z axes, respectively, on the 3D spectra. The signal intensity showed the amplitude of the peak [23, 31]. There were fewer VOCs on the spectrum of Z0 samples, while there were a higher variety and quantity of VOCs on the spectra of Z5, Z15, and Z30 samples steamed for 5, 15, and 30 min. When the meat was cooked, it could produce a greater variety and quantity of flavor substances. This is consistent with the results that the restewed chicken produced more variety and quantity of VOCs than the raw chicken [24] and the contents and types of VOCs of steamed sea bass were significantly higher than those of unsteamed sea bass [34]. However, it is difficult to tell the differences in VOCs on the 3D spectra.

To compare the variations of VOCs in the four breast meat samples more intuitively, 2D view spectra (Figure 2(b)) were converted by the 3D spectra in Figures 2(a), and 2D difference spectra (Figure 2(c)) were obtained by deducting Z0 spectrum as a base. Different VOCs have different horizontal migration times and vertical RT, and the same VOC content in different samples varied with an obvious trend of change (Figures 2(b) and 2(c)). The VOCs of Lueyang black chicken breast meat at different steaming times could be separated and distinguished effectively by GC-IMS, showing different characteristic GC-IMS spectra (Figures 2(b) and 2(c)).

3.3. Qualitative Analysis of VOCs in Four Chicken Breast Meat. The qualitative analysis of VOCs is conducted based on their DT and RT. Samples Z0 were taken as an example for qualitative analysis of VOCs (Figure 3), while those of other samples were not shown here. Each signal peak in Figure 3 represents a substance, marked with numbers. There were 60 signal peaks obtained from the breast meat of Lueyang black chicken at the different steaming time, from which 46 VOCs were identified, including 24 aldehydes (51.19–72.57%), 8 ketones (10.15–16.97%), 9 alcohols (7.98–13.16%), 2 furans (2.24–10.85%), 2 esters (0.54–1.56%) and 1 ether (0.05–2.47%) (Figure 3 and Table 2).

The RI, RT, DT, relative proportion, and flavor description of various VOCs are shown in Table 2. The relative proportion of hexanal was the highest among aldehydes, reaching 23.82%, 31.87%, 30.96%, and 29.95%, respectively, in the samples of Z0, Z5, Z15, and Z30 (Table 2), which can endow the breast meat of Lueyang black chicken with grass, tallow, and fat aroma, consistent with the results from Wang et al. [35]. Lueyang black chicken is a kind of free-range chicken in mountainous areas, and the hexanal content of free-range chickens is greater than that of cage-range chickens [36], and this may be one of the reasons for the high hexanal content of Lueyang black chicken breast meat. Research also showed that the *SLC27A1* gene and peroxisome proliferator-activated receptor (PPAR) pathway are closely related to hexal content in Chinese local chicken VOCs [37].

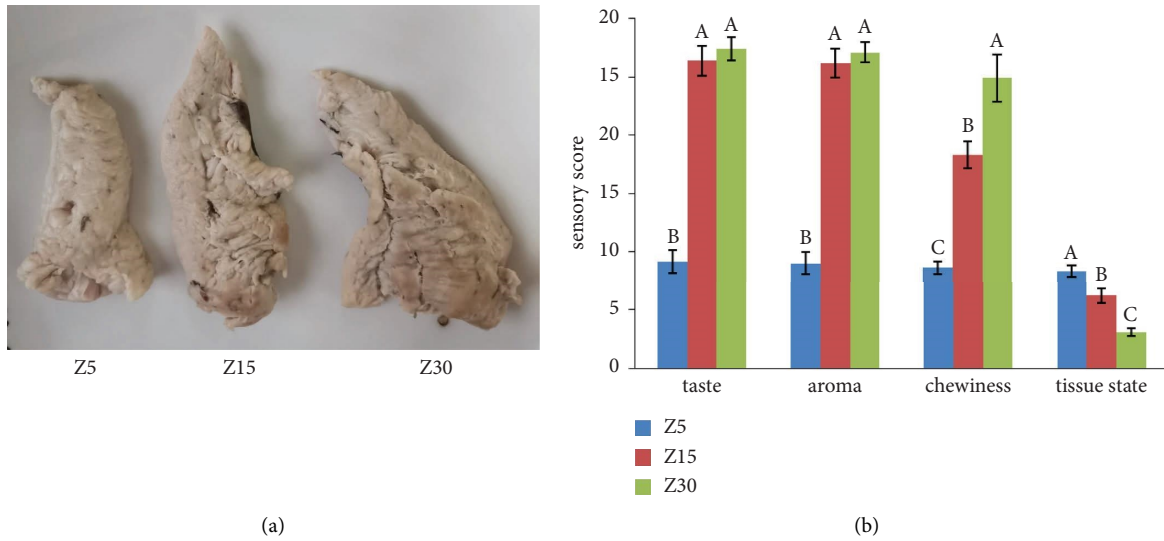


FIGURE 1: Appearance photo (a) and sensory scores (b) of Lueyang black chicken breast meat at different steaming times (note: Z5, Z15, and Z30 represent samples of Lueyang black chicken breast meat steamed for 5, 15, and 30 min, respectively).

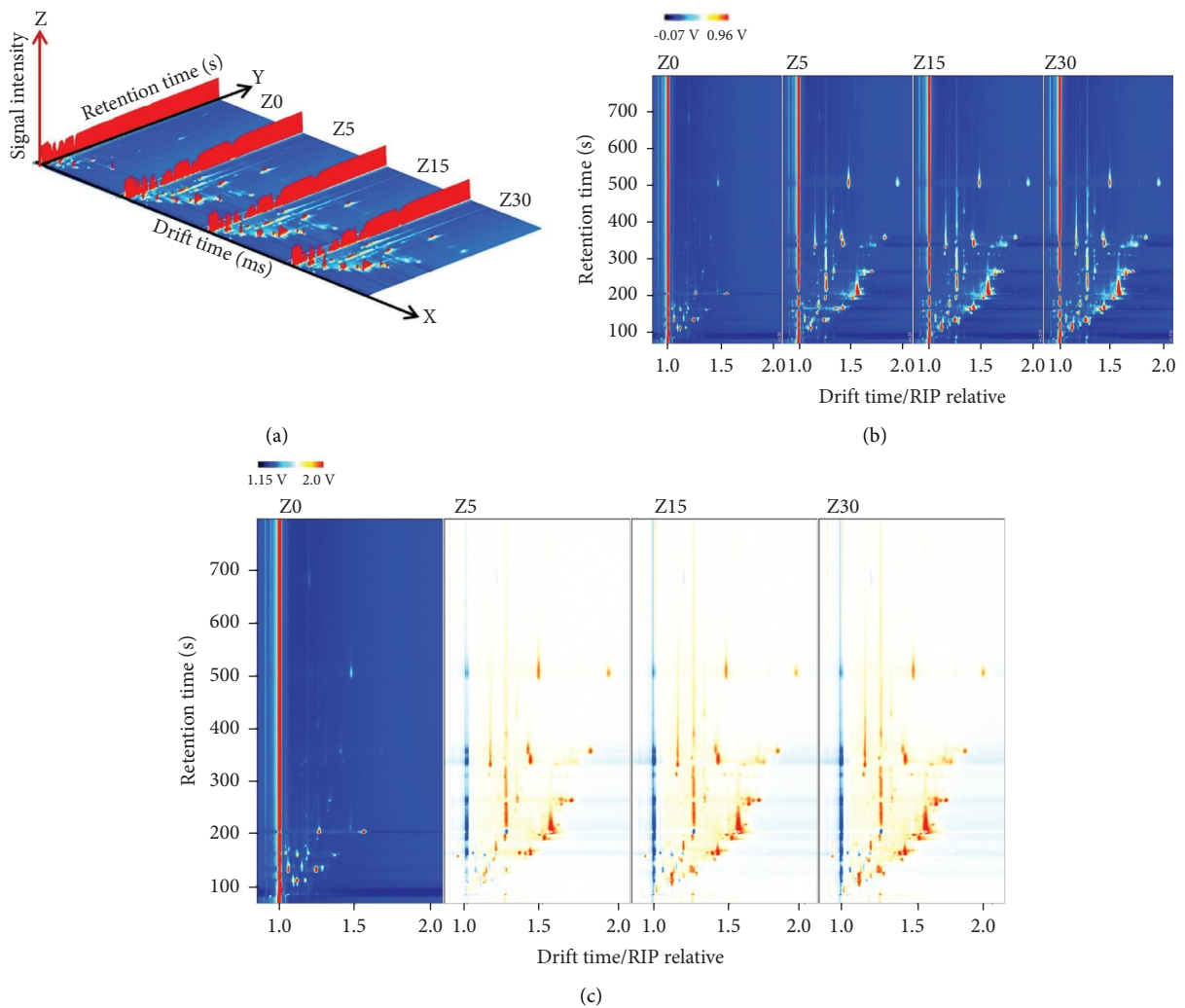


FIGURE 2: Spectra of GC-IMS in four breast meat (note: Z0, Z5, Z15, and Z30 represent samples of Lueyang black chicken breast meat steamed for 0, 5, 15, and 30 min, respectively). (a) 3D spectra. (b) 2D top view spectra. (c) 2D difference spectra.

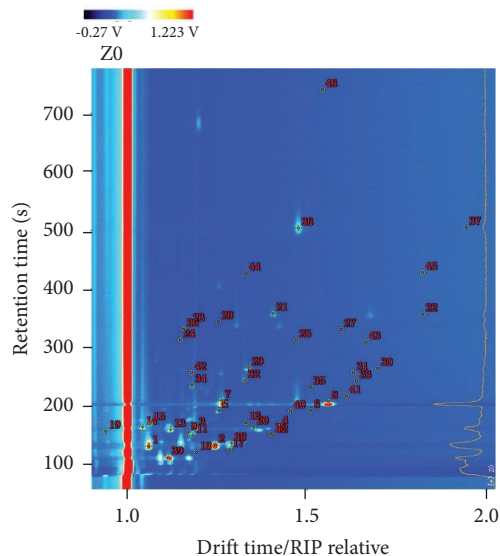


FIGURE 3: Qualitative spectrum of VOCs in the samples of Z0.

3.4. Fingerprint of VOCs in Four Chicken Breast Meat Samples.

The fingerprint was constructed to display the VOCs' differences in four breast meat samples, including Z0, Z5, Z15, and Z30, by three parallel tests (Figure 4). The horizontal represents four breast meat samples (Z0, Z5, Z15, and Z30) from top to bottom, while the vertical represents the same volatile compound at different steaming times. The redder and larger the spot is, the higher the VOC content [38, 39]. There are significant differences in VOCs in four breast meat samples (Figure 4). The relative content of VOCs in samples of Z0, including butanal, 2-pentanone, 1-propene-3-methylthio, 6-methyl-5-hepten-2-one, 3-hydroxy-2-butanone monomer, 2-butanone, hexanal, and n-hexanol, were relatively higher (Figure 4). The variety and content of VOCs in samples Z5, Z15, and Z30 exhibited a clear increase compared to that of samples Z0, which is consistent with the observed trend of VOCs variation in chicken following thermal processing [22, 24]. The concentration of VOCs, including 1-penten-3-ol, octanal, n-nonanal, pentanal dimer, 1-pentanol, 2-methylbutanal monomer, 1-octen-3-ol, 2-pentyl furan, heptanal dimer, 2-hexenal dimer, 2-penten-1-ol (Z), and butyl acetate, exhibited relatively higher levels as the steaming time increased from 0 min to 5 min. After increasing the steaming time from 5 min to 15 min, there was a decrease in the levels of 1-penten-3-ol dimer, octanal dimer, 1-pentanol monomer, hexanal monomer, heptanal, n-nonanal, decanal, and octanal monomer, while there was an increase in the content of benzaldehyde monomer, 2-heptanone, acetone, butyl acetate, pentanal-D, 3-methylbutanal dimer, 2-methyl-propanal, methyl acetate, benzaldehyde dimer, 1-octen-3-ol dimer, 2-pentyl furan, 2-penten-1-ol (Z), and 2-heptenal (E). The content of VOCs, including octanal monomer, and heptanal dimer content decreased, and the contents of pentanal dimer, 3-methylbutanal dimer, 2-methyl-propanal, methyl acetate,

benzaldehyde dimer, 1-octen-3-ol dimer, 2-pentyl furan, 2-penten-1-ol (Z), and 2-heptenal (E) showed relatively higher levels as the steaming time increased from 15 min to 30 min.

During the hot processing of meat, various VOCs are generated, such as alcohols, aldehydes, ketones, esters, furans, ethers, and other substances [21–24]. To demonstrate the various VOC changes, the relative content of various VOCs in Lueyang black chicken breast meat at different steaming times was calculated based on the signal intensity of various substances on the fingerprint spectrum (Figure 5). The results showed that aldehydes, ketones, and alcohols were the main VOCs in steamed Lueyang black chicken breast meat, which was consistent with the research results of Fan et al. [21]. The relative content of aldehyde in breast meat of Lueyang black chicken was the highest, ranging from 69.14% to 72.57%, composed of hexanal, heptanal, decanal, butanal, octanal, pentanal, n-nonanal, benzaldehyde, 2-heptenal (E), 2-hexenal, 2-methyl-propanal, 2-octenal (E), 2-methylbutanal, and 3-methylbutanal. The relative content of ketones was 10.14%–16.37%, including 2-pentanone, 2-butanone, 2-heptanone, 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, and acetone. The relative content of alcohols was 10.50%–13.16%, which was composed of 1-octen-3-ol, 1-pentanol, 1-penten-3-ol, n-hexanol, and 2-penten-1-ol (Z). The relative content of furans, including 2-butyl furan and 2-pentyl furan, was 2.24%–3.16%. The relative content of esters, namely, butyl acetate and methyl acetate, was 0.74%–1.56%. The relative content of ether 1-propene-3-methylthio was 0.05%–0.23%.

The aldehydes, ketones, alcohols, furans, esters, and ethers mainly generated after the oxidation and degradation of chicken fat are important for chicken flavor [40]. From Figure 5, it can be seen that before steaming, the VOCs of Lueyang black chicken breast meat are mainly composed of aldehydes (51.19%), ketones (26.97%), furans (10.85%), and

TABLE 2: Qualitative analysis and flavor description of VOCs in Lueyang black chicken breast meat during the steaming process.

| No. | Chemicals | RI | RT/s | DT/ms | Z0 | Relative content (%) | | | Z30 | Flavor description |
|-----|-------------------------|--------|---------|---------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|--------------------------|--------------------|
| | | | | | | Z5 | Z15 | Z30 | | |
| 1 | 2-Butanone-M | 568.7 | 130.791 | 1.06064 | 12.994 ± 1.449 ^a | 5.030 ± 0.528 ^c | 8.153 ± 0.189 ^b | 8.711 ± 0.205 ^b | Ether | |
| 2 | 2-Butanone-D | 575.7 | 132.439 | 1.24613 | 0.104 ± 0.011 ^c | 0.174 ± 0.028 ^c | 0.460 ± 0.012 ^b | 0.585 ± 0.070 ^a | Ether | |
| 3 | Pentanal-M | 687.6 | 162.092 | 1.19251 | 4.350 ± 0.740 ^a | 3.648 ± 0.211 ^{ab} | 3.278 ± 0.011 ^b | 3.086 ± 0.068 ^b | Almond, malt, pungent | |
| 4 | Pentanal-D | 694 | 164.438 | 1.42362 | 0.523 ± 0.124 ^d | 4.252 ± 0.345 ^c | 5.803 ± 0.095 ^a | 5.431 ± 0.060 ^b | Almond, malt, pungent | |
| 5 | 1-Pentanol-M | 761.1 | 192.16 | 1.25622 | 1.948 ± 0.346 ^b | 2.918 ± 0.302 ^a | 1.875 ± 0.011 ^b | 1.618 ± 0.039 ^b | Balsamic | |
| 6 | 1-Pentanol-D | 761.3 | 192.23 | 1.51238 | 0.390 ± 0.023 ^b | 3.686 ± 0.297 ^a | 3.404 ± 0.073 ^a | 3.537 ± 0.018 ^a | Balsamic | |
| 7 | Hexanal-M | 797.2 | 209.086 | 1.26376 | 11.318 ± 0.713 ^a | 11.229 ± 0.521 ^a | 8.513 ± 0.118 ^b | 7.776 ± 0.144 ^b | Grass, tallow, fat | |
| 8 | Hexanal-D | 792.6 | 206.824 | 1.56223 | 12.504 ± 2.674 ^b | 20.641 ± 0.487 ^a | 22.449 ± 0.099 ^a | 22.175 ± 0.160 ^a | Grass, tallow, fat | |
| 9 | 2-Methylbutanal-M | 658.1 | 153.69 | 1.16897 | 0.614 ± 0.036 ^a | 0.384 ± 0.014 ^c | 0.524 ± 0.008 ^b | 0.529 ± 0.016 ^b | Cocoa, almond | |
| 10 | 2-Methylbutanal-D | 651.9 | 151.976 | 1.40071 | 1.2855 ± 0.755 ^a | 0.376 ± 0.037 ^b | 0.255 ± 0.014 ^b | 0.230 ± 0.007 ^b | Cocoa, almond | |
| 11 | 3-Methylbutanal-M | 641.8 | 149.232 | 1.18336 | 1.550 ± 0.216 ^a | 1.208 ± 0.043 ^b | 1.171 ± 0.007 ^b | 1.164 ± 0.009 ^b | Malt | |
| 12 | 3-Methylbutanal-D | 642.4 | 149.404 | 1.40677 | 0.052 ± 0.006 ^c | 0.058 ± 0.009 ^c | 0.275 ± 0.018 ^b | 0.383 ± 0.030 ^a | Malt | |
| 13 | 2-Pentanone | 678.1 | 159.349 | 1.12126 | 3.018 ± 0.391 ^a | 0.393 ± 0.078 ^b | 0.199 ± 0.003 ^b | 0.185 ± 0.008 ^b | Ether, fruit | |
| 14 | 1-Propene-3-methylthio | 691.0 | 163.292 | 1.04401 | 2.471 ± 0.178 ^a | 0.230 ± 0.066 ^b | 0.046 ± 0.003 ^c | 0.060 ± 0.005 ^{bc} | Garlic, onion | |
| 15 | 3-Hydroxy-2-butanone-M | 710.0 | 170.665 | 1.06522 | 1.811 ± 0.373 ^a | 0.299 ± 0.066 ^b | 0.189 ± 0.003 ^b | 0.218 ± 0.005 ^b | Butter, cream | |
| 16 | 3-Hydroxy-2-butanone-D | 711.7 | 171.351 | 1.33104 | 0.121 ± 0.072 ^a | 0.073 ± 0.003 ^a | 0.092 ± 0.008 ^a | 0.092 ± 0.004 ^a | Butter, cream | |
| 17 | 2-Methyl-propanal | 543.5 | 124.967 | 1.28492 | 0.297 ± 0.005 ^c | 0.238 ± 0.043 ^c | 0.951 ± 0.046 ^b | 1.218 ± 0.094 ^a | Pungent, malt, green | |
| 18 | Methyl acetate | 520.8 | 119.959 | 1.19416 | 0.433 ± 0.096 ^c | 0.287 ± 0.025 ^d | 0.741 ± 0.008 ^b | 0.910 ± 0.050 ^a | Sweet, fruity | |
| 19 | 1-Penten-3-ol-M | 669.7 | 156.949 | 0.9404 | 0.534 ± 0.173 ^b | 0.904 ± 0.050 ^a | 0.838 ± 0.009 ^a | 0.834 ± 0.033 ^a | Butter, pungent | |
| 20 | 1-Penten-3-ol-D | 691 | 163.32 | 1.35354 | 1.470 ± 0.113 ^b | 1.853 ± 0.201 ^a | 0.774 ± 0.047 ^c | 0.721 ± 0.037 ^c | Butter, pungent | |
| 21 | Octanal-M | 1000.4 | 357.209 | 1.40732 | 2.301 ± 0.141 ^d | 4.911 ± 0.101 ^a | 3.905 ± 0.021 ^b | 3.489 ± 0.044 ^c | Fat, soap, lemon, green | |
| 22 | Octanal-D | 1000.1 | 356.829 | 1.82369 | 0.476 ± 0.018 ^c | 3.004 ± 0.359 ^a | 2.505 ± 0.033 ^b | 2.286 ± 0.056 ^b | Fat, soap, lemon, green | |
| 23 | 6-Methyl-5-hepten-2-one | 983.5 | 339.799 | 1.17573 | 0.378 ± 0.080 ^a | 0.157 ± 0.013 ^b | 0.124 ± 0.009 ^b | 0.128 ± 0.011 ^b | Pepper, mushroom, rubber | |
| 24 | Benzaldehyde-M | 953.7 | 312.134 | 1.14842 | 0.599 ± 0.169 ^b | 0.510 ± 0.037 ^b | 1.007 ± 0.025 ^a | 1.036 ± 0.012 ^a | Almond, burnt sugar | |
| 25 | Benzaldehyde-D | 953.6 | 312.078 | 1.47255 | 0.132 ± 0.033 ^c | 0.084 ± 0.012 ^d | 0.320 ± 0.007 ^b | 0.400 ± 0.023 ^a | Almond, burnt sugar | |
| 26 | 1-Octen-3-ol-M | 974.3 | 331.012 | 1.15835 | 0.657 ± 0.065 ^c | 2.283 ± 0.118 ^a | 2.063 ± 0.013 ^b | 2.098 ± 0.050 ^b | Mushroom | |
| 27 | 1-Octen-3-ol-D | 973.5 | 330.27 | 1.59704 | 0.194 ± 0.009 ^d | 0.437 ± 0.113 ^c | 0.655 ± 0.034 ^b | 0.808 ± 0.015 ^a | Mushroom | |
| 28 | 2-Pentyl furan | 987.1 | 343.264 | 1.25468 | 0.204 ± 0.018 ^d | 0.554 ± 0.020 ^c | 0.651 ± 0.013 ^b | 0.716 ± 0.026 ^a | Green bean, butter | |
| 29 | Heptanal-M | 895 | 264.184 | 1.33916 | 2.802 ± 1.004 ^a | 2.693 ± 0.279 ^a | 1.710 ± 0.036 ^b | 1.514 ± 0.022 ^b | Fat, citrus, rancid | |
| 30 | Heptanal-D | 894.5 | 263.813 | 1.69931 | 0.352 ± 0.075 ^d | 3.054 ± 0.086 ^a | 2.540 ± 0.006 ^b | 2.390 ± 0.005 ^c | Fat, citrus, rancid | |
| 31 | 2-Heptanone | 883.0 | 256.388 | 1.63113 | 0.134 ± 0.016 ^a | 0.077 ± 0.003 ^c | 0.120 ± 0.002 ^b | 0.123 ± 0.008 ^b | Soap | |
| 32 | n-Hexanol-M | 861.8 | 243.764 | 1.32879 | 2.399 ± 1.754 ^a | 0.785 ± 0.074 ^{ab} | 0.516 ± 0.017 ^b | 0.536 ± 0.007 ^b | Resin, flower, green | |
| 33 | n-Hexanol-D | 859.2 | 242.279 | 1.64002 | 0.340 ± 0.299 ^a | 0.186 ± 0.019 ^a | 0.216 ± 0.007 ^a | 0.275 ± 0.004 ^a | Resin, flower, green | |
| 34 | 2-Hexenal-M | 846.8 | 235.225 | 1.18058 | 0.213 ± 0.209 ^a | 0.129 ± 0.028 ^a | 0.118 ± 0.005 ^a | 0.142 ± 0.008 ^a | Fat, rancid | |
| 35 | 2-Hexenal-D | 840.8 | 231.906 | 1.51179 | 0.066 ± 0.003 ^b | 0.126 ± 0.042 ^a | 0.131 ± 0.006 ^a | 0.160 ± 0.009 ^a | Fat, rancid | |
| 36 | n-Nonanal-M | 1104.1 | 503.508 | 1.47787 | 6.149 ± 0.571 ^b | 8.331 ± 0.259 ^a | 6.493 ± 0.135 ^b | 6.215 ± 0.175 ^b | Fat, citrus, green | |
| 37 | n-Nonanal-D | 1104.5 | 504.205 | 1.94640 | 0.876 ± 0.096 ^c | 2.720 ± 0.323 ^a | 2.197 ± 0.130 ^b | 2.213 ± 0.127 ^b | Fat, citrus, green | |

TABLE 2: Continued.

| No. | Chemicals | RI | RT/s | DT/ms | Z0 | Relative content (%) | | | Flavor description |
|-----|-------------------|--------|---------|---------|-----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| | | | | | | Z5 | Z15 | Z30 | |
| 38 | Butanal | 590.3 | 135.976 | 1.29064 | 2.633 ± 0.085 ^a | 0.833 ± 0.019 ^b | 0.846 ± 0.049 ^b | 0.787 ± 0.057 ^b | Pungent, green |
| 39 | Acetone | 484.9 | 112.431 | 1.11614 | 8.387 ± 1.094 ^a | 3.952 ± 0.455 ^c | 5.636 ± 0.292 ^b | 6.328 ± 0.224 ^b | Apple, pear |
| 40 | 2-Penten-1-ol (Z) | 758.6 | 191.039 | 1.45654 | 0.0513 ± 0.005 ^d | 0.110 ± 0.027 ^c | 0.161 ± 0.008 ^b | 0.198 ± 0.006 ^a | Green, plastic, rubber |
| 41 | Butyl acetate | 813.3 | 217.232 | 1.61174 | 0.106 ± 0.018 ^c | 0.448 ± 0.088 ^b | 0.664 ± 0.020 ^a | 0.654 ± 0.012 ^a | Pear |
| 42 | 2-Butyl furan | 883.8 | 256.845 | 1.17934 | 10.648 ± 0.735 ^a | 2.602 ± 0.457 ^b | 1.671 ± 0.049 ^c | 1.522 ± 0.049 ^c | Fruity, wine, sweet, spicy |
| 43 | 2-Heptenal (E) | 949.9 | 308.789 | 1.66653 | 0.536 ± 0.167 ^d | 2.850 ± 0.540 ^c | 4.509 ± 0.056 ^b | 5.208 ± 0.083 ^a | Soap, fat, almond |
| 44 | 2-Octenal (E) -M | 1053.9 | 426.438 | 1.33121 | 0.623 ± 0.079 ^b | 0.752 ± 0.118 ^{ab} | 0.819 ± 0.016 ^a | 0.866 ± 0.043 ^a | Green, nut, fat |
| 45 | 2-Octenal (E) -D | 1054.5 | 427.217 | 1.82421 | 0.209 ± 0.047 ^a | 0.073 ± 0.011 ^b | 0.068 ± 0.007 ^b | 0.078 ± 0.005 ^b | Green, nut, fat |
| 46 | Decanal | 1220.2 | 739.579 | 1.54424 | 0.727 ± 0.026 ^a | 0.462 ± 0.031 ^b | 0.365 ± 0.015 ^c | 0.367 ± 0.008 ^c | Soap, orange peel, tallow |

The -M and -D following VOCs indicate monomer and dimer, respectively. Different lowercase letters in the same line indicate a significant difference ($p < 0.05$). The flavor description comes from <https://www.flavornet.org/flavornet.html> and <https://www.thegoodscentscompany.com/search2.html>.

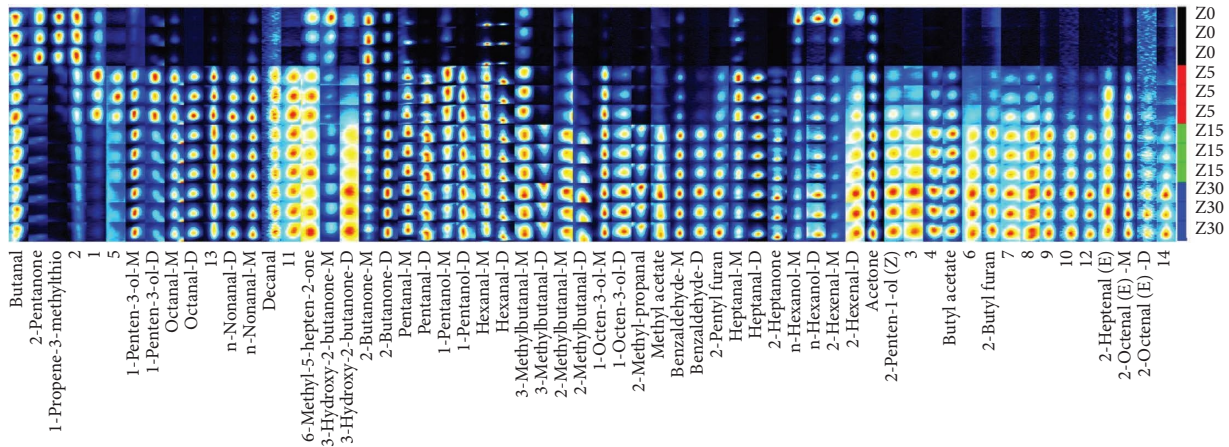


FIGURE 4: Gallery fingerprint of VOCs in four steamed chicken breast meat samples.

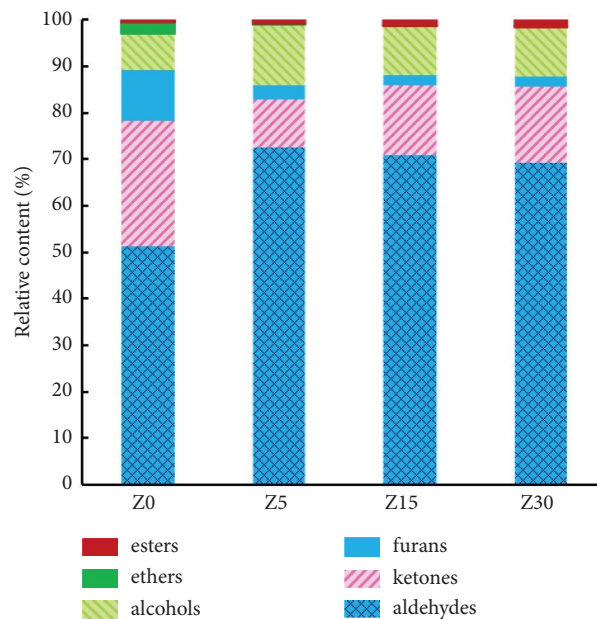


FIGURE 5: The relative content changes of VOCs in four chicken breast meat samples.

alcohols (7.98%), with a small amount of ethers (2.47%) and esters (0.54%). When the breast meat was steamed for 5 min, the content of aldehydes, esters, and alcohols increased, while the content of furans, ketones, and ethers decreased. With the steaming time extended from 5 min to 30 min, the content of aldehydes, alcohols, furans, and ethers in the breast meat of the steamed Lueyang black chicken gradually decreased, while the content of ketones and esters gradually increased. When steamed for 30 min, the VOCs in the breast meat of Lueyang black chicken are mainly aldehydes (69.14%), ketones (16.37%), and alcohol (10.625%).

Aldehydes have a low threshold, which is very important in the production of meat flavor [40]. They are the key VOCs in local Chinese chicken meat [41], which account for the highest proportion of VOCs in the unsteamed and steamed breast meats of Lueyang black chicken, and contribute grass, tallow, fat, almond, malt, pungent, citrus, green aromas to

the breast meat. Aldehydes also contributed the largest to the overall breast meat flavor of Piao chicken and Yanjin silky fowl [20], Chai hen, and black chicken [35]. The thresholds of ketones and alcohols are higher than those of aldehydes, with pleasant flavors such as floral and fruity. Ketones are few in variety; however, they are important in the formation of chicken's characteristic flavor. The contents of ketones and alcohols in the breast meat of Lueyang black chicken steamed for 30 min were higher than that in the breast meat of many Chinese native chickens [21, 35].

3.5. OPLS-DA and Model Validation. OPLS-DA, different from PCA, is a supervised statistical method for discriminant analysis. The relationship model between the substance expression and sample category was established by partial least squares regression to predict the sample category. $R^2 X$

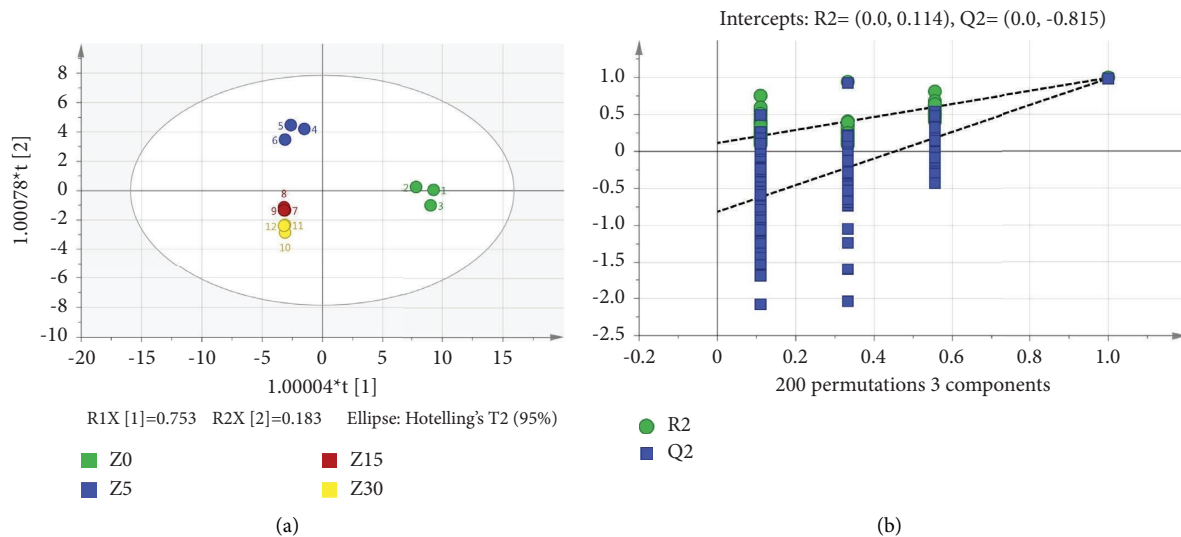


FIGURE 6: OPLS-DA scores of VOCs in breast meat of Lueyang black chicken at different steaming times (a) and displacement tests (b).

and R^2Y stand for the explanation rate of the constructed model for the X and Y matrices, respectively, and Q^2 denotes the prediction ability of the model. A scoring plot in OPLS-DA was used to classify VOCs of 4 kinds of breast meat, and the results are shown in Figure 6. Parameters of Q^2 greater than 0.5 and less than 1.0 are considered to be more accurate [38, 39, 42]. Most information on the VOCs of four breast meat was covered by this model with Q^2 (cum) = 0.648, R^2X (cum) = 0.96, and R^2Y (cum) = 0.786 (Figure 6). Most breast meat samples at different steaming times could be classified by the OPLS-DA map (Figure 6(a)). To prevent overfitting, a permutation test was used to confirm the OPLS-DA model reliability, and the result is shown in Figure 6(b). The regression line of Q^2 (-0.815) is less than 0 at the crossing point of the ordinate after 200 cross-validations. R^2 and Q^2 are lower than the raw values in all tests, showing that the established OPLS-DA model is not overfitting and is stable and reliable [43].

3.6. Screening of Characteristic VOCs in Four Breast Meat.

According to the VIP values in the OPLS-DA model, the importance of each variable for sample classification can be quantified. The characteristic VOCs were screened based on VIP values greater than 1.0 and significance levels less than 0.05. The screening method based on the OPLS-DA model and VIP values was used in many kinds of foods to select the characteristic flavor components [31, 34, 38, 39, 44]. The results showed that 18 characteristic VOCs were screened from 46 VOCs in four breast meat samples (Figure 7(a)), including 10 aldehydes (hexanal (monomer and dimer), pentanal (monomer and dimer), octanal (monomer and dimer), heptanal (monomer and dimer), 2-heptenal (*E*), and *n*-nonanal monomer), 3 ketones (acetone, 2-butanone monomer, and 2-pentanone), 3 alcohols (1-pentanol (monomer and dimer) and *n*-hexanol monomer), 1 furan (2-butyl furan), and 1 ether (1-propene-3-methylthio). Hexanal dimer (with aromas of grass, tallow, and fat) had the highest VIP value, followed by 2-

butanone monomer (with aromas of ether), 2-butyl furan (with aromas of fruity, wine, sweet, and spicy), hexanal monomer (with aromas of grass, tallow, and fat), 2-heptenal (*E*) (with aromas of soap, fat, and almond), acetone (with aromas of apple and pear), and pentanal dimer (with aromas of almond, malt, and pungent). Hexanal is the main VOC in steamed, stewed, air-fried, boiled, fried, and roasted chicken breast meat [21–23, 35]. 2-Butanone is present in the volatile flavor compounds of fried and roasted chicken breast meat [22]. 2-Heptenal (*E*) is an olefinic aldehyde with a very low threshold that plays an important role in chicken breast flavor.

The 18 characteristic VOCs were then subjected to PCA and cluster analysis (Figures 7(b) and 7(c)). PCA results showed that the cumulative contribution ratio of 18 characteristic substances was 92% (PC1 and PC2 were 68.7% and 23.3%, respectively) (Figure 7(b)), which could explain the variation in the samples. In addition, 18 kinds of characteristic VOCs in the breast meat samples of Lueyang black chicken at the same steaming time were relatively concentrated, which could better distinguish the breast meat samples of Lueyang black chicken at different steaming times. Based on the signal intensities of these VOCs, a clustering heatmap was created (Figure 7(c)), and it was found that there were differences in 18 characteristic VOCs in the breast meat samples of Lueyang black chicken at different steaming times. The flavor characteristics of Lueyang black chicken breast meat could be divided into three categories: before steaming (Z0), the early stage of steaming (Z5), and the late stage of steaming (Z15, Z30). The results of PCA and heat map of 18 characteristic flavor substances could distinguish the breast meat of Lueyang black chicken with different steaming times. In this study, 18 potential characteristic VOCs were screened by GC-IMS and the OPLS-DA model with $VIP > 1.0$ and $p < 0.05$. However, quantitative analysis was lacking. In the future, it is necessary to combine GC-MS, GC-O, and relative odor activity values to further reveal the fine changes of VOCs during the steaming process of Lueyang black chicken breast meat.

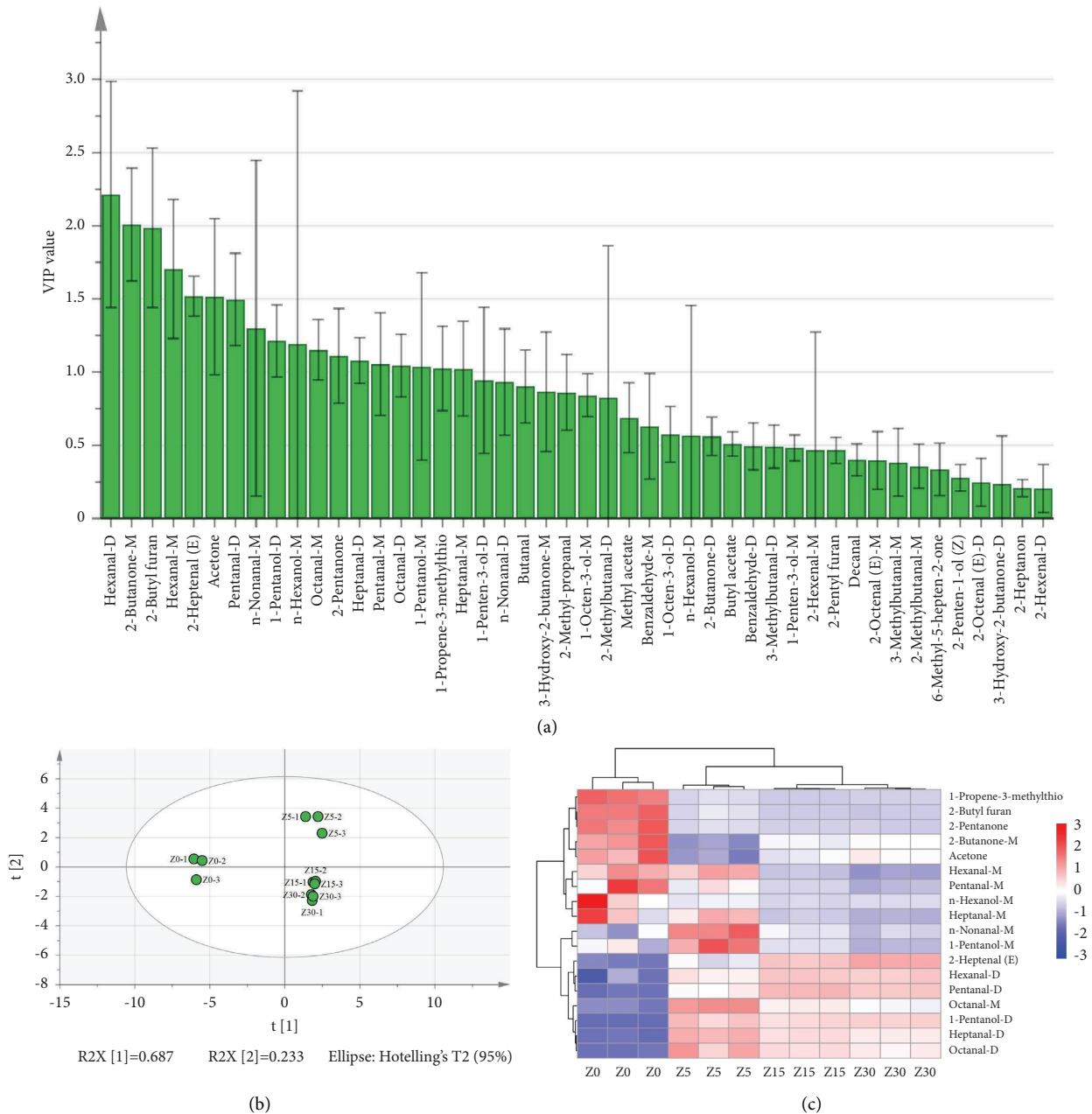


FIGURE 7: Screening of VOCs in breast meat of Lueyang black chicken at different steaming times. (a) VIP values. (b) Principal component score map. (c) Cluster heatmap.

4. Conclusions

In summary, a total of 46 VOCs were identified from the breast meat of Lueyang black chicken at different steaming times, mainly including aldehydes, ketones, alcohols, and furans. Compared with raw chicken breast meat, the relative contents of aldehydes and alcohols increased after steaming, whereas the relative contents of ketones and furans decreased. A stable and reliable OPLS-DA model was established, and 18 characteristic VOCs were screened including 10 aldehydes, 3 ketones, 3 alcohols, 1 furan, and 1 ether. Among them, the important substances affecting the flavor

of chicken breast meat during the steaming process were hexanal dimer, 2-butanone monomer, 2-butyl furan, hexanal monomer, 2-heptenal (E), acetone, and pentanal dimer (VIP > 1.50). Further, the PCA and cluster analysis of 18 characteristic VOCs showed that they could effectively distinguish the breast meat of Lueyang black chicken at different steaming times. Combined with sensory evaluation results, 15–30 min were recommended for steaming Lueyang black chicken breast meat. The GC-O aroma profiles, degradation of protein, and lipid of Lueyang black chicken breast during the steaming process will be reported elsewhere.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Linlin He and Rui Chen conducted investigation, wrote the original draft, and performed plot analysis. Fei Lan and Hui Yang performed partial analysis, visualization, and language check. Ruichang Gao reviewed and edited the manuscript. Wengang Jin reviewed and edited the manuscript, supervised the work, and acquired the funding. Linlin He and Rui Chen contributed equally to this work.

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