

Research Article

Characterization of Volatile Component Changes in Peas under Different Treatments by GC-IMS and GC-MS

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Volatile profiles of peas under 9 kinds of different treatments including native, washing, blanching, precooling, freezing, steaming, boiling, frying, and freeze-drying were characterized by GC-IMS and GC-MS. The differences of volatile compounds in different peas were observed from the characteristic fingerprints by GC-IMS. The Venn diagram found that the common flavor substances codetected by GC-IMS and GC-MS were n-hexanal, nonanal, 1-octene-3-ol, benzaldehyde, 6-methyl-5-hepten-2-one, trans-2-octenal, and 2-ethyl-3,5-dimethylpyrazine, which were speculated to be the key flavor substances of peas. The cluster analysis of the heat map conducted towards the differences of volatile components in peas under different treatments; the results indicated that peas could be mainly divided into four groups, which was consistent with the above conclusion of GC-IMS. Eight sensory descriptors were used to evaluate the aroma notes: sweet flowers, fat fragrance, waxy aldehydes, mushroom hay, roasted potato with nuts, vegetable-like bean, spicy dry tar, and bitter almond from the sensory analysis, and the sensory analysis also showed good agreement with the results of GC-IMS and GC-MS. The results indicated that the volatile compounds of peas under different treatments could be visualized and identified quickly via GC-IMS, and the samples could be clearly classified based on the difference of volatile compounds. *Practical Application.* In the study, fingerprints coupled with cluster analysis were a visualized method for the identification of volatile compounds. Meanwhile, a new method, the Venn diagram with OAV, was used to identify the key-aroma of products. Finally, a rapid method is established to classify products by GC-IMS. In future practical applications, GC-IMS can be used to classify products from different origins and different manufacturers. Similarly, it can identify fake and inferior products and whether the products have deteriorated. In addition, this research will provide a new strategy to find the relationship between flavor compounds and various processed technology towards different cereals.

1. Introduction

Peas (*Pisum sativum* L.) are the type of legume most widely used for human consumption, and they are widely cultivated around the world [1, 2]. Peas are increasingly recognized around the world as a healthy legume with good supplies of energy and protein for health-conscious diets. Peas are rich in calcium, vitamin A, carotene, potassium, and a variety of amino acids for the human body, which play an important role in enhancing the body's metabolic

function [3]. Peas are also beneficial to the spleen and stomach and have a certain therapeutic effect. Because of the abundant raw materials, low price, easy processing, storage, and transportation of peas, they are very popular in domestic and foreign markets [4]. With the continuous improvement of people's living standards, people's dietary structure has undergone great changes; the demand for fresh peas has changed from a single type to a diversified type. Applications of peas are in soup mixes, purees, and processed products.

Peas are rarely eaten at the raw state, and various methods were introduced to product processing before consumption. Deep-fried or hydrothermal treatments are the most used technologies applied in peas processing, and this may cause substantial changes in its nutritional value, chemical composition, and bioactive properties. Besides nutritional value, flavor is another important characteristic of food quality. The flavor reflects the olfactory and taste of food and can be influenced by volatile aroma compounds and nonvolatile taste compounds. During technological processing, except for nutritional components, the volatile compounds of food also changed due to various chemical reactions [5]. Identification of the volatile compounds of peas could be very important in selecting and commercializing the right cultivars for targeted food applications. Volatile compounds that characterize the flavor of foods such as peas can be affected by variety, processing conditions, ingredients, and storage [6]. Meanwhile, our team previously has studied the effects of three processing methods (oven drying after boiling, frying, and freeze-drying) on aroma components of fresh peas, and a comprehensive analysis showed that the fresh peas by freeze-drying and oven-drying after boiled had better retention of aromatic compounds. Therefore it is necessary to focus on the aroma compounds difference during peas product processing.

Ion mobility spectrometry (IMS) is a physical method that allows a mobility-based separation of volatile and semivolatile compounds in a weak electric field at ambient pressure [7]. Though IMS fundamentals have been developed since the beginning of the twentieth century, the utilization of IMS for food sample analysis is still quite new [8]. Moreover, combining IMS with other instruments is an efficient and useful method for enlarging its advantages and obtaining better analysis results [7, 9, 10]. In recent years, IMS coupled to GC has been extensively applied to evaluate volatile compounds in various food samples. GC-IMS is a new hybrid technology, which can analyze the test results quickly, simply, intuitively, and accurately. Another instrument, GC-MS, is a widespread and effective method based on solid-phase micro-extraction, gas phase separation, and mass spectrometry for analyzing volatile compounds in food samples. The combination of different techniques discloses more comprehensive, reliable, and scientific information on food aroma. However, to the best of our knowledge, monitoring the variations of volatile flavor during cereals processing via the combination of GC-MS and GC-IMS is rarely reported.

In this study, GC-IMS was used to analyze the variations in the volatile compositions of peas under different treatments, and the fingerprints were established to confirm the characteristic substance of the peas. The established fingerprints coupled with cluster analysis of GC-MS results would be a visualized and useful method for the identification of volatile compounds in peas and provide a novel alternative method for the classification of various peas products.

2. Materials and Methods

2.1. Materials. The peas used in the experiment were provided by Henan Academy of Agricultural Sciences (Zhengzhou, Henan, China), which was purchased from the local market (picking time is May 2019) and stored at -18°C . The basic physical and chemical indices of fresh peas: the dry-based protein content is 24.50%, crude fiber is 17.90%, total sugar is 3.03%, and starch is 50.86%. The different processing methods are as follows: native (untreated fresh peas), washing (rinsed gently with water three times), blanching (boiled in boiling water for two minutes), pre-cooling (refrigerated after blanching), freezing (froze after blanching), steaming (steamed for 15 minutes), boiling (boiled in boiling water for 15 minutes), frying (fried for 25 minutes until the peas produce flavor), and freeze-drying (vacuum freeze-drying).

Internal standards were used in this study to quantify volatile components; 2-octanol was purchased from Shanghai YuanYe Bio-Technology Co., Ltd (Shanghai, China) as an internal standard; the concentration of 2-octanol is 100 mg/L; and the volume of addition is 10 μL .

2.2. GC-IMS Determination Conditions. Analyses of peas were performed using the GC-IMS as described by Zhang et al. [11] with slight modifications. Specifically, peas samples (2.0 g) were weighed and placed into a 20 mL headspace glass sampling vial. The samples were incubated at 40°C for 15 min. After incubation, 500 μL of headspace was automatically injected into the injector under splitless injection mode with a syringe at 45°C . The GC was performed with an FS-SE-54-CB capillary column (15 mm \times 0.53 mm \times 1 μm ; column temperature: 60°C) to separate volatile components and coupled to IMS at 45°C . Nitrogen (99.999% purity) was used as the carrier gas; the drift gas (nitrogen gas) was set at 150 mL/min.

Data analysis of GC-IMS was carried out using functional software Laboratory Analytical Viewer and analysis software and three plug-ins Reporter, Gallery Plot, Dynamic PCA, and GC \times IMS Library Search (G.A.S. Gesellschaft für analytische Sensorenysteme embH., Dortmund, Germany). The built-in NIST 2014 gas-phase retention index database and G.A.S IMS migration time database were used for two-dimensional qualitative analysis.

2.3. GC-MS Measurement Conditions. Sample pretreatment: 2-octanol purchased from Shanghai were used as internal reference compounds for composition identification analysis; peas samples (6.0 g) of different treatments were placed into a 30 mL brown screw SPME vial and equilibrated at 80°C for 20 min. Then the SPME DVB/CAR/PDMS-coated SPME fiber (2 cm) was inserted into the balanced sample vial with a constant temperature water bath at 80°C for 50 min; the SPME fiber was thermally desorbed at 250°C for 6 min in a splitless injection port of the GC for analysis. After each run, the SPME fiber was aged by reheating for 20 min at 250°C in the injection port.

GC-MS conditions were according to the procedure described by Zhang et al. [12] with modifications; the specific conditions were as follows.

GC conditions: the chromatographic capillary column is DB-5MS (60 mm × 0.32 mm × 1 μm); the oven temperature program was as follows: initial temperature 40°C (held for 2 min), rising to 180°C at the rate of 5°C/min, then heated to 250°C at the rate of 10°C/min (held for 10 min). Helium was used as the carrier gas at a flow rate of 0.8 mL/min with the splitless GC inlet mode.

MS conditions: the MS fragmentation was performed by electronic impact (EI) mode (ionization energy, 70 eV; source temperature, 230°C). The quadrupole temperature was 150°C. The acquisition was full-scan mode and mass acquisition range of 35–450 *m/z*. The chromatogram obtained was analyzed, and each peak was checked by determining the internal standard area on the chromatogram, the retention time, the spectrum, and the base peak and then referring to the characteristic mass spectra of compounds listed on the National Institute of Standards and Technologies (NIST) [13].

2.4. Sensory Evaluation. Sensory evaluation of the aroma strength of the samples was performed by combining the flavor characteristics of flavor compounds with OAV values greater than 100 in peas under 9 kinds of different treatments; a panel of 10 trained assessors (age: 20–26; 4 females and 6 males) was invited to evaluate the aroma of peas. The sensory attributes used were: sweet flowers, fat fragrance, waxy aldehydes, mushroom hay, roasted potato with nuts, vegetable-like bean, spicy dry tar, and bitter almond. The intensities of the various sensory attributes were evaluated based on a balanced 9-point hedonic rating. The 0–9 scores represent from “no perception or extremely weak” to “strong perception” [14]. The data were presented as the mean value of the scores of each odor note and plotted in the radar charts. The sensory evaluation protocols complied with the guidelines of the declaration of Helsinki for studies on human subjects and were approved by the experiment ethics committee of the Shanghai Institute of Technology. All subjects had studied the protocol and provided their written informed consent before the sensory evaluation.

2.5. Statistical Procedures. Experimental data were analyzed statistically using SPSS 16.0 (IBM, Armonk, NY, USA); radar chart and cluster heat map analysis were performed by Origin Pro 8.0 software (Origin Lab Inc., USA). The results were expressed as the mean ± SD (standard deviation) of triple measurements. The statistical analyses were calculated using a one-way analysis of variance (ANOVA); differences were considered as significant at a level of $p < 0.05$.

3. Result and Discussion

3.1. Volatile Profile of Peas Characterized by GC-IMS

3.1.1. Differential Analysis of the Topographic Plots of Volatile Components in Peas under Different Treatments by GC-IMS. The volatile compounds of various peas were analyzed by GC-IMS, which combines the advantages of the high

separation efficiency of gas chromatography with the fast response and high sensitivity of ion mobility spectrometry [8, 15].

The ion migration time and the position of the reactive ion peak (RIP) were normalized; the ordinate represents the retention time of the gas chromatography; the abscissa represents the ion migration time, and the vertical line at the abscissa 1.0 is the RIP peak. This revealed the total headspace compounds of the samples. Each point on the right of RIP represented a volatile compound extracted from the samples. Color represented the signal intensity of the substance. White indicated lower intensity, and red indicated higher intensity. Figure 1(a) shows that most of the signals appeared in the retention time of 100–400 s and the drift time of 1.0–1.5. Combining the above description and the two-dimensional top view (Figure 1(a)) of GC-IMS can visually compare the composition of volatile substances among different samples. Compared with the native peas, the flavor profiles of the washed, blanched, and precooled peas were similar, and the concentration of flavor substances is relatively close. The number of flavor substances of frozen peas had increased, and the concentration of a few flavor substances had also increased. The number of flavor substances of peas after steaming, boiling, and freeze-drying was significantly reduced, and the changes were more obvious; the spectrum of the fried sample showed the most abundant types of volatile substances, which was the most obvious difference from other processing methods of green peas.

The difference comparison model was applied to compare the aroma variety of peas samples (Figure 1(b)). The topographic plot of native peas was selected as a reference, and the topographic plot of other samples was deducted from the reference. If the volatile compounds were consistent, the background after deduction was white, while red indicated that the concentration of the substance was higher than the reference, and blue indicated that the concentration of the substance was lower than the reference. As shown in Figure 1(b), most of the signals in the topographic plot of heated samples (steamed peas, boiled peas, and fried peas) and control (native peas) appeared within a range of 100 and 400 s of retention time, and significant differences in aroma profile were obtained according to the signals and colors variation. Heat treatments obviously improved the signals of aroma compounds (Figure 1(b)), which were consistent with the previously reported experimental phenomena [16]. Moreover, the freezing processed peas (frozen peas and freeze-dried peas) obtained more volatile components as well as compared to native peas (Figure 1(b)). Under pre-frozen conditions, the material was frozen to form ice crystals, which promoted the dispersion of water, and the volatile compounds were lost with the water. On the other hand, the pressure difference in the vacuum state caused the volatile compounds in the material to escape from the cells [17, 18]. As for native, washed, blanched, and precooled peas, most of the peaks were similar but different in concentration (Figure 1(b)). This result demonstrated that the flavor of the four samples was similar but different in regard to odor strength.

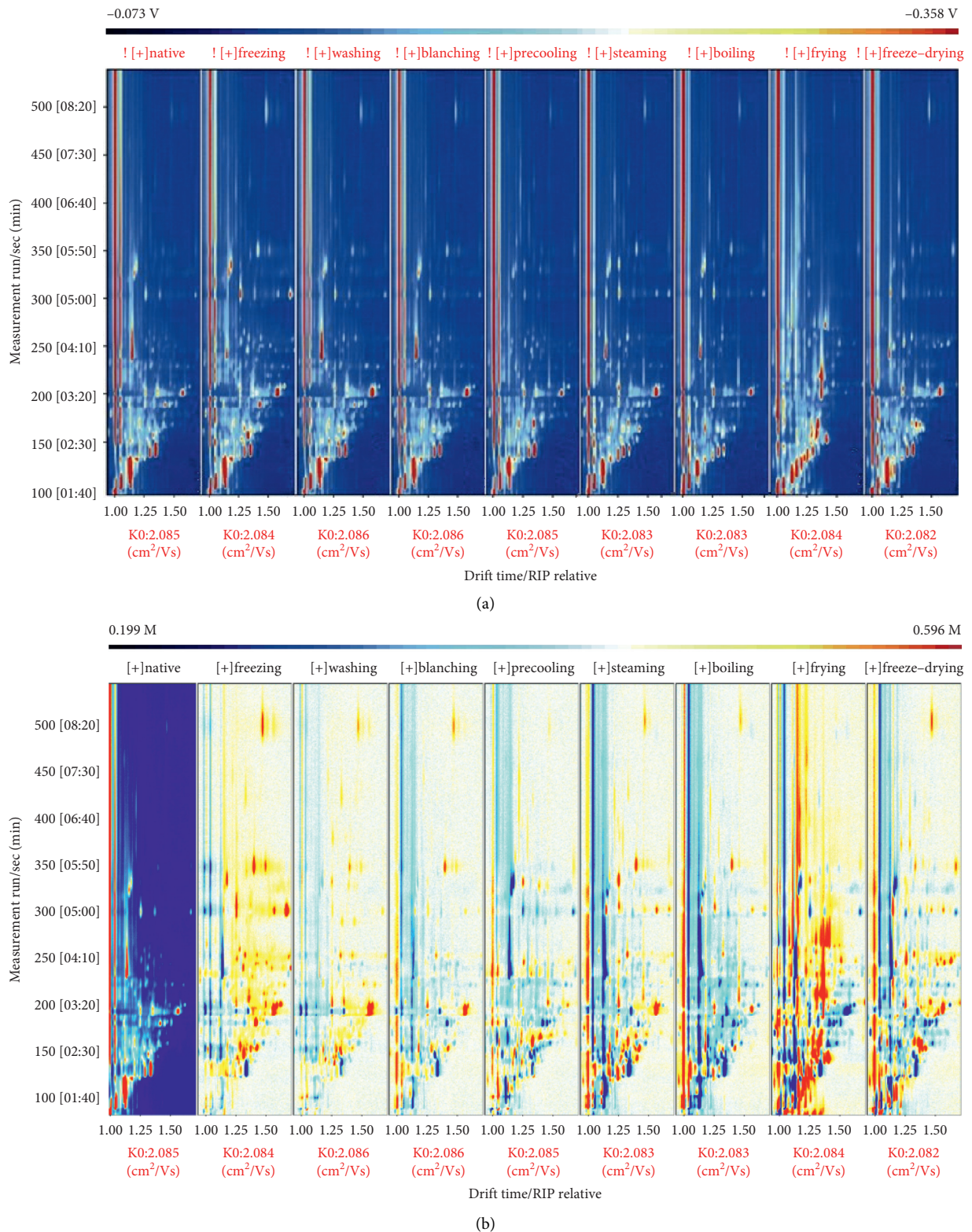


FIGURE 1: GC-IMS topographic plots of peas samples: (a) 2D-topographic plot and (b) differentiation plot of volatile compounds.

3.1.2. *Differences of Volatile Compounds of Peas under Different Treatments by GC-IMS.* In order to show the changing regularities and relative content of volatile compounds of peas under different treatments more specifically

and intuitively, the Gallery Plot analysis as fingerprinting technique was used to solve this problem [9, 10]. Changes of volatile compounds of processed peas were analyzed by the Gallery Plot analysis, which revealed that each sample had its

own characteristic aroma components (Figure 2). In the fingerprints, each row showed the entire signal intensities of one sample, and each column revealed the selected compound in different samples. Furthermore, colors represented the signal intensity (compound concentration) of volatile molecules. The low intensity was expressed in white color; the high intensity was red; and the deeper color indicated higher signal intensity. Accordingly, the differences of volatile compounds in different peas were observed, and the characteristic fingerprints corresponding to each sample were established (Figure 2). A total of 34 volatile components have been accurately qualitative (Table 1) and classified according to alcohols, aldehydes, ketones, and so on; the same series of substances were put together in order to analyze the changing regularities of flavor substances in the peas under different treatments.

As shown in the red box (Figure 2), the content of alcohols in peas was higher under different treatment methods, especially native, washing, blanching, and freezing peas, such as 1-octen-3-ol, 2-propanol, pentanol, and so on, and the content of alcohols were significantly reduced in fried and freeze-dried peas. This may be due to the continuous heating process made the small molecular alcohols with lower boiling points are continuously taken away with the water vapor. The research of Zeng [19] also confirmed that the concentration of small molecule alcohols with low boiling points such as pentanol and 2-propanol decreased with the extension of cooking time. Moreover, high-temperature conditions easily promote the esterification of some alcohols, and alcohols were also easily oxidized to aldehydes and ketones under high-temperature conditions. As shown in the yellow box, the content of aldehydes such as nonanal, trans-2-hexenal, octyl aldehyde was highest in frozen peas, followed by cooking methods. The threshold of aldehydes is low, but the ability to impart aroma is strong, and lower concentration can have a greater impact on the appearance of flavor. The aldehydes of freeze-dried green wheat can be retained to the greatest extent; however, other treatment methods may cause aldehydes to volatilize or convert to pyrazines and other substances due to long-term heating or it produced a strong binding force with the sample components without sufficient vapor pressure to form a characteristic flavor, resulting in a lower concentration of volatile aldehydes [20]. As shown in the green box, the content of ketones such as 2-heptanone and 3-hydroxy-2-butanone has been greatly increased in fried and freeze-dried peas. As shown in the white box, the content of ethyl acetate, butyl acetate, and isoamyl acetate was highest in the frozen peas, and the concentration of those flavor compounds was less than those after steaming, boiling, frying, and freeze-drying.

3.2. Volatile Profile of Peas Characterized by GC-MS

3.2.1. Differences of Flavor Substances in Peas with Different Treatment Methods. Volatile compounds isolated from peas were identified with GC-MS; the total concentration of volatile compounds differed depending on the processing methods. Among the flavor substances analyzed by GC-MS (Table 2), the following conclusions were drawn.

Among aldehydes, the kinds of aldehydes compounds in frozen peas were more, and OAV value of n-hexanal was significantly higher than that in other treatments, followed by precooling, steaming, and boiling. Taking n-hexanal and benzaldehyde as an example, the kinds and content of aldehydes were significantly less than those of freezing, precooling, steaming, and boiling, which was basically consistent with the conclusion in the fingerprints above. In the study of the influence of different treatments on the flavor of corn, we found that aldehydes were mainly produced in large quantities during the cooking process, and for peas, the content and kinds of aldehydes in the freezing and precooling treatments at lower temperatures were much more, which were considered to be mainly generated from the products of auto-oxidation and hydrolysis of unsaturated fatty acids [21].

Ketones were generally considered to present tallow and burnt aromas and also present an enhanced floral aroma as the carbon chain grows. Under the freeze-drying process, 3,5-octadienone and beta-ionone were obviously produced. The ketones were probably produced by the oxidation reaction and condensation reaction of carotenoids (such as phytoalkenes or phytofluoroalkenes) [22].

Among alcohols, taking 1-octen-3-ol as an example, it was obvious that the content of alcohols in peas dropped sharply after steaming, boiling, and frying, which was consistent with the above conclusion. This may be due to the oxidation of some fatty acids by lipoxygenase, the reduction of some aldehydes by alcohol dehydrogenase, and the hydrolysis of esters. Comparable results were also conveyed in shiitake mushrooms by Selli et al. [23].

Esters were possibly formed by the chemical reaction between alcohols and free fatty acids, which were produced by fat oxidation [24]. Due to their small contribution to the aroma, the OAV value was low, and the difference was not obvious. Although the relative contents of methyl caproate, methyl benzoate, and isopropyl myristate were relatively low, they were found in different treatments.

In summary, the processing methods of peas could be preliminarily judged based on the overall content of different compounds.

3.2.2. Cluster Analysis of Volatile Components of Peas under Different Methods Based on the Heat Map. To further understand the differences in volatile components of peas under different treatments, cluster analysis was performed using a heat map (Figure 3). According to the vertical direction of the heat map, all samples were classified into four main categories. Native, washed, blanched, and pre-cooled peas were clustered together to form the first group; steamed and boiled peas were clustered together to form the second group; fried peas alone formed the third group; and the rest of the samples were clustered together to form the fourth group (frozen and freeze-dried peas in Figure 3). According to the above results, we might infer that the volatile components of peas largely differed due to the differences in their treatments, and high-temperature frying and low-temperature conditions had a large influence on the volatile

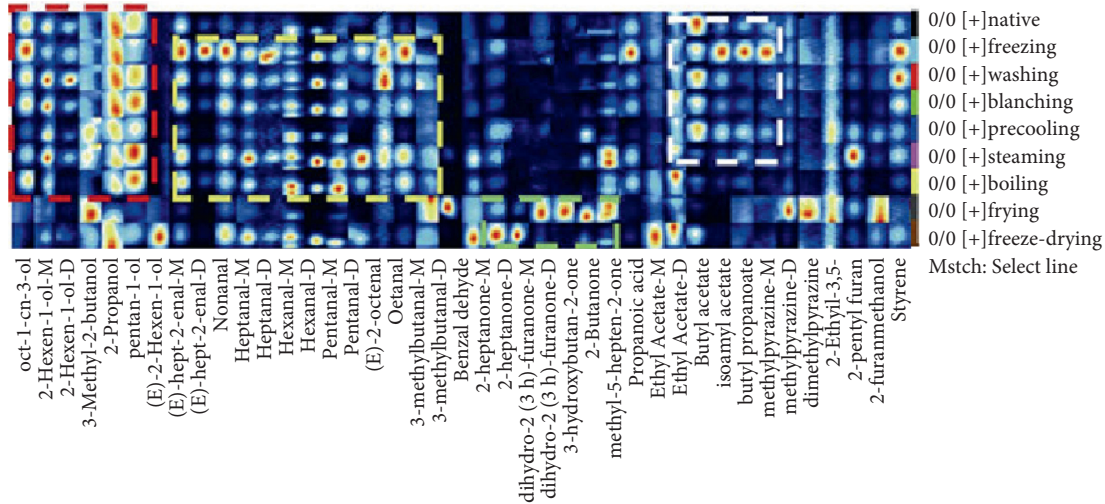


FIGURE 2: Gallery plot fingerprint of peas samples with different treatment methods.

TABLE 1: Peak area of volatile compounds of peas under 9 different treating methods via GC-IMS.

| # | Compounds | RI | Peak area of peas samples | | | | | | | | |
|----|------------------------------|----------|---------------------------|----------|-----------|------------|----------|----------|----------|----------|---------------|
| | | | Native | Washing | Blanching | Precooling | Freezing | Steaming | Boiling | Frying | Freeze-drying |
| 1 | Nonanal | 1,105.70 | 138.88 | 266.07 | 282.77 | 166.19 | 365.70 | 270.85 | 232.14 | 134.79 | 326.36 |
| 2 | 2-Pentyl furan | 990.10 | 39.68 | 81.52 | 51.65 | 138.67 | 67.77 | 258.78 | 64.65 | 109.55 | 75.28 |
| 3 | Oct-1-en-3-ol | 979.20 | 268.55 | 256.48 | 275.21 | 115.70 | 343.91 | 195.68 | 280.51 | 112.33 | 113.56 |
| 4 | (E)-Hept-2-ena | 952.20 | 325.91 | 373.45 | 379.64 | 191.86 | 595.89 | 433.44 | 419.22 | 83.27 | 236.77 |
| 5 | Heptanal | 901.00 | 63.62 | 118.21 | 129.66 | 75.75 | 124.08 | 185.53 | 122.11 | 55.83 | 154.90 |
| 6 | 2-Heptanone | 888.70 | 59.15 | 62.89 | 58.28 | 102.50 | 101.47 | 104.08 | 59.28 | 86.25 | 240.79 |
| 7 | 2-Hexen-1-ol | 846.30 | 133.17 | 170.85 | 171.22 | 152.22 | 114.79 | 198.27 | 162.94 | 111.60 | 138.78 |
| 8 | Hexanal | 791.00 | 776.29 | 1,865.98 | 1,543.11 | 766.14 | 1,609.41 | 1,998.21 | 1,086.15 | 102.28 | 1,606.15 |
| 9 | 3-Methylbutanal | 646.50 | 74.91 | 49.64 | 69.86 | 53.29 | 71.96 | 211.90 | 56.56 | 1,122.64 | 76.64 |
| 10 | Ethyl acetate | 596.80 | 2,453.45 | 1,940.43 | 1,458.70 | 1,987.80 | 1,505.24 | 513.29 | 544.36 | 286.92 | 1,244.53 |
| 11 | 2-Butanone | 580.70 | 200.59 | 197.36 | 221.28 | 144.38 | 253.36 | 634.48 | 353.05 | 709.21 | 210.71 |
| 12 | Pentanal | 702.40 | 202.48 | 293.61 | 301.72 | 258.45 | 292.28 | 381.63 | 453.27 | 141.08 | 193.06 |
| 13 | Ethyl acetate | 593.20 | 234.58 | 245.26 | 282.71 | 283.16 | 220.38 | 242.83 | 316.60 | 194.70 | 295.74 |
| 14 | 2-Propanol | 509.10 | 168.20 | 203.12 | 203.77 | 172.30 | 190.57 | 197.48 | 188.70 | 129.27 | 218.66 |
| 15 | 3-Methylbutanal | 650.90 | 57.12 | 78.96 | 54.55 | 43.86 | 69.53 | 104.24 | 66.90 | 148.71 | 77.77 |
| 16 | 3-Hydroxybutan-2-one | 713.00 | 195.55 | 244.47 | 214.37 | 600.81 | 285.87 | 417.69 | 159.98 | 1,056.22 | 427.12 |
| 17 | 3-Methyl-2-butanol | 706.60 | 71.72 | 91.17 | 74.90 | 115.29 | 72.51 | 120.27 | 54.08 | 162.34 | 92.06 |
| 18 | Benzaldehyde | 955.70 | 99.91 | 86.05 | 105.01 | 58.54 | 138.19 | 217.78 | 159.75 | 179.99 | 350.48 |
| 19 | 2-Furanmethanol | 890.10 | 40.80 | 39.76 | 25.59 | 56.02 | 48.31 | 30.47 | 18.11 | 144.92 | 66.74 |
| 20 | (E)-2-Octenal | 1,055.60 | 48.08 | 98.24 | 54.07 | 55.60 | 79.54 | 64.71 | 46.42 | 38.22 | 27.68 |
| 21 | Octanal | 1,002.80 | 54.81 | 116.58 | 177.67 | 46.11 | 273.07 | 232.16 | 206.30 | 35.10 | 72.89 |
| 22 | Dihydro-2 (3h)-furanone | 917.50 | 79.64 | 79.55 | 81.07 | 81.32 | 101.87 | 71.77 | 65.06 | 295.89 | 67.34 |
| 23 | Methylpyrazine | 825.80 | 163.55 | 150.52 | 132.56 | 120.79 | 171.20 | 130.85 | 99.75 | 364.10 | 59.02 |
| 24 | Hexanal | 796.60 | 407.05 | 423.47 | 513.14 | 438.73 | 418.16 | 587.98 | 634.73 | 204.18 | 564.68 |
| 25 | Butyl acetate | 801.20 | 60.38 | 81.70 | 63.45 | 96.45 | 180.26 | 39.90 | 29.10 | 11.73 | 19.45 |
| 26 | Pentan-1-ol | 756.40 | 129.00 | 134.03 | 155.48 | 112.72 | 97.53 | 168.28 | 163.54 | 26.46 | 87.33 |
| 27 | 2-Ethyl-3,5-dimethylpyrazine | 1,067.20 | 102.51 | 111.48 | 127.07 | 157.66 | 109.76 | 122.38 | 105.79 | 157.41 | 118.07 |
| 28 | Propanoic acid | 706.90 | 48.16 | 61.95 | 49.81 | 28.46 | 67.93 | 40.03 | 79.90 | 56.22 | 142.46 |
| 29 | Methyl-5-hepten-2-one | 989.70 | 250.18 | 195.28 | 216.69 | 100.66 | 494.13 | 264.84 | 264.98 | 65.45 | 85.87 |
| 30 | Isoamyl acetate | 876.30 | 66.38 | 48.84 | 60.77 | 112.02 | 156.57 | 78.49 | 70.90 | 51.21 | 42.18 |
| 31 | Butyl propanoate | 904.80 | 33.98 | 31.07 | 26.64 | 35.29 | 70.84 | 27.69 | 16.76 | 19.48 | 19.91 |
| 32 | Styrene | 896.20 | 32.96 | 68.13 | 53.18 | 35.22 | 76.69 | 44.87 | 24.58 | 21.43 | 42.32 |

TABLE 2: GC-MS analysis shows the relative content of main flavor compounds (relative internal standard *n*-octanol concentration) in different treatment methods.

| # | Compounds | Volatile compounds ($\mu\text{g/g}$) | | | | | | | | |
|----|---|--|---------|-----------|------------|----------|----------|---------|--------|---------------|
| | | Native | Washing | Blanching | Precooling | Freezing | Steaming | Boiling | Frying | Freeze-drying |
| 1 | Hexanal | 2.49 | 2.29 | 3.56 | 5.88 | 10.02 | 9.31 | 3.79 | 1.82 | 2.89 |
| 2 | 2-Heptenal, (E)- | 0.88 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.10 |
| 3 | Benzaldehyde | 2.56 | 2.40 | 3.54 | 3.92 | 7.32 | 5.62 | 3.30 | 14.70 | 5.04 |
| 4 | Phenylacetaldehyde | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 |
| 5 | Nonanal | 10.24 | 11.02 | 9.74 | 10.83 | 25.48 | 11.54 | 13.11 | 6.45 | 11.06 |
| 6 | Benzaldehyde, 4-ethyl- | 11.69 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 7 | Benzaldehyde, 3-ethyl- | 0.00 | 0.19 | 0.24 | 0.40 | 0.69 | 0.50 | 0.30 | 0.00 | 0.00 |
| 8 | Decanal | 2.79 | 2.68 | 3.86 | 4.83 | 8.80 | 3.00 | 3.72 | 2.65 | 0.00 |
| 9 | 2-Decenal, (E)- | 0.00 | 0.00 | 0.51 | 0.00 | 0.00 | 0.21 | 0.00 | 0.22 | 0.00 |
| 10 | Heptanal | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.01 | 0.00 | 0.00 | 0.00 |
| 11 | 2,4-Nonadienal, (E, E)- | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 |
| 12 | 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl- | 0.30 | 0.21 | 0.21 | 0.38 | 0.66 | 0.26 | 0.33 | 0.00 | 1.05 |
| 13 | 2-Undecenal | 0.00 | 0.00 | 0.00 | 0.61 | 0.00 | 0.60 | 0.00 | 0.00 | 0.00 |
| 14 | 2-Octenal, (E)- | 0.00 | 0.00 | 0.00 | 1.95 | 2.68 | 1.72 | 1.45 | 0.00 | 0.00 |
| 15 | 2-Decenal, (E)- | 0.00 | 0.20 | 0.00 | 0.82 | 1.19 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16 | Trans-2-undecen-1-ol | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.18 | 0.00 | 0.00 |
| 17 | 1-Octanol | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.76 | 0.00 | 0.00 |
| 18 | 1-Octen-3-ol | 4.87 | 3.51 | 2.44 | 6.07 | 8.79 | 2.91 | 4.10 | 0.00 | 5.29 |
| 19 | 2-Octen-1-ol, (Z)- | 1.94 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20 | Cedrol | 0.00 | 0.00 | 0.00 | 0.79 | 0.92 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21 | 1-Hexanol, 2-ethyl- | 0.00 | 0.00 | 0.00 | 0.00 | 2.21 | 0.00 | 0.00 | 0.00 | 0.00 |
| 22 | 5,9-Undecadien-2-one, 6,10-dimethyl- | 0.00 | 1.04 | 0.00 | 1.62 | 2.61 | 0.00 | 1.35 | 0.80 | 0.00 |
| 23 | 3,5-Octadien-2-one | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.14 |
| 24 | 5-Hepten-2-one, 6-methyl- | 0.79 | 0.82 | 0.44 | 0.00 | 0.97 | 0.00 | 0.00 | 0.00 | 0.00 |
| 25 | 2-Decanone | 0.23 | 0.00 | 0.20 | 0.32 | 0.00 | 0.28 | 0.19 | 0.00 | 3.16 |
| 26 | 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- | 0.59 | 0.55 | 0.51 | 0.98 | 1.47 | 0.58 | 0.69 | 1.01 | 1.80 |
| 27 | Butylated hydroxytoluene | 0.79 | 1.25 | 0.79 | 0.83 | 1.77 | 0.49 | 0.59 | 0.00 | 1.41 |
| 28 | Hexanoic acid, methyl ester | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.26 |
| 29 | Benzoic acid, methyl ester | 0.56 | 0.48 | 0.00 | 0.65 | 0.00 | 0.00 | 0.46 | 0.79 | 0.00 |
| 30 | Isopropyl myristate | 0.00 | 0.47 | 0.16 | 3.42 | 1.03 | 0.20 | 0.00 | 0.32 | 0.00 |
| 31 | Hexadecanoic acid, methyl ester | 0.13 | 0.18 | 0.05 | 0.13 | 0.00 | 0.09 | 0.21 | 0.07 | 0.00 |
| 32 | Hexadecanoic acid, ethyl ester | 0.00 | 0.05 | 0.00 | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.00 |
| 33 | Dibutyl phthalate | 0.56 | 0.67 | 0.25 | 0.62 | 0.23 | 0.79 | 1.03 | 0.42 | 0.00 |
| 34 | Cyclohexene, 1-methyl-4-(1-methylethylidene)- | 0.12 | 1.55 | 0.98 | 0.71 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 35 | D-Limonene | 0.84 | 0.00 | 0.87 | 0.43 | 0.00 | 0.00 | 0.30 | 0.85 | 8.15 |
| 36 | Cyclopropane, pentyl- | 1.53 | 1.32 | 1.12 | 1.90 | 3.67 | 1.57 | 0.00 | 0.00 | 0.00 |
| 37 | Styrene | 0.00 | 0.00 | 0.00 | 0.00 | 1.52 | 0.00 | 0.00 | 0.00 | 10.96 |
| 38 | (+)-4-Carene | 0.95 | 0.13 | 0.00 | 0.00 | 1.06 | 0.45 | 0.58 | 1.69 | 0.00 |
| 39 | Undecane | 1.13 | 1.25 | 1.29 | 1.44 | 2.38 | 1.04 | 1.14 | 1.87 | 3.67 |
| 40 | Dodecane | 0.33 | 0.28 | 0.27 | 0.43 | 0.72 | 0.31 | 0.29 | 0.36 | 10.27 |
| 41 | 1,3-Butadiene, 1,1,2,3,4,4-hexachloro- | 0.15 | 0.15 | 0.11 | 0.14 | 0.26 | 0.00 | 0.11 | 0.15 | 0.00 |
| 42 | 3-Tridecene, (E)- | 0.10 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.07 | 0.00 | 0.00 |
| 43 | Tridecane | 0.34 | 0.29 | 0.28 | 0.42 | 0.81 | 0.23 | 0.24 | 0.75 | 2.13 |
| 44 | 5-Tetradecene, (E)- | 1.91 | 1.30 | 0.00 | 2.44 | 4.36 | 0.72 | 0.00 | 4.60 | 1.05 |
| 45 | Tetradecane | 1.32 | 1.11 | 1.27 | 1.67 | 3.35 | 0.61 | 0.85 | 2.79 | 6.20 |
| 46 | Pentadecane | 2.96 | 2.98 | 3.13 | 4.01 | 7.11 | 1.23 | 2.49 | 4.49 | 3.01 |
| 47 | Hexadecane | 1.12 | 1.62 | 1.69 | 1.97 | 0.00 | 1.60 | 1.54 | 1.81 | 1.75 |
| 48 | 8-Heptadecene | 0.75 | 0.67 | 0.72 | 0.80 | 0.00 | 0.00 | 0.61 | 0.74 | 0.00 |
| 49 | Heptadecane | 3.41 | 3.06 | 4.64 | 4.89 | 8.38 | 3.02 | 3.71 | 3.48 | 4.68 |
| 50 | Octadecane | 0.26 | 0.45 | 0.28 | 0.41 | 0.64 | 0.43 | 0.54 | 0.21 | 0.00 |
| 51 | Z-5-Nonadecene | 0.81 | 1.26 | 0.92 | 1.38 | 2.56 | 1.12 | 0.00 | 0.85 | 0.68 |
| 52 | Eicosane | 0.07 | 0.08 | 0.08 | 0.11 | 0.16 | 0.20 | 0.74 | 0.07 | 0.00 |
| 53 | 1-Nonadecene | 0.11 | 0.32 | 0.20 | 0.00 | 0.37 | 0.26 | 0.33 | 0.00 | 0.00 |
| 54 | Heneicosane | 0.07 | 0.06 | 0.06 | 0.09 | 0.21 | 0.06 | 0.11 | 0.07 | 0.00 |

TABLE 2: Continued.

| # | Compounds | Volatile compounds (µg/g) | | | | | | | | |
|----|---|---------------------------|---------|-----------|------------|----------|----------|---------|--------|---------------|
| | | Native | Washing | Blanching | Precooling | Freezing | Steaming | Boiling | Frying | Freeze-drying |
| 55 | Furan, 2-pentyl- | 2.79 | 2.10 | 3.41 | 4.89 | 7.41 | 4.80 | 2.65 | 4.14 | 6.89 |
| 56 | Benzene, 1-methyl-2-(1-methylethyl)- | 0.41 | 0.55 | 0.00 | 0.16 | 0.52 | 0.18 | 0.25 | 0.00 | 0.00 |
| 57 | Naphthalene | 0.54 | 0.65 | 0.32 | 0.72 | 1.37 | 0.15 | 0.60 | 0.00 | 0.00 |
| 58 | Naphthalene, 1-methyl- | 0.35 | 0.00 | 0.00 | 0.11 | 0.27 | 0.21 | 0.35 | 0.00 | 0.00 |
| 59 | Naphthalene, 2-methyl- | 0.00 | 0.35 | 0.18 | 0.32 | 0.65 | 0.00 | 0.00 | 0.00 | 1.87 |
| 60 | Naphthalene, 2,3-dimethyl- | 0.00 | 0.15 | 0.00 | 0.19 | 0.39 | 0.00 | 0.15 | 0.00 | 0.00 |
| 61 | Pyrazine, 2-methoxy-3-(1-methylpropyl)- | 0.47 | 0.47 | 0.35 | 0.00 | 0.88 | 0.29 | 0.41 | 0.30 | 0.00 |
| 62 | Hexadecane, 2,6,10,14-tetramethyl- | 0.00 | 0.51 | 0.16 | 0.24 | 0.35 | 0.36 | 0.00 | 0.00 | 0.73 |
| 63 | Toluene | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.21 |
| 64 | Oxime-, methoxy-phenyl- | 0.00 | 1.74 | 3.86 | 1.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 65 | 5-Tetradecene, (E)- | 0.00 | 0.00 | 1.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 66 | N, N-Dimethyl-m-phenylenediamine | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.49 | 0.00 |
| 67 | Pyrazine, 3-ethyl-2,5-dimethyl- | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.25 | 0.00 |
| 68 | Pyrazine, 2-ethyl-3,5-dimethyl- | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.01 | 0.00 |
| 69 | 2,3,5-Trimethyl-6-ethylpyrazine | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.08 | 0.00 |
| 70 | Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)- | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.08 | 0.00 |

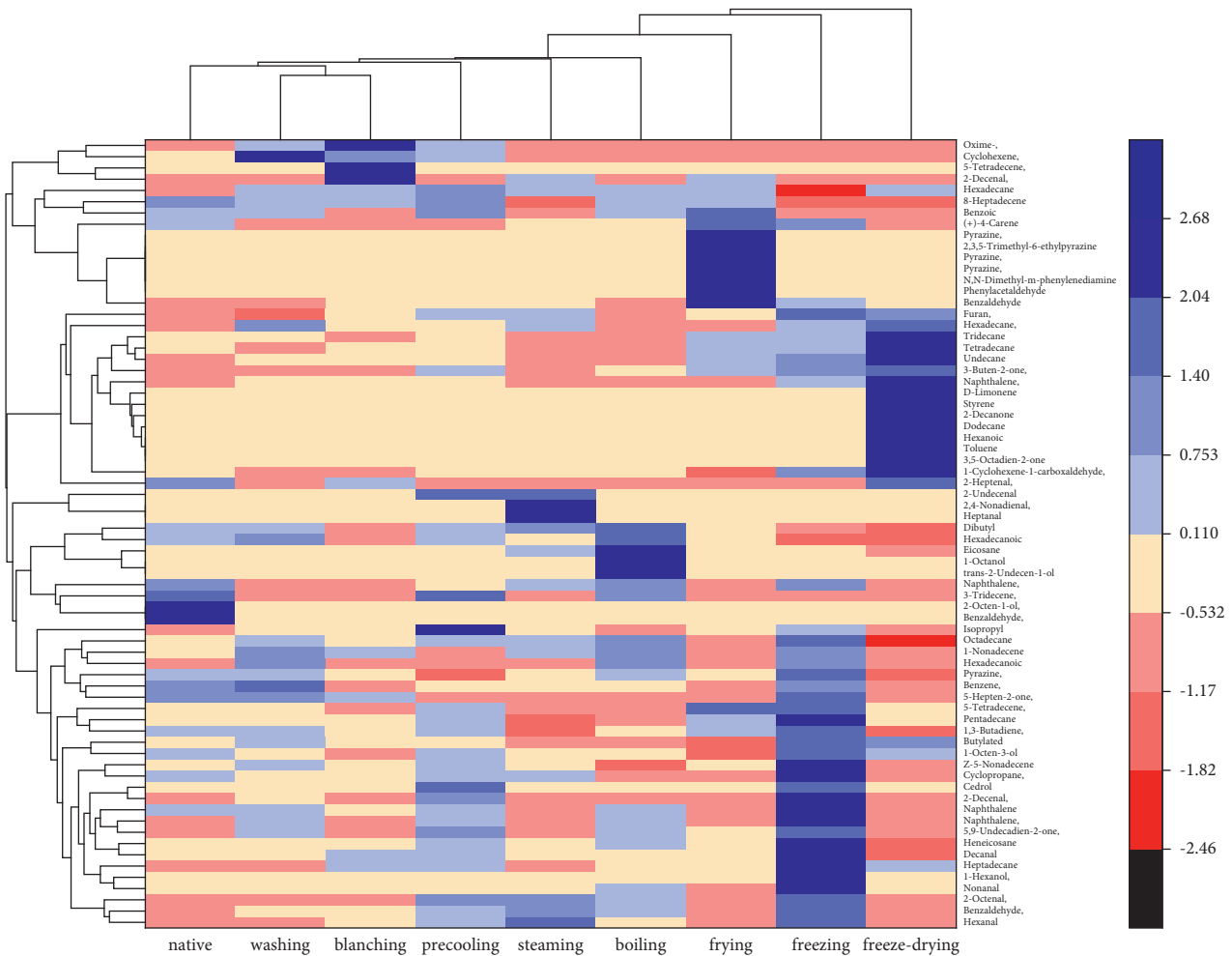


FIGURE 3: Heat map and cluster analysis of peas under nine kinds of different treatments.

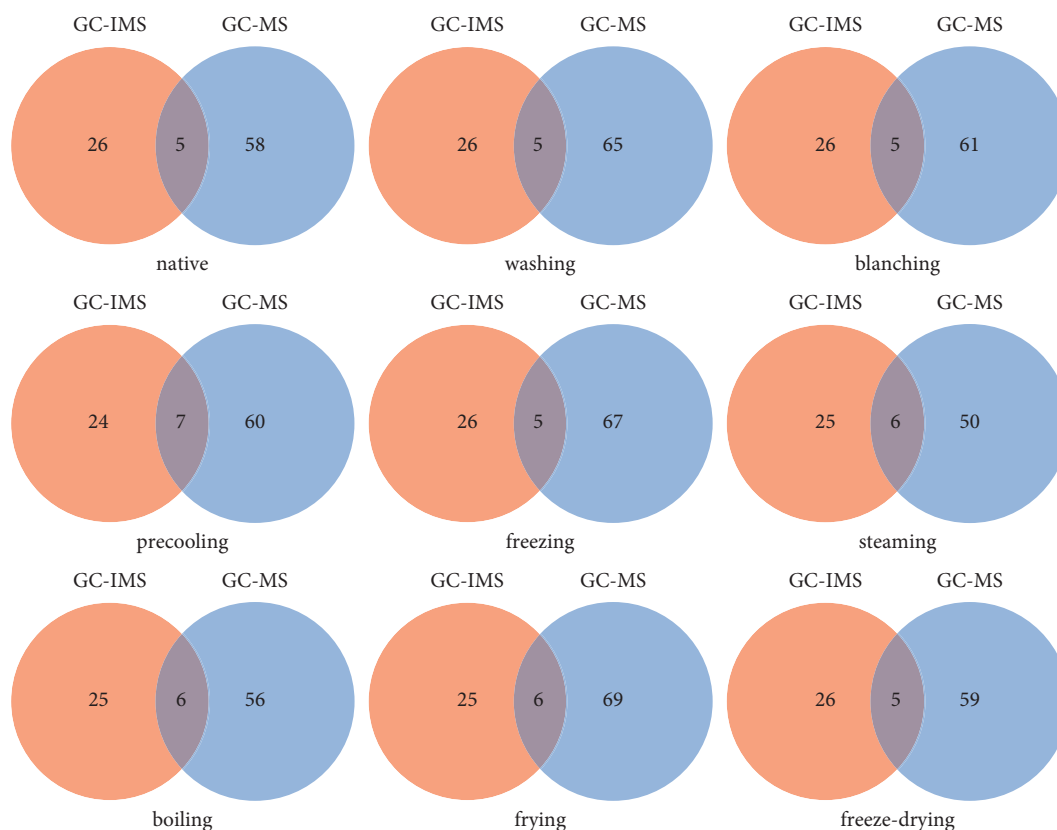


FIGURE 4: Wayne diagram of the flavor of GC-MS and GC-IMS under different treatment methods.

components of peas. The content of pyrazines was higher in fried peas; the content of aldehydes was higher in frozen peas; and the content of ketones was higher in freeze-dried peas, which was consistent with the above conclusion of GC-IMS.

3.3. Comparison of Flavor Substances in Peas by GC-IMS Coupled with GC-MS. Figure 4 shows the main flavor compounds of peas under 9 kinds of different treatments analyzed by GC-IMS and GC-MS.

By comparing and analyzing the common flavor substances of peas under different processing methods, the Venn diagram found that the common flavor substances codetected by GC-IMS and GC-MS were generally 5–7 kinds; mostly, nonanal, 1-octene-3-ol, n-hexanal, benzaldehyde, 6-methyl-5-hepten-2-one, trans-2-octenal, and 2-ethyl-3,5-dimethylpyrazine were speculated to be the key flavor substances of peas (the mass spectra of the key components are shown in Figure 5). Specifically, n-hexanal, benzaldehyde, nonanal, and 1-octene-3-ol were the flavor substances codetected under 9 different treatments by GC-IMS and GC-MS; styrene was codetected in frozen and freeze-dried peas by GC-IMS and GC-MS; and 6-methyl-5-hepten-2-one was also codetected in control, washed, and frozen peas by GC-IMS and GC-MS, which was consistent with the conclusion that the content of ketone was increased after lyophilization only by GC-IMS. Trans-2-octenal was codetected by GC-IMS and GC-MS except for the control

and washed peas, which was consistent with the conclusion that the content of aldehydes was lowest in native peas by GC-IMS. The results indicated that the content and kinds of aldehydes in peas would increase after processing. 2-ethyl-3,5-dimethylpyrazine with a heavier roasted flavor was codetected in fried peas by GC-IMS and GC-MS, which was the key flavor of fried peas. 2-methyl-6-vinylpyrazine and 3-ethyl-2,5-methylpyrazine were detected by GC-MS, and the percentages of 3-ethyl-2,5-methylpyrazine and 2,5-dimethyl-3-(3-methylbutyl) pyrazine were higher. It can be seen that heterocyclic compounds had a large impact on the flavor of fried peas, which was consistent with the conclusion of Murray et al. [25].

3.4. Key-Aroma Analysis by OAV and Sensory Evaluation. Studies showed that 3% of the volatile compounds had odor activity values (OAV) and contributed most to the overall aroma or flavor of the food [26]. In order to identify these volatiles in peas, OAV combined with sensory evaluation was conducted for all compounds. Generally, volatile compounds with a higher OAV were considered as the major contributors to the overall aroma of the grain [27, 28]. The flavor compounds with OAV value greater than 100 in peas under 9 different treatments were combined with their flavor characteristics (Table 3); eight sensory descriptors were used to evaluate the aroma notes: sweet flowers, fat fragrance, waxy aldehydes, mushroom hay, roasted potato with nuts, vegetable-like bean, spicy dry tar, and bitter

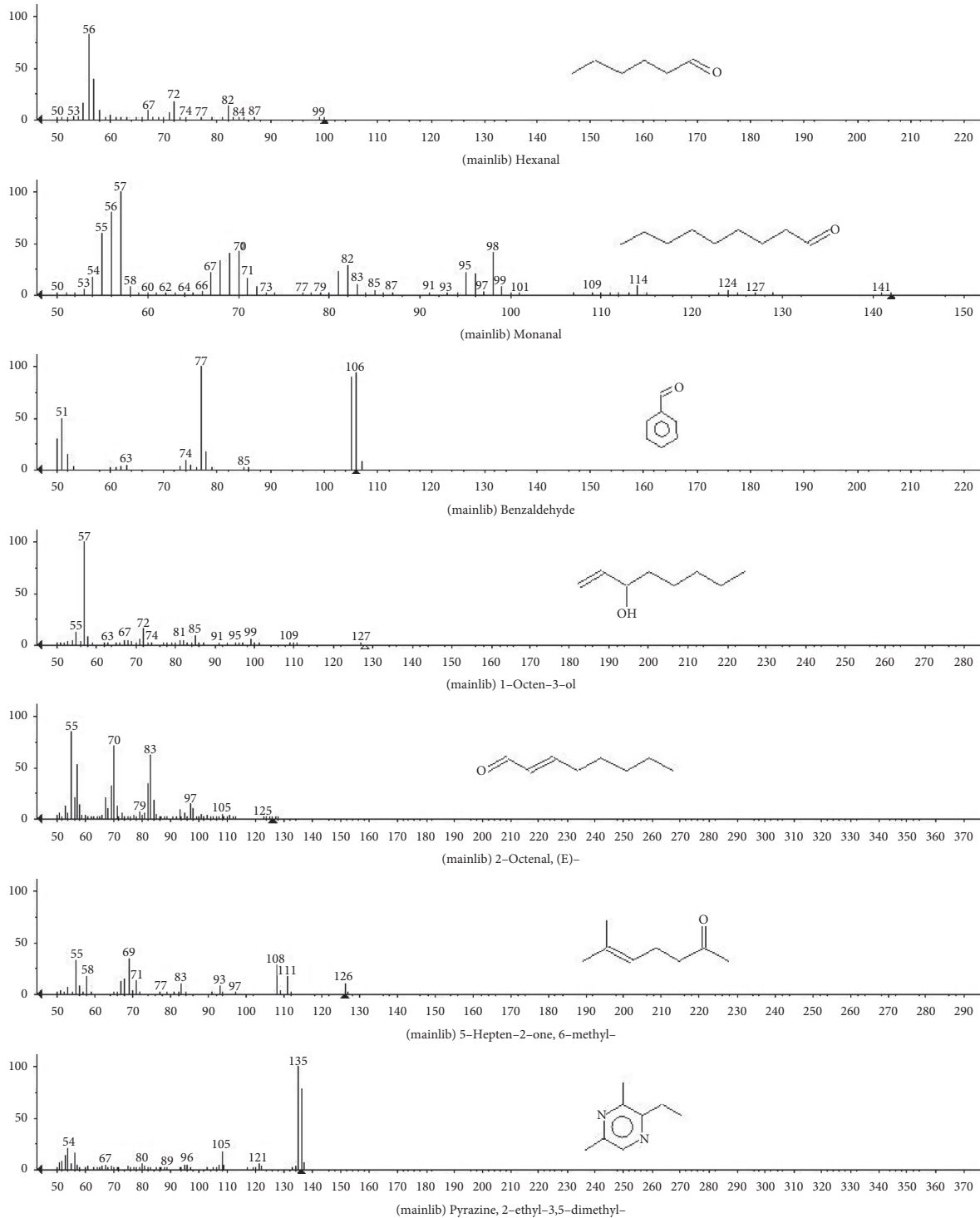


FIGURE 5: The mass spectra of the key components.

almond, and the aroma profiles (Figure 6) were drawn based on sensory evaluation results.

Waxy aldehydes notes played an important role in the flavor of peas especially, and it was mainly contributed by *trans*-2-decenal, nonanal, decanal, and *trans*-2-hexenal. Moreover, the flavor profiles of the control, blanching, washing, and precooling peas were similar; waxy aldehydes were still the main aroma notes. Considering that it was

caused by the increase of aldehydes in the processing process, it was consistent with the above conclusion. However, the intensity of the fat fragrance, mushroom hay, and vegetable-like bean had increased, and the contribution was equivalent. Considering that it was mainly caused by the increase in the content of 2-n-pentylfuran, 2-n-pentylfuran was a typical product of Maillard reaction in food thermal processing [29], which was consistent with the changing

TABLE 3: Flavor compounds with OAV value greater than 100 detected by GC-MS.

| # | Compounds | OAV | | | | | | | | |
|----|-----------------------------|------------|------------|------------|------------|--------------|---------------|------------|------------|------------|
| | | Native | Washing | Blanching | Precooling | Freezing | Freeze-drying | Steaming | Boiling | Frying |
| 1 | Hexanal | 3,311.13 | 3,043.59 | 4,740.30 | 7,817.07 | 13,328.12 | 3,848.20 | 12,383.83 | 5,043.53 | 2,415.97 |
| 2 | Trans-2-heptenal | 406.52 | — | 229.11 | — | — | 504.85 | — | — | — |
| 3 | Benzaldehyde | 5,100.96 | 4,790.13 | 7,072.47 | 7,817.98 | 14,609.04 | 10,059.21 | 11,228.19 | 6,585.38 | 29,332.54 |
| 4 | Phenylacetaldehyde | — | — | — | — | — | - | - | - | 744.31 |
| 5 | 1-Nonanal | 7,662.86 | 8,247.45 | 7,288.29 | 8,106.13 | 19,070.08 | 8,277.58 | 8,634.94 | 9,812.56 | 4,829.43 |
| 6 | 3-Ethylbenzaldehyde | — | — | — | — | — | — | — | — | — |
| 7 | Ethylbenzaldehyde | — | — | — | — | — | — | — | — | — |
| 8 | Decyl aldehyde | 333,588.34 | 321,029.55 | 462,830.73 | 578,427.40 | 1,053,600.36 | — | 359,859.55 | 445,091.10 | 317,457.81 |
| 9 | Beta-cyclocitral | 8,912.76 | — | 6,415.31 | 11,358.52 | 19,680.98 | 31,493.61 | 7,686.87 | 9,820.03 | — |
| 10 | 2-Undecenal | — | — | — | 2,598.67 | — | — | — | — | — |
| 11 | (E)-2-Octenal | — | — | — | 2,918.11 | 4,013.71 | — | 2,580.51 | 2,165.64 | — |
| 12 | 3-Heptylacrolein | — | 3,895.86 | — | 16,437.12 | 23,766.36 | — | — | — | — |
| 13 | Trans-2-hexenal | — | — | — | — | — | — | 73.89 | — | — |
| 14 | Heptaldehyde | — | — | — | — | — | — | 2,023.42 | — | — |
| 15 | Trans, trans-2,4-nonadienal | — | — | — | — | — | — | 19,875.53 | — | — |
| 16 | 1-Octen-3-ol | 29,141.84 | 21,008.68 | 14,643.29 | 36,370.60 | 52,608.48 | 31,684.53 | 17,414.77 | 24,556.79 | — |
| 17 | Irisone | — | 10.36 | — | 18.43 | — | 33.91 | — | — | 19.08 |
| 18 | (+)-Dipentene | 505.14 | — | 521.19 | 257.71 | — | 4,878.31 | — | 182.36 | 507.54 |
| 19 | 2-Pentylfuran | 2,782.88 | 2,091.41 | 3,405.71 | 4,880.11 | 7,399.90 | 6,872.49 | 4,792.13 | 2,645.99 | 4,237.39 |
| 20 | Naphthalene | 9.76 | 11.77 | 5.84 | 13.11 | 24.93 | — | 2.75 | 10.94 | — |
| 21 | 3,5-Octadien-2-one | — | — | — | — | — | 384,573.50 | — | — | — |
| 22 | Methyl hexanoate | — | — | — | — | — | 22.31 | — | — | — |
| 23 | Toluene | — | — | — | — | — | 51.59 | — | — | — |

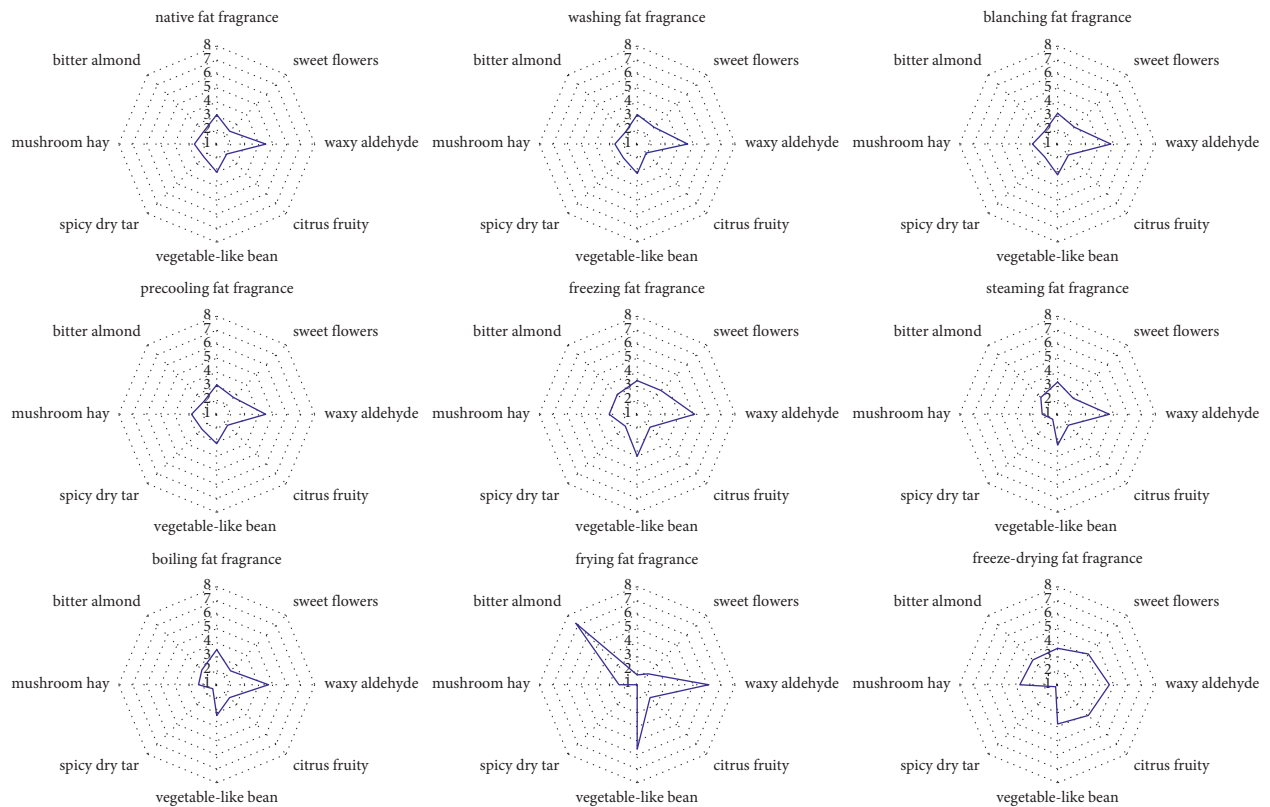


FIGURE 6: Radar charts of the sensory analysis of peas with scores ranging from 0 (no perception) to 9 (strong perception).

trend of the OAV value above. Compared with the native peas, the aroma profile of frozen peas slightly changed, which may be caused by the low temperature, but the main aroma note was still waxy aldehydes. The intensity of the mushroom hay aroma of steamed and boiled peas tended to decrease, which was due to the fact that the content of 1-octen-3-ol decreased. The intensity of citrus fruit in freeze-dried peas increased, mainly due to the influence of toluene, methyl caproate, (+)-limonene, and 2-undecenal. The bitter almonds note brought by benzaldehyde in fried peas was more obvious, and the intensity of waxy aldehydes aroma was significantly weakened, which was consistent with the conclusion that the frying process could lead to the reduction of aldehydes.

In this study, the main factor influencing peas aromas include processing treatments. By comparing the key aroma components or flavor characteristics of different processing treatments with that of untreated peas, we can clearly understand the specific impact of processing treatments on peas aromas. Therefore, studying the aroma components of untreated peas and the other eight processing treatments of peas can provide a basis for future research to determine whether the peas are fresh (untreated) or which processing treatment has been passed through the aroma components.

4. Conclusion

The volatile profile of nine varieties of peas was identified by GC-IMS coupled with GC-MS, and the key aroma compounds of peas were n-hexanal, nonanal, 1-octene-3-ol, benzaldehyde, 6-methyl-5-hepten-2-one, trans-2-octenal, and 2-ethyl-3,5-dimethylpyrazine. In addition, the cluster analysis of the heat map and the sensory analysis indicated that peas could be mainly divided into four groups, which was consistent with the conclusion of GC-IMS and GC-MS; it was proved that GC-IMS coupled with GC-MS could be a fast and accurate method to identify the general flavor difference in different varieties of peas. However, according to the differences of the key aroma substances under different treatments, the precise processing and refinement research of peas can be effectively studied in the future.

Data Availability

All data, models, and code generated or used during the study appear in the submitted article.

Disclosure

Kangyi Zhang and Can Zhang should be considered co-first authors.

Conflicts of Interest

The authors declare that they have no known competing financial interests.

Authors' Contributions

Kangyi Zhang and Can Zhang contributed equally to this work. Can Zhang and Haining Zhuang contributed to conceptualization; Kangyi Zhang, Tao Feng, Haining Zhuang, and Bin Nie contributed to data curation; Kangyi Zhang and Haining Zhuang contributed to funding acquisition; Kangyi Zhang, Can Zhang, Yue Liu, Tao Feng, Haining Zhuang, and Bin Nie contributed to the investigation; Kangyi Zhang, Can Zhang, Yue Liu, and Tao Feng contributed to methodology; Haining Zhuang contributed to project administration; Can Zhang and Tao Feng performed study supervision; Kangyi Zhang, Can Zhang, Yue Liu, Tao Feng, Haining Zhuang, and Bin Nie prepared the original draft; and Can Zhang and Tao Feng reviewed and edited the manuscript.

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