Hindawi
Journal of Food Quality
Volume 2022, Article ID 3961593, 12 pages
https://doi.org/10.1155/2022/3961593

Research Article

GC-IMS-Based Preliminary Analysis of Volatile Flavor Compounds in Ejiao at Different Processing Stages

Ning Ma, Rui Guan, Rentang Zhao, and Yue Geng

Key Laboratory of Food Nutrition and Safety of SDNU, Provincial Key Laboratory of Animal Resistant Biology, College of Life Science, Shandong Normal University, Jinan 250014, China

Correspondence should be addressed to Yue Geng; gengy@sdnu.edu.cn

Received 5 August 2022; Accepted 19 October 2022; Published 10 November 2022

Academic Editor: Gianfranco Picone

In order to find out the changes of flavor substances in the processing of *Colla corii asini* (Ejiao) and provide reference for the flavor and quality control in the production of Ejiao, gas chromatography-ion mobility spectrometry (GC-IMS) was used to analyze the Ejiao products in different processing stages and establish the fingerprint. The differences among these stages were analyzed using multivariate statistical analysis, and the mechanism underlying volatile flavor compound formation was explored by discriminant analysis of Ejiao at different processing stages. The results indicated that Ejiao contains 47 volatile flavor compounds at different processing stages; they mainly include aldehydes, alcohols, esters, ketones, dimethyl disulfide, thiazole, and pyrazines. During Ejiao processing, the formation of these substances is mainly attributable to the Maillard reaction, amino acid or protein-oxidized lipid interaction, lipid oxidation and degradation, and long-chain compound degradation during heating. Principal component analysis results showed that volatile flavor compounds could be used to distinguish different Ejiao processing stages. The current results provide some reference for flavor and quality control of Ejiao products.

1. Introduction

Ejiao, also known as *Colla corii asini* (CCA), as a medicinal and edible homologous product, is solid glue made from the dried or fresh skin of *Equus asinus* L (i.e., donkey) by boiling and concentrating [1]. Its effects include nourishment of blood and yin, moistening, and hemostasis; therefore, it has high clinical application value. Because Ejiao is prepared from the donkey skin, it has a strong animal-like smell and fatty flavor, which may seem unpleasant when eating. Therefore, although Ejiao has many benefits to the human body, it is not accepted by most consumers; this is a factor that has been restricting further development of Ejiao. Thus, exploring volatile flavor compounds in Ejiao processing and tracing their source are highly important for improving the flavor of Ejiao products.

At present, the techniques for analyzing the volatile flavor compounds of Ejiao include gas chromatography-mass spectrometry (GC-MS) [2, 3], gas chromatography-olfactometry (GC-O) (Yu et al., 2016; [4], and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME- GC-MS) [5–7]. Mao et al. [2] detected 23 volatile flavor compounds in Ejiao through GC-MS; of these, methyl isothiocyanate showed the highest content, indicating that methyl isothiocyanate may play a major role in odor development in Ejiao. Zhang et al. [3] used GC-MS, GC-O, and sensory evaluation to analyze Ejiao from 19 origins and determined that its main aromatic active compounds are methyl thioacetate, 2,6-dimethylpyrazine, and 2-ethyl-3,6-dimethylpyrazine. The authors also pointed out that hexanal, 2-pentylfuran, and 2-ethyl-3,6-dimethylpyrazine are representative aromatic compounds in Dong-E Ejiao. At the same time, Wang et al. (Wang et al., 2016) used simultaneous distillation extraction (SDE) to extract volatile compounds from three Ejiao brands. A total of 47 volatile compounds were identified through GC-MS and mainly included aldehydes, pyrazines, ketones, and acids, while 14 active aroma components were identified through GC-O. Of these, 2-ethyl-3,6-dimethyl pyrazine, 2,6-dimethyl pyrazine, hexanal, 2,3-dimethyl pyrazine, and

dimethyl disulfide had a higher dilution factor (FD) of aroma extract dilution analysis. Zhang et al. [7] established a method for determining volatile flavor compounds in Ejiao by HS-SPME-GC-MS combined with an automatic deconvolution technique and identified 41 volatile compounds of Ejiao, including pyrazines, aldehydes, esters, and ketones. Among them, the compounds with relatively high content were 2,5-dimethylpyrazine, nonanal, 2,3,5-trimethylpyrazine, 3-ethyl-2,5-methylpyrazine, diisobutyl phthalate, hexanal, 2-decanone, benzaldehyde, 2-nonanone, and 2,2,4-trimethylpentanediol isobutyl ester. Sui et al. [5] further found that the flavor profile of Ejiao was mainly composed of gum odor, coke paste, and meat flavor, with compounds including pyrazines, acids, and sulfur compounds.

To prepare Ejiao, the donkey skin is dissolved to a gluey consistency with water and then boiled and concentrated repeatedly; this is followed by the addition of rock sugar, soybean oil, and other ingredients [8]. The production process is long and complex, and volatile flavor compounds keep changing constantly. These compounds have a significant impact on the flavor of Ejiao, affecting the overall flavor evaluation of Ejiao. However, research thus far has focused only on the qualitative study of the aroma of Ejiao products, and no study has evaluated the changes in volatile flavor compounds over the stages of Ejiao processing.

Gas chromatography-ion mobility spectrometry (GC-IMS) is a new technology, which has facilitated separate and sensitive detection of volatile flavor compounds in recent years. This modality combines the high separation ability of the gas phase with the rapid response of IMS [9]. Its advantages include low detection limit, good selectivity, short analysis time, simple operation under atmospheric pressure, and no need for sample pretreatment. GC-IMS is widely used for the qualitative analysis of food flavor [10], adulteration identification [11], processing monitoring [12], and other food fields [13], but it has not been reported that it is applied to the analysis of flavor substances in Ejiao at different processing stages.

In this study, GC-IMS technology was used for the first time to analyze the changes and differences in volatile flavor compounds in Ejiao at different processing stages so as to explore the changes and mechanism underlying volatile flavor compound formation in Ejiao processing. The current result may provide a reference for understanding flavor formation and regulation during Ejiao processing in the future.

2. Materials and Methods

2.1. Materials and Instruments. The samples used at different processing stages were kindly gifted by Shandong Dong'e Ejiao Co., Ltd. A FlavourSpec® flavor analyzer was obtained from G.A.S., Germany.

2.2. Test Method

2.2.1. Sample Collection. From May 2021 to June 2021, a complete processing process of Ejiao was tracked and collected. Ejiao samples were collected at the end of seven points over the processing stages of Ejiao production: dry donkey skin (DDS), wet donkey skin (WDS), donkey skin dissolving (DSD), concentration of glue (COG), deforming of glue (DOG), adding accessory (AAC), and Colla corii asini (CCA).The specific sampling information is shown in Figure 1. Three batches of parallel samples were taken from each sampling point. After numbering, it was stored in a −20°C freezer before analysis.

2.2.2. GC-IMS Analysis. The samples were taken out of the refrigerator and thawed at room temperature. Before GC-IMS, solid DDS, WDS, AAC, and CCA samples were crushed by using a pulverizer, whereas the remaining liquid samples were directly sampled without pretreatment. Next, 1 g of a sample was placed it in a 20 mL headspace glass-sampling vial, labeled, and inserted into the sampling tank of the automatic sampler.

The chromatography column used was an MXT-5 chromatographic column (15 mL, 0.53 mm ID, 1 μm FT), and the column temperature was 60°C. Headspace sampling was selected as the injection method, with an injection volume of 200 μL, incubation time of 15 min, incubation temperature of 60°C, injection needle temperature of 85°C, oscillation rate of 500 rpm, and N_2 as the carrier or drift gas. The carrier gas flow rate was programmed as follows: the initial flow rate reached 2.0 mL/min within 2 min; internal linearity was increased to 100.0 mL/min within 18 min. The drift gas flow rate in a drift tube was 150 mL/min. The total running time was 20 min. All analyses were performed in triplicate.

2.3. Statistical Analysis. The built-in supporting analysis software program of the GC-IMS instrument includes VOCal and three plug-ins, and it can analyze samples from different angles.

We performed qualitative analysis of volatile flavor compounds by using VOCal with the built-in NIST and IMS databases. The plug-in reporter was used to compare the spectral differences of the two-dimensional top view and three-dimensional spectra between different samples. We used the gallery plot plug-in to intuitively and quantitatively compare the fingerprints of volatile flavor compounds between different samples. Next, the dynamic PCA plug-in was used to perform dynamic principal component analysis and cluster analysis. We used imageGP to draw the heatmap by data clustering analysis.

The GC-IMS data were imported into SIMCA (Umtrics, Sweden version 14.1) for further multivariate statistical analysis. The differences at different processing stages were compared through PCA, PLS-DA, OPLS-DA analysis, and permutation test.

3. Results and Analysis

3.1. Analysis of GC-IMS Spectra of Ejiao at Different Processing Stages. VOCal automatically generated three-dimensional and two-dimensional top view spectra of sample volatile flavor compounds. It can be clearly seen from the three-

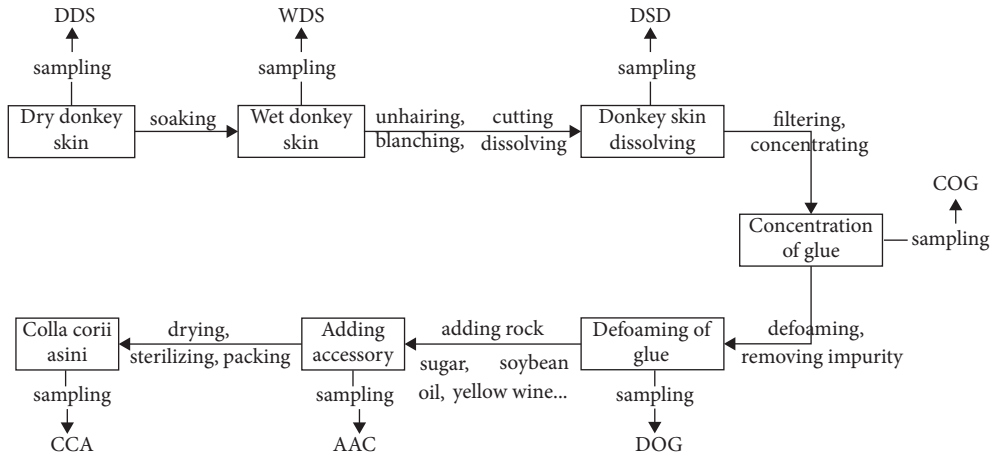


Figure 1: Specific information of the sampling point and the processing stage.

dimensional spectrum (Figure 2(a)) that during processing, the content of some compounds decreased and new substances formed. Some compounds disappeared in the WDS stage, and a new peak appeared in DSD and disappeared in AAC. In combination with the two-dimensional top view (Figure 2(b)), the differences and changes in volatile flavor compounds can be further reflected.

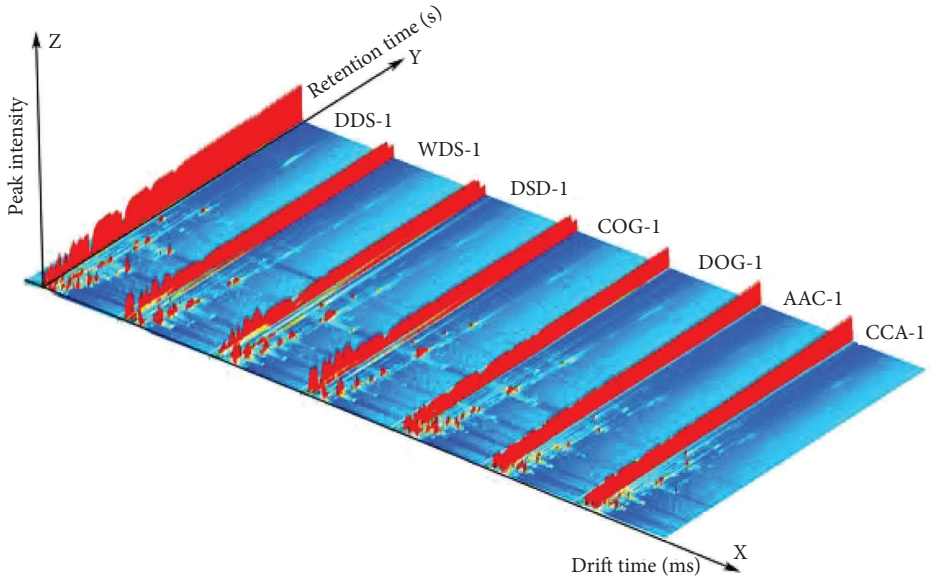
To observe the differences in the two-dimensional GC-IMS spectra of Ejiao at different processing stages, DDS samples were selected as reference samples, and the spectra of other samples were deducted from the reference samples (Figure 2(c)). The differences in the signal intensity are shown in different colors: red indicates a higher concentration of the substance in the sample than in the reference sample (i.e., DDS), whereas blue indicates a lower concentration and the white areas indicate that substances between the two samples are identical. Most of the signal peaks appeared during the retention time (0–400 s) and drift time (1–1.7 ms), and the number of red spots decreased gradually during Ejiao processing.

3.2. Qualitative Analysis of Volatile Flavor Compounds in Ejiao at Different Processing Stages. To extract the effective feature variables from the original atlas, the built-in LAV software of the instrument was used to extract effective feature peaks (Figure 2(c)). Finally, 60 feature regions were selected to characterize the flavor information of the samples, and each region of the signal qualitative analysis is shown in Figure 3. The right side of the gallery plot fingerprint presents the serial numbers of the Ejiao samples at different processing stages (i.e., one fingerprint spectrum of the Ejiao sample for each line and three parallel batches for each sample). The x-axis denotes the names of compounds or the number of unidentified compounds in the selected 60 characteristic regions.

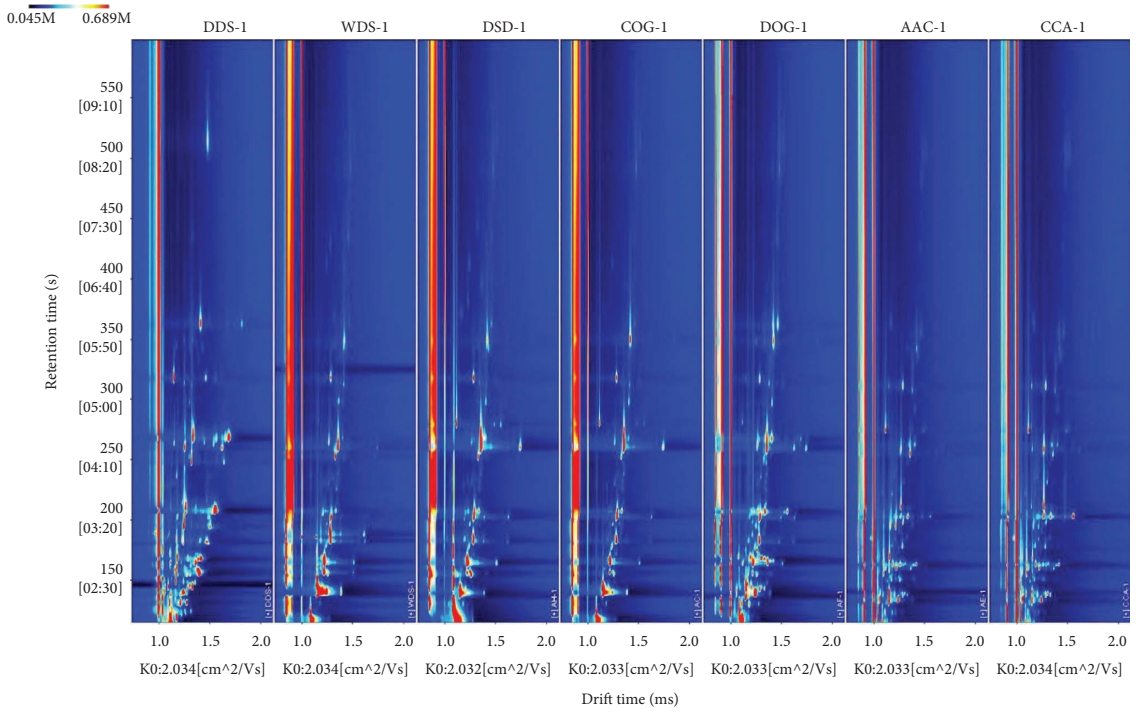
As shown in Figure 3, the species in the DSD and COG samples are very similar. The corresponding compounds 8 and 9 did not appear in the samples before the AAC stage, but they began to appear in AAC and CCA samples, possibly due to the addition of related ingredients resulting in corresponding substances. DDS samples contained about 50 substances, which subsequently decreased to 20 due to blistering in the WDS sample. Differences were also noted in the types of substances; this may be because in the process of immersion in water, some volatile flavor compounds decomposed into other substances, and their concentration decreased to negligible concentrations in some cases. As shown in the red box in Figure 3, aldehydes and ketones in the DDS samples included nonanal, octanal, benzaldehyde, 2-methylbutanal, 2-hexenal, cyclohexanone, and octanone. After soaking, these substances disappeared. The WDS, DSD, COG, and DOG samples demonstrated some similarity, and some new compounds, such as trimethylpyrazine, 2,3-dimethylpyrazine, ethyl 2-methylpropanoate, and furfuryl propionate, were noted after processing. In the DOG samples, the addition of soybean oil, rock sugar, yellow rice wine, and other ingredients led to changes in the compound species in AAC again; moreover, AAC and CCA samples also demonstrated high similarity, with composition dominated by esters. The results demonstrated that the addition of other ingredients promoted final flavor compound formation in Ejiao.

The qualitative analysis of these compounds was carried out based on the built-in NIST and IMS databases. During the processing of Ejiao, 47 monomers and dimers of some compounds were identified; they included 14 alcohols, 9 ketones, 9 aldehydes, 9 esters, and 3 pyrazines as well as pyrrole, thiazole, and dimethyl disulfide. Some single compounds demonstrated multiple signal peaks, such as monomers, dimers, and even polymers, due to the difference in their concentrations [14]. The identified compounds are listed in Table 1.

3.3. Cluster Analysis of Ejiao at Different Processing Stages. To further determine the characteristics of various volatile flavor compounds in each period, flavor compounds were clustered, and the results are shown in Figure 4(a). The composition and content of volatile flavor compounds in samples at different processing stages differed considerably. According to the cluster analysis of volatile flavor compounds in the different stages of the heatmap, the AAC, CCA, and

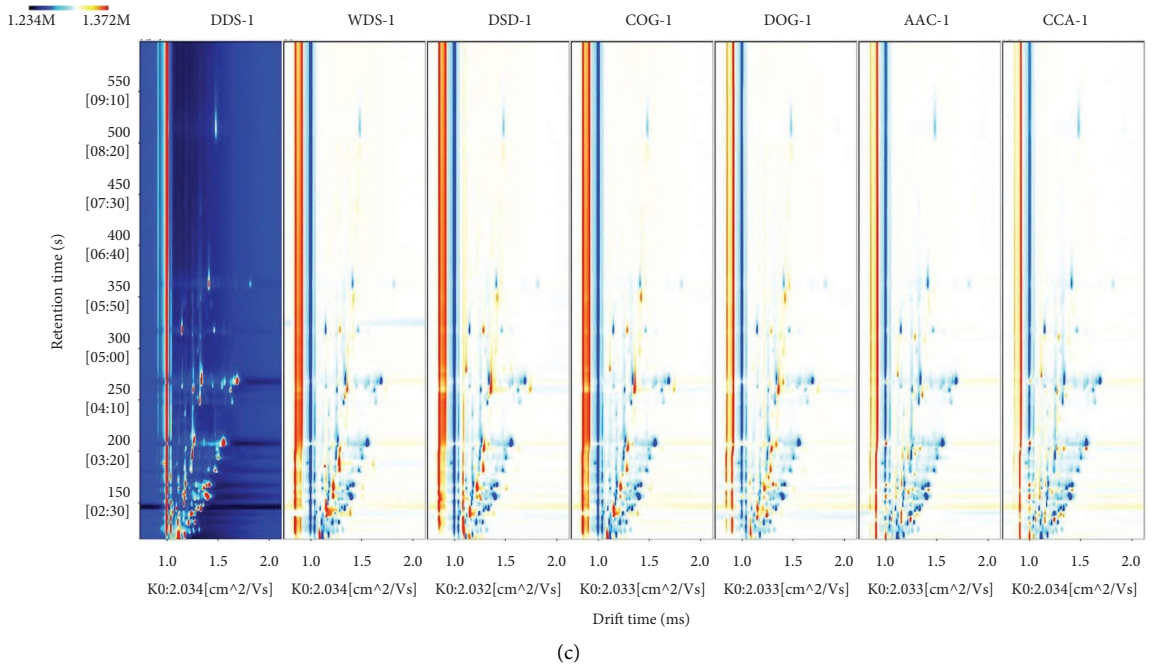


(a)



(b)

Figure 2: Continued.



(c)

Figure 2: (a) 3D top view spectra and (b) 2D spectra of volatile flavor compounds in Ejiao at different processing stages. (c) Difference spectra of volatile flavor compounds in Ejiao at different processing stages.

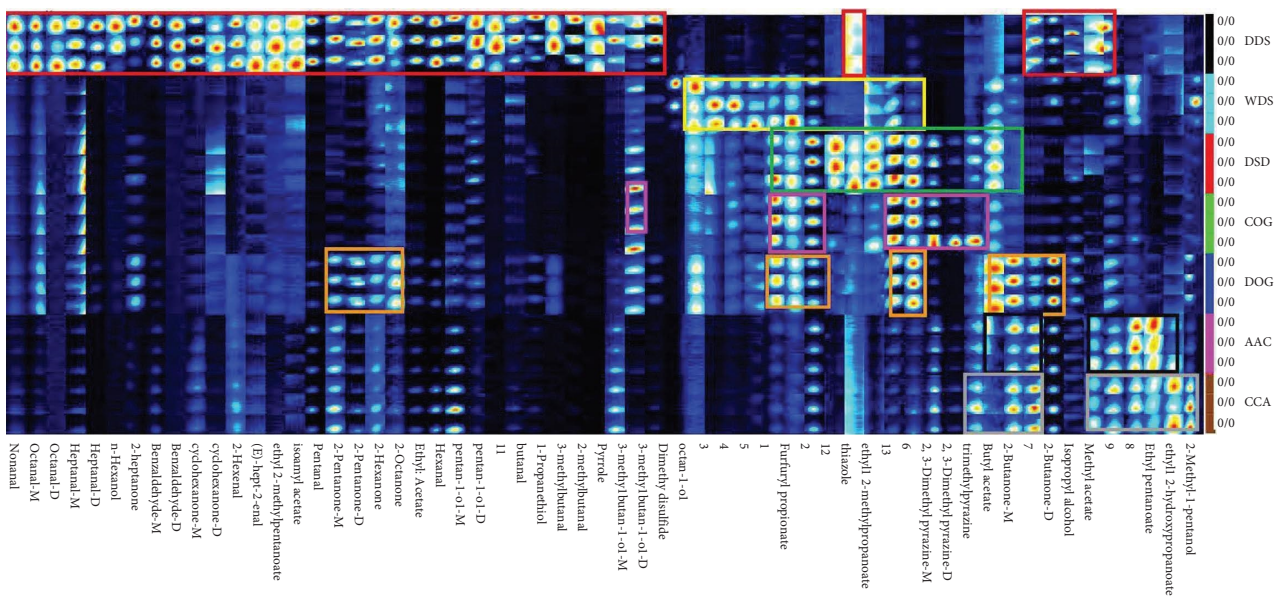


Figure 3: Gallery plot fingerprint of volatile flavor compounds in Ejiao at different processing stages.

WDS samples were clustered together, indicating that volatile flavor compound composition and content in the sample after adding ingredients were similar to those in the finished product of Ejiao and WDS. The samples of other stages, except for the DDS samples, were clustered into one category, indicating that DDS was quite different from the samples of other stages.

The Euclidean distance diagram was plotted to demonstrate the differences and similarities of volatile flavor compounds between samples. Here, the smaller the distance between samples, the greater was the similarity between samples. As shown in Figure 4(b), the distance between DDS and other samples was the largest, indicating that the difference between the DDS and other samples was the largest. This is consistent with the later fingerprint analysis result.

3.4. Multivariate Statistical Analysis of Ejiao at Different Processing Stages. GC-IMS data were imported into SIMCA (version 14.1), and further multivariate statistical analysis was performed using PCA, PLS-DA, and OPLS-DA.

Table 1: Qualitative analysis of volatile flavor compounds.

No.	Compound	CAS	Formula	Mw	RI	Rt (min)	Dt (ms)	Odor description[a]
1	Nonanal	C124-19-6	$C_9H_{18}O$	142.2	1113.1	517.484	1.48666	Waxy, aldehydic, citrus, fresh, green, lemon, peel, cucumber, fatty
2	Octanal-M	C124-13-0	$C_8H_{16}O$	128.2	1005.1	363.689	1.4182	Aldehydic, waxy, citrus, orange, green, peely
3	Octanal-D	C124-13-0	$C_8H_{16}O$	128.2	1004.8	363.267	1.82268	Aldehydic, waxy, citrus, orange, green, peely
4	Heptanal-M	C111-71-7	$C_7H_{14}O$	114.2	899.7	269.266	1.33963	Fresh, aldehydic, fatty, green, herbal, cognac, ozone
5	Heptanal-D	C111-71-7	$C_7H_{14}O$	114.2	899.2	268.844	1.69319	Fresh, aldehydic, fatty, green, herbal, cognac, ozone
6	n-Hexanol	C111-27-3	$C_6H_{14}O$	102.2	866.5	248.85	1.64517	Ethereal, fusel, oily, fruity, alcoholic, sweet, green
7	2-Heptanone	C110-43-0	$C_7H_{14}O$	114.2	888.9	261.071	1.62745	Cheesy, fruity, ketonic, green, banana, creamy
8	Benzaldehyde-M	C100-52-7	C_7H_6O	106.1	956.5	317.475	1.14785	Almond, fruity, powdery, nutty, cherry, maraschino
9	Benzaldehyde-D	C100-52-7	C_7H_6O	106.1	956.8	317.789	1.46804	Almond, fruity, powdery, nutty, cherry, maraschino
10	Cyclohexanone-M	C108-94-1	$C_6H_{10}O$	98.1	892.3	262.951	1.15194	Minty, acetone
11	Cyclohexanone-D	C108-94-1	$C_6H_{10}O$	98.1	894.5	264.831	1.45305	Minty, acetone
12	2-Hexenal	C505-57-7	$C_6H_{10}O$	98.1	841.8	235.376	1.17783	Sweet, almond, bitter, almond, fruity, green, leafy, apple, plum, vegetable
13	(E)-Hept-2-enal	C18829-55-5	$C_7H_{12}O$	112.2	952.4	314.029	1.25685	Pungent, green, vegetable, fresh, fatty
14	Ethyl 2-methylpentanoate	C39255-32-8	$C_8H_{16}O_2$	144.2	932.5	297.107	1.30454	Fresh, fruity, green, melon, apple, skin, pineapple, natural, waxy
15	Isoamyl acetate	C123-92-2	$C_7H_{14}O_2$	130.2	872.2	251.984	1.30045	Sweet, fruity, banana, solvent
16	Pentanal	C110-62-3	$C_5H_{10}O$	86.1	686.1	165.052	1.42351	Fermented, bready, fruity, nutty, berry
17	2-Pentanone-M	C107-87-9	$C_5H_{10}O$	86.1	681.5	163.903	1.11971	Sweet, fruity, ethereal, winey, banana, woody
18	2-Pentanone-D	C107-87-9	$C_5H_{10}O$	86.1	678.2	163.065	1.3664	Sweet, fruity, ethereal, winey, banana, woody
19	2-Hexanone	C591-78-6	$C_6H_{12}O$	100.2	774.4	200.069	1.18928	Fruity, fungal, meaty, buttery
20	2-Octanone	C111-13-7	$C_8H_{16}O$	128.2	989.7	345.751	1.33054	Musty, ketonic, cheesy, blue, cheese, cheesy, parmesan, cheese, earthy, dairy
21	Ethyl acetate	C141-78-6	$C_4H_8O_2$	88.1	604	144.406	1.33489	Ethereal, fruity, sweet, weedy, green
22	Hexanal	C66-25-1	$C_6H_{12}O$	100.2	792	208.254	1.55752	Fresh, green, fatty, aldehydic, grassy, leafy, fruity, sweaty
23	Pentan-1-ol-M	C71-41-0	$C_5H_{12}O$	88.1	754.4	192.065	1.25259	Pungent, fermented, bready, yeasty, fusel, winey, solvent
24	Pentan-1-ol-D	C71-41-0	$C_5H_{12}O$	88.1	762.2	195.157	1.50793	Pungent, fermented, bready, yeasty, fusel, winey, solvent
25	Butanal	C123-72-8	C_4H_8O	72.1	547.5	130.218	1.27791	Pungent, cocoa, musty, green, malty, bready
26	1-Propanethiol	C107-03-9	C_3H_8S	76.2	619.4	148.299	1.35946	Cabbage, gassy, sweet, onion
27	3-Methylbutanal	C590-86-3	$C_5H_{10}O$	86.1	646.9	155.207	1.40344	Ethereal, aldehydic, chocolate, peach, fatty
28	2-Methylbutanal	C96-17-3	$C_5H_{10}O$	86.1	662.6	159.145	1.38911	Musty, chocolate, nutty, malty, fermented
29	Pyrrole	C109-97-7	C_4H_5N	67.1	742.5	187.287	0.96643	Sweet, warm, nutty, ethereal
30	3-Methylbutan-1-ol-M	C123-51-3	$C_5H_{12}O$	88.1	722.2	179.15	1.24098	Fusel, alcoholic, whiskey, fruity, banana
31	3-Methylbutan-1-ol-D	C123-51-3	$C_5H_{12}O$	88.1	724.5	180.059	1.47838	Fusel, alcoholic, whiskey, fruity, banana
32	Octan-1-ol	C111-87-5	$C_8H_{18}O$	130.2	1056.1	436.343	1.45961	Waxy, green, citrus, aldehydic, floral, sweet, fatty, coconut
33	Furfuryl propionate	C623-19-8	$C_8H_{10}O_3$	154.2	1092.6	488.29	1.48231	Sweet, fruity, green, banana, oily, coffee, spicy
34	Dimethyl disulfide	C624-92-0	$C_2H_6S_2$	94.2	738.1	185.515	1.1386	Sulfurous vegetable cabbage onion
35	Thiazole	C288-47-1	C_3H_3NS	85.1	734.8	184.191	1.02283	Pyridine nutty meaty
36	Ethyl 2-methylpropanoate	C97-62-1	$C_6H_{12}O_2$	116.2	735.2	184.367	1.20069	Sweet, ethereal, fruity, alcoholic, fusel, rummy
37	2,3-Dimethyl pyrazine-M	C5910-89-4	$C_6H_8N_2$	108.1	911	278.809	1.11649	Musty, nut, skin, cocoa, powdery, roasted, potato, coffee
38	2,3-Dimethyl pyrazine-D	C5910-89-4	$C_6H_8N_2$	108.1	908.4	276.639	1.48669	Musty, nut, skin, cocoa, powdery, roasted, potato, coffee
39	Trimethylpyrazine	C146675-5-1	$C_7H_{10}N_2$	122.2	1007.3	366.839	1.16619	Nutty, musty, powdery, cocoa, potato, musty
40	Butyl acetate	C123-86-4	$C_6H_{12}O_2$	116.2	791	207.674	1.232	Ethereal, solvent, fruity, banana
41	2-Butanone-M	C78-93-3	C_4H_8O	72.1	589.5	140.768	1.05739	Acetone, ethereal, fruity, camphoraceous

Table 1: Continued.

No.	Compound	CAS	Formula	Mw	RI	Rt (min)	Dt (ms)	Odor description[a]
42	2-Butanone-D	C78-93-3	C_4H_8O	72.1	583.7	139.313	1.24731	Acetone, ethereal, fruity, camphoraceous
43	Methyl acetate	C79-20-9	$C_3H_6O_2$	74.1	550.1	130.867	1.1819	Ethereal, sweet, fruity
44	Isopropyl alcohol	C67-63-0	C_3H_8O	60.1	520.6	123.451	1.21948	Alcohol musty woody
45	Ethyl pentanoate	C539-82-2	$C_7H_{14}O_2$	130.2	890.6	262.011	1.27048	Sweet, fruity, acidic, pineapple, apple, green, berry, tropical
46	Ethyl 2-hydroxypropanoate	C97-64-3	$C_5H_{10}O_3$	118.1	797.6	211.304	1.14163	Sweet, fruity, acidic, pineapple, apple, green, berry, tropical
47	2-Methyl-1-pentanol	C105-30-6	$C_6H_{14}O$	102.2	837.8	233.182	1.30863	—

MW: molecular weight, RI: retention index, Rt: retention time, Dt: drift time. [a]Aroma source: Perflavory information system https://www.perflavory.com/.

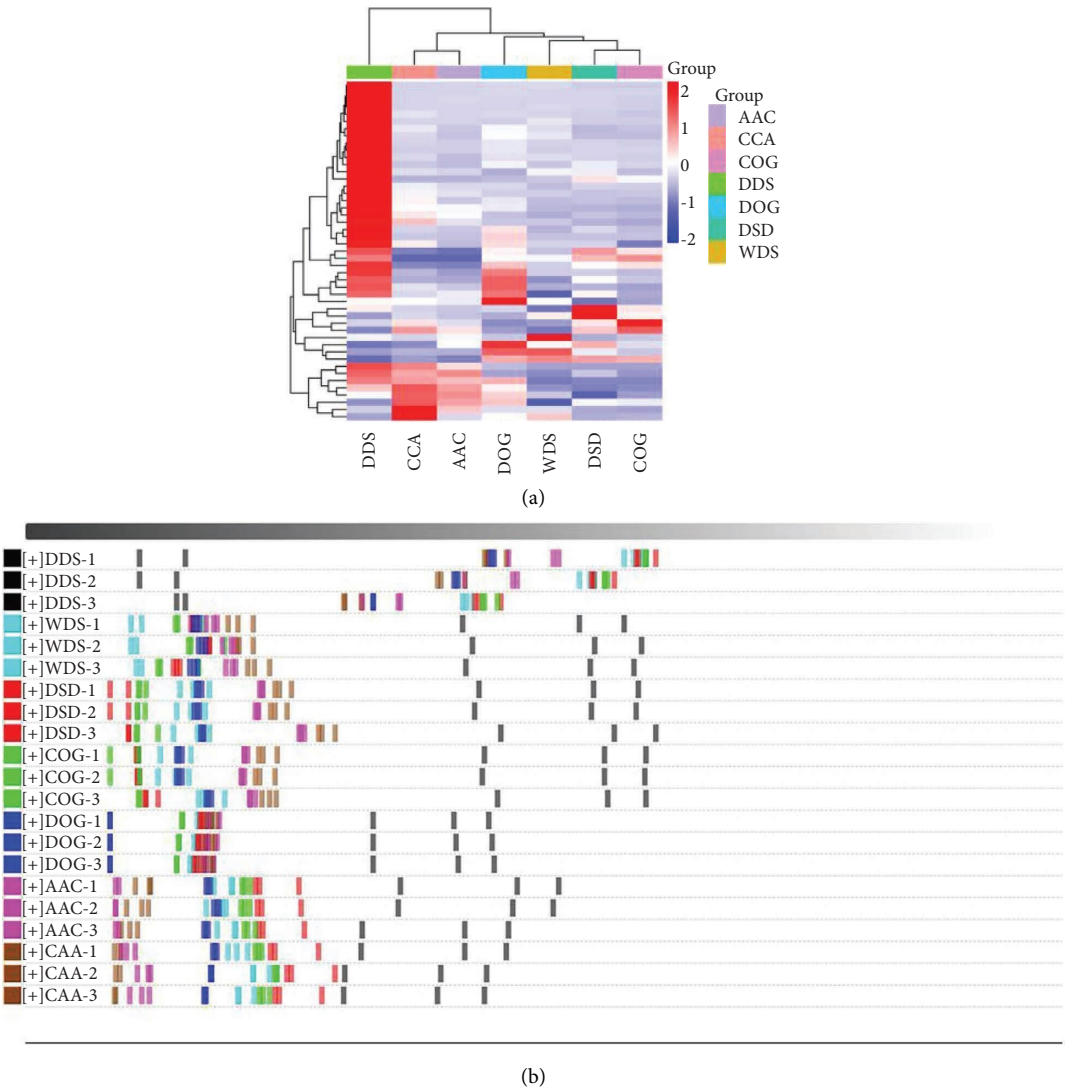


Figure 4: (a) Heatmap and (b) Euclidean distance diagram of volatile flavor compounds in Ejiao at different processing stages.

The PCA, PLS-DA, and OPLS-DA results demonstrated that the separation degree was the highest between DDS and other samples. Samples from the other stages were separated, but the distance was small. We performed 200 permutation tests on OPLS-DA. Figure 5(d) illustrates the permutation test results, where the intercepts of both the $R2$ and $Q2$ regression line on the y-axis are <0.5. The model demonstrated no overfitting, with desirable permutation test results.

PCA can be used to explain the differences between multidimensional samples and further perform key comprehensive analysis of samples with many complex relational

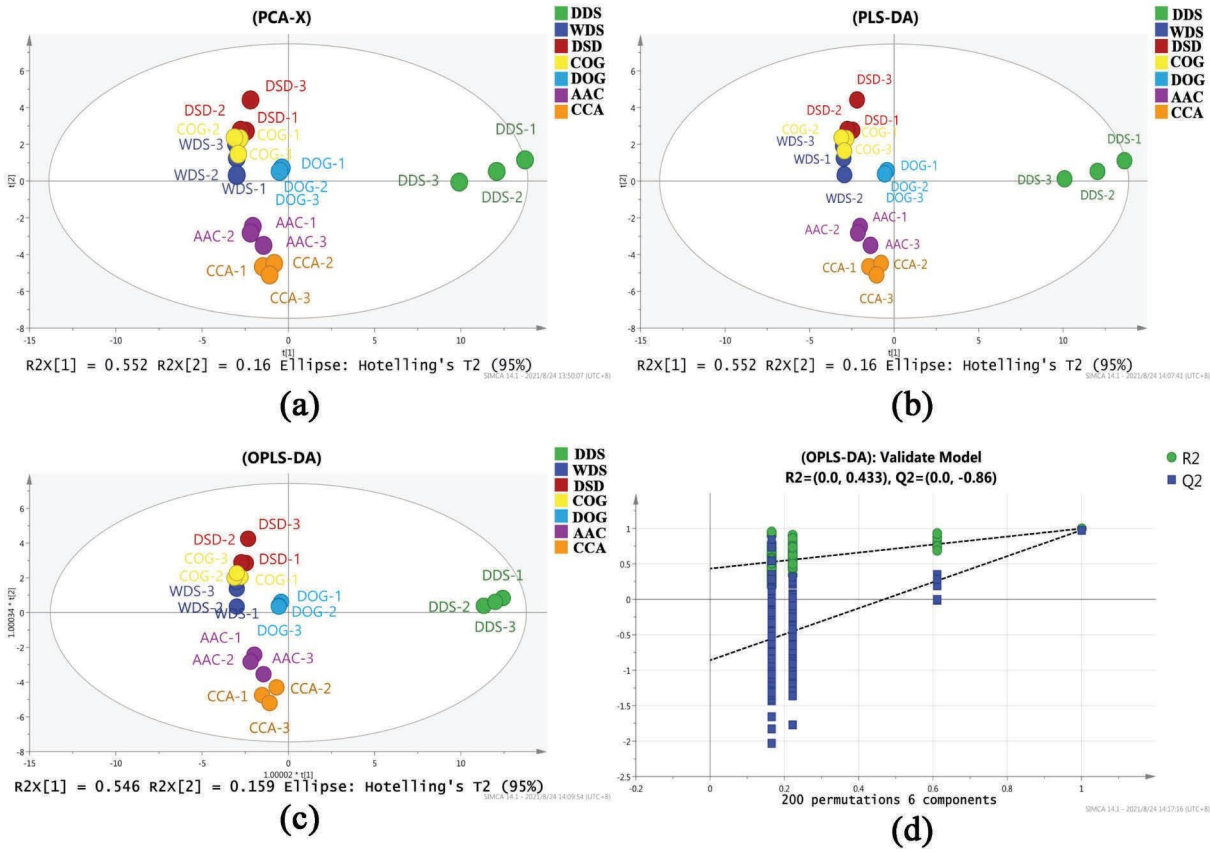


Figure 5: (a) PCA score chart. (b) PLS-DA score chart. (c) OPLS-DA score chart. (d) Permutation test results of volatile flavor compounds in Ejiao at different processing stages.

factors [14]. The PCA plot is illustrated in Figure 5(a): the contribution rates of the first principal component PC1 and second principal component PC2 were 55% and 16%, respectively, and the cumulative contribution rate of PC1 and PC2 after dimension reduction is 71%, indicating that it could better characterize the characteristics of the original data. According to Figure 5, all samples can be divided into three clusters: the DDS sample is located on the right side of PC1, the WDS, DSD, COG, and DOG samples are concentrated on the left upper part of PC1, and the AAC and CCA samples are located on the left lower part of PC1. The same separation trend is shown in PLS analysis (Figure 5(b)) and OPLS-DA analysis (Figure 5(c)). All these findings indicated significant indigenous differences in volatile flavor compounds in Ejiao at different processing stages. The spatial distribution of sample points showed that volatile flavor compounds changed significantly as processing progressed. Therefore, the volatile flavor compound composition can be divided into three stages: the first stage is DDS, the second is WDS, DSD, COG, and DOG, and the third is AAC and CCA.

3.5. Changes in Volatile Flavor Compounds during Ejiao Processing. For the classification of qualitative substances, the changes in different processing stages were analyzed. The relative contents of various substances in Ejiao at different processing stages are presented in Figure 6(a).

The changes in each volatile flavor compounds at different processing stages were analyzed further in detail. The broken line graphs for the changes in each flavor compound in Ejiao at different processing stages are illustrated in Figure 6.

The graphs indicate that from the DDS to WDS stages, which involved water infiltration in the donkey skin, the change in each compound was the most obvious. In the subsequent processing stage, each compound content increased or decreased to a varying degree, but the change was much smaller than that in this process. This result indicated that the difference in volatile flavor compounds between the DDS and other samples at various stages was the most obvious, which is consistent with the PCA results.

In the subsequent processing, DSD, DOG, and AAC may be the key processing stage. In these three processes, physical and chemical changes occurred to varying degrees.

As shown in Figures 6(b)–6(f), during the donkey skin dissolving process, heptanal-M, octanal-M, pentan-1-ol-M, 3-methylbutan-1-ol-M, methyl acetate, and 2,3-dimethyl pyrazine-M contents increased significantly, whereas those of 2-butanone-D, ethyl acetate, ethyl pentanoate, and dimethyl disulfide decreased significantly.

With the removal of foam impurities, the contents of hexanal, pentan-1-ol-M, 2-butanone, 2-pentanone-D, methyl acetate, ethyl pentanoate, ethyl acetate, and butyl acetate increased significantly after deformation, whereas the content of 3-methylbutan-1-ol-M decreased significantly.

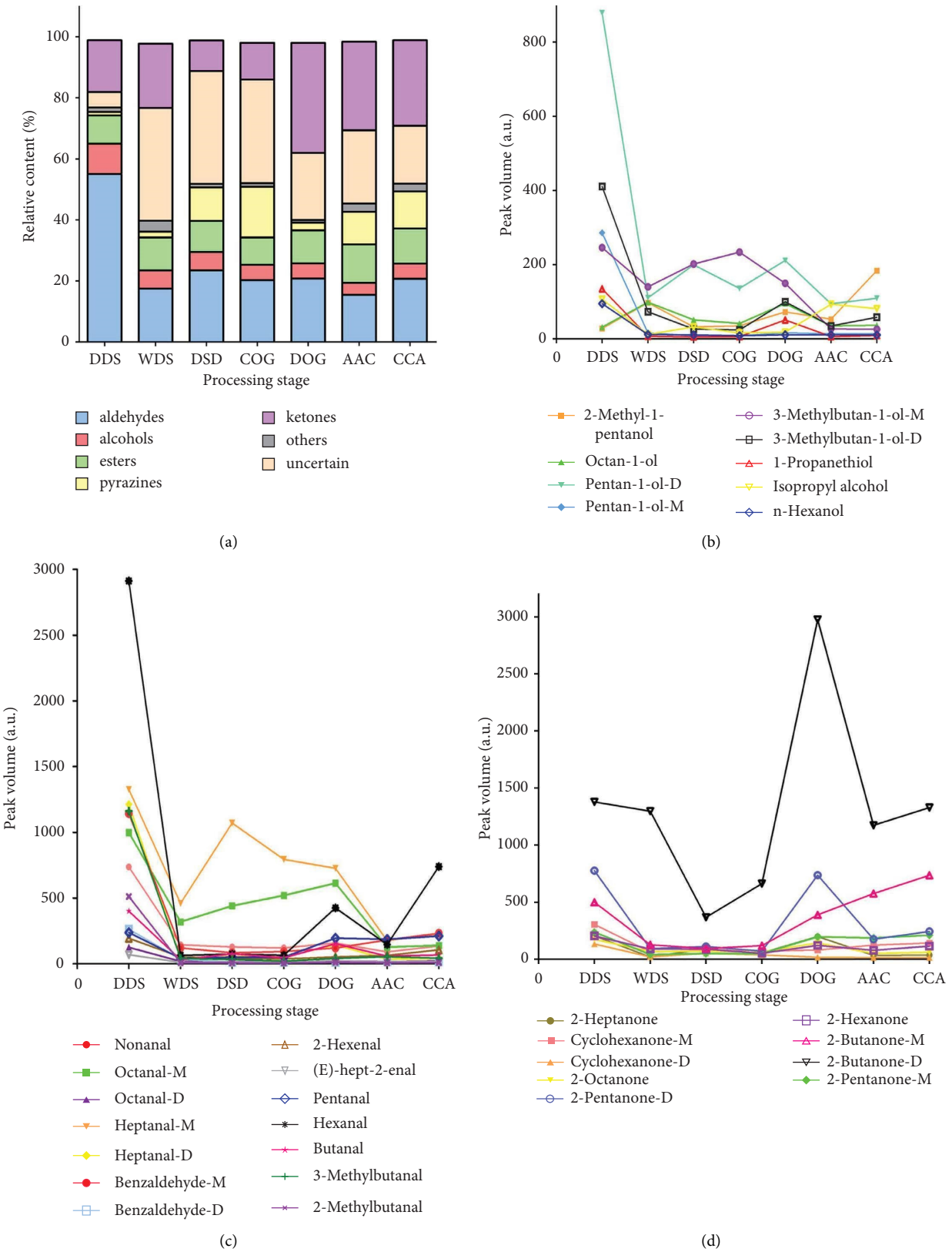


Figure 6: Continued.

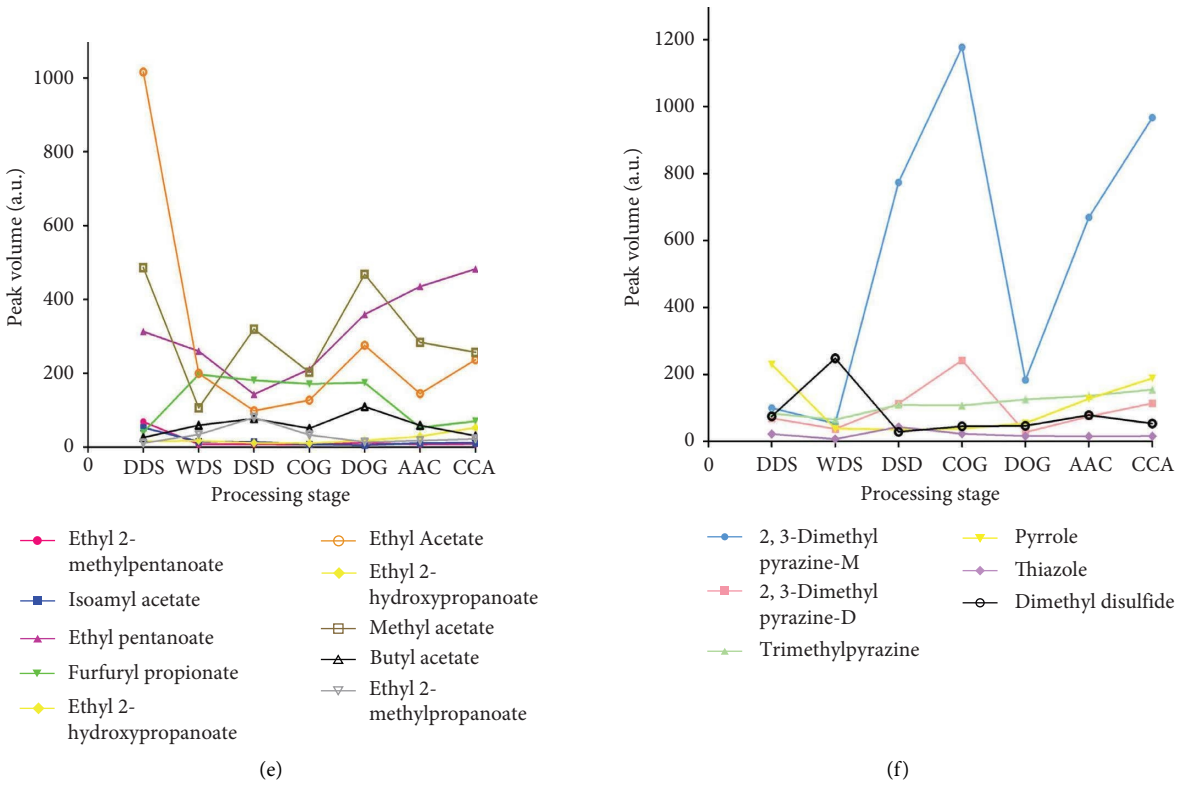


Figure 6: Relative contents and change curves of volatile flavor compounds in Ejiao at different processing stages. (a, b) Alcohols, (c) aldehydes, (d) ketones, (e) esters, and (f) pyrazines, pyrrole, thiazole, and dimethyl disulfide.

After rock sugar, soybean oil, yellow rice wine, and other ingredients were added, aldehyde contents were reduced to varying degrees. Except for a slight increase in isopropyl alcohol content, the contents of other alcohols decreased. Moreover, the contents of ketones such as 2-butanone-D and 2-pentanone-D decreased significantly, whereas 2-butanone-M content increased slightly. Of esters, ethyl pentanoate content increased, whereas methyl acetate, ethyl acetate, butyl acetate, and furfuryl propionate decreased. Pyrazine compound contents also increased at varying degrees; pyrrole and dimethyl disulfide contents also increased significantly.

4. Discussion

A total of 47 volatile flavor compounds were identified in Ejiao through GC-IMS, which mainly included aldehydes, alcohols, esters, ketones, and pyrazines as well as dimethyl disulfide, thiazole, and pyrrole.

During the processing of Ejiao, the contents of aldehydes and alcohols gradually decreased, while the contents of ketones, esters, pyrazines, and other compounds increased. Many volatile flavor compound contents decreased sharply in the WDS stage, which may be due to many reasons. The sharp decrease in many volatile flavor substances in the WDS stage may be caused by many reasons. In the process of soaking DDS into WDS, the decrease may be caused by the decrease of its concentration, the volatilization of volatile substances, and their dissolution by water.

In the dissolving stage of WDS, degradation was mainly concentrated in the dermal layer of the donkey skin. Collagen is abundant in the dermis of mammals, but processing degraded it only partially [15].

Aldehydes are the main volatile flavor compounds formed during the processing of Ejiao, and they mainly included nonanal, octanal, heptanal, benzaldehyde, 2-hexenal, pentanal, hexanal, butanal, 3-methylbutanal, 2-methylbutanal, and (E)-hept-2-enal. Aldehydes, especially unsaturated aldehydes, are important intermediates in the Maillard reactions and lipid oxidation and are involved in interactions between amino acids and carbonyl compounds [16]. Fatty acids in the donkey skin mainly included oleic acid, palmitic acid, linoleic acid, palmitoleic acid, stearic acid, myristic acid, and transoleic acid [17]. Hexanal is the oxidation product of linoleic acid, whereas heptanal, octanal, and nonanal are the oxidation products of oleic acid, all contributing to the green grass aroma of Ejiao [18, 19], which is desirable. The degradation product of phenylalanine was converted to benzaldehyde by the Strecker reaction. However, benzaldehyde produces bad taste, which in turn affects the aroma of Ejiao [20]. Unsaturated aldehydes are usually considered the products of unsaturated fatty acid oxidation. 3-Methylbutanal and 2-methylbutanal may be compounds obtained via the Strecker degradation of amino acids or microbial

degradation of amino acids [18, 21]. Most aldehydes were detected only in the DDS samples, and most of them disappeared in the subsequent processing stage, possibly due to lipid oxidation during the processing of fresh donkey skin toward the DDS stage and subsequent transformation into other substances.

The main ketones detected during processing were 2-heptanone, 2-octanone, 2-pentanone, 2-hexanone, 2-butanone, and cyclohexanone. All these ketones, except 2-butanone, were detected in the DDS sample and disappeared in the subsequent processing stages. Ketones are considered precursors to produce fat flavor, which may be formed by decarboxylation or β-oxidation of saturated fatty acid β-ketoacid [22], contributing to the fruit and cream aroma in Ejiao [19]. Among them, 2-heptanone may lead to flavor changes in meat and meat products, which can be used as a marker of product deterioration [21].

Esters are usually synthesized through the esterification of fat or protein with acids produced via alcohol degradation or via transesterification (alcoholysis) of fatty acids in triglycerides and ethanol [19]. Esters produced during the gelatin process mainly included ethyl pentanoate, ethyl 2-methylpentanoate, isoamyl acetate, furfuryl propionate, ethyl lactate, ethyl acetate, methyl acetate, butyl acetate, and ethyl 2-methylpropanoate. Alcohols, originating from fatty acid degradation, mainly included hexanol, octan-1-ol, pentan-1-ol, 2-methyl-1-pentanol, 3-methylbutan-1-ol, 1-propanethiol, and isopropyl alcohol.

2,3-Dimethylpyrazine and trimethylpyrazine contributed to "roasted nut" and "burnt" odor in Ejiao, respectively. Pyrazine is mainly formed during the Maillard reaction between sugar and amino acid residues. In the long-term high-temperature boiling process, higher temperature is conducive to the Maillard reaction and more pyrazine is produced [3]. The Maillard reaction is complex, leading to the production of pyrazine, furan, aldehyde, ketone, and other flavor compounds and is affected by many factors. In the DOG and COG samples, pyrazine compound production was promoted and the content decreased after deformation. After the addition of rock sugar and other ingredients, these contents increased.

Dimethyl disulfide contents increased at the WDS stage but decreased significantly thereafter. Then, they gradually increased during processing. Dimethyl disulfide is a sulfur-containing compound, mainly derived from the degradation of sulfur-containing amino acids such as cysteine and methionine in the donkey skin [23]. Methionine is converted to methyl mercaptan and some sulfur-containing compounds via transamination and decarboxylation, and methyl mercaptan is oxidized to produce dimethyl disulfide [24]. Although the concentration of sulfur-containing compounds in Ejiao is low, their extremely low threshold makes them crucial compounds contributing to Ejiao's flavor. They contribute to unpleasant smells such as cooked cabbage and roasted onion smells in Ejiao.

When the glue solution is concentrated to a certain extent, impurities will float on the surface and gather in the center of the glue solution [8]. At this time, the glue solution is concentrated to the maximum extent, and impurities are removed through deformation. As the concentration reached the maximum, the oxidative degradation of fat and amino acids also increased, thus increasing aldehyde, alcohol, ketone, and ester contents at the DOG stage. After rock sugar and other ingredients were added, the Maillard reaction promotes the formation of pyrazines and other new compounds. Simultaneously, it may somewhat dilute glue, reducing aldehyde, alcohol, ketone, and ester proportion at the AAC stage.

The AAC and CCA samples had high similarity, and the addition of ingredients eventually promoted flavor formation in Ejiao products and thus played an important role in Ejiao processing. In the final Ejiao products, compounds such as 2,3-dimethylpyrazine, 2-butanone, hexanal, 2-pentanone, ethyl pentanoate, pentanal, heptanal, and benzaldehyde have a great contribution to its flavor, which together constitute the overall composition of volatile flavor compounds in Ejiao. This result is similar to that reported by Wang (Wang et al., 2016) and Zhang et al. [7]; however, their analysis of flavor substances in the final Ejiao products was qualitative and did not involve the flavor and source analysis at different Ejiao processing stages.

5. Conclusion

Here, GC-IMS was used to dynamically detect and analyze the changes in volatile flavor compounds during the processing of traditional Ejiao. In total, 47 volatile flavor compounds were identified, which included aldehydes, esters, alcohols, ketones, and pyrazines. At different processing stages of Ejiao production (i.e., DDS, WDS, DSD, COG, DOG, AAC, and CCA), the contents of each volatile flavor compound demonstrated a certain trend for change. Volatile flavor compound formation during Ejiao processing is attributable to the Maillard reaction, amino acid or protein-oxidized lipid interaction, lipid oxidation and degradation, and long-chain compound degradation during heating. The DDS sample was mainly composed of aldehydes and ketones, such as nonanal, octanal, benzaldehyde, 2-methylbutanal, 2-hexenal, cyclohexanone, and octanone. After processing, these substances disappeared. The WDS, DSD, COG, and DOG samples had some similarity. After processing, some new compounds, such as trimethylpyrazine, 2,3-dimethyl pyrazine, ethyl 2-methylpropanoate, and furfuryl propionate, were produced. The AAC sample also had high similarity with the finished CCA products, and it mainly contained esters. In other words, the addition of other ingredients played a crucial role in the formation of final flavor in Ejiao.

Because the GC-IMS database is incomplete, some volatile flavor compounds remain unknown. In the future, GC-MS combined with other methods can be used to further analyze the fine changes in volatile flavor compounds in Ejiao. The results can provide data support for formulating quality control standards in the future.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Chinese Pharmacopocia Commission, *Pharmacopoela of the People's Republic of China*, China Medical Science Press, Beijing, China, 2020.

[2] G. Mao, Q. Guo, X. Li, and J. Qu, "Anaysis of flavor ingredients in colla corii asini with GC-MS," *Progress in Veterinary Medicine*, vol. 31, no. 12, pp. 72–75, 2010.

[3] S. Zhang, L. Xu, Y. X. Liu, H. Y. Fu, Z. B. Xiao, and Y. B. She, "Characterization of aroma-active components and antioxidant activity analysis of E-jiao (*colla corii asini*) from different geographical origins," *Natural Products and Bioprospecting*, vol. 8, no. 2, pp. 71–82, 2018.

[4] Y. Yu, C. Shu, and Z. Xiao, "Investigations on key aroma compounds in colla corii asini by GC-MS/GC-O combined with chemometrics," *Modern Food Science and Technology*, vol. 32, no. 2, pp. 269–275, 2016.

[5] X. Sui, Q. Zhu, N. Zhang et al., "Analysis of aroma active compounds of Ejiao," *Journal of Food Science & Technology*, vol. 39, no. 03, pp. 89–100, 2021.

[6] J. Zhang, J. Zhu, J. Zen, Y. Huang, Z. Yu, and J. Guo, "Developing excellence: Chinese university reform in three steps," *Nature*, vol. 514, no. 7522, pp. 295-296, 2014.

[7] P. Zhang, D. Li, W. Zhang, P. Zhang, Q. Kan, and J. Sun, "Anslysis of volatile compounds from *colla corii asini* by HS-SPME-GC-MS combined with AMDIS," *Asian Journal of Pharmaceutical Sciences*, vol. 14, no. 1, pp. 52–62, 2019.

[8] C. Yao, L. Cao, F. Guang, Z. Chen, and H. Liu, "Study on the quality control of Ejiao by introducing the HACCP System," *Herald of Medicine*, vol. 40, no. 4, pp. 509–514, 2021.

[9] C. Capitain and P. Weller, "Non-targeted screening approaches for profiling of volatile organic compounds based on gas chromatography-ion mobility spectroscopy (GC-IMS) and machine learning," *Molecules*, vol. 26, no. 18, p. 5457, 2021.

[10] X. Wang, K. M. Rogers, Y. Li, S. Yang, L. Chen, and J. Zhou, "Untargeted and targeted discrimination of honey collected by *Apis cerana* and *Apis mellifera* based on volatiles using HS-GC-IMS and HS-SPME-GC-MS," *Journal of Agricultural and Food Chemistry*, vol. 67, no. 43, pp. 12144–12152, 2019.

[11] T. Chen, X. Chen, D. Lu, and B. Chen, "Detection of adulteration in canola oil by using GC-IMS and chemometric analysis," *International Journal of Analytical Chemistry*, vol. 2018, Article ID 3160265, 8 pages, 2018.

[12] Y. Yang, B. Wang, Y. Fu et al., "HS-GC-IMS with PCA to analyze volatile flavor compounds across different production stages of fermented soybean whey tofu," *Food Chemistry*, vol. 346, Article ID 128880, 2021.

[13] S. Wang, H. Chen, and B. Sun, "Recent progress in food flavor analysis using gas chromatography-ion mobility spectrometry (GC-IMS)," *Food Chemistry*, vol. 315, Article ID 126158, 2020.

[14] M. Li, R. Yang, H. Zhang, S. Wang, D. Chen, and S. Lin, "Development of a flavor fingerprint by HS-GC-IMS with PCA for volatile compounds of tricholoma matsutake singer," *Food Chemistry*, vol. 290, pp. 32–39, 2019.

[15] G. Zhang, T. Liu, Q. Wang et al., "[Investigation on degradation process of collagens during manufacturing procedure of E'jiao]," *China Journal of Chinese Materia Medica*, vol. 34, no. 10, pp. 1211–1215, 2009.

[16] A. Adams, V. Kitrytė, R. Venskutonis, and N. De Kimpe, "Formation and characterisation of melanoidin-like polycondensation products from amino acids and lipid oxidation products," *Food Chemistry*, vol. 115, no. 3, pp. 904–911, 2009.

[17] L. Li, H. Zhang, J. Shi, Z. Wei, N. Zhang, and G. Liu, "Rapid identification of donkey skin based on gas chromatography," *Modern Food Science and Technology*, vol. 37, no. 12, pp. 1–6, 2021.

[18] L. R. Beldarrain, L. Morán, M. Á. Sentandreu, L. J. R. Barron, and N. Aldai, "Effect of ageing time on the volatile compounds from cooked horse meat," *Meat Science*, vol. 184, Article ID 108692, 2022.

[19] Q. Huang, K. Dong, Q. Wang et al., "Changes in volatile flavor of yak meat during oxidation based on multi-omics," *Food Chemistry*, vol. 371, Article ID 131103, 2022.

[20] A. Watanabe, G. Kamada, M. Imanari, N. Shiba, M. Yonai, and T. Muramoto, "Effect of aging on volatile compounds in cooked beef," *Meat Science*, vol. 107, pp. 12–19, 2015.

[21] Q. Zhang, Y. Ding, S. Gu, S. Zhu, X. Zhou, and Y. Ding, "Identification of changes in volatile compounds in dry-cured fish during storage using HS-GC-IMS," *Food Research International*, vol. 137, Article ID 109339, 2020.

[22] Y. Shi, X. Li, and A. Huang, "A metabolomics-based approach investigates volatile flavor formation and characteristic compounds of the dahe black pig dry-cured ham," *Meat Science*, vol. 158, Article ID 107904, 2019.

[23] X. Pan, W. Zhang, F. Lao et al., "Isolation and identification of putative precursors of the volatile sulfur compounds and their inhibition methods in heat-sterilized melon juices," *Food Chemistry*, vol. 343, Article ID 128459, 2021.

[24] C. Li, J. Wu, Y. Li, and Z. Dai, "Identification of the aroma compounds in stinky mandarin fish (*Siniperca chuatsi*) and comparison of volatiles during fermentation and storage," *International Journal of Food Science and Technology*, vol. 48, pp. 2429–2437, 2013.