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Effects of Low-Temperature and Low-Salt Fermentation on the Physicochemical Properties and Volatile Flavor Substances of Chinese Kohlrabi Using Gas Chromatography–Ion Mobility Spectrometry

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Abstract: To explore the effect of low-temperature and low-salt fermentation on the volatile flavor substances of Chinese kohlrabi, low-temperature and low-salt fermented Chinese kohlrabi (LSCK) and traditional high-salt fermented Chinese kohlrabi (HSCK) were produced. The physicochemical and texture properties of the two kinds of Chinese kohlrabies were evaluated. Headspace gas chromatography-ion mobility spectrometry (GC-IMS) and electronic nose (E-nose) were used to analyze the volatile flavor substances of the kohlrabi. The results showed that the total acid content significantly decreased ($p < 0.05$), while protein and reducing sugar contents significantly increased ($p < 0.05$) by low-temperature and low-salt fermentation. A total of 114 volatile flavor substances were identified. The alcohol, ketone, pyrazine, ether, and nitrile contents in LSCK were significantly higher than those in HSCK ($p < 0.05$). Moreover, the unpleasant flavor from the 3-methylbutyric acid formation was effectively depressed in LSCK. The principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) models established by multivariate statistical analysis significantly distinguished the two types of kohlrabies. Multivariate statistical analysis suggested that 16 volatile flavor substances with VIP > 1, including tetrahydrothiophene, ethyl 3-(methylthio)propanoate, 3-methylbutyric acid, hexanenitrile, and 3-methyl-3-buten-1-ol, could be used as potential biomarkers for identifying LSCK and HSCK. The E-nose analysis further demonstrated that there was a significant difference in overall flavor between the LSCK and HSCK. The present study provides support for the development of green processing technology and new low-salt Chinese kohlrabi products.

Keywords: kohlrabi; low-temperature and low-salt fermentation; high-salt fermentation; volatile flavor substances; GC-IMS; E-nose

1. Introduction

Kohlrabi (*Brassica juncea* var. *megarrhiza* Tsen et Lee), which originates from China, is a root-type variant of mustard, belonging to the cruciferous *Brassica* annual herb, which is rich in vitamins, amino acid, protein, mineral elements, and other nutrients [1]. Raw kohlrabi normally has a strong mustard spicy taste, which will be removed after 3–5 months fermentation. Furthermore, the kohlrabi becomes crisp and tender, with a rich aroma and a delicious taste after fermentation, making it a very popular side dish all over China. Salt is considered one of the most important additives in the processing of fermented foods to inhibit the growth of spoilage related to microorganisms and improve food quality. Desalination is normally required in the high-salt fermentation procedure. Although the

salt content in the finished products will be decreased by desalination, a lot of nutrients are lost during desalination [2]. Low-salt and green processing technology for pickles are attracting increasingly more attention from researchers all over the world. Low-temperature and low-salt fermentation have more advantages over high-salt fermentation, including the significantly decreased salt usage, without desalination and significantly reduced nutrient loss. Research on the effects of low-temperature and low-salt fermentation on the volatile flavor substances of Chinese industrially produced kohlrabi is rare.

Flavor is one of the most important qualities for fermented vegetables, affecting consumers' purchasing decisions [3]. The volatile flavor substances of fermented vegetables are regulated by salt, fermentation temperature, microorganisms, and other factors. It has been well demonstrated that salt is the most common condiment in food processing, which plays an important role in the formation of food taste and flavor [4]. Appropriate salt concentrations can alter the microbial community composition and metabolite profile in a specific food product, which thereby positively affect food quality [5]. He et al. found that 20 °C was the optimal temperature for Chinese suancai fermentation because 20 °C could accelerate the ripening of suancai and enhance suancai sensory quality [6]. Microorganisms are the key factors affecting the quality and flavor of fermented vegetables, which metabolize amino acids into various flavor compounds and participate in the biosynthesis of flavor amino acids [7]. The application of lactic acid bacteria (LAB) is the most commonly used methods for preserving dairy and vegetables, due to its simplicity, low cost, and sustainability to preserve [8]. In addition to extending the shelf life, lactic acid (LA) fermentation also modifies the sensory properties of products and increases their health-promoting effects [9]. On the other hand, yeast has been reported to play essential roles in the fermentation process which contributes to the taste and flavor of fermented vegetables [10].

In the present study, low-temperature and low-salt fermented and traditional high-salt fermented Chinese kohlrabies were produced. The price for the LSCK is four times higher than that of the HSKC, which greatly promotes the development of the local kohlrabi industry. The flavor probably plays an important role for the acceptance of consumers. Whether the low-temperature and low-salt fermentation will greatly affect the volatile flavor substances of Chinese kohlrabi is still an open problem. The volatile flavor substances of the two different kinds of industrially produced Chinese kohlrabies were analyzed by GC-IMS combined with E-nose. The two kohlrabi flavors were further analyzed by multivariate statistical analysis including PCA and OPLS-DA. The volatile flavor substance fingerprints of HSKC and LSCK were established. This study provides supports for the green processing of Chinese kohlrabi and the development of new low-salt Chinese kohlrabi products.

2. Materials and Methods

2.1. Production of HSKC and LSCK

The HSKC was produced as described in our previous study [2]. After harvest, the kohlrabies were washed with water and subsequently air-dried outside for about 30 days (average temperature 7–12 °C). After air-drying, the kohlrabies were transferred into fermentation tanks. Eight percent (*w/w*) salt (upper layer 60%, middle layer 30%, and lower layer 10%) was added to the kohlrabies and they were pickled for 5 days. Then, 5% (*w/w*) salt (upper layer 10%, middle layer 30%, and lower layer 60%) was added to the treated kohlrabies and they were pickled for a further 4 days. Finally, the pickled kohlrabies were fermented for 5 months at room temperature.

For production of LSCK, after natural air-drying outside (average temperature 7–12 °C), the kohlrabies were washed in 60 ± 2 °C water for 10 min and dried at 35–40 °C for 15 min. For the first round of curing, a 2.5% (*w/w*) salt was added to the kohlrabies and they were pickled for 2 days at 4 ± 1 °C. Then, a 1.5% (*w/w*) salt and 4% yellow sugar were added to the treated kohlrabies and fermented for 5 months at 4 ± 1 °C.

2.2. GC-IMS Analysis

The kohlrabi was ground and vacuum-stored in aluminum foil bags at $-80\text{ }^{\circ}\text{C}$ for volatile flavor analysis. GC-IMS analysis was performed in a FlavourSpec[®] from G.A.S. (Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany) equipped with a syringe and an autosampler unit for headspace analysis. After 15 min incubation at $40\text{ }^{\circ}\text{C}$, 500 μL of the headspace content was automatically injected by the heated syringe ($85\text{ }^{\circ}\text{C}$). Chromatographic separation was performed on an MXT-1 capillary column ($15\text{ m} \times 0.53\text{ mm}$) at $60\text{ }^{\circ}\text{C}$ with nitrogen (N_2) as carrier gas (purity $\geq 99.999\%$) at the following flow rate: 2 mL/min for 2 min, then linearly increased to 10 mL/min for 3 min, increased the flow rate to 15 mL/min for 10 min, then increased the flow rate to 50 mL/min for 5 min, and finally increased the flow rate to 100 mL/min for 10 min. The total GC run time was 30 min. After separation in the capillary column, the headspace content was first injected into the ionization chamber for ionization, then through the shutter grid into the drift zone, and finally into the IMS detector. Drift tube was 98 mm long and kept at $45\text{ }^{\circ}\text{C}$. Drift gas (N_2 , purity $\geq 99.999\%$) flow was set at 150 mL/min. The experiment was carried out in triplicate.

2.3. E-Nose Analysis

A Fox 4000 Sensory Array Fingerprint Analyzer (Alpha M.O.S., Toulouse, France) was employed for analyzing the odor of kohlrabi samples. The E-nose system consists of a sampling apparatus, a detector unit with an array of 18 different metal oxide sensors, and pattern recognition software for data recording and analysis. Precisely 0.5 g of the kohlrabi sample was put into the sample vials and placed at $70\text{ }^{\circ}\text{C}$ in an air bath for 5 min to equilibrate prior to the start of the experiment. Clean dry air was used with a flow rate of 150 mL/min as the carrier gas, an injection speed of 500 $\mu\text{L/s}$, an injection period of 1 s, and odor data, which were recorded every second for 120 s. Five parallel experiments were performed for every sample.

2.4. Physicochemical Analysis

The total acid, pH, protein, reducing sugar, redness (a^*), yellowness (b^*), and brightness (L^*) were determined as described in our previous study [2]. The pH of the kohlrabi was measured by a pH-3C meter (Yidian, Shanghai, China). For color measurement, the kohlrabi was homogenized and color coordinates (L^* , a^* , and b^* values) were measured using a colorimeter (RC-10, Konica Minolta, Tokyo, Japan). Total acid was determined by titrating up to pH 8.3 with NaOH (0.05 M) and expressed as percent (w/v) of lactic acid. The reducing sugar contents were determined by the direct titration of the alkaline copper tartrate solution method. The experiment was carried out in triplicate.

2.5. Microbiological Analysis

Precisely 25 g of fermented kohlrabi was diluted with 225 mL of 0.9% saline solution and homogenized for 2 min. After appropriate gradient dilution, the total number of colonies was determined on a Plate Count Agar (PCA) plate (Solarbio, Beijing, China) incubated at $37\text{ }^{\circ}\text{C}$ for 48 h [11]. Lactic acid bacteria count was determined on de Man Rogosa and Sharpe (MRS) agar plates (Solarbio, Beijing, China). Plates were incubated at $36\text{ }^{\circ}\text{C}$ for 72 h [12]. The experiment was carried out in triplicate.

2.6. Texture Analysis

The frangibility of kohlrabi was determined by a TA.XT.plus texture analyzer (Stable Micro System, Surrey, England, UK) fitted with a cylindrical probe (P/2, 0.2 cm in diameter) [13]. Frangibility was determined at room temperature using a puncture test. The measurement parameters were set as follows: pre-test speed of 5 mm/s, test speed of 2.5 mm/s, post-test speed of 5 mm/s, probe travel distance of 12 mm, and trigger force of 5.0 g. The experiment was carried out in triplicate.

2.7. Data Analysis

Data processing was performed by Microsoft office 2021. The PCA and OPLS-DA were carried out by SIMCA (14.1, Umetrics, Umea, Sweden). Peak volume histograms of volatile flavor substances and electronic nose radar plots were plotted by Origin 2021. The qualitative analysis of the volatile compounds was carried out based on retention time and drift time using the software VOCal included in the GC-IMS system. The two-dimensional spectra of the volatile flavor substances were automatically generated by the built-in Reporter plug-in of the GC-IMS system and the fingerprint profiles of volatile flavor substances were generated by the built-in Gallery Plot plug-in.

3. Results

3.1. Physicochemical Properties and Microbial Indicators Analysis

The physicochemical properties of the LSK and HSK are shown in Table 1. No significant difference in redness and brightness between HSK and LSK was observed ($p > 0.05$). However, the yellowness value of LSK with the value of 22.65 was significantly higher than that of HSK with the value of 20.78 ($p < 0.05$). The pH for LSK was 4.76, which was significantly higher than that of HSK with the pH value of 4.27 ($p < 0.05$), suggesting that the lactic acid formation was somewhat inhibited by low temperature. Furthermore, the reducing sugar content in LSK was 3.55 g/100 g, which was significantly declined to 3.10 g/100 g ($p < 0.05$) in HSK. Similarly, the protein content in HSK was 2.13 g/100 g, which was significantly increased to (2.54 g/100 g) ($p < 0.05$) in LSK. Brittleness is considered a very important indicator for evaluating the quality of fermented vegetables, and is a measurement of frangibility. As shown in Table 1, the brittleness value for LSK was 0.14 mm^{-1} , which was significantly decreased to 0.11 mm^{-1} ($p < 0.05$) in HSK. The total number of colonies and lactic acid bacteria in LSK were significantly lower than those in HSK ($p < 0.05$), indicating that temperature reduction significantly inhibited the microbial growth.

Table 1. The physicochemical and microbial properties between LSK and HSK.

Indicator	LSK	HSK
Lightness	57.89 ± 1.70^a	56.22 ± 1.20^a
Redness	3.63 ± 0.50^a	3.57 ± 0.17^a
Yellowness	22.65 ± 0.41^a	20.78 ± 0.65^b
pH	4.76 ± 0.01^a	4.27 ± 0.01^b
Total acid (%)	0.39 ± 0.01^a	0.59 ± 0.02^b
Reducing sugar (g/100 g)	3.55 ± 0.03^a	3.10 ± 0.07^b
Protein content (g/100 g)	2.54 ± 0.09^a	2.13 ± 0.10^b
Brittleness (mm^{-1})	0.14 ± 0.01^a	0.11 ± 0.01^b
Colonies number ($\log_{10} \text{CFU/g}$)	4.18 ± 0.08^a	5.60 ± 0.04^b
Lactic acid bacteria count ($\log_{10} \text{CFU/g}$)	4.40 ± 0.17^a	5.23 ± 0.02^b

Different superscript letters in the same row indicate significant differences ($p < 0.05$).

3.2. GC-IMS Analysis for Volatile Flavor Substances

The original two-dimensional (2D) GC-IMS profiles of the LSK and HSK are shown in Figure 1A. The X- and Y-axes represent the ion migration time for identification and the retention time for GC separation [14]. Each point on either side of the RIP indicates a volatile flavor substance. The colors distinguished from the blue background representing the concentration of the compounds, where the white color suggests lower concentration, red suggests higher concentration, and darker colors implies heavy concentration. The entire spectrum indicates the volatile composition of the fermented kohlrabies. It can be seen obviously from Figure 1A that the volatile flavor substances of LSK and HSK were well separated. To compare the differences in volatile flavor substances between the LSK and HSK more conveniently, the topographical plot of LSK-1 was taken as the reference and the topographical plots of HSK were deducted from the reference [15]. The white

color after deduction represents the same concentration of a volatile flavor substances in LSCK and HSCK, while the red dot suggests a higher concentration of a volatile flavor substance than that in the reference and the blue color indicates a lower concentration of a compound as compared to that in the reference. As can be seen in Figure 1B, the differences in volatile flavor substances between LSCK and HSCK were significant attributing to more blue dots were observed in Figure 1B. Moreover, the total volatile flavor substances contents in LSCK were higher than those in HSCK, which agreed well with the results in Figure 2.

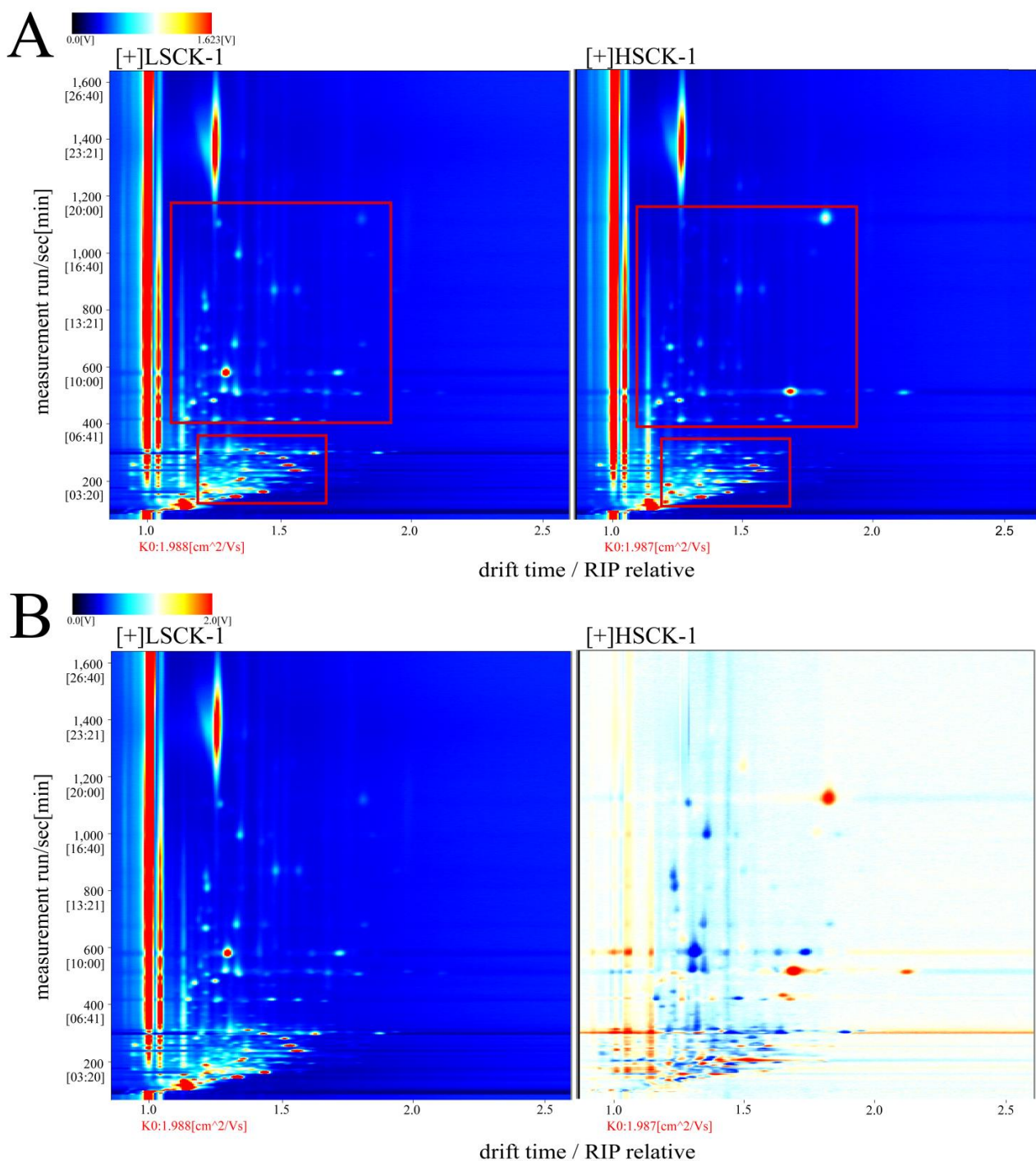


Figure 1. GC-IMS plots of volatile flavor substances of LSCK and HSCK in 3D (A) and 2D (B).

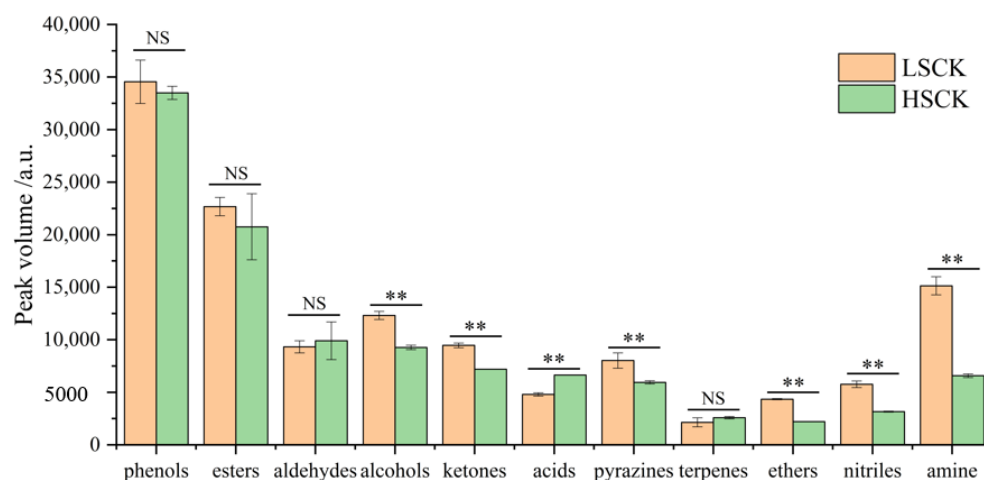


Figure 2. Peak volume of volatile flavor substances in LSK and HSK. NS indicates no significant difference ($p > 0.05$), ** indicates extremely significant difference ($p < 0.01$).

3.3. Qualitative and Quantitative Analysis of Volatile Flavor Substances

The qualitative and quantitative analysis results are listed in Table S1. There were 124 peaks detected from LSK and HSK by GC-IMS. After removal of the signals possibly caused by dimers or trimers [16], a total of 114 volatile flavor substance were identified from LSK and HSK. These compounds were composed of 30 esters, 21 alcohols, 13 aldehydes, 10 ketones, 8 pyrazines, 7 acids, 6 ethers, 4 terpenes, 3 phenols, 2 nitriles, and 10 other substances. The qualitative analysis for volatile flavor substances from LSK and HSK is shown in Figure 2. There was no significant difference in phenols, esters, aldehydes, and terpenes between LSK and HSK ($p > 0.05$). The acids in LSK were significantly lower than those in HSK ($p < 0.01$), while alcohols, ketones, pyrazines, terpenes, ethers, and nitriles in LSK were significantly higher than those in HSK.

Phenols were the volatile flavor substances with the highest content in LSK and HSK, followed by esters and aldehydes. Three phenols were identified in LSK and HSK, namely carvacrol, p-methyl guaiacol, and hexanenitrile, among which carvacrol was the phenol with the highest content. On the other hand, the phenol content in LSK was significantly higher than that in HSK ($p < 0.01$). Thirty esters were identified from LSK and HSK. Moreover, the ester content in LSK was slightly higher than that in HSK, suggesting that low temperature and low salt promotes the formation of esters. However, ethyl dihydrocinnamate, benzyl propionate, ethyl acrylate, hexyl acetate, (Z)-3-hexenyl acetate, ethyl 3-hydroxybutanoate, 2-furanmethanol and δ -heptanolide in LSK were significantly higher than those in HSK ($p < 0.05$). Thirteen aldehydes were identified from LSK and HSK. The total aldehyde content in LSK was slightly lower than that in HSK, while the benzaldehyde, vanillin, salicylaldehyde, furfural, and (E,E)-2,4-hexadienal contents in LSK were significantly higher than those in HSK ($p < 0.05$).

In this study, 21 alcohols were identified from LSK and HSK, with 7 unsaturated alcohols termed (Z)-2-penten-1-ol, dihydromyrcenol, (E)-3-hexen-1-ol, 3-methyl-3-buten-1-ol, 4-hexen-1-ol, 2-hexen-1-ol, and 2-furfurylthiol. Acids are dominant sources for fermented vegetables and seven acids were detected from LSK and HSK. The acids content including 3-methylbutyric acid in LSK was significantly lower than that in HSK ($p < 0.01$). Natural terpenes and their derivatives have diversified aroma characteristics and thus are important food flavor sources [17]. Four terpenes including limonene, α -terpinolene, α -phellandrene, and α -terpinene were identified from LSK and HSK. On the other hand, the α -terpinene content in HSK was significantly higher than that in LSK ($p < 0.01$).

3.4. Fingerprints of Volatile Flavor Substances for LSK and HSK

To further identify the differences in volatile flavor substances between LSK and HSK, all the volatile flavor substances detected in the GC-IMS spectra were selected to

generate the fingerprints using the Gallery Plot plug-in, as shown in Figure 3. Each row in the gallery plot indicates the entire signal peak of a kohlrabi sample and each column reveals the signal intensity of the same compounds presented in different kohlrabies. Area A showed the volatile flavor substances with no significant differences between LSK and HSK, including heptyl acetate, pentanoic acid, 4-hexen-1-ol and 2-propanol. Meanwhile area B represented the volatile flavor substances with higher contents in LSK, mainly including hexyl acetate, methylpyrazine, benzaldehyde, 2-acetylpyrazine, and dihydromyrcenol. However, area C indicated the volatile flavor substances with higher contents in HSK, including limonene, 2-methyl-1-propanol, 3-methylbutyric acid and (E)-2-octenal.

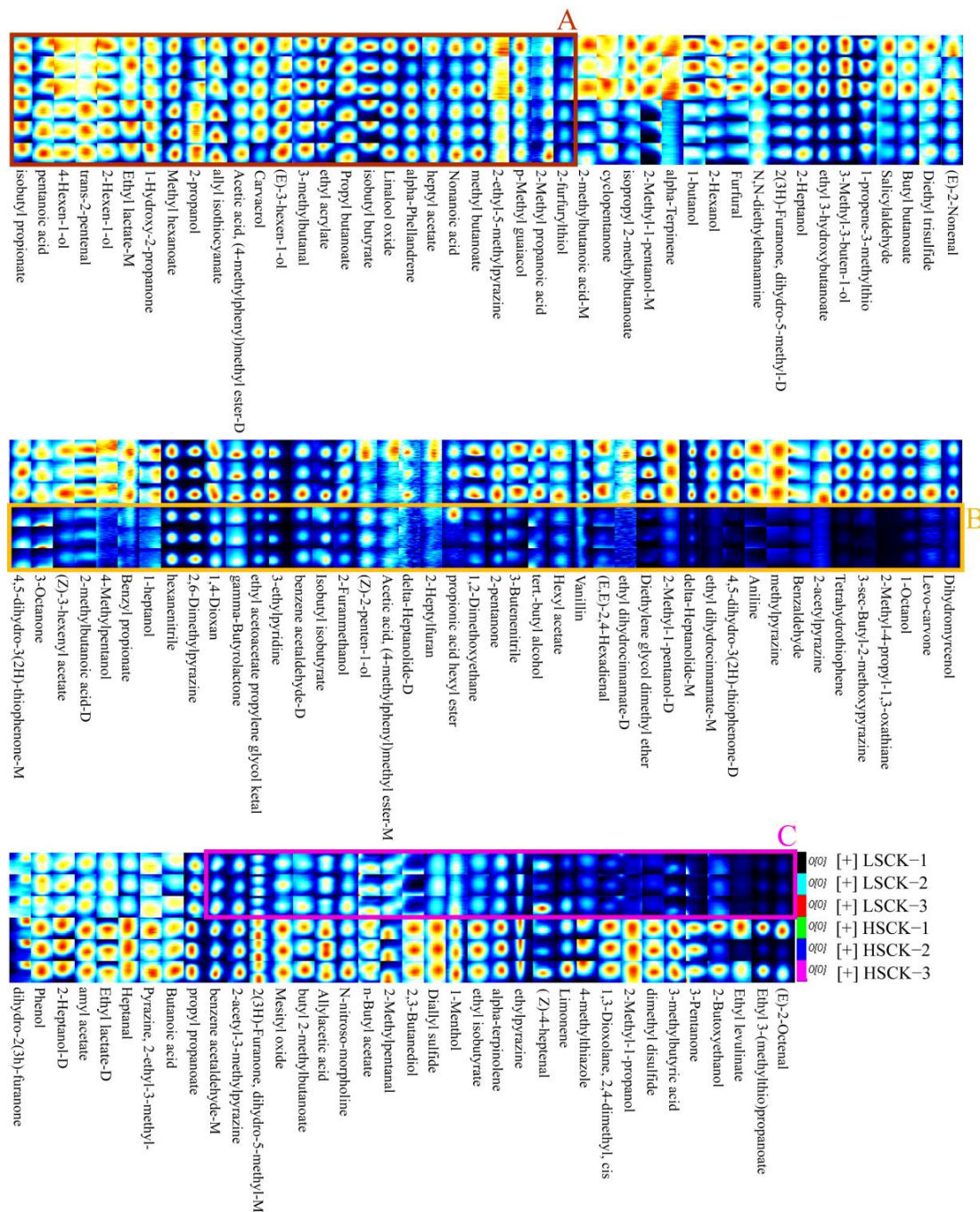


Figure 3. The fingerprint profiles of the labelled peaks for volatile flavor substances from LSK and HSK. LSK-1, LSK-2, and LSK-3 indicated the three parallel experiments for LSK. Similarly, HSK-1, HSK-2, and HSK-3 indicated the three parallel experiments for HSK.

To further demonstrate the differences in volatile flavor substances between LSCK and HSKC, the PCA and OPLS-DA were employed, as shown in Figure 4. The scatter plots for PCA and OPLS-DA of LSCK and HSKC distributed well, suggesting that the differences in volatile flavor substances between LSCK and HSKC were significantly different [18], which agreed well with the GC-IMS results. The quality parameters of the PCA and OPLS-DA model are shown in Tables 2 and 3.

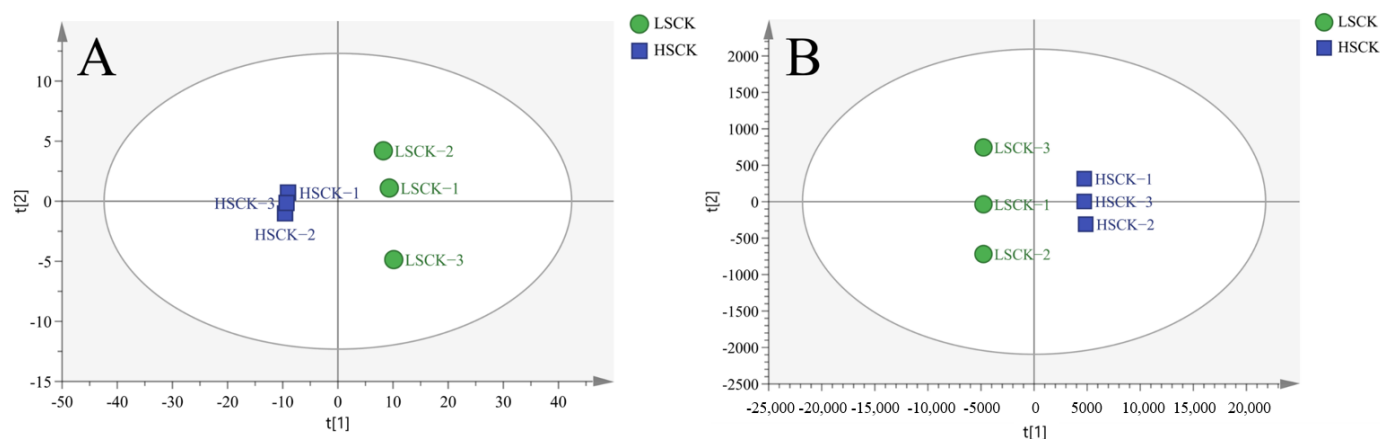


Figure 4. The scatter plots for PCA (A) and OPLS-DA (B) of LSCK and HSKC.

Table 2. Quality parameters of PCA models.

Data Origin	The Principal Components	R^2_X	R^2_X (cum)
GC-IMS	PC1	0.8350	0.835
	PC2	0.0704	0.905

R^2_X represents the interpretation of variable differences by the model, R^2_X (cum) represents the interpretation of cumulative differences.

Table 3. Quality Parameters of OPLS-DA models.

Data Origin	R^2_X (cum)	R^2_Y (cum)	Q^2 (cum)
GC-IMS	0.989	1	0.997

R^2_X (cum) represents the interpretation of variable X by the model, R^2_Y (cum) represents the interpretation of variable Y by the model, Q^2 (cum) represents the predictive capacity of the model.

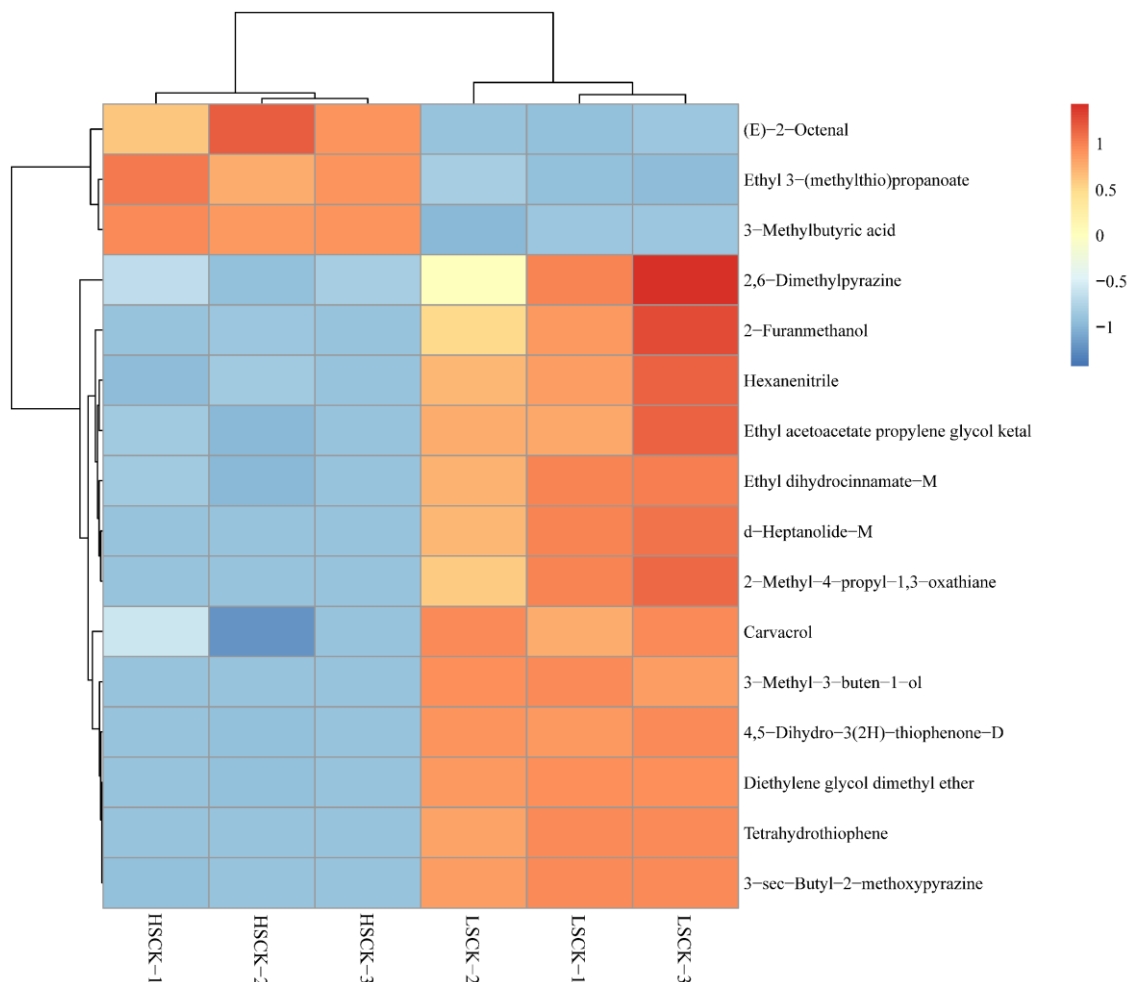
3.5. Differential Volatile Flavor Substances between LSCK and HSKC

Based on variable importance for the projection (VIP) of OPLS-DA, the differential volatile flavor substances between LSCK and HSKC were selected, as revealed in Table 4. The $VIP > 1$ was set as the standard [19], and consequently 16 differential volatile flavor substances were screened, including tetrahydrothiophene, ethyl 3-(methylthio)propanoate, 3-methylbutyric acid, hexanenitrile, 3-methyl-3-buten-1-ol, diethylene glycol dimethyl ether, (E)-2-octenal, 4,5-dihydro-3(2H)-thiophenone-D, 3-sec-butyl-2-methoxypyrazine, δ -heptanolide-M, 2,6-dimethylpyrazine, ethyl dihydrocinnamate-M, carvacrol, 2-methyl-4-propyl-1,3-oxathiane, ethyl acetoacetate propylene glycol ketal, and 2-furanmethanol. Most of the compounds have pleasant flower and fruit aroma and strong spice flavor. However, 3-methylbutyric acid has rotten and sweaty odor.

In order to better evaluate the differences of the differential volatile flavor substances in LSCK and HSKC, the relative contents of the 16 differential volatile flavor substances in LSCK and HSKC were analyzed by hierarchical clustering analysis and displayed in the form of a heat map, as shown in Figure 5. Hierarchical cluster analysis intuitively classified 16 differential volatile flavor substances into two categories, which showed that LSCK and HSKC could be effectively distinguished through the 16 differential volatile flavor substances.

Table 4. The VIP values for differential volatile flavor substances between LSCK and HSCK.

	Flavor Substance	CAS	VIP	Aroma Descriptions
1	Tetrahydrothiophene	110-01-0	7.28553	Cabbage
2	Ethyl 3-(methylthio)propanoate	13327-56-5	3.57834	Meat, onion, and garlic
3	3-Methylbutyric acid	503-74-2	2.44778	Putrid and sweaty smell
4	Hexanenitrile	628-73-9	2.35979	
5	3-Methyl-3-buten-1-ol	763-32-6	2.24654	Sweet fruit
6	Diethylene glycol dimethyl ether	111-96-6	2.14249	
7	(E)-2-Octenal	2548-87-0	2.10519	Citrus
8	4,5-Dihydro-3(2H)-thiophenone-D	1003-04-9	2.09335	Garlic and Onion
9	3-sec-Butyl-2-methoxypyrazine	24168-70-5	1.91523	Fresh green pea
10	δ -Heptanolide-M	713-95-1	1.77461	Peach
11	2,6-Dimethylpyrazine	108-50-9	1.42207	Coffee and fried peanut
12	Ethyl dihydrocinnamate-M	103-36-6	1.38867	Orange and grape
13	2-Methyl-4-propyl-1,3-oxathiane	59323-76-1	1.38086	Spicy herb
14	Carvacrol	499-75-2	1.23141	
15	Ethyl acetoacetate propylene glycol ketal	6290-17-1	1.15615	Fruit
16	2-Furanmethanol	623-19-8	1.12531	Green and burnt

**Figure 5.** Hierarchical clustering analysis heat map of the differential volatile flavor substances between LSCK and HSCK.

3.6. E-Nose Analysis

The E-nose was used to analyze the overall flavor of the LSCK and HSCK, as revealed in Figure 6A. Both LSCK and HSCK had almost no response to six sensors LY2/LG, LY2/G,

LY2/AA, LY2/Gh, LY2/gCTI, and LY2/gCT, indicating that nitrogen oxides, amine compounds, sulfide, and acetone were rarely produced during LSKC and HSKC fermentation. The HSKC had higher response values to T30/1, P10/1, P10/2, P40/1, T70/2, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, and TA/2 than those in LSKC, suggesting that polar compounds, aromatic compounds, and strong oxidizing substances in HSKC were significantly higher than those in LSKC. The PCA analysis was further used to analyze the overall flavor of HSKC and LSKC, as shown in Figure 6B. The first two components (PC1 and PC2 were 99.400% and 0.323%, respectively) explained nearly 100% of the total variance, indicating that the PCA model can better reflect the overall flavor [20,21]. Additionally, the PC1 contributed much more than PC2, indicating the farther the sample was on the horizontal axis, the greater the odor difference [22], which was consistent with the GC-IMS results.

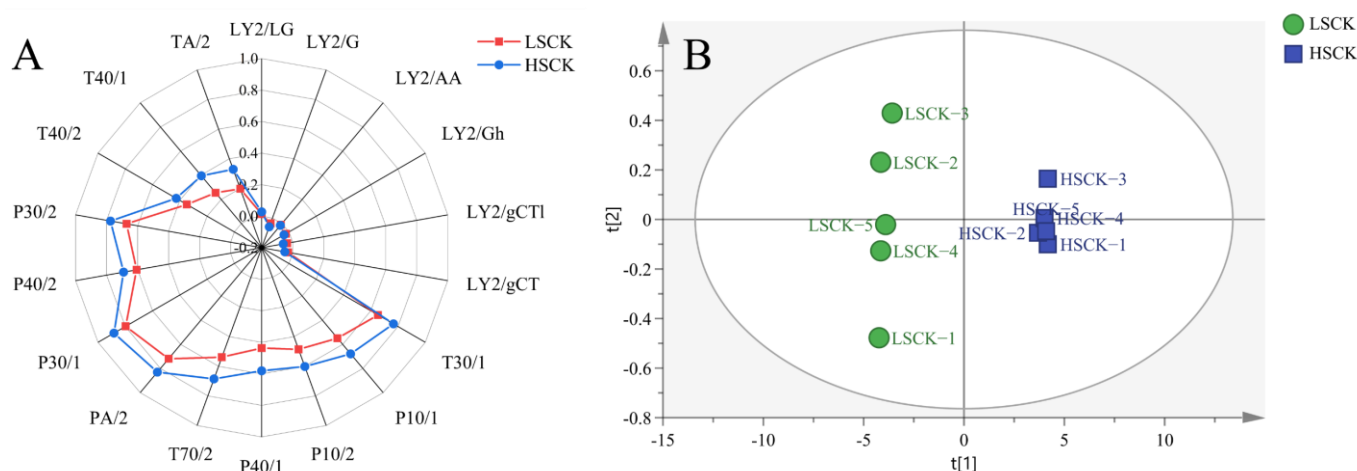


Figure 6. E-nose analysis. (A) Radar graph of the LSKC and HSKC. (B) PCA plot of the two fermented kohlrabies.

4. Discussion

The physicochemical properties of LSKC and HSKC were analyzed, as shown in Table 1. There was no significant difference in brightness and redness between LSKC and HSKC ($p > 0.05$), while the yellowness value of LSKC was significantly higher than that of HSKC ($p < 0.05$), which might be due to the changed kohlrabi tissue structure caused by moisture migration during high-salt fermentation [23]. On the other hand, microbes generate acid substances such as organic acids by utilizing reducing sugars and proteins in fermentation systems during kohlrabi fermentation and thereby the pH of the fermentation system dropped [24]. In the present study, the pH value, reducing sugar, and protein contents of LSKC were significantly higher than those of HSKC ($p < 0.05$) and the total acid content of LSKC was significantly lower than that of HSKC ($p < 0.05$), which was inconsistent with Mi's study [25], that could be owing to the low temperature inhibiting the growth of lactic acid bacteria and related enzyme activities. As for the texture, the brittleness value of LSKC was significantly higher than that of HSKC, which might be owing to the pectinase activity, which was inhibited under the low-temperature condition [26]. Furthermore, moisture migration caused by high salt was also one of the important factors leading to the difference in brittleness [27].

A total of 114 volatile flavor substances were identified by GC-IMS in LSKC and HSKC. The total volatile flavor substances content in LSKC was significantly higher than that in HSKC ($p < 0.05$), suggesting that low temperature and low salt could promote the volatile flavor substances formation, which agreed well with Chun's result [28]. Under low-salt condition, the growth and metabolism of microorganisms were stronger and thus more volatile flavor substances were produced [29]. It has been well demonstrated that *Lactobacillus*, *Microbacterium*, *Lactococcus*, *Staphylococcus*, and *Weissella* are considered as the

microbial genera significantly affected by salt content [25]. On the other hand, the decreased salt also contributes to shorten the cumulative time of volatile flavor substances [28].

Phenols are important secondary metabolites widely distributed in plant roots, stems, leaves, and fruits. They participate in life activities of plants, contributing to the plants defense against foreign pathogens, providing special tastes that are sweet, bitter, and astringent [30]. Carvacrol was one of the phenolic substances with the highest content in kohlrabi, which has a pungent woody smell and brings a unique spicy taste to food. The phenols will be hydrolyzed under higher temperature conditions [31]. Therefore, the phenol content in LSK was significantly higher than that in HSK.

Esters have a low threshold and fruity and floral flavor, which are important sources for food flavors [32]. Four esters with acid odor were identified in LSK and HSK, including ethyl acrylate, allyl isothiocyanate, 2-furanmethanol, and ethyl isobutyrate. Isothiocyanates in cruciferous plants mainly come from the metabolism of glucosinolates, leading to pungency and bitterness of kohlrabi [33]. On the other hand, they play an important role in the formation of mustard spicy taste in raw kohlrabi [34]. The glucosinolates in kohlrabi are hydrolyzed into isothiocyanates and nitriles under the catalysis of black mustard enzyme, and isothiocyanates can be further degraded into nitriles under the catalysis of Fe^{2+} and epithelial-specific protein [35]. In the present study, the isothiocyanates content in LSK was significantly lower than that in HSK ($p < 0.05$), while the nitriles content in LSK was significantly higher than that in HSK ($p < 0.01$), suggesting that low temperature and low salt promoted the degradation of glucosinolates and isothiocyanates, which agreed well with the previous study [36].

Aldehydes are mainly derived from lipid oxidation with low threshold and the aldehydes flavor have an overlapping effect [37]. Aldehydes normally have a fruit aroma, contributing to the overall flavor of foods [38]. The aldehyde content in LSK was slightly lower than that in HSK, which could be explained by the high salt promoting the halophiles and becoming the dominant microorganism, producing more aldehydes by the Ehrlich pathway and the branched chain amino acid biosynthetic pathway [39]. On the other hand, the low temperature during LSK processing also inhibits the aldehydes formation [40]. The benzaldehyde, vanillin, salicylaldehyde, furfural, and (E,E)-2,4-hexadienal contents in HSK were significantly higher than those in LSK ($p < 0.05$), which was possibly due to the higher salt and temperature accelerating the lipid oxidation [41].

Alcohols are the essential flavor components mainly generated from lipid oxidation, amino acid metabolism, and microbial reproduction [42], which generally have a high threshold resulting in a little contribution to the overall flavor. However, the unsaturated alcohols have a lower threshold and contribute greatly to the overall flavor. On the other hand, yeast is the second important factor participating in the production of alcohols [43]. The dihydromyrcenol, 3-methyl-3-buten-1-ol, has a fresh green fruit taste [44], with the content in LSK significantly higher than those in HSK. The alcohols in HSK were significantly lower than those in LSK, which might result from the yeast metabolism and lipid oxidation being depressed under high-salt conditions [45]. The growth-associated metabolites of yeast tend to be affected by low-temperature fermentation. Low temperature can regulate yeast metabolism by stimulating low-temperature enzymes in the metabolic process, thus affecting the content of alcohols in fermented food [46].

Acids are important flavor substances in fermented foods [47], which are mainly produced by phospholipid hydrolysis and lipid oxidation. Besides their direct contribution to fermented food flavors, acids can be generated into esters, alcohols, and aldehydes by lactic acid bacteria. The acids in HSK were significantly higher than those in LSK. It has been reported that the *Lactobacillus casei* and *Bifidobacterium lactis* participate in acid production in fermented foods [48] and their activity is depressed under lower temperature conditions. The 3-methylbutyric acid has a putrid and sweaty odor [49], which is mainly obtained from propionibacterium through leucine degradation during fermentation [50]. The 3-methylbutyric acid in LSK was significantly lower than that in HSK, which could be attributed to the growth of propionibacterium depressed by lower temperature [51]. On

the other hand, Castada found that butanoic acid could efficiently depress the production of 3-methylbutyric acid [52], which agreed well with the finding that the butanoic acid in LSCK was higher than that in HSKC.

Pyrazines are considered the substances closely related to sauce flavor in fermented foods [29]. The pyrazine content in LSCK was relatively higher than that in HSKC, which was opposite to Wu's findings [53]. Low-temperature and low-salt fermentation significantly increased the alcohol, ketone, pyrazine, and ether production ($p < 0.01$) and thus changed the flavor composition of LSCK, indicating that the metabolic pathways in traditional fermented kohlrabies were possibly changed by low-temperature and low-salt fermentation [54].

Sixteen volatile flavor substances with VIP > 1 were selected, which can be used as the potential biomarkers for identifying the LSCK and HSKC. Among which, the content of tetrahydrothiophene, hexanenitrile, 3-methyl-3-buten-1-ol, carvacrol, diethylene glycol dimethyl ether, 4,5-dihydro-3(2H)-thiophenone-D, 3-sec-butyl-2-methoxypyrazine, δ -heptanolide-M, 2,6-dimethylpyrazine, ethyl dihydrocinnamate-M, 2-methyl-4-propyl-1,3-oxathiane, ethyl acetoacetate propylene glycol ketal, and 2-furanmethanol in LSCK were significantly higher than those in HSKC ($p < 0.05$). However, the ethyl 3-(methylthio)propanoate, 3-methylbutyric acid, and (E)-2-octenal contents in HSKC were significantly higher than those in LSCK ($p < 0.01$). The ethyl 3-(methylthio)propanoate and 3-methylbutyric acid mainly generated from amino degradation [55,56], while (E)-2-octenal normally produced by linoleic acid degradation [57]. The relatively high temperature is probably the reason caused the three substances with higher contents in HSKC [58]. Furthermore, polar compounds and strong oxidizing substances in HSKC were significantly higher than those in LSCK, which was consistent with that for the 2-propanol, α -terpinolene, allylacetic acid, 1-propene-3-methylthio, and dimethyl disulfide contents in HSKC, which were significantly higher than those in LSCK ($p < 0.05$).

5. Conclusions

The physicochemical properties, texture, and volatile flavor substances of LSCK and HSKC were analyzed. Compared to HSKC, the LSCK was more brittle and had more reducing sugar and protein. Volatile flavor substance contents including alcohols and ketones were increased in LSCK as demonstrated by GC-IMS. In addition, low-temperature and low-salt fermentation inhibited the formation of the unpleasant flavor of 3-methylbutyric acid. The multivariate statistical analysis including PCA and OPLS-DA suggested the significant difference in flavor between the HSKC and LSCK. On the other hand, the 16 identified volatile flavor substances with VIP > 1 can be used as potential biomarkers for identifying the LSCK and HSKC. The difference in overall flavor between the LSCK and HSKC was significant based on E-nose analysis. The present study describes the difference of volatile flavor substances between LSCK and HSKC and explains why the LSCK was more expensive and acceptable than the HSKC in the aspects of flavor. This work provides support for developing kohlrabi green processing technology and new Chinese kohlrabi products. In the future, the dynamics of metabolites and microbial diversity in LSCK during fermentation and their correlation will be described to better understand the effects of low-temperature and low-salt fermentation on the quality of Chinese industrially produced kohlrabi.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fermentation9020146/s1>, Table S1: Volatile flavor substances in LSCK and HSKC.

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