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Research Article

Correlation of Volatile Compounds and Sensory Attributes of Chinese Traditional Sweet Fermented Flour Pastes Using Hierarchical Cluster Analysis and Partial Least Squares-Discriminant Analysis

Meigui Huang,[1] Yulin Li,[2] Ping Zhan,[3] Ping Liu,[4] Honglei Tian,[3] and Jie Fan[1]

[1] *College of Light Industry and Food Engineering, Nanjing Forestry University, Nanjing 210037, China*
[2] *Hubei Key Laboratory of Edible Wild Plants Conservation and Utilization, 11 Cihu Road, Huangshi 435002, China*
[3] *College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an, Shaanxi 710119, China*
[4] *School of Food and Bioengineering, Xihua University, Chengdu 610039, China*

Correspondence should be addressed to Honglei Tian; tianhonglei1979@163.com

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The aroma compositions, sensory attributes, and their correlations of various traditional Chinese sweet fermented flour pastes (SFFPs) were investigated. SFFPs, including LEEJ, LEEH, and XH6, showed high overall acceptance scores of 8.00, 8.21, and 7.50, respectively. Ninety-six volatile compounds were detected using solid-phase microextraction gas chromatography mass spectrometry. Hierarchical cluster analysis grouped SFFPs into three clusters according to their concentrations and compositions of volatile components. Partial least squares-discriminant analysis showed that volatile compounds, including ethyl phenylacetate, 5-methyl furfural, amyl cinnamal, ethyl myristate, decyl aldehyde, 1-phenylethyl acetate, 1-octen-3-ol, 3-buten-2-ol, butanoic acid, and caproaldehyde, were highly negatively correlated with saltiness, sourness, and bitterness, while they were positively correlated with sweetness, umami, richness, and acceptance. The obvious correlation between flavor profiles and sensory attributes could help online monitoring of SFFPs' flavor quality during production.

1. Introduction

Sweet fermented flour pastes (SFFPs) are a wheat-based food, mainly made using wheat flour (or sometimes soybean flour), which is mixed with salt. The mixture is processed by grinding and steaming, followed by fermentation for 30 days by microorganisms such as *Aspergillus oryzae*. Subsequently, the mixture is sterilized at an industrial level [1]. SFFPs are widely consumed in Eastern countries because of their complex aroma characteristics and significant health benefits, such as anticancer, antihypertension, and antioxidative activities [2]. The flavors and taste varieties of fermented flour pastes include sweetness, saltiness, umami, and many other complex flavors, such as garlic, seafood, and mushroom [3, 4]. Arabinoxylans (AX) constitutes the predominant part of the nonstarch polysaccharides of wheat flour which originate in the cell walls. During fermentation process of flour paste (miso paste in Japan), many flavor compounds including alcohols and volatile phenols are produced. 4-Vinylphenols as the characteristic flavors of miso paste could be formed from the proposed precursors, such as lignin, shakuchirin, and esters with arabinoxylan [5]. However, the traditional method of processing SFFPs has been adapted for mass production using different fermentation conditions for *Aspergillus oryzae*; thus, different manufacturing companies produce commercial SFFPs with different flavor profiles.

Recently, both consumers and the fermented paste industry have focused on aroma as a quality criterion, and studies have been published on the volatile compounds in various fermented soybean and wheat flour products [6–9]. SFFPs aromas are formed during steaming and fermentation. During the fermentation phase, secondary metabolic activities of microorganisms result in the release a variety of volatile components, which are strongly affected by the fermentation time and temperature. Volatile components of commercially and traditionally manufactured fermented soybean/flour pastes are vital contributors to quality parameters [10, 11]. Researchers have focused on the aroma-active compounds of SFFPs [10] and the changes of microflora and flavor [12, 13]. However, the role of volatile components on SFFPs sensory qualities, as affected by different manufacturing processing, has not been studied before.

Multivariate analysis, such as hierarchical cluster analysis (HCA) and principal component analysis (PCA), is a powerful tool to summarize and explain large datasets statistically and visually [14, 15]. Cluster analysis relies on minimization of distances between groups of variables, so that a large number of variables can result in sphericity, an instance where the cumulative variances in multidimensional datasets fully fill the lower-order dimensional space, making clusters hard to distinguish in a two- or three-dimensional visualization. Cluster analysis can also be used as a useful statistical tool to identify the clusters of SFFPs based on a volatile variation hierarchical algorithm. PCA can simplify the analysis by displaying similarities and differences among different samples by compressing the number of dimensions without loss of information. PCA was used successfully to correlate the aroma compounds' sensory perception and chemical characteristics in Chinese famous liquors [16]. One of the most popular methods is partial least squares-discriminant analysis (PLS-DA), which is also called PLS2. PLS-DA is particularly appropriate for the analysis of large, highly complex, and noisy datasets that are common output(s) where the number of variables often far exceeds the number of samples. This chemometrics technique is used to optimize separation between different groups of samples, which is accomplished by linking two data matrices X (i.e., raw data) and Y (i.e., groups and class membership). PLS-DA can handle multiple dependent categorical variables. This approach aims to maximize the covariance between the independent variables X (sample readings) and the corresponding dependent variable Y of highly multidimensional data by finding a linear subspace of the explanatory variables. These factors describe the behavior of dependent variables Y and they span the subspace onto which the independent variables X are projected [17]. In addition, one of the perceived advantages of PLS-DA is its widespread availability among the most well-known statistical software packages, where its implementation is very easy if the default settings are used [17]. If a clear correlation and statistically significant discrimination among samples can be found, the method can be applied to the experimental data collected using different instrumental techniques and strategies [14, 18–20]. PLS-DA has been widely used to evaluate relationships between macronutrients and mineral composition of diet, health, and disease in food [21, 22].

Different brands of SFFPs, including Lijinji (LEE), Liubiju (LS), Juancheng (PS), and Xinhe (XH), are well known in their corresponding geographic regions; however, their flavor profiles are quite different because of varying quality standards under different processes used in their manufacture. With the rapidly growing popularity of these unique tastes and flavors, it is necessary to control the flavor quality and to develop appropriate methodologies for online monitoring of SFFPs' flavor. Therefore, the objectives of this study were as follows: (1) to examine the aroma composition and sensory attributes of various SFFPs and (2) to determine the correlations among sensory attributes and flavor characteristics of SFFPs from different geographical regions.

2. Materials and Methods

2.1. Materials. Phenethyl acetate and n-alkanes (C8–C20) were purchased from Sigma-Aldrich (St Louis, MO, USA). Sucrose, sodium chloride, citric acid, caffeine, and monosodium glutamate (MSG) were of food grade and were acquired from commercial sources. Seven kinds of SFFP from representative locations of origin (with different flavors) were purchased from Suguo Supermarket (Nanjing, China). LEEH and LEEJ were from Guangdong, LS was from Beijing, PS was from Sichuan, XH6 was from Shandong, DFS was from shanghai, and HJ was from Hunan, respectively. SFFPs were made of wheat flour and/or soybean flour, mixed with salt and sugar. The mixture was processed by steaming for 1 hour, followed by fermentation at $40 \pm 2^{\circ}$C for 30 days using microorganisms such as *Aspergillus oryzae*. Subsequently, the mixture was sterilized at 90°C for 30 min to achieve commercial sterility. In this study, SFFPs were randomly selected in two batches and labeled according to their different processing dates.

2.2. Identifying Volatile Composition

2.2.1. Extraction of Volatile Compounds. The volatile compounds were extracted using a solid-phase microextraction fiber (SPME, 75 μm, Divinylbenzene/Carboxen/Polydimethylsiloxane) in the samples' headspace and determined by headspace-gas chromatographic-mass spectrometry (GC-MS, Finnigan Trace GC-MS, Finnigan, USA). The sample (5.0 g) was weighed and placed in a 15 mL vial. Immediately, 10 μL of phenethyl acetate solution (5 μg/mL in methanol) as an internal standard was added to each sample before the trap. The vial was sealed with a Polytetrafluoroethylene (PTFE)/BUTL septum and equilibrated at 40°C for 20 min in the sample headspace. After equilibrium, the SPME fiber was desorbed into the injector port at 250°C for 3 min in splitless mode.

2.2.2. GC-MS Analysis. The volatile compounds were separated using a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technology, Inc., CA, USA). The temperature program employed was as follows: maintenance at 35°C for 4 min, and programming from 35 to 70°C at 15°C/min, then increasing to 110°C at a rate of 3°C/min, followed by increasing to 160°C at 6°C/min, and ramping to 220°C at 10°C/min. Helium was used as the carrier gas at a constant velocity of 1.8 mL/min. To obtain the linear retention index values of the volatile compounds, a series of n-alkanes (C8–C20) was run under the same conditions. Mass spectra were obtained in an electron impact mode, with an energy voltage of 70 eV and emission current of 35 μA. The detector was set at a scanning range of 35 to 450 m/z at a rate of 4.45 scans/s.

Table 1: Basic compositions of SFFPs (mean ± Std., $n = 2$).

	LS	XH6	LEEH	LEEJ	HJ	PS	DFS
Salt (%)	5.69 ± 0.38	4.48 ± 0.08	3.61 ± 0.40	3.32 ± 0.13	4.13 ± 0.69	4.43 ± 0.00	4.44 ± 0.27
Amino-nitrogen (g/ml)	0.79 ± 0.01	1.13 ± 0.01	0.47 ± 0.01	0.30 ± 0.01	0.72 ± 0.01	0.58 ± 0.01	0.48 ± 0.01
Protein (%)	8.01 ± 1.26	7.59 ± 1.37	10.71 ± 0.73	10.92 ± 1.45	7.43 ± 1.35	6.36 ± 1.92	6.12 ± 2.18
Crude fat (mg/100 g)	0.91 ± 0.08	0.53 ± 0.07	0.65 ± 0.031	0.37 ± 0.08	0.34 ± 0.01	0.48 ± 0.08	0.47 ± 0.14
Total acid (g/kg)	2.17 ± 0.04	3.41 ± 0.03	1.02 ± 0.01	1.01 ± 0.01	0.99 ± 0.07	1.04 ± 0.02	0.66 ± 0.02
Reducing sugar (g/100 g)	2.09 ± 0.60	1.81 ± 0.35	1.80 ± 0.77	1.76 ± 0.83	2.06 ± 0.71	2.11 ± 0.92	2.38 ± 0.70

2.2.3. Identification and Quantification of Volatile Compounds. Volatile compounds were either identified by comparison of ion spectra using the NIST and WILEY libraries (Scientific Instrument Services, Inc., Ringoes, NJ) or the Kovats index (http://www.flavornet.org). Approximate quantities of the volatile compounds were estimated by comparison of their peak areas with that of internal standard, phenethyl acetate, as obtained from the total ion chromatograms.

2.3. Sensory Evaluation. A sensory panel comprising 11 individuals (20–50 years of age, seven females and four males) was employed to evaluate the sensory attributes of SFFPs. The panelists were trained or had previous experience in sensory evaluation of various food products, including similar fermented paste products. Before sensory evaluation, all panelists were trained at least for 4 weeks (three times per week) and for up to 1 h in each training session. Seven taste attributes were generated to characterize the sensory properties of the SFFPs from the descriptive analysis. The descriptions and reference samples for the attributes were as follows: sweet (2% sucrose solution), salty (0.5% sodium chloride solution); sour (0.03% citric acid solution); bitter (0.03% caffeine solution), umami (0.3% MSG solution) [23].

To perform the sensory analysis by mimicking normal consumption, 2 h before the experiment, 5 g of each sample of SFFP was dissolved in 90 mL of water and warmed at 40°C. The samples were served to every panelist in a randomized manner during each session [15, 20]. The samples were individually labeled with three-digit random numbers and were served one at a time in a random order. To rate the samples, the panelists used a nine-point scale with word anchors at each end. The samples were evaluated for overall acceptability (1 = very unacceptable; 9 = very acceptable), sweetness, umami, saltiness, and richness intensity (1 = very weak; 9 = very strong). Sourness and bitterness were considered as undesirable tastes (1 = very strong; 9 = very weak). There was a 5 min interval between the evaluations of each sample. Panelists were served distilled water (30°C) and salt-free plain sliced bread before the first sample and between each sample to cleanse the mouth.

2.4. Statistical Methods. The data were analyzed by ANOVA using the SPSS statistical program (SPSS13.0, SPSS Inc., New York, USA). Differences among the means were compared using Duncan's multiple range test ($p < 0.05$) in origin 8.0 (OriginLab Corporation, Northampton, MA, USA). All determinations were repeated more than twice. PCA and HCA were performed using Unscrambler version 9.7 (CAMO ASA, Oslo, Norway) to analyze the relationship between different SFFPs samples based on volatile compounds and sensory properties. PLS-DA handles several responses simultaneously with GC-MS data as the X- or Y-matrix, and sensory data averaged across measurements as the Y- or X-matrix. All data responses by autoscaling (1/Sdev) were used, which gave all the variables the same variance.

3. Results and Discussion

3.1. Sensory Evaluation. The sensory characteristics of SFFPs including saltiness, sourness, bitterness, sweetness, umami, richness, and overall acceptance were evaluated. Five basic tastes (saltiness, sourness, bitterness, sweetness, and umami) are recognized by the taste buds. As shown in Figure 1, all attributes were significantly different among the samples ($p < 0.05$). LEEJ, LEEH, and XH6 showed high overall acceptance scores of 8.00, 8.21, and 7.50, respectively. The sweetness of LEEJ, LEEH, XH6, and DFS was significantly higher than that of HJ, LS, and PS. Moreover, LEEH showed the strongest umami and richness taste, but weak saltiness, sourness, and bitterness, producing high overall acceptance. However, LS showed much lower umami and richness, but higher bitterness, sourness, and saltiness compared with the other samples, resulting in lower overall acceptance. Sourness is mainly caused by organic acids [24]. Bitterness and sourness of SFFPs are often considered distasteful. Saltiness is mainly caused by sodium salt, a good indicator of electrolyte balance in foods, acting as "flavor enhancers" by releasing sweetness and suppressing bitterness [25]. Umami indicates the presence of amino acids and flavor peptide, and sweetness involves nutrient sources, such as sugars or sugar alcohols. Richness is mainly produced by unique peptides and is considered a good indicator of taste quality in the broad sense [26, 27]. The presence of diverse tastes in SFFPs might be attributed to their basic composition (Table 1) and volatile compounds.

3.2. Volatile Compounds and Their Contribution to the Aroma Characteristics of SFFPs. A total of 96 volatile compounds were detected by GC-MS coupled with SPME (supplementary Table S1) and were subdivided into different classes, including 12 acids, 17 alcohols, 23 aldehydes, 33 esters, four ketones, three nitrogenous compounds, and four lactones. All the compounds were consistently quantified using the internal standard method. As presented in Figure 2, LEEJ showed the highest concentrations of acids, aldehydes, esters, and lactones compared with the other SFFPs, while the

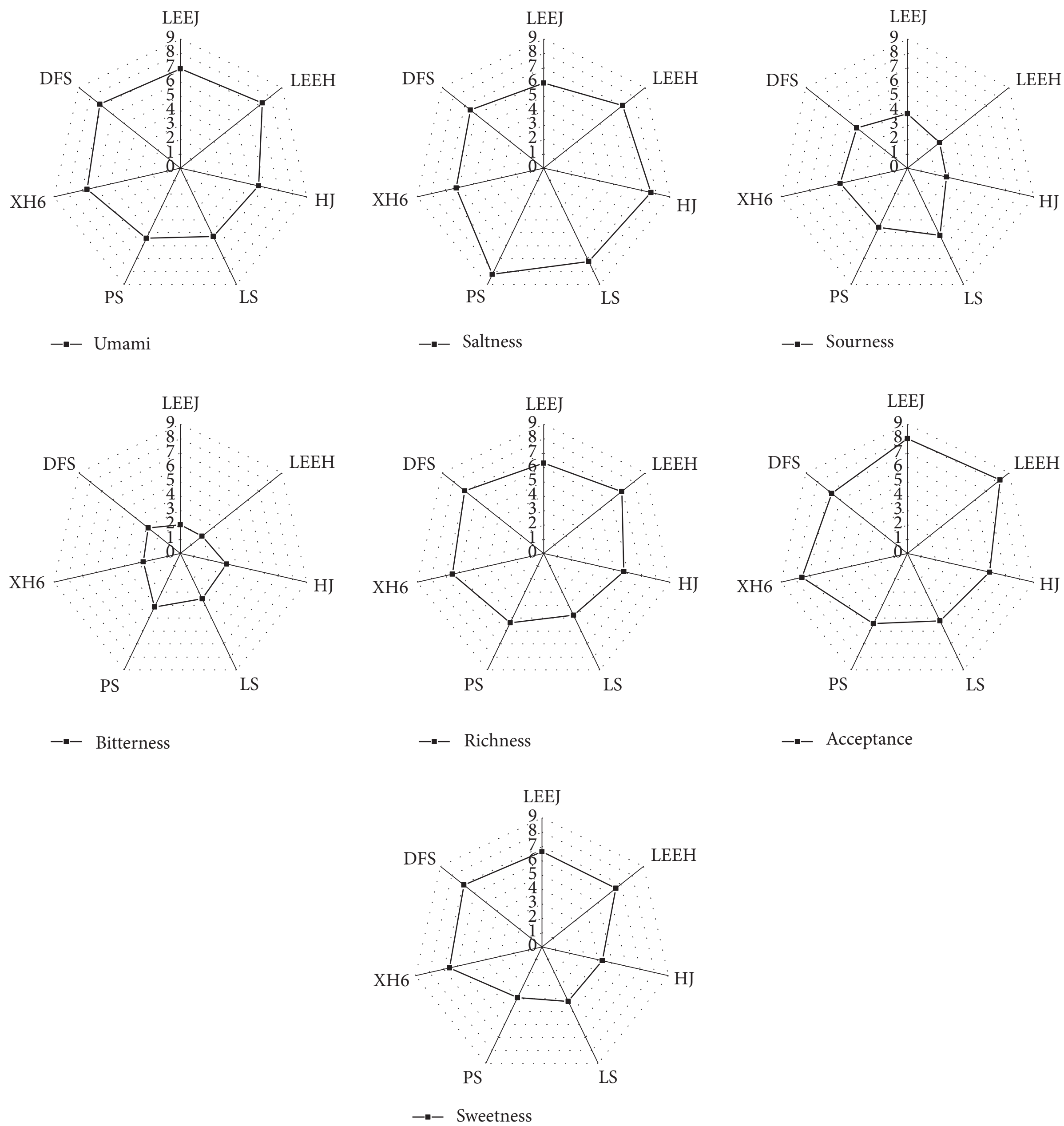


Figure 1: Radar plot of the SFFPs sensory descriptors, as assessed by trained panelists for each attribute. Sensory evaluation scores in the plot are means of triplicate analysis by 11 panelists.

concentrations of ketones and nitrogenous compounds were higher in LEEH. DFS exhibited the highest concentration of alcohols ($p < 0.05$). Aldehydes are important aroma-active compounds in foods because of their low threshold values, which contribute to desirable aromas, as well as rancid odors and flavors [28]. During aging of a paste, transformation of liberated amino acids from peptides and proteins into Strecker aldehydes and other flavor and aroma compounds plays an important role in the development of aged flavors [29]. The main aldehydes identified included phenylacetaldehyde, 2-methylbutyraldehyde, 5-methyl furfural, and nonylaldehyde. Their concentrations were higher in LEEJ compared with those in the other SFFPs. Esters were the second most prominent contributors to the aroma of SFFPs. LEEJ also showed a higher esters concentration than the other SFFPs. Certain esters, such as *n*-butyl acetate, but notanoic acid ethyl ester, and amyl-2-methylbutyrate, at higher concentrations might impart sweet and fruity flavors to SFFPs [30].

In addition, the alcohols accounted around the half of the total volatiles in all samples. Among the detected alcohols, 2-methyl-1-butanol, 3-methylthiopropanol, 2-methyl-3- phenyl-2-propen-1-ol, and 3-furanmethanolmight make significant contributions to the aroma of SFFPs because of their low threshold values [31, 32]. Alcohol might be formed during the fermentation process by secondary decomposition

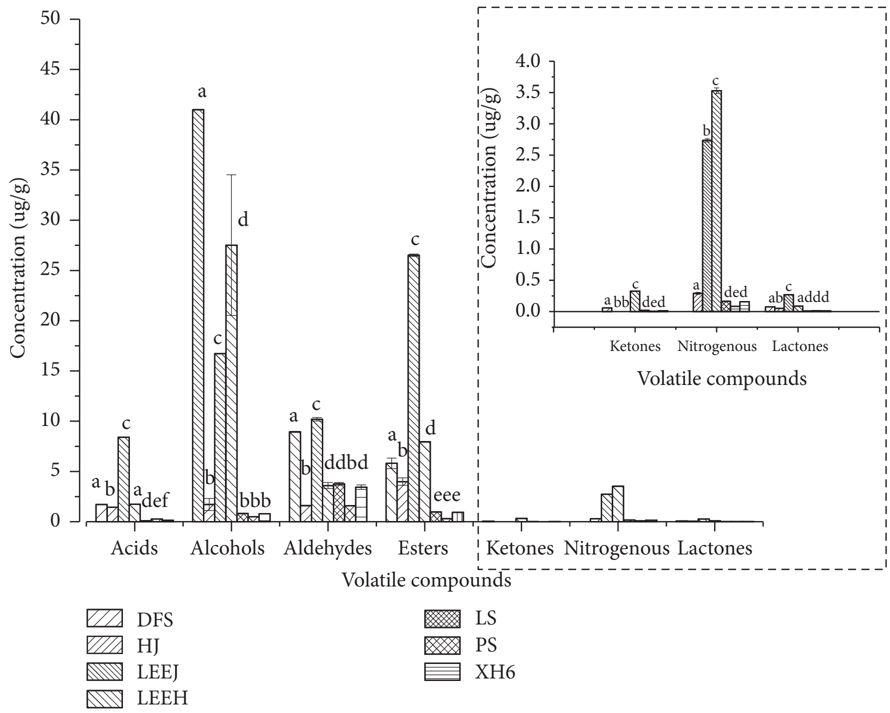


Figure 2: The compositions of volatile compounds in SFFPs. Bars show the mean (±SD) of duplicate analysis from GC-MS data. Different letters near the bar indicate significant differences ($p < 0.05$). Enlargement of the box (dashed line) is displayed for the differences in ketones, nitrogenous compounds, and lactones. Different letters represent a significant difference ($p < 0.05$).

of hydroperoxide of fatty acids via two different pathways, including the Embden–Meyerhof–Parnas pathway and the Ehrlich pathway [33]. Nitrogenous compounds are known to provide a strong flavor to fermented products. A high concentration of nitrogenous compounds was detected in LEEH and LEEJ, at 3.53 μg/g and 2.74 μg/g, respectively. PS showed the lowest nitrogenous compounds concentration (0.83 μg/g) among the SFFPs. Fermentation from nonstarch polysaccharides impacts the aroma compounds types and yield. Among the various classes of volatile compounds, nitrogenous heterocyclic compounds, such as furans and pyrroles, have been reported to provide odor attributes to fermented foods. Furans can be formed through the Amadori rearrangement pathways in dehydrated or fermented condensates of carbohydrates [34]. *Aspergillus oryzae* showed convertibility of ferulic acid (FA) of arabinoxylan to 4-vinylguaiacol (4-VG) and to 4-ethylguaiacol (4-EG), which are characteristic flavors of miso paste [35]. In addition, ketones and lactones showed relatively low concentrations, suggesting that they might have little effect on the aroma of SFFPs.

However, from the aroma composition and sensory characteristic results, it could be speculated that the overall taste and aroma of SFFPs were dependent on particular components, such as the "critical balance" and "weighted concentration ratio" of all the present components. Odorants, inducing olfactory perception, could strongly modulate taste perception, such as enhancing or reducing bitterness, sweetness, and sourness [6, 36]. Therefore, to demonstrate the differences in flavor characteristics of SFFPs, the relationship between volatile components and sensory properties was evaluated using multivariate analysis.

3.3. Multivariate Analysis

3.3.1. Relationship among SFFPs by Cluster Analysis. Cluster analysis was applied to the response of concentration of volatile compositions by squared Euclidean measurement. The derived normalization of the data set substantially improved the classification of SFFPs. Three distinct clusters were separated out in the resulting dendrogram after performing cluster analysis, enabling a correct classification for all samples. Figure 3 shows the HCA of seven SFFPs with the 96 characteristic volatile compounds as variables. Distinct clusters were identified, indicating that LEEJ and LEEH were markedly different from other SFFPs. Samples were divided into three main branches: A, B, and C. Interestingly, LEEJ and LEEH were grouped in the same cluster, which suggested that they were closely related. In addition, HJ, PS, XH6, and LS were grouped in the same cluster, confirming their similarities. By contrast, DFS was the only SFFP in separated cluster B. These distinct differences might be caused by the volatile compounds compositions. These results are also consistent with their sensory properties. Ongoing follow-up will allow us to confirm whether these clusters correspond to sensory characteristics.

3.3.2. Correlations between Volatile Compounds and Sensory Attributes. PCA, as one of the multivariate statistical techniques, has the advantage of capturing the interactions among

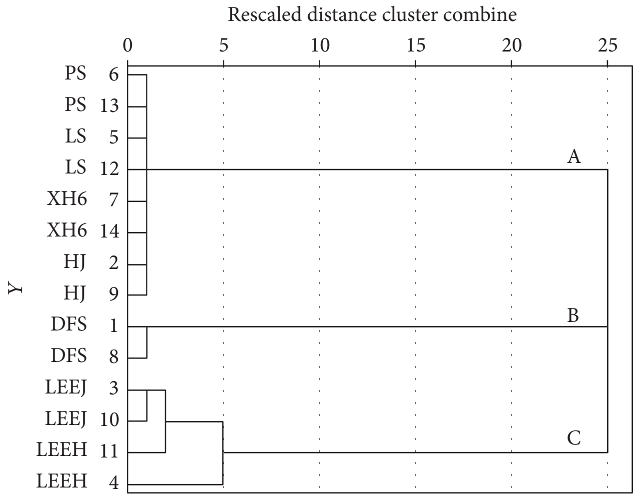


Figure 3: Dendrogram from the hierarchical cluster analysis using centroid clustering of seven SFFPs samples with the relative abundances of 96 volatile compounds as variables. The different clusters are marked as A, B, and C. x-axis means rescaled distance cluster; y-axis means the samples measured.

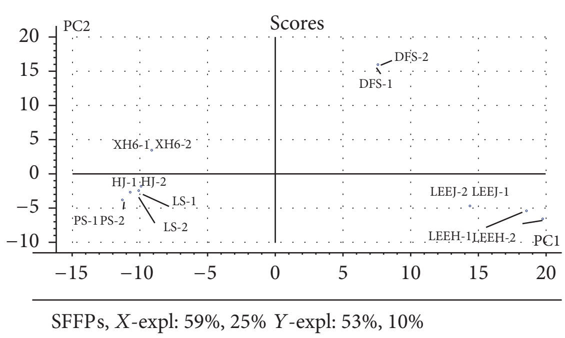
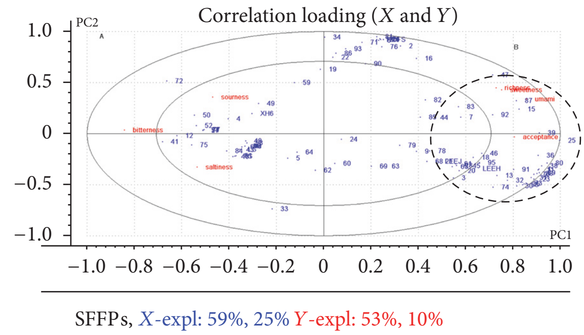
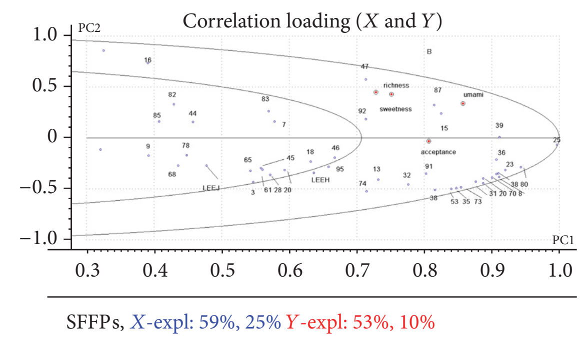


Figure 4: PCA scores derived using the average concentration of volatiles detected by GC-MS in different SFFPs samples.



(a)



(b)

Figure 5: (a) Correlation loadings plot for sensory attributes (Y-matrix) and GC-MS data (X-matrix), PC1 versus PC2. Significant variables are marked with circles. Ellipses show $R^2 = 50\%$ and 100%, respectively. (b) Enlargement of the ellipse (dashed line) labeled in (a). The Arabic numbers marked for X variables correspond to the volatile compound names in Table S1.

parameters. To simplify the interpretation of relationships between volatile compounds detected by GC-MS and sensory attributes in the different samples, PCA was carried out with 96 volatile compounds and the sensory attributes of the seven SFFPs samples. As shown in Figure 4, the scores of the variable values were plotted and showed a clear discrimination of the observations. The 96 volatile compounds were designated as the X-matrix, while the Y-matrix consisted of seven sensory attributes data. The model included three significant PCs explaining 84% of the cross-validated variance. The first principal component (PC1) and second principal component (PC2) are shown in Figure 5. LEEJ and LEEH were located in the right side of the X-matrix, far from HJ, LS, and PS in the PC1 axis, indicating their distinct difference from the other SFFPs. These results agreed with the findings of HCA. In addition, XH6 and DFS seemed to possess almost similar characteristics, because they were loaded closer to each other in the PCA plot. PC1 accounted for 53% and its principal component loadings (Eigen vectors) relatively crossed the volatile constituents (Figure 5(a)). Therefore, the majority of the variation captured by PC1 for all the volatiles and sensory characteristics served to distinguish the different SFFPs samples. The inner and outer concentric circles indicate 50% and 100% of the explained variance. The correlation loadings plot of the first two components (PC1 and PC2) in Figure 5(b) shows that the GC-MS variables are highly correlated with sensory attributes. In detail, sweetness, umami, richness, and acceptance, marked with small circles, were closely located with ethyl phenylacetate, 5-methyl furfural, amyl cinnamal, ethyl myristate, decyl aldehyde, 1-phenylethyl acetate, 1-octen-3-ol, 3-buten-2-ol, butanoic acid, and caproaldehyde on the right side along the X-matrix. However, these compounds were negatively correlated with saltiness, sourness, and bitterness. Therefore, the results produced from PLS-DA were highly consistent with those from the HCA.

4. Conclusions

In the present study, different data fusion strategies were used to discriminate the flavor difference among SFFPs to understand the potential correlation between the sensory profiles and volatile compounds. The volatile compounds responsible for the sensory profiles were successfully highlighted. Hierarchical clustering analyses grouped SFFPs based on their composition and concentration of volatile compounds, which could help to determine the sensory difference among samples. The correlations between the sensory attributes and the GC-MS variables were demonstrated according to the PLS loading plot. Thus, the correlation between characteristic compositions and jagged qualities of products can be useful for online monitoring of the flavor quality of SFFPs during production. Furthermore, it represents a great step toward achieving a standard procedure and aroma criteria for SFFP production, in contrast to the current situation, which mainly relies on local experience.

Abbreviations

HCA:	Hierarchical cluster analysis
PCA:	Principal component analysis
PLS-DA:	Partial least squares-discriminant analysis
SPME-GC-MS:	Solid-phase microextraction gas chromatography mass spectrometry
SFFPs:	Sweet fermented flour pastes.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Meigui Huang and Yulin Li contributed equally to this study.

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Supplementary Materials

Table S1. Concentration of volatile compounds of seven SFFPs by GC-MS (means ($n = 2$), μg/g). #Each compound was separated with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and identified by comparing it with an authentic standard based on the following criteria: (A) matching retention time on the same column from http://www.flavornet.org, (B) mass spectrum using the NIST and WILEY library. †Values expressed as phenethyl acetate equivalent (5 μg/mL) and given means ($n = 2$); n.d., not detected. *(Supplementary Materials)*

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