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Relationship between Fungal Communities and Volatile Flavor Components during the Traditional Chinese Fermentation of *Capsicum annuum* L. Var. *Dactylus* M

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Abstract: Microbial diversity and dynamic changes play an important role in the production of fermented peppers. In this study, the relationship between fungal communities and the volatile flavor compounds of traditional Chinese fermented peppers was investigated by high-throughput sequencing technology. The results showed that *Hanseniaspora* was a dominant fungus during the whole fermentation course and accounted for 82.22% of the fungal community on average (ranging from 50.44% to 98.15%). Bidirectional orthogonal partial least squares (O2PLS) analysis between fungal community and volatile flavor compounds showed that *Pichia*, *Hanseniaspora*, *Cryptococcus*, *Debarvomvces*, and *Trichosporon* were closely correlated with the concentrations of the volatile flavor components such as α-terpineol, trans-3-tetradecene, 4-methylpentyl 3-methylbutanoate, and 11 other volatile flavor compounds. This study elucidated the dynamics of fungal communities and volatile flavor compounds during pepper fermentation and the correlation between them. Our analysis of the relationships between fungal communities and volatile flavor compounds advanced our understanding of the formation mechanism of volatile flavor compounds in fermented peppers.

Keywords: *Capsicum annuum* L. Var. *Dactylus* M; fungal diversity; volatile favor compounds; correlation analysis

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1. Introduction

Pepper (*Capsicum* spp.) is one of the most important fruit crops worldwide. It is cultivated all over the world, primarily in tropical and subtropical countries. The *Capsicum* genus belongs to the Solanaceae family and includes 27 recognized species. There are five distinct cultivated species: *C. annuum* L., *C. frutescens* L., *C. Chinese* Jacq., *C. baccatum* L., and *C. pubescens* Ruiz et Pav [1]. *C. annuum* L. is the most widely cultivated pepper species in the world. Its variants are mainly var. *cerasiforme* Irish, var. *conoides* Irish, var. *fasciculatum* Sturt, var. *longum* Sendt, var. *grossum* Sendt, and var. *Dactylus* M [2]. Pepper (*Capsicum* spp.) is not only an important vegetable, but it can also be processed into condiments, spices, coloring agents, etc. [3]. According to the amount of salt used for fermentation, fermented peppers can be divided into high-salt-content and low-salt-content fermented peppers [4]. Fermented peppers with a high salt content can be stored for about one year after desalination, seasoning, bottling, vacuum sealing, and sterilization [5]. Most low-salt-content fermented peppers are currently made from high-salt-content fermented peppers that have been desalted, seasoned, bottled, vacuum sealed, and sterilized to

become low-salt-content fermented pepper products. However, this method produces products with poor flavor and generates large amounts of brine and pepper juice during the desalination process, resulting in environmental pollution and resource waste [6]. On the other hand, fermentation with a low salt content of 5–12% is difficult to control due to the abundance of microbial species [7].

The fermentation, flavor, safety, and preservation of fermented peppers are strongly dependent on microorganisms. In our previous study, the fungal communities in fresh and fermented *C. annuum* L. var. *fasciculatum* Sturt were investigated by 454 pyrosequencing. A wide variety of fungi such as *Hanseniaspora*, *Pichia*, and *Debaryomyces*, which are known to influence the flavor and maturation of fermented peppers, were isolated and identified [8]. *Capsicum annuum* L. var. *fasciculatum* Sturt and *Capsicum annuum* L. Var. *Dactylus* M are two pepper varieties that are very different in their pungency and water content. There may be different species and abundances of endophytic bacteria and fungi on the surface of different varieties of fresh pepper due to their varying water content, capsaicin content, and growth environments [9]. The microbial composition and relative abundance of different pepper varieties may be further complicated by the process of fermentation. However, the association between these microbiota and flavor components is poorly understood. In recent years, a large number of studies have been conducted to explore the correlation between microorganisms and the flavor components and microorganisms of fermented foods. Yang, et al. [10] elucidated the relationship between microbial genera and fresh taste peptide formation during the fermentation of stinky cinnamon by peptidomic and macrogenomic analyses, and the results showed that *Vagococcus*, *Peptostreptococcus*, *Acinetobacter*, *Psychrobacter*, and *Enterococcus* play a major role in the formation of fresh taste peptides. Zhang, et al. [11] characterized the aroma profile, including the key aroma compounds and bacterial community, of tempeh after fermentation and investigated the correlation between the dominant bacterial genera and the key aroma compounds, showing that five dominant bacterial genera were positively correlated with more than six key volatile compounds. Therefore, in order to standardize fermentation, it is crucial to elucidate the key microbial communities in traditional Chinese fermented peppers.

The aim of this study was to identify the succession of fungal communities during the fermentation of *Capsicum annuum* L. Var. *Dactylus* M by high-throughput sequencing technology. Changes in the volatile components during fermentation were identified using GC–MS. Based on this information, the relationship between fungal communities and volatile flavor compounds was revealed by two-way orthogonal partial least squares (O2PLS). Our results can provide a reference for studying the interactions between microbial communities and metabolites in fermentation systems.

2. Materials and Methods

2.1. Sample Preparation and Collection

The pepper species used in this study was *Capsicum annuum* L. var. *Dactylus* M, which is slender and has a low pungency degree (about 2600 SHU) and high water content (about 84%, *w*/*w*). Fresh *Capsicum annuum* L. Var. *Dactylus* M was cleaned, chopped, salted with 8% (*w*/*w*) salt, placed in 8 sterile pickle jars with the same mass, covered, sealed with water, and fermented in a 30 °C incubator. In order to reveal the fungal communities in the fermented pepper, one pickle jar was removed from the incubator on the 3rd, 5th, 7th, 9th, 11th, 14th, 17th, and 20th fermentation days for sampling. Samples were marked X_3, X_5, X_7, X_9, X_11, X_14, X_17, and X_20, respectively. X_0 was cleaned fresh *Capsicum annuum* L. Var. *Dactylus* M. All samples were stored at −20 °C.

2.2. Fungal Community Analyses by High-Throughput Sequencing

2.2.1. DNA Extraction

Total genomic DNA was extracted from the samples by using a previously reported method [8]. Total microbial DNA was extracted from 0.2-1.0 g pepper using the E.Z.N.A Soil DNA kit (OMEGA, Bio-Tek, Norcross, GA, USA) according to the manufacturer's

protocol. The extracted DNA was purified using an AxyPrep™ DNA Gel Extraction Kit (OMEGA, Bio-Tek, Norcross, GA, USA), and the DNA concentration and quality were checked on 2% agarose gel. The purified DNA was stored at −20 °C until quantitative PCR and 454 pyrosequencing analysis.

2.2.2. PCR Amplification

Fungal ITS rDNA genes were amplified using the forward primer ITS1 (5′-TCCGTAG GTGAACCTGCGG-3′) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR amplifications were carried out according to the previously reported method [8]. The amplification products were visualized on 2.0% agarose gels and purified using an AxyPrep™ DNA Gel Extraction Kit (OMEGA, Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. All PCR reactions were repeated in triplicate using the DNA extracted from each pepper sample.

2.2.3. Pyrosequencing Analysis

After being purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison, Wis, USA), a mixture of amplicons of each sample was used for pyrosequencing on a Roche 454 GS FLX + Titanium platform (Roche 454 Life Sciences, Branford, CT, USA) according to standard protocols.

2.2.4. Bioinformatics Analysis

After sequencing, low-quality sequences were filtered, and high-quality sequences were clustered into operational taxonomic units (OTUs). All sequences were classified from phylum to genus at the 97% sequence similarity level according to silva (version 115 http://www.arb-silva.de accessed 20 July 2021) using the RDP Classifier (version 2.2 http://sourceforge.net/projects/rdp-classifier/ accessed 20 July 2021) of the Qiime platform. Sequences that could not be classified into any known group are labeled "unclassified". Taxa in proportions <1% were grouped as "Others". Rarefaction analysis, OTU cluster analysis, and principal component analysis (PCA) were performed using Mothur, as reported in our previously study [8].

2.3. Qualitative and Semi-Quantitative Determination Analysis of Volatile Components

A 3.0 g sample was placed into a headspace injection bottle, 2 mL deionized water and internal standard o-dichlorobenzene were added, and the bottle cap was tightened. The extraction head was inserted into the injection port of the GC–MS, and the fiber head was activated at 270 °C for 60 min. The headspace injection bottle was preheated at 70 °C for 15 min on a constant temperature magnetic stirrer. The aged extraction head was inserted after preheating, and the fiber head was pushed out for extraction for 30 min. After extraction, the fiber head was retracted and inserted into the GC–MS injection port. The fiber head was pushed out for 5 min. The column temperature was 40 °C. The initial temperature of the first stage was 40 °C, which was maintained for 3 min. In the second stage, the temperature was raised to 150 °C for 7 min at a rate of 5 °C/min. In the third stage, the temperature was raised to 270 °C for 2 min at a rate of 10 °C/min. In the splitless mode, the flow rate had a pressure of 33.8 kPa, total flow was 124.1 mL/min, column flow was 0.8 mL/min, linear velocity was 32.3 cm/s, and purge flow was 3.0 mL/min. Mass spectrometry conditions were as follows: interface temperature 220 °C, ion source temperature 200 °C, ionization mode EI, and ionization voltage 70 ev. The scanning range was 45–500 (M/z). The identification of VOCs was performed by comparing the retention index and matching mass spectra fragment with the NIST14, NIST17 databases (matching degree > 80).

2.4. Statistical Analysis

Significant differences were calculated with one-way ANOVA in SPSS 20.0 (International Business Machines Corp., Armonk, NY, USA). The line graph was created using OriginPro 2019 (OriginLab Corp., Northampton, Mass, USA). To study the dynamic succession of the microbial community, hierarchical cluster analysis (HCA) was carried out using OriginPro 2019 (OriginLab Corp., Northampton, Mass, USA). The heatmaps and stacked histogram of the relative abundance at the microbial genus level were created using OriginPro 2019 (OriginLab Corp., Northampton, Mass, USA). Bidirectional partial least squares (O2PLS) modeling was used to estimate the relationship between microbiota and volatile compounds. The visualized network planning of the Pearson correlation coefficient was conducted using Cytoscape 3.8.2.

3. Results

3.1. PCR Amplification of Fungal 18S rDNA Genes

The PCR amplification results for the fungal ITS genes are shown in Figure 1. All the amplicons of fermented pepper were bright and clear, and there were few nonspecific bands. The concentration of PCR products was greater than 5 ng/μL, and the $OD_{260/280}$ was between 1.8 and 2.0. The PCR amplification products were of good quality for pyrosequencing analysis.

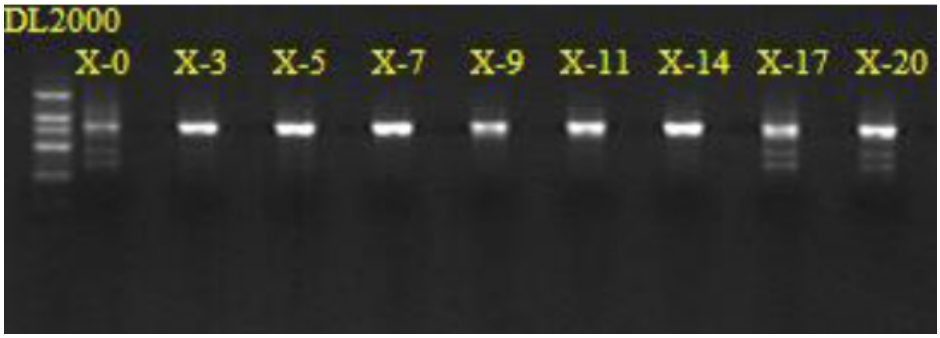

Figure 1. 2% agarose gel electrophoresis of PCR amplification result for fungal 18S rDNA genes using universal primers ITS1/ITS4. DL2000: DL2000 marker (from top to bottom: 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp, and 100 bp). Target DNA bands of samples (about 800 bp).

3.2. Fungal Abundance and Diversity in the Fresh and Fermented Capsicum annuum L. Var. Dactylus M

The rarefaction curve and the Shannon diversity curve of the fungi for each sample are presented in Figures 2 and 3, respectively. When the sequencing amount increased to about 1000, the rarefaction curves and the Shannon diversity curves of all samples tended to be flat, and the sequencing amount reached saturation. The maximum sequencing amount of the experiment was 5000, which met the requirement of sequencing and implied that the sequencing depth was reasonable. The fungal abundance and diversity of each sample are shown in Table 1. The nine samples yielded 53,282 high-quality fungal 18S rDNA gene sequences with an average length of about 447 bp. After the low-quality sequences were filtered out, 10,887 trimmed sequences with an average length of about 430 bp were obtained and clustered into OTUs. In total, 225 OTUs were identified at a 97% similarity level for fresh *Capsicum annuum* L. Var. *Dactylus* M, and 82 OTUs on average (from 47 to 163) were identified for fermented *Capsicum annuum* L. Var. *Dactylus* M. Shannon diversity (H) and Simpson diversity (D) were calculated to describe the microbial diversity, while the Chao1 and ACE indices were positively correlated with the change in species richness. As shown in Table 1, sample X_0 exhibited the highest fungal diversity (ACE = 413, Chao 1 = 325, H = 3.87, D = 0.0536). During the fermentation process, the fungal diversity of the peppers decreased, which may have been due to the increase in acid

inhibiting the growth of fungi. Fungal diversity decreased to the minimum level on the seventh fermentation day (ACE = 96, Chao 1 = 68, H = 1.22, D = 0.6161) and then increased.

Table 1. Fungal diversity and richness estimators of fresh and fermented *Capsicum annuum* L. Var. *Dactylus* M based on the 454 pyrosequencing data.

Sample ID	Valid Reads	OTU	Estimator (ACE)	Chao1 Diversity	Shannon Diversity (H)	Simpson Diversity (D)
X_0	6931	225	413	325	3.87	0.0536
X_3	3750	54	140	90	2.04	0.3535
X_5	6024	61	166	123	1.49	0.5342
X_7	6151	47	96	68	1.22	0.6161
X_9	5664	98	431	264	2.06	0.366
X_11	5932	101	203	257	2.3	0.3047
X_14	6200	55	154	89	1.16	0.6345
X_17	6852	163	484	335	2.79	0.1905
X_20	5778	83	146	169	1.72	0.4583

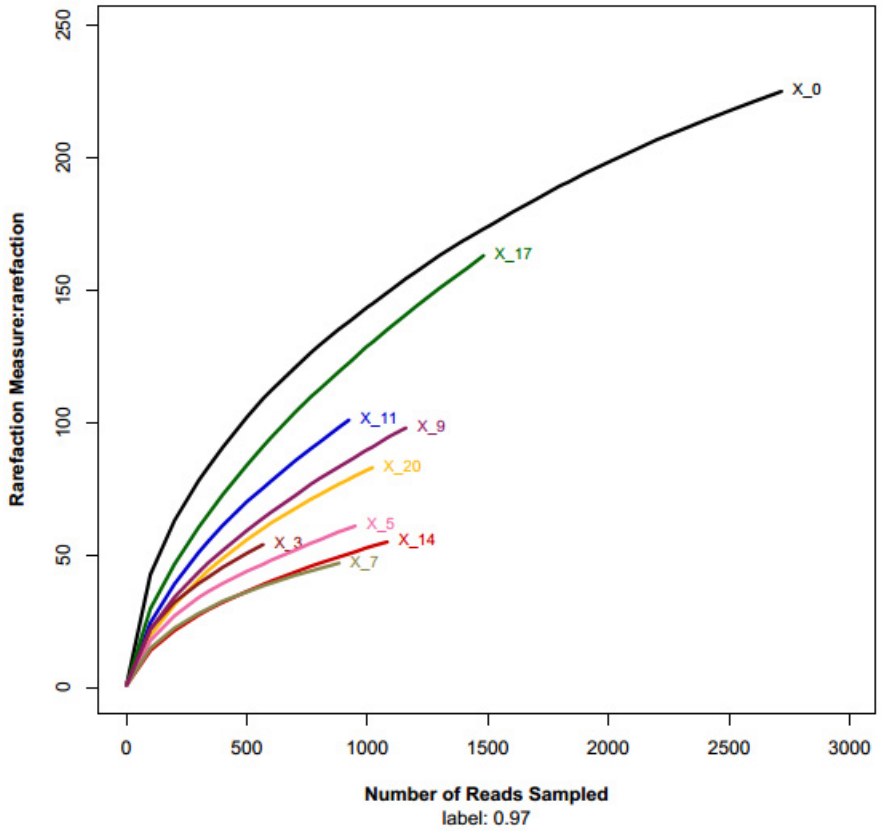

Figure 2. Rarefaction analysis at the 97% sequence similarity level. The rarefaction curve, plotting the number of observed OTUs as a function of the number of sequences, was computed using the RDP Pyrosequencing Pipeline Rarefaction tool. The samples were arranged in descending order based on the numbers of OTUs.

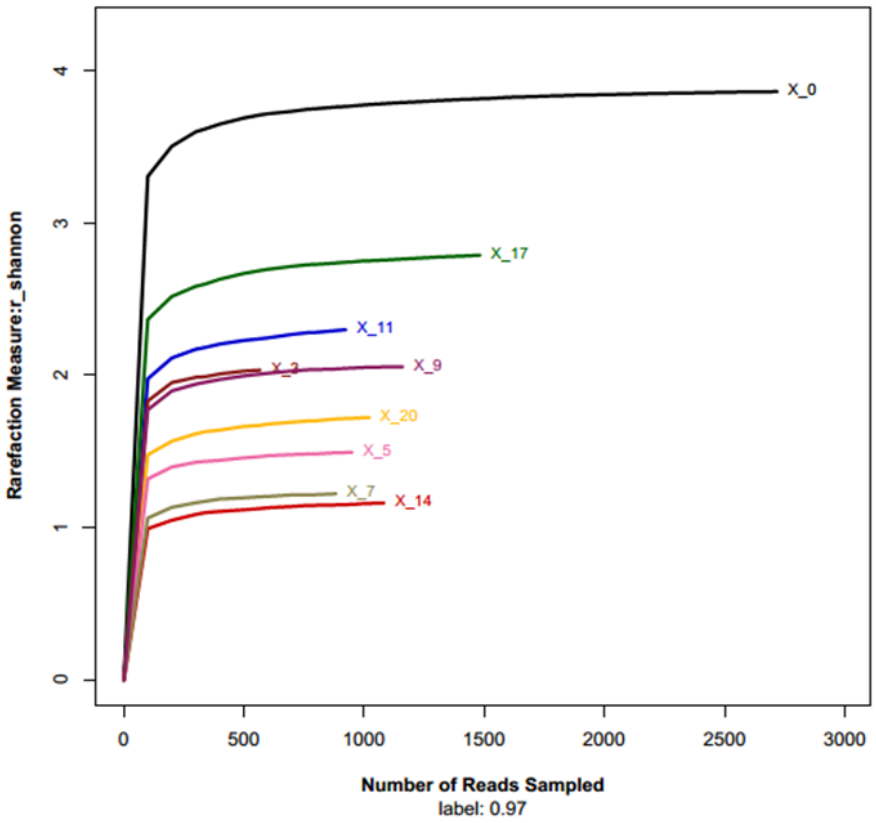

Figure 3. Shannon diversity index curves. The Shannon diversity index reached saturation, suggesting that the observed sequences were a good representation of the fungal community in the nine samples.

3.3. Fungal Community Dynamics of Capsicum annuum L. Var. Dactylus M in Different Fermentation Stages

The relative fungal community abundance at the genus level for each sample is summarized in Figure 4. The results show that only eight genera had relative abundances >1% during fermentation. In sample X_0, taxa with known taxonomic statuses in proportions >0.01% were *Debarvomvces* 2.69%, *Rhodotorula* 2.39%, *Trichosporon* 2.28%, *Cladosporium* 0.77%, *Pichia* 0.63%, *Guehomyces* 0.33%, *Cryptococcus* 0.26%, *Hanseniaspora* 0.26%, *Candida* 0.04%, and others 1.62%. About 88.59% of the sequences of sample X_0 could not be classified into any known group because of the inadequate fungal genome database, the limitation of reading length, experimental error, etc. When fresh *Capsicum annuum* L. Var. *Dactylus* M was chopped, salted, and sealed in a pickle jar to be fermented, the acid content increased, and the oxygen content decreased gradually. Since salt, acid, and hypoxia inhibit the growth of fungi, the fungal richness and diversity decreased. Taxa in proportions >0.01% in samples X_3, X_5, X_7, X_9, X_11, X_14, X_17, and X_20 were only assigned to 7, 4, 5, 9, 9, 3, 13, and 7 different genera, respectively. *Hanseniaspora* was a dominant fungus during the whole fermentation course, accounting for 82.22% of the fungal community on average (ranging from 50.44% to 98.15%).

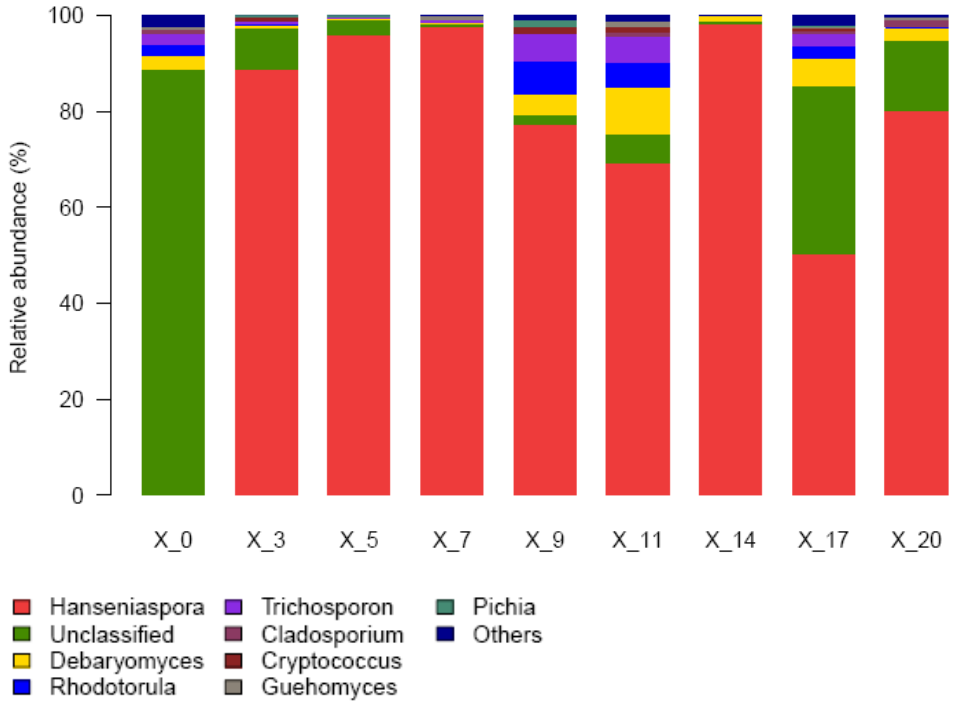

Figure 4. Analysis of fungal genera in proportions >1% in the fresh *Capsicum annuum* L. Var. *Dactylus* M and different fermentation stages of *Capsicum annuum* L. Var. *Dactylus* M using 454 pyrosequencing.

3.4. Volatile Flavor Compounds Analysis

According to Figure 5, 64 main volatile flavor compounds were detected in the natural fermentation of *Capsicum annuum* L. Var. *Dactylus* M. Esters, alcohols, and alkanes were the main volatile flavor compounds, including 21 esters, 12 alcohols, and 10 alkanes. In addition, 8 aldehydes, 7 olefins, and 5 ketones were detected. The number of different volatile flavor compounds gradually increased along with the fermentation time. The numbers of volatile flavor compounds in X_3, X_5, X_7, X_9, X_11, X_14, X_17, and X_20 were 32, 44, 45, 55, 40, 47, 51, and 46, respectively, which were greater than that (24) before fermentation (X_0). The volatile flavor compounds in X_0 were mainly alcohols and aldehydes, which came from the metabolism of yeast attached to the surface of the pepper and were important substrates for the synthesis of esters. The alcohol compounds mainly included linalool, α-terpineol, and n-hexanol. The main aldehydes in X_0 were benzaldehyde and octanal. Benzaldehyde has almond, cherry, and nut aromas and is the most commonly used aromatic aldehyde in industry. Octanal has a strong fruit flavor and can be used as an intermediate in spices and organic synthesis. In the fermented peppers, the aldehydes were mainly benzaldehyde and octanal; the alcohols were mainly linalool, α-terpineol, and 4-methyl-1-pentanol; the esters were mainly methyl salicylate, 4-methylpentyl 2-methylbutanoate, and 4-methylpentyl 3-methylbutanoate; the olefins were mainly β-guaiene; the alkanes were mainly 2-methyltetradecane, 2-methyltridecane, and 2-isobutyl-3-methoxypyrazine; and the ketones were mainly trans-β-ionone and methyl pentyl ketone.

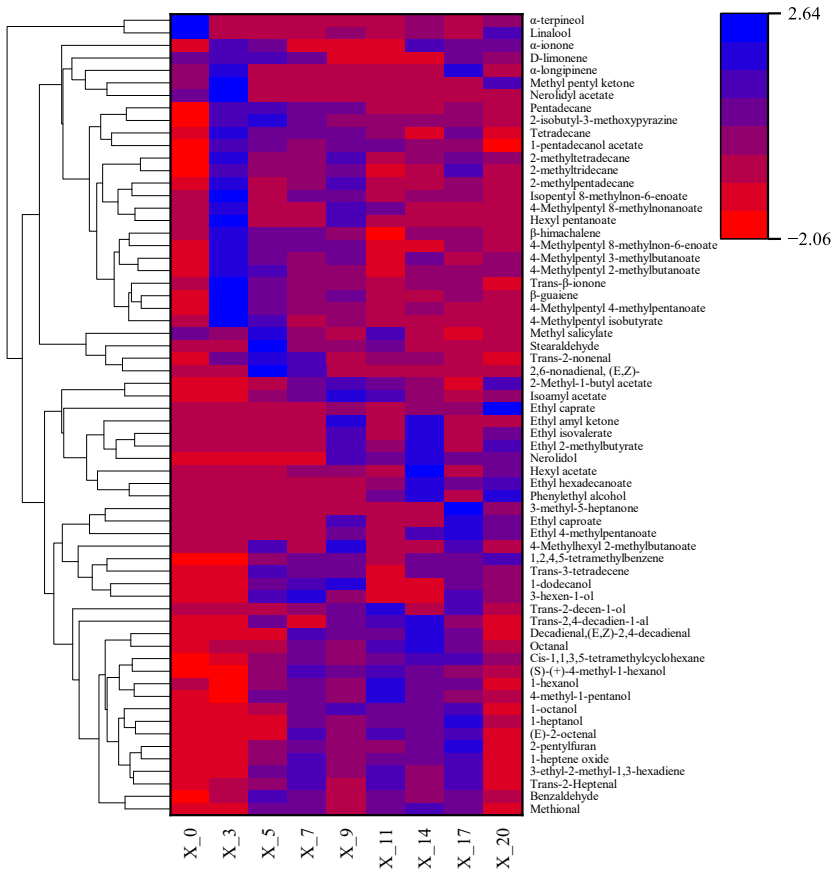

Figure 5. The content distribution of the VOCs in fresh *Capsicum annuum* L. Var. *Dactylus* M and different fermentation stages of *Capsicum annuum* L. Var. *Dactylus* M. The clustering was performed with OriginPro 2019. The colors correspond to normalized mean levels from low (red) to high (purple).

3.5. Correlation Analysis between Fungi and Volatile Flavor Compounds

In the fermentation process of the peppers, five kinds of fungi were identified as the core functional fungi because of their high abundances throughout the whole fermentation process, high VIP values (VIP > 1.0), and the high absolute values of the linear correlation coefficient between the concentration profile of the volatile flavor compounds and the fungi (R > 0.7). The significant linear correlation coefficient (with a significance $p < 0.05$) between the selected microorganisms and the flavor compounds was calculated and is shown in Figure 6. Five kinds of fungi, *Pichia*, *Hanseniaspora*, *Cryptococcus*, *Debarvomvces*, and *Trichosporon*, were closely correlated with the concentrations of the flavor compounds. The abundance of *Pichia* was positively correlated with 4-methylhexyl 2-methylbutanoate and1-dodecanol. The abundance of *Hanseniaspora* was positively correlated with 1,2,4,5-tetramethylbenzene, trans-3-tetradecene, Cis-1,1,3,5-tetramethylcyclohexane, and (S)-(+)-4-methyl-1-hexanol ($p < 0.05$, R > 0.73) and negatively correlated with α-terpineol ($p < 0.05$, R < −0.83). The abundance of *Cryptococcus* was positively correlated with trans-2-decen-1-ol ($p < 0.05$, R > 0.78). The abundance of *Debarvomvces* was positively correlated with trans-2-decen-1-ol ($p < 0.05$, R > 0.82) and negatively correlated with 4-methylpentyl 3-methylbutanoate and 4-methylpentyl 2-methylbutanoate ($p < 0.05$, R < −0.77). The abundance of *Trichosporon* was negatively correlated with α-ionone ($p < 0.05$, R < −0.72).

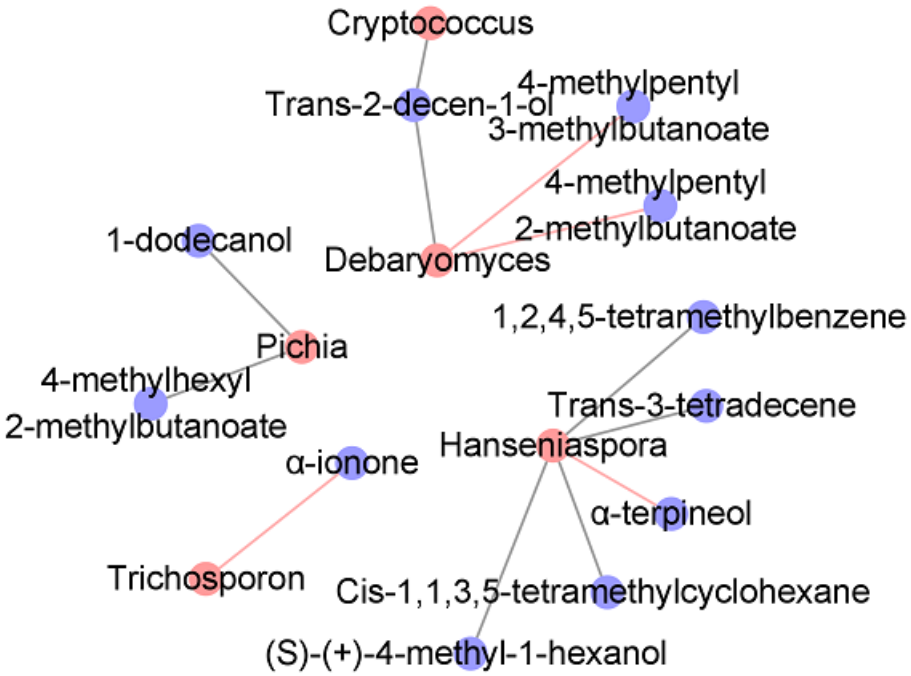

Figure 6. Correlation analysis between fungi and volatile components by O2PLS modeling. The fungi are shown in orange, and the VOCs are shown in blue. The grey and orange lines represent positive and negative correlation, respectively.

4. Discussion

Traditional Chinese fermented peppers are mostly spontaneously fermented with a complex mixture of participating microorganisms, which makes it difficult to control the process of fermentation and produce a product of uniform quality [12,13]. Therefore, it is crucial to study the diversity of the microbial communities involved in fermentation in order to control the whole fermentation process. Most previous studies have used culture-dependent or culture-independent approaches to understand microbial communities in vegetable fermentation [14,15], but to our knowledge, little is known about the correlation between fungi and the main volatile flavor components of fermented peppers. In this study, we revealed the structure of the fungal communities at different fermentation stages, the main volatile flavor compounds, and the correlation between the fungi and these compounds. The results showed that in fresh *Capsicum annuum* L. Var. *Dactylus* M, there were eight fungi with a relative abundance greater than 1% of the known genus-level classification, of which the most abundant fungus was *Debarvomvces*. The fungal community structure of *Capsicum annuum* L. Var. *Dactylus* M at different stages of fermentation was relatively stable, being dominated by *Hanseniaspora*, which accounted for 82.22% of the fungal community on average (ranging from 50.44% to 98.15%), indicating that *Hanseniaspora* may play an important role in product quality. The abundance and diversity of the fungal communities differed greatly before and after fermentation, with the abundance of "Unclassified" fungi decreasing rapidly after fermentation, probably due to the inhibition of the growth of these fungi by salt, acid, and anoxia [16]. In addition, only *Hanseniaspora* showed a significant increase in abundance compared to the unfermented samples, while the abundance of *Debarvomvces*, *Rhodotorula*, *Trichosporon*, *Cladosporium*, *Pichia*, *Guehomyces*, *Cryptococcus*, and *Candida* significantly decreased after fermentation. The results of the analysis of fungal community structure and abundance were consistent with the previous analysis of fungal community diversity in the fermentation of *Capsicum annuum* L. var. *Fasciculatum* Sturt [8]. However, few studies have focused on the dominant fungal genera. *Cladosporium* was found to be the main microorganism in the early stages of vegetable fermentation as an endophyte of vegetable tissues, and its abundance gradually decreased as fermentation progressed, being strongly influenced by the salt concentration during

vegetable fermentation [17–19]. *Mucor* has been reported to be the primary fermentation starter microorganism in traditional Chinese fermented tofu, giving the fermented tofu its creamy consistency and unique flavor while reducing biogenic amine production during the fermentation process [20,21]. However, the contribution of microorganisms to the flavor production of fermented products is rarely considered when selecting the ideal culture. *Candida* has been reported to be used in the production of flavor compounds, namely geraniol and propionic acid [22,23]. Therefore, further research will focus on predicting the mechanisms of production between microorganisms and flavor substances.

Flavor compounds are a key factor in consumer acceptance and product identification. Volatile substances are the main contributors to the flavor of fermented peppers and, when present in different combinations, they influence the aroma of these products [24]. Flavor components in different fermented foods have been systematically investigated [25]. However, studies on the relationship between microbiota and flavor have been scarce. In this study, 21 ester, 12 alcohol, 10 alkane, 8 aldehyde, 7 olefin, and 5 ketone volatile compounds were detected during spontaneous fermentation, and these volatile components changed dynamically during fermentation, with some aromas increasing and others decreasing, implying that the overall flavor profile also changed during fermentation. In addition, the O2PLS method was used to determine the association between fungal flora and volatile components during the fermentation process. *Hanseniaspora* was the predominant fungus during fermentation and was positively correlated with 1,2,4,5-tetramethylbenzene, trans-3-tetradecene, cis-1,1,3,5-tetramethylcyclohexane, and (S)-(+)-4-methyl-1-hexanol ($p < 0.05$, $R > 0.73$) and negatively correlated with α-pinoresinol ($p < 0.05$, $R < -0.83$). *Hanseniaspora* has been reported to be the main microorganism in the fermentation process of other foods, including the fermentation of apple juice, cocoa beans, vegetables, and wine [26–31]. Several studies have shown that *Hanseniaspora* produces maximum concentrations of isoamyl acetate and isobutyl acetate during wine fermentation, as well as some common short-chain ethyl esters that contribute banana and strawberry aromas to the wine [32–34]. *Debaryomyces* produces alcohols, acids, esters, aldehydes, and other flavor compounds that sweeten and significantly improve the quality of fermented peppers. During the fermentation of *Capsicum annuum* L. Var. *Dactylus* M, *Debaryomyces* was associated with three volatile components: trans-2-decen-1-ol, 4-methylpentyl 3-methylbutanoate, and 4-methylpentyl 2-methylbutanoate [35]. *Pichia* has been reported to be one of the most dominant fungal genera during the fermentation of light and strong white wines, producing large amounts of alcohols, organic acids, and esters as a non-saccharide fungal genus [36–39]. *Pichia* was also found to be associated with the production of 1-dodecanol during the fermentation of *Capsicum annuum* L. Var. *Dactylus* M. However, this study was based on DNA, which may also detect dead or inactive cells. Therefore, third-generation sequencing technologies with improved performance are promising and will be used in future studies [40].

In summary, comprehensive information on the composition and dynamics of the fungal communities at different fermentation stages of *Capsicum annuum* L. Var. *Dactylus* M was revealed by high-throughput sequencing, and *Hanseniaspora* was found to be the most abundant fungus during the fermentation of *Capsicum annuum* L. Var. *Dactylus* M. The flavor compounds were characterized and identified using GC–MS. Moreover, O2PLS was applied to determine the correlation between fungal communities and flavor, and *Pichia*, *Hanseniaspora*, *Cryptococcus*, *Debarvomvces*, and *Trichosporon* were found to be associated with the formation of volatile flavors. These findings provide new insights into the variability of fungal communities and increase our understanding of the core aroma-related microbiota involved in the manufacture of fermented *Capsicum annuum* L. Var. *Dactylus* M with unique flavor profiles.

Author Contributions: F.D. and L.Z. designed work; D.M. performed experimental work and prepared initial draft of manuscript; Y.L., J.W., L.P. and W.K. analyzed data; Z.W. (Zengguang Wang), Z.W. (Zhongkun Wu) and Z.D. critically revised manuscript. All authors have read and agreed to the published version of the manuscript.

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