ACS OMEGA

Open Access

This article is licensed under CC-BY-NC-ND 4.0

http://pubs.acs.org/journal/acsodf

Article

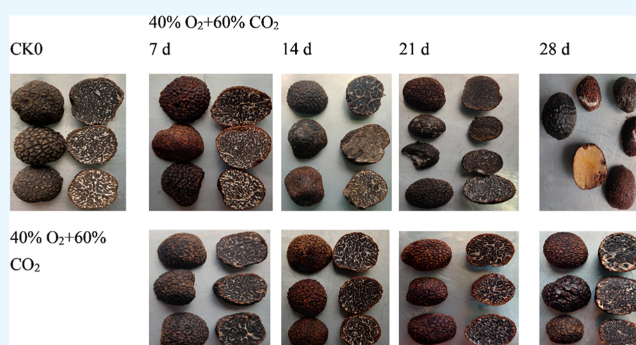
Effects of Storage in an Active and Spontaneous Controlled O_2/CO_2 Atmosphere on Volatile Flavor Components and the Microbiome of Truffles

Qiang Li, Haiyang Hu, Xingyi Tan, Jianhui Wang, Ruhuai Mei, Fangguo Jiang, Yunkun Ling, and Xiang Li*

Cite This: *ACS Omega* 2024, 9, 9331−9347

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: This study explored the potential to improve the storage quality and prolong the shelf life of truffles by storing them in a modified atmosphere fresh-keeping box with sealed gas components of Active Modified Atmosphere Packaging (AMAP, 40% O_2 + 60% CO_2) at 4 °C. During the storage period, a total of 63 volatile components in 10 categories were detected, with aldehydes being the most abundant and the relative content of ethers being the highest. The relative odor activity value and principal component analysis revealed that isovaleraldehyde, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide were the characteristic flavor components of fresh truffles. However, 3-methylthiopropionaldehyde and (E, E)-2,4-nonadienal were the components that caused the deterioration of truffle flavor and could potentially serve as markers of truffle decay characteristics. 16S rDNA high-throughput sequencing showed that *Leuconostoc* and *Lactococcus* were dominant in the truffle samples stored for 14 days, but the abundance of putrefactive pathogenic bacteria showed an increasing trend in the truffle samples stored for 28 days. During the whole storage period, the common fungi detected in the different treatment groups were *Candida* and *Aspergillus*. The relative abundance of the former decreased, while the relative abundance of the latter decreased initially and then increased. The correlation between volatile components and the microbial flora was further analyzed, which indicated that *Lactococcus* and *Lactobacillus* had the same contributions to the same flavor, while *Pseudomonas* and *Glutamicibacter* had the opposite contributions to the same flavor. The results provide a reference for the storage and preservation of truffles.

1. INTRODUCTION

Truffles (*Tuber* spp.) belong to the phylum Ascomycete, the order Pezizales, and the family Tuberaceae. Approximately 180 species of truffle have been identified in the world.[1] The most famous species, such as *T. melanosporum* and *T. magnatum*, with a market price of 500−5000 euros/kg, enjoy the international reputation of a "black diamond" and "underground gold".[2] China is rich in wild black truffle resources, accounting for approximately half of the total output of wild black truffles in the world. The main varieties include Chinese summer truffle (*T. sinoaestivum*), false Himalayan truffle (*T. pseudohimalayense*), Panzhihua white truffle (*T. panzhihuanese*), Yunnan black truffle (*T. melanosporum*), Indian truffle (*T. indicum*), and false sunken truffle (*T. pseudoexcavatum*).[3]

Truffle has a strong aroma, contains a variety of volatile metabolites, and is widely welcomed by consumers. In addition, truffles contain rich nutritional active ingredients and exert certain antioxidant, anti-inflammatory, antitumor, and other effects. However, at present, the storage and preservation technology of wild truffles lags, resulting in short shelf life of truffles and a deterioration in the quality of truffles, such as texture changes, aroma loss, and nutrient degradation, will occur to different degrees during the storage process. The loss caused by improper preservation methods is up to 30%.[4] Bellesia et al. found that the concentration of sulfur-containing volatiles (thiophene derivatives) in the fruiting body of *T. borchii* was almost unchanged when stored at 0 °C; however, when the storage temperature was 25 °C, the concentration of thiophene derivatives increased in a few weeks.[4] According to Strojnik et al.,[5] the content of volatile organic compounds (VOCs) in fresh truffle nigra stored at 4 °C for 7 days changed little compared with that before storage, while dimethyl sulfide

Received: October 30, 2023
Revised: January 29, 2024
Accepted: January 31, 2024
Published: February 13, 2024

© 2024 The Authors. Published by American Chemical Society

and 3-methylbutanol were no longer detected. Feng et al.[6] reported that vacuum freeze-drying increased the types of alcohols, phenols, ketones, and nitrogen- and sulfur-containing substances in truffles. Based on an OAV (odor activity value (oAV) calculation, 46 substances in dried truffles had OAVs greater than 1, while only 20 substances in fresh truffles had OAVs greater than 1, indicating that the aroma composition of dried truffles was more complex than that of fresh truffles, and the smell was also more intense. Culleré et al.[7] showed that the contents of diacetyl, 1-octen-3-one, 1-octen-3-alcohol, 2-methylisobornyl, and dimethyl trisulfide in truffles increased only after 24 h of freezing (independent of temperature), while the contents of isoamyl alcohol, ethyl-3-methylbutyrate, and methyl mercaptan decreased. Researchers speculated that the changes in these compounds may be related to the destruction of truffle tissue or the degradation of related enzymes caused by long-term freezing.

To date, studies have used low temperatures,[8] controlled atmospheres,[9] irradiation,[10] and preservatives[11] for truffle preservation, but some chemical preservatives cause environmental pollution and exert toxic side effects.[12] Guo et al.[13] found that O_2/CO_2 active spontaneous controlled atmosphere storage has unique advantages in the preservation of fruits and vegetables.[13] Through the combined action of O_2 and CO_2, a high concentration of CO_2 inhibits the respiratory intensity of fruits and vegetables, while a high concentration of O_2 alleviates the odor and physiological toxicity caused by anaerobic metabolism. In terms of microbial prevention and control, active spontaneous O_2/CO_2 gas regulation may increase the release of spore contents by destroying the integrity of cell membranes and ultimately weaken the environmental adaptability and pathogenic ability of molds, thus inhibiting the ability of molds to invade fruits and vegetables.[14] Jiang et al.[15] showed that 40% O_2 + 60% CO_2 modified atmosphere packaging at 4 °C could delay the respiratory peak of Indian truffle (*T. indicum*) to 21 days, effectively reducing the loss of nutrients and extending its shelf life to 28 days. The modified atmosphere treatment potentially reduced the damage to the cell wall membrane caused by cellulase and malondialdehyde, and the microstructure of the truffle fruiting body remained intact and full, thus maintaining normal physiological activities.

The number of bacteria on the surface and inside the truffle fruiting body ranges from 10^7 to 10^8 CFU/g.[16] These bacteria are derived from soil, host plants, and the surrounding environment,[17] mainly including Proteobacteria,[18] Actinobacteria,[19] Bacteroides,[20] and Firmicutes.[21] Monitoring the dynamic change in the microbial community on the surface of truffles has important guiding significance for the evaluation of the freshness-keeping effect of truffles after harvest. According to Vandatzadeh et al.,[22] the dominant fungi characterized on fresh truffle (α-Proteobacteria, β-Proteobacteria, and Sphingobacteria classes) are gradually corrupted by bacteria (γ-Proteobacteria and Bacilli classes) after nine d of storage at room temperature. In addition to participating in the development and maturation of fruiting bodies, the bacteria in truffles also promote the growth of truffles and prevent the establishment of harmful microorganisms. However, the dynamic changes in surface flora in the postharvest fresh-keeping stage of truffle are unknown.

This study intends to study the dynamic changes in volatile components of truffles in different stages of controlled atmosphere storage at 4 °C in cooperation with 40% O_2 + 60% CO_2 using HS-HPME-GC–MS and screen the characteristic markers of spoilage. 16S rDNA and ITS high-throughput sequencing were used to analyze the microbial community structure on the surface of truffle fruit bodies, and the correlations between volatile substances and microbial communities were analyzed by calculating Spearman's correlation coefficients, providing a reference to further reveal the biological function of the characteristic aroma substances regulated by bacteria on the surface of truffle during storage. This study provides technical support for the safe storage and preservation of truffles.

2. MATERIALS AND METHODS

2.1. Raw Materials and Pretreatment.

In January 2021, the fruiting body of *T. indicum* (S grade specification: 2 cm < the maximum diameter of the ascocarp <3 cm, weight ≥3 g) was collected in the natural truffle-producing area of Panzhihua City, Sichuan Province, by manual excavation and immediately placed in an ice bag in a low-temperature box for temporary storage and quickly transported to the laboratory of Chengdu University for treatment. Using the truffle decontamination procedure described by Rivera et al.,[23] with the only difference that the selection of truffle maturity is increased (the surface of the ascocarp is light brown–black to tea-brown black; the cut flesh is tea-brown to black, and 80–90% has marbling), precooling at 4 °C for 1 day was shortened to 12 h. Then, the truffle sample was weighed and placed in a PP fresh-keeping box (22 × 13 × 4 cm, weighing 20 g) (Zhucheng Wanrui Plastic Co., Ltd., China) with a loading capacity of 1/4 and a layer of moisture-absorbing paper at the bottom and inflated with a MAP-JY260 modified atmosphere packaging machine (Shanghai Jiyi Machinery Co., Ltd.).

The gas regulation procedure was as follows: the gas regulation ratio was 40% O_2 + 60% CO_2, the pumping time was 4.00 s, the inflation time was 4.00 s, the heat-sealing time was 2.00 s, the heat-sealing temperature was 152 °C, and a double-layer 0.02 mm PE film was used for sealing (Shenzhen HSBC Packaging Technology Co., Ltd.). The storage temperature was 4 °C, and the fresh-keeping box was placed in cold storage with a relative humidity of 90–95%. The weight of truffles in each fresh-keeping box was 300 ± 5.00 g, three replicates (the same fresh-keeping box) were used at each sampling point, and the total number of fresh-keeping boxes was 3; namely, samples were collected at 0, 14, and 28 days of storage for the volatile compounds analysis and 16S rDNA and ITS analysis.

2.2. Extraction of Volatile Substances from Truffles.

The 75 μM CAR/PDMS extraction head (Supelco Company, United States) was inserted into the gas chromatography sample inlet and conditioned at 260 °C for 20 min. The truffle sample was ground with a CQF-02C crusher (Zhejiang Chaoqun Mechanical Equipment Co., Ltd.) through a 60-mesh sieve, and 2.000 g were removed and transferred into a 20 mL headspace bottle that had been preheated at 55 °C for 10 min. Then, the extraction head was inserted into the headspace bottle for extraction in a 60 °C water bath for 40 min. Afterward, the extraction head was inserted into the sample inlet and desorbed at 260 °C for 2 min.

2.2.1. GC–MS Conditions.

GC was performed using a 7890B-5977B gas chromatograph (Agilent Company, USA) and HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm). The following conditions were used: the injection port temperature was maintained at 260 °C, and the temperature

program was initially 50 °C for 1 min, an increase to 160 °C at a rate of 5 °C/min, hold for 0 min, an increase to 200 °C at *r* rate of 10 °C/min, and hold for 0 min. Finally, the temperature was increased to 280 °C at a rate of 15 °C/min, with hold for 5 min. He was used as the carrier gas; the column flow rate was 1 mL/min, and the nonsplit injection mode was used.

MS conditions included an EI ion source, energy of 70 eV, temperature of 230 °C, four-stage rod temperature of 150 °C, interface temperature of 280 °C, scanning range of 20−450 amu/s, and full scanning mode.

2.2.2. Determination of Key Volatile Substances during Truffle Storage. The relative odor activity value (ROAV) was used to evaluate the contribution of each volatile component to the flavor of the truffle during different storage periods. The volatile component with the largest contribution to the flavor of the truffle sample was specified as having an ROAV of 100, and the ROAV of other components was calculated using the following formula:[24]

$$\mathrm{ROAV}_i \approx \frac{C_i}{C_{\mathrm{stan}}} \times \frac{T_{\mathrm{stan}}}{T_i} \times 100$$

C_i, the relative content of volatile components in the sample; C_{stan}, the relative content of volatile substances with the greatest contribution to the overall flavor of the sample; T_i, the aroma threshold of each volatile component in the sample (μg/kg); and T_{stan} is the aroma threshold of volatile substances with the greatest contribution to the overall flavor of the sample (μg/kg).

2.2.3. Evaluation of Aroma Characteristics. Based on the calculated results for ROAV and the method described by Wang et al.[25], quantitative descriptive analysis (QDA) was used to evaluate the sensory properties of truffles in different storage periods. Ten food professionals were organized to form an evaluation group after sensory training, such as smell and taste. After a discussion on consistency, seven aroma characteristic sensory words were selected: soil aroma, mushroom flavor, flower and fruit flavors, green grass flavor, hala flavor, and cooking flavor. The team members were familiarized with the characteristics of each aroma and were able to accurately identify the aroma and the strength it represented. The samples in each storage period were cut into 0.5 cm thick slices and smelled. The sensory score ranged from 0 to 10 points (0 to 2 points = extremely weak or no, 3 to 5 points = average, 6 to 8 points = moderate, and 9 to 10 points = prominent). The higher the score, the more prominent the aroma. Finally, a radar chart depicting the average score of the aroma was drawn.

2.3. Bacterial and Fungal Community Tests during Storage. *2.3.1. Total DNA Extraction and PCR Amplification.* The truffles stored for 0, 14, and 28 days were collected, and the total microbial DNA on the surface of the truffles was extracted according to the instructions of the BIOMICS DNA Microprep Kit (Zymo Research, USA). The 16S rDNA V4 region of bacteria was amplified using primers 515F (5′-GTGYCAGCMGCGGTAA-3′) and 806R (5′-GACTACHVGGGTWTCTAAT-3′),[26] and the amplification procedure was predenaturation at 94 °C for 60 s; 30 cycles of denaturation at 94 °C for 5 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s; and extension at 72 °C for 5 min. Each sample was analyzed in three replicates with PCR technology, and the PCR products were mixed in equal amounts for subsequent database construction.[27] Fungal sequencing uses specific primers to amplify the ITS2 region, and the primer sequences used for amplification were as follows (5′-3′): ITS3 (5′-GATGAAGAACGYAGRAA-3′) and ITS4 (5′-TCTCCGCTTATTGTGC-3′). The PCR procedure was predenaturation at 94 °C for 1 min; 25−30 cycles of denaturation at 94 °C for 20 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s; and an incubation at 72 °C for 5 min.[28] The 50 μL reaction system (10× PCR Buffer for KOD-Plus-Neo, 5 μL) was used. Each sample was analyzed in three replicates using PCR technology, and the linear PCR products were mixed in equal amounts for subsequent database construction. The PCR product was mixed with 6× loading buffer, and then the target fragment was detected by electrophoresis on a 2% agarose gel. The samples passing the test were recycled with the target strip, and the Zymoclean Gel Recovery Kit (D4008) was used; samples were then quantified with a Qubit 2.0 Fluorometer (Thermo Scientific). finally, samples were mixed in equal molar weights.

2.3.2. Library Construction and 16S rDNA High-Throughput Sequencing. PCR products in qualified target bands were collected using the Zymoclean Gel Recovery Kit (Zymo Research, USA), quantified with a Qubit 2.0 Fluorometer (Thermo Scientific, USA) and finally mixed in equal molar amounts for detection. The NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs) was used to build the library. PE250 sequencing was adopted, and the HiSeq Rapid SBS Kit v2 sequencing kit (Illumina, USA) was used for high-throughput sequencing.[29]

The NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB#E7645L) were used for library construction (New England Biolabs, USA). Then, the constructed libraries were sequenced by using the PE250 platform.

2.4. Data Processing. The scans and results for the identification of the volatile components of truffles were retrieved through the NIST/Filey computer spectrum library. At the same time, by performing a manual analysis of the spectrum, substances with positive and negative matching degrees above 80% were selected for the qualitative analysis. Excel 2016 software was used to process the data and draw a Wayne diagram in the analysis of volatile components. TBtools was used to draw a heatmap. Origin 2017 software was used to conduct the principal component analysis and mapping.

FLASH was used to splice the double-ended sequences in 16S rDNA and ITS sequencing data and sequences from each sample were separated from reads based on barcodes. Barcode sequences were interpreted to obtain the original data, and then, QIIME quality control was used to obtain effective data tags. Usearch software was used to cluster the OTUs at a 97% consistency level according to the UPARSE algorithm. The sequence with the highest frequency of occurrence in each OTU was selected as the representative sequence of the OTUs. The UCLUST classification method and the SILVA database were used for the annotation analysis,[30] PyNAST was used for multiple comparisons of the representative sequences, and the R language was used to analyze the alpha diversity and community composition. The correlations between the volatile compounds of truffles and the top ten microbial populations of the truffle body surface abundance were analyzed by calculating the Pearson correlation coefficients using IBM SPSS Statistics 26.0.

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

3. RESULTS

3.1. Analysis of the Morphological Changes in Truffle Appearance. As shown in Figure 1, the appearance of truffles

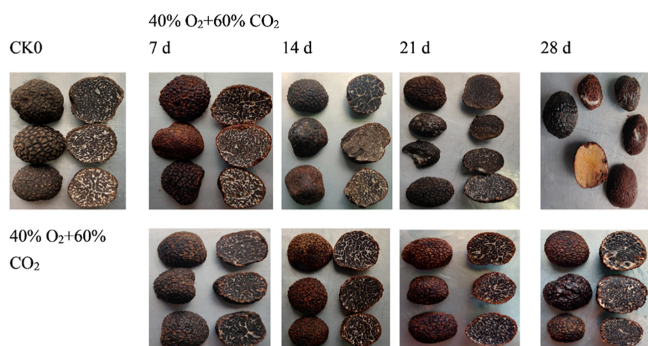


Figure 1. Morphological changes of truffles during different storage times.

changed to varying degrees during storage, and the appearance scores of each treatment group showed significant differences after 7 days ($p < 0.05$). In particular, in the middle and late storage periods (14−28 days), the control group mainly showed a softening texture, blurred cross-section texture, severe odor, stickiness, and a large number of molds. The sensory score at 14 days had decreased to 20.5 points, and the sample had completely lost its commodity value. However, storage with 40% O_2 + 60% CO_2 produced good sensory properties at 14 days. With the further extension of the storage time, no colony growth was observed on the surface. The AMAP treatment maintained a high level of sensory properties throughout the storage period, along with the highest sensory score, indicating that an appropriate modified atmosphere treatment can effectively delay the sensory deterioration of truffles.

3.2. Changes in the Types and Relative Contents of Volatile Compounds. Sixty-three volatile substances in 10 categories were detected during the three storage periods (Table 1), including 20 aldehydes, 12 alcohols, 5 ketones, 7 esters, 7 ethers, 2 furans, one olefin, one acid, and 9 others. At 0, 14, and 28 days of storage, 45, 48, and 48 volatile substances were detected, respectively. Figure 2A shows that 34 types of volatile substances were detected in each period, and four, five, and ten unique volatile substances were detected at 0, 14, and 28 days, respectively, indicating that truffles were actively spontaneously aerating. During storage, new volatile substances were produced. As shown in Figure 2B, the types of furans, olefins, and other substances did not change; the types of aldehydes and ketones increased initially and then decreased; the types of alcohols increased at 14 and 28 days; esters and ethers remained unchanged at 0 and 14 days but increased at 28 days; the types of ethers remained unchanged at 0 and 14 days but decreased at 28 days; and an acid species appeared at 28 days. The relative contents of aldehydes, alcohols, and ketones initially increased and then decreased during the storage period; the relative contents of esters, furans, and acids increased throughout the storage period; and the relative contents of ethers and olefins during storage first decreased and then increased. Taken together, AMAP storage delayed the decay of aldehydes, alcohols, esters, furans, acids, and olefins in truffles and slowed the increase in the levels of ketones, ethers, and other substances.

3.3. Cluster Analysis of Volatile Compounds. The relative content of each volatile substance was calculated as the logarithm of base 10, processed into a heatmap, and a cluster analysis was conducted to further clarify the changes in volatile substances in postharvest truffles during storage, where the columns represented samples from different storage periods. The rows represent different volatile components, as shown in Figure 2C. The types and relative contents of truffle volatile substances were significantly different at different storage periods. In terms of the clustering relationship, the volatile substances of truffles stored at 0 and 14 days were the first to cluster, indicating that the types and levels of volatile substances in these two periods were similar. Regarding the red and black areas, aldehydes, ethers, and other (benzene) substances were the main substances detected after storage for 0 days; aldehydes, alcohols, ketones, and ethers were mainly detected after storage for 14 days; aldehydes, alcohols, ethers, and esters were the main substances observed. According to these results, the relative contents of aldehydes, alcohols, and ethers were higher than those of other substances throughout the storage period, suggesting that they may play a more important role in contributing to the flavor of truffles.

The aroma threshold of volatile components in water was analyzed by calculating the ROAV, and the aroma threshold of 1-octen-3-one was the lowest, and the relative content during storage was relatively high, indicating that 1-octen-3-one is an active component. The component with the greatest contribution to the flavor of truffles in each period during spontaneous modified atmosphere storage was defined as having a relative odor activity value (ROAV) of 100, while the ROAV scores of other components ranged from 0 to 100. According to the relative definition of the ROAV,[24] the ROAV of a substance ranging from 0.1 to 1 indicates that it has a modification or coordination effect on the flavor of the sample, and an ROAV between 1 and 100 indicates that this substance is a key volatile substance in the sample, which has an important contribution or a significant effect on the flavor. The larger the ROAV is, the more obvious the effect. An ROAV between 0.01 and 0.1 indicated that this substance may have a potential contribution to the flavor of the sample. In this study, volatile substances with ROAVs greater than 0.01 were selected and are shown in Table 2.

During the entire storage period, seven types of volatile substances with ROAVs between 1 and 100 were identified, 11 with values ranging from 0.1 to 1 and 10 with values ranging from 0.01 to 0.1.

At 0 days of storage, the components with important contributions to the flavor of truffles were isovaleraldehyde, (E,E)-2,4-decadien-1-al, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide. The ingredients that potentially modified the flavor of truffles were 2-methylbutyraldehyde, hexanal, 3-(methylthio) propionaldehyde, benzaldehyde, octanal, (E)-2-octenal, nonanal, decyl aldehyde, and (E,E)-2,4-nonadienal.

After 14 days of storage, the important components that contributed to the flavor of truffles were isovaleraldehyde, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide. The ingredients that modified the flavor of truffles were 2-methylbutyraldehyde, hexanal, 3-(methylthio) propionaldehyde, octanal, (E)-2-octenal, nonanal, 1-nonanal, decyl aldehyde, (E,E)-2,4-nonadienal, and trans-2,4-decadienal.

After 28 days of storage, the components with important contributions to the flavor of truffles were isovaleraldehyde, 3-methylthiopropionaldehyde, (E,E)-2,4-nonadienal, trans-2,4-

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

Table 1. GC−MS Results for Volatile Substances Detected in Truffles during Different Storage Periods[a]

				relative content (%)		
compound	*RT* (min)	name	CAS	0 day	14 days	28 days
Aldehydes						
1	2.64	isovaleraldehyde	590−86−3	2.706 ± 0.036^{b}	4.134 ± 0.002^{a}	2.328 ± 0.001^{b}
2	2.73	2-methylbutyraldehyde	96−17−3	1.167 ± 0.007^{b}	4.271 ± 0.012^{a}	
3	5.22	hexanal	66−25−1	2.189 ± 0.027^{b}	3.376 ± 0.103^{a}	1.981 ± 0.041^{b}
4	8.74	heptanal	111−71−7	0.130 ± 0.002^{b}	0.237 ± 0.003^{a}	0.240 ± 0.003^{a}
5	8.89	3-(methylthio)propionaldehyde	3268−49−3	0.559 ± 0.017^{a}	0.577 ± 0.006^{a}	0.729 ± 0.021^{b}
6	11.08	(E)-2-heptenal	18829−55−5	0.272 ± 0.110^{a}	0.261 ± 0.005^{a}	0.333 ± 0.004^{a}
7	11.19	benzaldehyde	100−52−7	1.419 ± 0.201^{a}	0.639 ± 0.015^{c}	1.059 ± 0.056^{b}
8	13.04	2-thiophenecarboxaldehyde	98−03−3	0.216 ± 0.013^{b}	1.937 ± 0.208^{a}	
9	13.26	octanal	124−13−0	0.564 ± 0.004^{b}	0.913 ± 0.011^{a}	0.174 ± 0.001^{c}
10	15.11	phenylacetaldehyde	122−78−1	0.939 ± 0.008^{c}	1.331 ± 0.017^{b}	2.590 ± 0.009^{a}
11	15.85	(E)-2-octenal	2548−87−0	2.227 ± 0.049^{bc}	4.321 ± 0.020^{a}	2.658 ± 0.016^{b}
12	18.10	1-nonanal	124−19−6	0.394 ± 0.083^{b}	0.546 ± 0.053^{a}	0.387 ± 0.003^{b}
13	20.52	2-phenyl-acrylaldehyde	4432−63−7			0.121 ± 0.001^{a}
14	20.71	(Z)-2-nonenal	60784−31−8	0.103 ± 0.002^{a}	0.105 ± 0.001^{a}	0.094 ± 0.002^{a}
15	22.83	decyl aldehyde	112−31−2	0.171 ± 0.013^{a}	0.114 ± 0.007^{b}	0.066 ± 0.001^{c}
16	23.12	(E,E)-2,4-nonadienal	5910−87−2	0.130 ± 0.011^{c}	0.175 ± 0.008^{b}	0.213 ± 0.013^{a}
17	25.01	(2E)-2-decenal	3913−81−3	0.107 ± 0.001^{b}	0.150 ± 0.015^{a}	0.079 ± 0.002^{c}
18	25.39	2-phenyl-2-butenal	4411−89−6		0.207 ± 0.032^{b}	0.430 ± 0.008^{a}
19	26.91	(E,E)-2,4-decadien-1-al	25152−84−5	0.183 ± 0.027^{a}	0.179 ± 0.051^{a}	0.146 ± 0.005^{ab}
20	28.42	2-undecenal	2463−77−6	0.105 ± 0.001^{b}	0.207 ± 0.002^{a}	0.086 ± 0.001^{b}
alcohols						
1	1.73	ethanol	64−17−5	1.246 ± 0.026^{c}	4.201 ± 0.334^{b}	6.441 ± 0.237^{a}
2	2.39	isobutanol	78−83−1	0.190 ± 0.020^{c}	0.650 ± 0.014^{a}	0.404 ± 0.007^{b}
3	3.72	isoamyl alcohol	123−51−3			0.290 ± 0.018^{a}
4	3.79	2-methyl butanol	137−32−6		1.703 ± 0.041^{a}	0.694 ± 0.011^{b}
5	4.42	1-pentanol	71−41−0	0.100 ± 0.001^{a}		
6	7.49	1-Hexanol	111−27−3		2.402 ± 0.036^{a}	
7	12.20	1-octen-3-ol	3391−86−4	7.520 ± 0.536^{b}	16.889 ± 1.243^{a}	9.898 ± 0.079^{b}
8	12.92	3-octanol	589−98−0	0.293 ± 0.034^{b}	0.275 ± 0.004^{b}	2.587 ± 0.024^{a}
9	16.36	(E)-2-octen-1-ol	18409−17−1	1.057 ± 0.113^{a}		
10	16.37	cyclo-octanol	696−71−9		2.509 ± 0.121^{a}	1.314 ± 0.009^{b}
11	16.51	1-octanol	111−87−5	0.274 ± 0.083^{b}	0.429 ± 0.051^{a}	0.503 ± 0.027^{a}
12	18.44	phenylethyl alcohol	60−12−8	0.129 ± 0.027^{c}	1.119 ± 0.036^{b}	3.492 ± 0.245^{a}
ketones						
1	2.20	2-butanone	78−93−3	0.330 ± 0.012^{b}	0.654 ± 0.005^{a}	
2	12.12	1-octen-3-one	4312−99−6	1.813 ± 0.257^{b}	2.30 ± 0.048^{a}	1.156 ± 0.016^{c}
3	12.51	3-octanone	106−68−3	0.739 ± 0.007^{b}	1.391 ± 0.004^{a}	1.327 ± 0.003^{a}
4	26.17	2-undecanone	112−12−9	0.177 ± 0.010^{c}	0.357 ± 0.014^{a}	0.297 ± 0.002^{ab}
5	27.74	2-pentyl-2H-furan-5-one	21963−26−8		0.084 ± 0.001^{a}	
esters						
1	6.96	ethyl isovalerate	108−64−5			0.122 ± 0.008^{a}
2	7.18	methyl dodecanoate	111−82−0	0.056 ± 0.001^{a}		
3	13.16	ethyl hexanoate	123−66−0			0.126 ± 0.031^{a}
4	22.53	ethyl caprylate	106−32−1			0.080 ± 0.017^{a}
5	24.85	1-phenylethyl acetate	93−92−5			0.076 ± 0.006^{a}
6	28.38	4-nonanolide	104−61−0			0.181 ± 0.028^{a}
7	30.99	geraniol acetate	105−87−3		0.271 ± 0.004^{a}	
ethers						
1	1.89	dimethyl sulfide	75−18−3	5.231 ± 0.075^{a}	2.849 ± 0.112^{c}	4.325 ± 0.244^{ab}
2	2.01	2-ethoxyethanol	110−80−5			0.116 ± 0.010^{a}
3	9.35	anisole	100−66−3	0.182 ± 0.001^{b}	0.230 ± 0.019^{a}	
4	14.15	4-methylanisole	104−93−8	59.403 ± 3.564^{a}	29.601 ± 1.352^{c}	43.658 ± 3.211^{b}
5	18.34	2-ethylanisole	14804−32−1	0.094 ± 0.013^{b}	0.170 ± 0.009^{a}	
6	20.14	1,2-dimethoxybenzene	91−16−7	0.136 ± 0.027^{a}	0.157 ± 0.014^{a}	
7	21.03	1,3-dimethoxybenzene	151−10−0	1.001 ± 0.003^{a}	0.131 ± 0.011^{b}	0.187 ± 0.007^{b}
furans						
1	12.72	2-pentylfuran	3777−69−3	0.231 ± 0.004^{b}	0.387 ± 0.016^{a}	0.420 ± 0.031^{a}
1	8.27	styrene	100−42−5	1.175 ± 0.037^{b}	0.747 ± 0.127^{c}	3.457 ± 0.452^{a}

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

Table 1. continued

compound	RT (min)	name	CAS	relative content (%) 0 day	14 days	28 days
acids						
1	2.18	acetic acid	64−19−7			0.487 ± 0.034^{a}
other						
1	7.49	1,4-dimethyl-benzene	106−42−3	0.568 ± 0.071^{a}		0.752 ± 0.035^{a}
2	21.42	1,2-dimethoxy-3-methyl-benzen	4463−33−6	0.437 ± 0.022^{a}	0.351 ± 0.017^{ab}	0.243 ± 0.043^{c}
3	24.19	3,4-dimethoxy-toluene	494−99−5	1.131 ± 0.094^{a}	0.291 ± 0.026^{c}	0.845 ± 0.005^{ab}
4	24.56	2,5-dimethoxytoluene	24599−58−4	0.679 ± 0.028^{a}	0.194 ± 0.031^{b}	0.206 ± 0.013^{b}
5	24.73	1,3-di-*tert*-butylbenzene	1014−60−4		0.119 ± 0.007^{a}	
6	30.45	3,4,5-trimethoxy toluene	6443−69−2	0.230 ± 0.014^{a}	0.082 ± 0.010^{b}	0.100 ± 0.001^{b}
7	30.86	1,2,4-trimethoxybenzene	135−77−3	0.884 ± 0.102^{a}		
8	2.92	*sec*-butyl ethyl ether	2679−87−0		2.402 ± 0.164^{a}	
9	15.32	1-ethyl-1-methylcyclopentane	16747−50−5			0.175 ± 0.020^{a}

[a]Notes: Data are presented as the means ± SD from triplicate determinations. Different letters in the same column indicate significant differences ($P < 0.05$), $n = 3$. "-" indicates that no data are available.

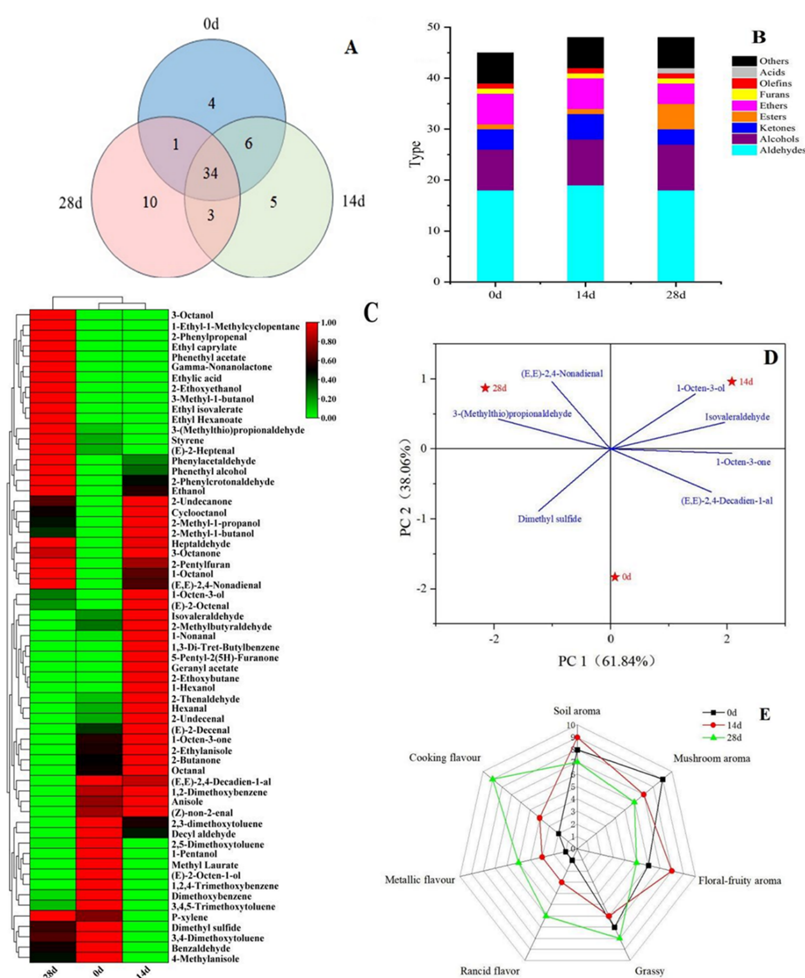


Figure 2. Analysis of volatile compounds in truffles stored at different times. Venn diagram of volatile substances during different storage periods (A); changes in the types of volatile compounds during different storage periods of truffles (B); thermographic analysis of the relative contents of volatile flavor components in truffles during different storage periods (C); principal component analysis (PCA) loading plot of key volatile substances (D); evaluation of aroma characteristics in different storage periods of truffles (E).

decadienal (E)-2-decenal, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide. The ingredients that modified the flavor of truffles were hexanal, benzaldehyde, octanal, phenylacetaldehyde, (E)-2-octenal, nonanal, 1-nonanal, and decanal aldehyde. At the same time, we should emphasize that none of the new volatile substances produced during the entire storage period had any significant contribution or modified the flavor of truffles, indicating that their effects on the flavor can be ignored, and thus, no further analysis was performed in the follow-up experiment. Therefore, during the active and spontaneous atmosphere storage of truffles, substances with

Table 2. Determination of Key Volatile Substances in Truffles during Different Storage Periods

chemical compound	relative content			odor threshold	ROAV			aroma description
	0 day	14 days	28 days		0 day	14 days	28 days	
isovaleraldehyde	2.706 ± 0.036^{b}	4.134 ± 0.002^{a}	2.328 ± 0.001^{b}	0.4	1.87	2.25	2.52	malty, cocoa aroma, fruity
2-methylbutyraldehyde	1.167 ± 0.007^{b}	4.271 ± 0.012^{a}		1	0.32	0.928		malty, cocoa aroma, fruity
hexanal	2.189 ± 0.027^{b}	3.376 ± 0.103^{a}	1.981 ± 0.041^{b}	4.5	0.13	0.16	0.20	grassy,. grease flavor, fishy smell
3-(methylthio)propionaldehyde	0.559 ± 0.017^{a}	0.577 ± 0.006^{a}	0.729 ± 0.021^{b}	0.2	0.77	0.63	1.58	cooked potato, pungent
(E)-2-heptenal	0.272 ± 0.110^{a}	0.261 ± 0.005^{a}	0.333 ± 0.004^{a}	4.2	0.02	0.01	0.03	grassy
benzaldehyde	1.419 ± 0.201^{a}	0.639 ± 0.015^{c}	1.059 ± 0.056^{b}	3	0.13	0.05	0.15	almond odor, caramel, nut aroma
octanal	0.564 ± 0.004^{b}	0.913 ± 0.011^{a}	0.174 ± 0.001^{c}	0.7	0.22	0.28	0.11	rancid flavor, lemon
phenylacetaldehyde	0.939 ± 0.008^{c}	1.331 ± 0.017^{b}	2.590 ± 0.009^{a}	4	0.06	0.07	0.28	hyacinth, nut aroma, honey aroma
(E)-2-octenal	2.227 ± 0.049^{bc}	4.321 ± 0.020^{a}	2.658 ± 0.016^{b}	3	0.20	0.31	0.38	grassy, fruity, grease flavor
1-nonanal	0.394 ± 0.083^{b}	0.546 ± 0.053^{a}	0.387 ± 0.003^{b}	1	0.11	0.12	0.17	rose-scented, citrus flavor
decyl aldehyde	0.171 ± 0.013^{a}	0.114 ± 0.007^{b}	0.066 ± 0.001^{c}	0.1	0.47	0.25	0.29	grassy, soapy aroma
(E,E)-2,4-nonadienal	0.130 ± 0.011^{c}	0.175 ± 0.008^{b}	0.213 ± 0.013^{a}	0.09	0.4	0.42	1.01	coriander, grease flavor, grassy
(E)-2-decenal	0.107 ± 0.001^{b}	0.150 ± 0.015^{a}	0.079 ± 0.002^{c}	2.7	0.01	0.01	0.01	grassy, orange flavor, fishy smell
(E,E)-2,4-decadien-1-al	0.183 ± 0.027^{a}	0.179 ± 0.051^{a}	0.146 ± 0.005^{ab}	0.05	1.01	0.78	1.26	grease flavor, grassy, citrus flavor
2-undecenal	0.105 ± 0.001^{b}	0.207 ± 0.002^{a}	0.086 ± 0.001^{b}	0.5	0.06	0.09	0.07	grease flavor, grassy, citrus flavor
1-octen-3-ol	7.520 ± 0.536^{b}	16.889 ± 1.243^{a}	9.898 ± 0.079^{b}	1	2.07	3.67	4.28	soil aroma, mushroom aroma
3-octanol	0.293 ± 0.034^{b}	0.275 ± 0.004^{b}	2.587 ± 0.024^{a}	18	<0.01	<0.01	0.06	nut aroma, citrus flavor, herb fragrance
1-octen-3-one	1.813 ± 0.257^{b}	2.30 ± 0.048^{a}	1.156 ± 0.016^{c}	0.005	100	100	100	soil aroma, mushroom aroma, metallic flavor
3-octanone	0.739 ± 0.007^{b}	1.391 ± 0.004^{a}	1.327 ± 0.003^{a}	28	<0.01	0.01	0.02	fat-waxy, vegetable flavor, fruity
2-undecanone	0.177 ± 0.010^{c}	0.357 ± 0.014^{a}	0.297 ± 0.002^{ab}	7	<0.01	0.01	0.02	fruity, grease flavor, rue
dimethyl sulfide	5.231 ± 0.075^{a}	2.849 ± 0.112^{c}	4.325 ± 0.244^{ab}	0.3	4.81	2.06	6.24	fish flavor, garlic flavor, onion flavor
4-methylanisole	59.403 ± 3.564^{a}	29.601 ± 1.352^{c}	43.658 ± 3.211^{b}	560	0.03	0.01	0.03	*Cananga odorata*, fruity, meat flavor
2-pentylfuran	0.231 ± 0.004^{b}	0.387 ± 0.016^{a}	0.420 ± 0.031^{a}	5.8	0.01	0.01	0.03	grease flavor, metallic flavor, fruity

an important contribution or modification effect on the change in flavor are aldehydes, alcohols, ketones, and ethers.

Throughout the storage period, the total relative content of volatile substances with an important contribution or modification effect on truffle flavor (0.1 < ROAV < 100) on the 14th day of storage was 40.644%, which was significantly higher than the values of 26.273% on the zeroth day and 27.71% on the 28th day. This increase in the content of volatile substances disturbed the balance of truffle flavor, resulting in new flavor characteristics of truffles and indicating that storage for 14 d was the inflection point of drastic changes in truffle flavor and possibly represented the inflection point of freshness changes. Among them, isovaleraldehyde, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide are the most important ingredients contributing to truffle flavor. They were also detected in Yunnan black truffles (*T. melanosporum*) and contributed to flavor,[31] indicating that they are characteristic volatile compounds in fresh truffles. After 28 days of storage, two aldehydes, 3-(methylthio) propionaldehyde and (E,E)-2,4-nonadienal, were substances that modified the flavor of truffles in the early and middle periods of storage and suddenly exerted a significant effect on the flavor. The ROAV increased from 0.77 and 0.4 to 1.58 and 1.01, respectively, and the relative contents also showed an increasing trend during the storage period, from 0.559 and 0.130% to 0.729 and 0.213%, respectively. These two compounds were the key volatile substances that gradually caused the odor of truffles during storage.

3.4. Principal Component Analysis of Key Volatile Compounds. The relative contents of the seven key volatile compounds (ROAV > 1) that were commonly detected during storage were used as the original information, and eigenvalues greater than 1 were used as factors for principal component analysis to determine the effects of key volatile compounds on the flavor of truffles during each storage period. The results are shown in Table S1. The variance contribution rates of Principal Component One (PC1) and Principal Component Two (PC2) were 61.84 and 38.16%, respectively, and the accumulation reached 100%, which fully expressed the basic information on the original data.

Figure 2 shows the principal component loading diagram of key volatile substances in truffles during different storage periods. First, the three storage periods were located in different quadrants, indicating that they were well differentiated. Second, in terms of key volatile substances, 1-octen-3-one had the largest positive semiaxis load coefficient on PC1;

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

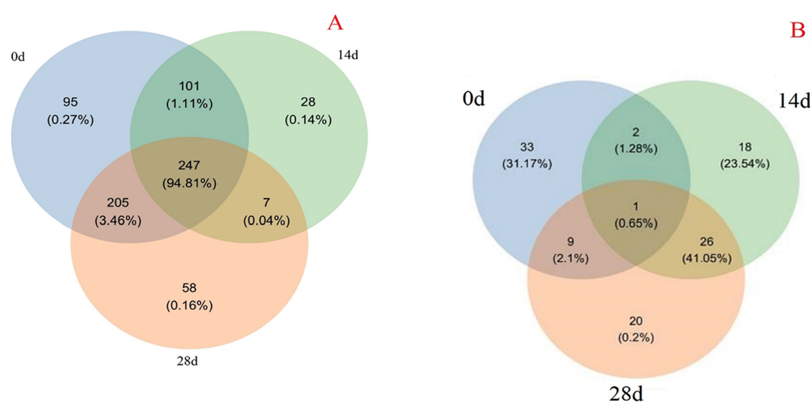


Figure 3. Venn diagram of the bacterial (A) and fungal (B) flora on the surface of truffles during different storage periods.

3-methylthiopropanal 3-(Methylthio) had the smallest load coefficient on the negative semiaxis, propionaldehyde; on PC2, (E,E)-2,4-nonadienal had the largest load coefficient on the positive semiaxis, and dimethyl aldehyde had the smallest load coefficient on the negative semiaxis. The larger the absolute value of the positive load coefficient, the stronger the correlation between this index and the corresponding principal component axis. Therefore, ketones, aldehydes, and ethers exert the greatest effect on flavor, and the relative contents of the four key volatile substances described above change significantly during the storage of truffles. 3-(Methylthio)-propionaldehyde was one of these volatile compounds. The relative content of (E,E)-2,4-nonadienal increased with a prolonged storage time, and thus it can be used as a marker for the spoilage period of truffles. The changes in the aforementioned substances can be monitored to distinguish samples collected after different storage periods.

3.5. Results from the Evaluation of the Aroma Characteristics of Truffles. Human sensory organs can capture trace components that cannot even be detected by chemical instruments with extremely high sensitivity and can truly and effectively describe and evaluate the color, aroma, taste, shape, and texture of food.[32] In this paper, QDA was conducted on the aroma characteristics of truffles detected based on smell, and the final results obtained are shown in Figure 2E. The flavor profiles of storage for 0 days (early stage) and 14 days (middle stage) compared with 28 days (late stage) were highly similar, indicating that the aroma characteristics of truffles analyzed in the first two periods were relatively close. In the evaluation of specific aroma characteristics, the mushroom aroma (1-octen-3-ol) was the most prominent aroma of truffles stored for 0 d. The 1-octen-3-one and fruity (isovaleraldehyde) aromas were more prominent, and the cooking smell (3-(methylthio) propionaldehyde) was the most prominent after storage for 28 days. The aromas of prominent metallic (2-pentylfuran), halal (octanal), and grassy ((E,E)-2,4-non-adienal) components increased continuously, indicating that the volatile substances in truffles changed significantly over different storage periods. At the end of the storage period, the altered aroma of truffles may be caused by factors such as nutrient consumption, an increase in enzyme activity, excessive oxidation of fatty acids, and microbial invasion. The overall flavor of the product is shifting toward a deteriorating trend. Therefore, each storage period may be distinguished according to the difference in the main aroma characteristics detected for each period.

3.6. Data Quality Control and Diversity Analysis. *3.6.1. 16S rDNA Bacterial Diversity Analysis.* The sequencing statistics are shown in Table S2. After splicing and quality control of 112,320 original sequences, 99,163 high-quality valid sequences with an average length of 297 nt were obtained, and the quality control efficiency rate was 88.28%. Sequences accounted for more than 86% of the total, and the Q30 values of the valid sequences were all greater than 93%, all of which met the requirements for the subsequent bioinformatics analysis.[33] The alpha diversity analysis of bacteria revealed that the species diversity and richness index of the bacterial community in truffles decreased first and then increased with a prolonged storage time. On day 0 of storage, the bacterial diversity and abundance in the samples were the highest due to the large amount of soil attached to the surface of the truffles after harvest, which contained many bacterial groups. On the 14th day of storage, the Shannon index and Simpson index of colony diversity decreased to their lowest values, which were 3.069 and 0.866, respectively. At the same time, the Chao1 and ACE colony richness indices in the storage environment also decreased to their lowest values at 14 days, which were 414.109 and 417.794, respectively. The decreases in colony diversity and abundance may be related to the effect of the storage microenvironment formed by the active and spontaneous controlled atmosphere at a low temperature on microorganisms. A total of 1548 OTUs were detected during the storage of truffles. By analysis of a Venn diagram of the entire storage period, 247 OTUs existed in each period, and 95, 28, and 58 OTUs were unique to each period, showing that the bacterial community structure in the active and spontaneous controlled atmosphere storage environment was quite different. The OTUs detected in the samples during the storage period were further classified into phyla, classes, orders, families, genera, and species, and 12 bacterial phyla, 49 bacterial orders, 96 bacterial families, 195 bacterial genera, and 443 bacterial species were identified, indicating that the bacterial community structure of truffles during storage was relatively complex (Figure 3A).

3.6.2. ITS Fungal Diversity Analysis. The sequencing statistics are shown in Table S3. After splicing and quality control of 51,252 original sequences, 47,380 high-quality valid sequences were obtained, with an average length greater than 351 nt. The quality control efficiency rate was 92.45%, with a Q30 value of all sequences greater than 94%, all of which met the requirements for the subsequent bioinformatics analysis.[33] After the number of sequences reached 5000, the OTU dilution curve became flat, indicating that the sequencing

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

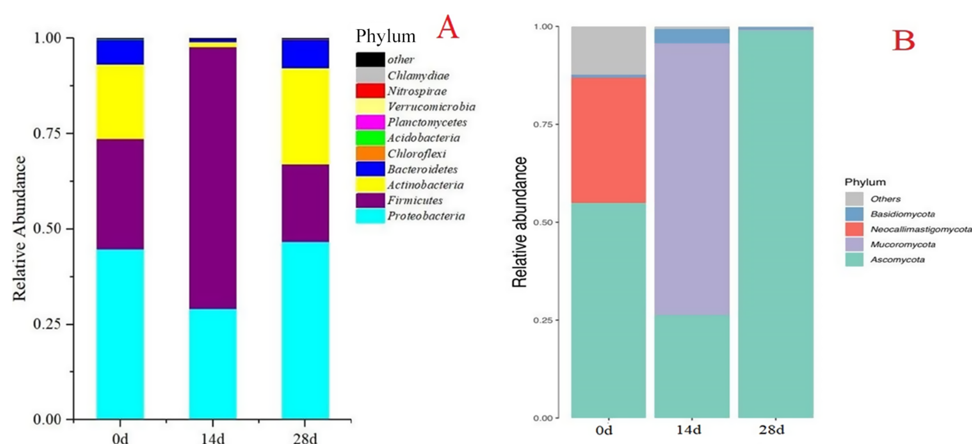


Figure 4. Community taxonomic composition and abundance distribution of bacteria (A) and fungi (B) at the phylum level on the surface of truffles during different storage periods.

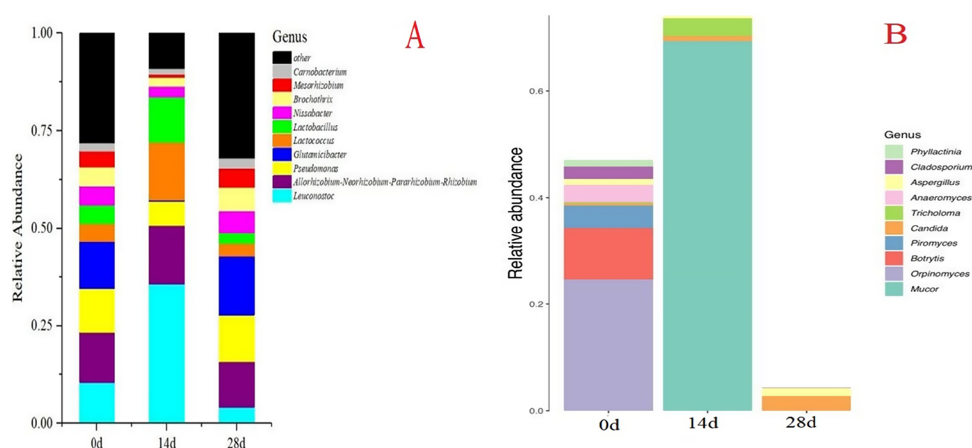


Figure 5. Community taxonomic composition and abundance distribution of bacteria (A) and fungi (B) at the genus level on the surface of truffles during different storage periods.

depth had basically covered all species in the sample, and the amount of sequencing data for this truffle sample was reasonable. The highest species richness and diversity of fungi were observed in the samples treated with CK (AMAP storage 0 days), and the species distribution was relatively uniform. The species richness and diversity of fungi were lower in HOAP-treated (AMAP storage for 14 days) and HOAP2-treated (AMAP storage for 28 days) samples.

A cluster analysis was performed on the test samples to obtain the OTU values of the samples, and the common and unique high-abundance OTUs (average abundance of >1) from different groups of samples were combined to construct the Venn diagram shown in Figure 3B. Differences in the OTUs of samples from different storage periods were compared. Figure 2 shows that 45 OTUs were present in the sample collected at 0 days of storage, 47 OTUs in the sample collected at 14 days of storage, and 56 OTUs in the sample collected at 28 days of storage. Throughout the storage period, only one OTU existed in each period, and 33, 18, and 20 OTUs were unique to each period. As shown in Table S4, with the prolongation of storage time, the Chao1 index, ACE index, Shannon index, and Simpson index of truffle samples initially decreased and then increased, indicating that the total abundance and diversity of fungi in the samples increased with storage time. The results also showed a trend of first decreasing and then increasing. Coverage indices were all >99%, again indicating that the sequencing depth was appropriate.

3.7. Changes in the Microbial Community on the Fruiting Body Surface.

3.7.1. Changes in the Bacterial Community on the Fruiting Body Surface at the Phylum and Genus Levels. At the phylum level, the top 10 species with the greatest abundance were selected to generate a cumulative histogram (Figure 4A). From the beginning to the end of storage, the main colonies identified in the truffles were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, and the total bacterial abundance of these four phyla was always >99%. In the present study, the bacterial flora with a relative abundance ≥10% was defined as the dominant flora, and Proteobacteria, Firmicutes, and Actinobacteria were the dominant bacterial phyla identified at 0 and 28 days of storage. On the 14th day of storage, the sum of the abundances of Proteobacteria and Firmicutes was 97.86%, indicating that AMAP treatment had certain selectivity for bacteria. The trends in bacterial abundance at the genus level during truffle storage are listed in Figure 4B. The top ten bacterial genera on the surface of truffles during storage in terms of relative abundance were *Bacillus* (25.02% at 0 day, 28.02% at 14 days, and 24.45% at 28 days), *Staphylococcus* (17.45% at 0 day, 16.02% at 14 days, and 22.45% at 28 days), and *Leuconostoc* (15.02% at 0 day, 35.76% at 14 days, and 4.26% at 28 days), among others, accounting for a total of 91.08% (0 day),

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

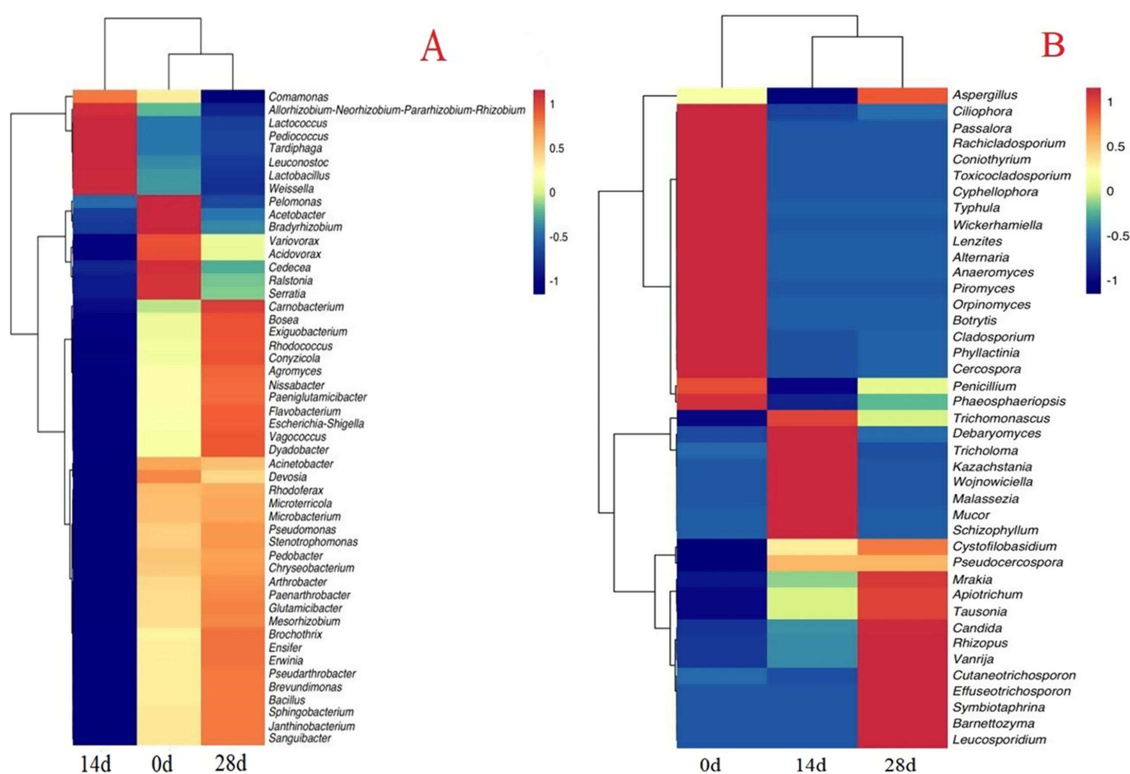


Figure 6. Heatmap analysis of the constitution of the bacterial (A) and fungal communities (B) on the surfaces of truffles during different storage periods.

97.86% (14 days), and 68.07% (28 days) of identified bacteria. The genus *Leuconostoc* was present at the highest abundance in the samples stored for 14 days, accounting for 35.76%, but it was detected at the lowest abundance in the samples stored for 28 days, accounting for only 4.26%. The abundance of *Lactococcus* and *Lactobacillus* increased to their highest levels on the 14th day of storage, accounting for 14.68 and 11.65%, respectively. The trend for the abundance of *Allorhizobium*, *Neorhizobium*, *Pararhizobium*, and *Rhizobium* was consistent with that of *Leuconostoc*, accounting for 12.77, 15.15, and 11.56% of the species detected in each period, respectively. The abundance of *Pseudomonas* showed a trend of first decreasing and then increasing during storage. The abundance of *Pseudomonas* was the lowest at 14 days, accounting for 6.07%, and the highest at 28 days, accounting for 11.99%. The trend for the abundance of *Glutamicibacter* was consistent with that of *Pseudomonas*, accounting for 12.04, 4.43, and 15.08% of species detected in each period, respectively.

3.7.2. Changes in Surface Fungal Communities at the Phylum and Genus Levels. The method of random resampling was used to analyze the valid sequences from fungal phyla (Basidiomycota, Neocallimastigomycota, Mucoromycota, and Ascomycota) in three samples of truffles collected after different storage periods, as shown in Figure 5A. The relative abundance of Ascomycota showed a trend of first decreasing and then increasing, and the relative abundance of Basidiomycota showed a trend of first increasing and then decreasing. The phyla Neoflagellate and Basidiomycota only appeared at 0 and 14 days of storage, respectively. Among them, the important phyla in the samples stored on day 0 were Ascomycota (54.92%) and the phylum Neoflagellate (32.02%); the phyla identified in the samples stored for 14 days were mainly Ascomycota (26.30%) and Mucoromycota (69.39%); and the phyla detected in the samples stored for 28 days mainly included Ascomycota (98.85%). With the prolongation of storage time, the fungal community composition at the phylum level became increasingly singular, indicating that the modified atmosphere treatment exerted a profound effect on the composition of fungi on the surface of truffles during storage.

Random resampling was also used to study the proportion of fungal genera sequences during the storage of truffles. Fungal genera accounted for 0.23% or more of the truffle samples stored on the zeroth day and mainly included nine genera Figure 5B For *Orpinomyces*, *Botrytis*, *Piromyces*, *Candida*, *Tricholoma*, *Anaeromyces* spp., *Aspergillus*, *Cladosporium*, and *Phyllactinia*, the relative abundances were 24.59, 9.74, 4.19, 0.42, 0.23, 3.24, 1.12, 2.30, and 1.23%, respectively. The fungal genera in the samples stored for 14 days mainly included four genera, namely, *Mucor*, *Candida*, *Trichoderma*, and *Aspergillus*, with relative contents of 69.30, 0.96, 3.30, and 0.50%, respectively. The fungal genera detected on the samples stored for 28 days mainly included four genera, namely, *Candida*, *Aspergillus*, *Cladosporium*, and *Spheroides*, with relative abundances of 2.77, 1.49, 0.06, and 0.04%, respectively.

3.8. Cluster Analysis of Microbial Communities on the Surface of Truffle Fruiting Bodies at the Genus Level. *3.8.1. Cluster Analysis of Bacteria Detected on the Surface of Fruiting Bodies at the Genus Level.* The relative abundance of each bacterial community was calculated as the base 10 logarithm, a heatmap was generated, and a cluster analysis was performed to further clarify the changes in the bacterial community on the body surface of the postharvest truffles at the genus level during storage, where the columns represent samples from different storage periods, the rows represent different bacterial communities, and the heatmap

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

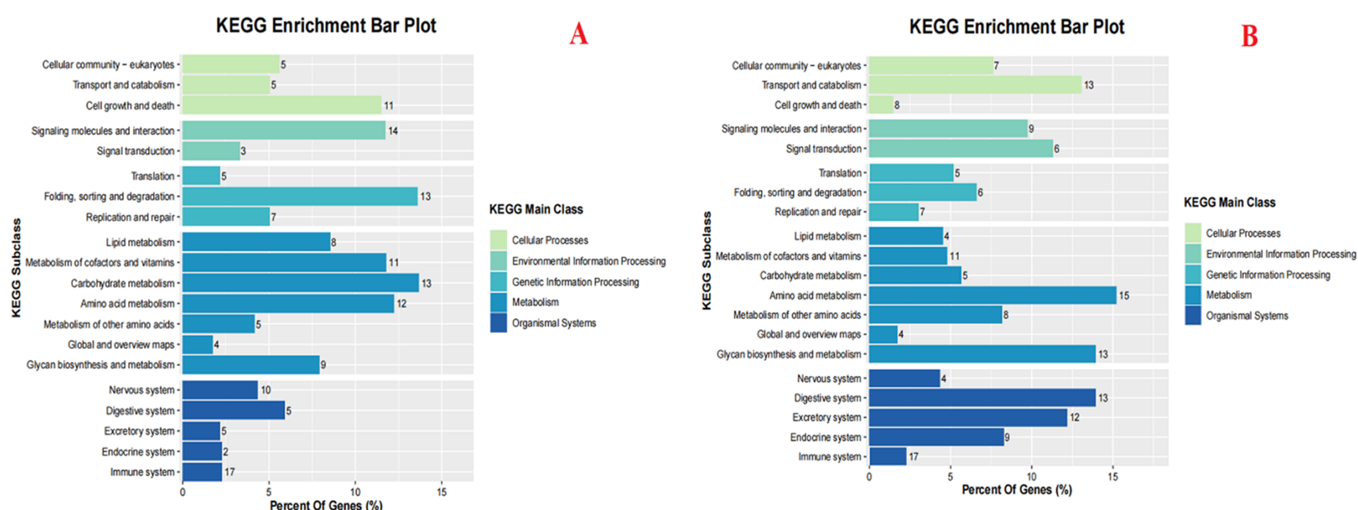


Figure 7. Annotation analysis of KEGG pathways of bacterial flora (A) and fungal flora (B) detected on the surface of truffles during different storage periods.

illustrating the abundances of the top 50 bacteria at the genus level is shown in Figure 6A. The columns represent samples from different storage periods, and the rows represent different bacterial species: the hue indicates relative abundance, with warmer colors indicating a higher species abundance. As shown in the figure, the main genera identified throughout the storage period were *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Leuconostoc*, *Pseudomonas*, *Lactococcus*, and *Lactobacillus*, which belong to phyla Proteobacteria and Firmicutes. Except for *Pseudomonas*, the proportions of other genera initially increased and then decreased during storage. *Leuconostoc*, *Lactobacillus*, and *Lactococcus* formed the acidic environment of the truffle, which has a certain contribution to the maintenance of food safety, but it may have a potential effect on the food flavor. Additionally, some bacteria in the genus *Lactococcus* are more putrefactive, which will accelerate the deterioration of the quality of truffles. At the same time, the growth of spoilage-causing pathogens such as *Pseudomonas*, *Brochothrix*, and *Carnobacterium* was significantly suppressed by the dominant bacterium *Leuconostoc* after 14 days of storage but gradually resumed growth after 28 days of storage, consistent with the results shown in the histogram of the genus-level relative abundance accumulation.

3.8.2. Cluster Analysis of Fungi on the Surface of Fruiting Bodies at the Genus Level. The diversity of genera was found to decrease during the whole process of truffle storage. Throughout the storage period, the common genera detected were *Candida* and *Aspergillus*. The relative abundance of the former showed a decreasing trend, while the relative abundance of the latter showed a trend of first decreasing and then increasing. Using R software, a cluster analysis was performed on the top 50 genera ranked by abundance, and a heatmap was drawn, as shown in Figure 6B. Each cell in the heatmap represents the relative abundance of a certain bacteria in the sample, and the redder the color, the higher the relative abundance.[34] At the genus level, the high-abundance species detected in the samples stored for 14 and for 28 days were relatively consistent and clustered first and then clustered with the samples stored for 0 day. At the same time, the coefficient of variation (CV) was calculated to characterize the fluctuation of species between samples,[35] and here, only the genus level was calculated to represent the 10 smallest and largest CVs of 10 genera. The results revealed seven genera with the largest species fluctuation among the samples, namely, *Mucor*, *Orpinomyces*, *Piromyces*, *Anaeromyces*, *Alternaria*, *Botrytis*, and *Lenzites*, and the *Aspergillus* genus exhibited the smallest species fluctuation.

3.9. KEGG Analysis of the Microbial Community on the Surface of Truffle Fruiting Bodies. In the primary classification of "Cellular Processes", "Cell Growth and Death" is the most representative pathway, accounting for the largest proportion identified during the whole period of active and spontaneous controlled atmosphere storage at low temperatures, indicating that bacterial communities on truffle surfaces activate cell signaling in response to low-temperature environments. In addition, as the most representative pathway in the category of "Environmental Information Processing", "Signal Transduction" exhibited the greatest enrichment throughout the period of air-conditioning storage in an ice-temperature microenvironment, which may help bacteria on the truffle surface adapt to cold conditions. Figure 7A shows that carbohydrate metabolism and amino acid metabolism were the most enriched pathways in metabolism, which may also provide one explanation for the change in the flavor of truffles during storage.[36]

The KEGG enrichment analysis of fungi is shown in Figure 7B, unlike bacteria, in the primary classification of "Cellular Processes", "Transport and Catabolism" is the most important pathway. Representatively, this pathway accounted for the largest proportion, indicating that the fungi present on the surface of truffles during active and spontaneously controlled atmosphere storage at low temperatures affect the biological activity of truffles, resulting in the degradation of nutrients and spoilage and may be involved in flavor substances produced during the storage of truffles. Some fungi also secrete adenosine to block the production and release of oxygen free radicals by neutrophils or produce aspartyl protease to degrade the extracellular matrix, causing tissue damage (0). Amino acid metabolism was the main enriched pathway in the primary classification of metabolism, which may be related to the role of bacteria in this classification and the flavor change that occurs during the storage of truffles.

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

4. DISCUSSION

Truffles are a rare edible fungus and are very perishable. They will lose their nutritional value and typical aroma due to water loss and a high load of microorganisms within 1 week after harvest without fresh-keeping measures. Therefore, in this study, truffles were placed at 4 °C with 40% O_2 and 60% CO_2 under active and spontaneous controlled atmosphere conditions to explore the changes in volatile components and the bacterial community structure during storage and to clarify the effects of storage conditions on truffle volatile components and microorganisms.

4.1. Analysis of Flavor Substances in Truffle during AMAP Storage.

During the active and spontaneous storage of truffles in the O_2/CO_2, 63 volatile components in 10 categories were detected using GC−MS, and aldehydes, alcohols, ketones, and ethers were identified in the ROAV analysis. Four major categories of substances exert an important effect on or modify the flavor of truffles.

Aldehydes are the most volatile substances in truffles, mainly containing C_5−C_9 aldehydes. These aldehydes are mainly derived from the oxidation and degradation of fat, producing a fruity and fatty odor and have a relatively high quality. Aldehydes with high carbon numbers also have the aroma of citrus peel,[37] and other aldehydes are formed through the Strecker degradation pathway, in which isovaleraldehyde is derived from the degradation of leucine and is produced in the form of malt and malt cocoa aroma.[38] Benzaldehyde is the degradation product of phenylalanine and has almond and nutty aromas.[39] Aldehydes are volatile and have low thresholds, which may work with other compounds to produce stronger flavor overlap effects,[40] such as (E,E)-2,4-nonadienal and hexanal, which have oily and grassy tastes. Some linear aldehydes, such as hexanal and octanal, may be the main source of a fishy smell.[41] During storage, the types and relative contents of aldehydes change first. The increase and subsequent decrease may be related to the fact that the initial high oxygen content accelerates the absorption of oxygen by the free radicals produced by fat and leads to fatty acid oxidation. Later, with an increase in carbon dioxide concentration, the inhibitory effect reduces the degree of fat oxidation. Aldehydes with an important contribution to the flavor of truffles during storage include isovaleraldehyde, 3-(methylthio)propionaldehyde, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, and (E,E)-2,4-decadien-1-al, of which 3-(methylthio) propionaldehyde is a sulfur-containing substance with cooking odor properties similar to boiled potatoes that is derived from the degradation of methionine or the Strecker degradation of methionine,[42] releasing an unpleasant odor and provoking irritation at high concentrations, which can deteriorate the aroma quality of truffles.

Among all the alcohols detected during the storage of truffles, the relative content of 1-octen-3-ol, known as "mushroom alcohol", accounted for the highest proportion, and its reaction is catalyzed by lipoxygenase. It is an oily liquid octacarbon compound ($C_8H_{16}O$) formed from linoleic acid, which, in addition to possessing a strong mushroom aroma, also has wet wood and herbal notes. It is presumed to be involved in the interaction between host plants and truffles and is involved in the mycorrhizal process in the truffle life cycle.[43] Throughout the storage period, the relative content of 1-octen-3-ol initially increased and then decreased, which may be related to the various oxidoreductase systems that are active in truffle fruiting bodies at different storage stages. The expressed enzyme activity is correlated.[44] In addition, the relative content of ethanol showed an increasing trend during storage, but its threshold was high (100,000 μg/kg), and its effect on flavor was basically negligible according to the ROAV evaluation.

Ketones made up the fourth largest class of compounds detected. They have floral, fruity, creamy, and long-lasting aromas. During the three storage periods, four, five, and three ketones were detected, respectively, and the greatest total amount of ketones was detected in the middle of the storage period, which may be caused by the autoxidation of unsaturated fatty acids and Strecker degradation.[45] In this study, 1-octen-3-one was detected in the highest quantity, and its aroma threshold was extremely low (0.005 μg/kg), which endowed the truffle with a strong earthy aroma. Its relative content increased from 1.813% in the early storage period to 2.30% in the middle storage period but finally decreased to 1.156% in the late storage period. The substances detected in higher amounts during the storage period were 3-octen-one and 2-undecanone, and the trend for the changes in their relative contents was consistent with that of 1-octen-3-one. 3-Octen-one has ketone, waxy, vegetable, and fruity aroma characteristics and 2-undecanone has fruity, oily, and cheese-like aromas, but the aroma thresholds of these two substances are much higher than that of 1-octen-3-one, suggesting that they may only potentially contribute to flavor.

Ethers were the most abundant volatile components detected in terms of the relative content. Among them, the relative contents of *p*-methylanisole and 4-methylanisole were the highest. Anisole naturally exists in ylang−ylang, violets, and tomatoes and provides sweet floral aromas to truffles. Except for Yunnan black truffles (*T. melanosporum*), no other reports have documented this compound in truffles. However, its threshold value was high (560 μg/kg); although it had the highest relative content, its final contribution to the truffle flavor was very small and it may be a potential contributor to flavor. Dimethyl sulfide, a lower-threshold sulfur-containing compound produced by the Maillard reaction induced by methionine, was detected during storage,[46] providing truffles with dimethyl sulfide. It has a strong fishy, onion, garlic, and other spicy taste. This compound has been shown to be a characteristic flavor component in other truffle species.[47,48]

Controlled atmosphere storage at 4 °C with 40% O_2 + 60% CO_2 delayed the decay of truffle aldehydes, alcohols, esters, furans, acids, and olefins and slowed the degradation of ketones, ethers, and other substances (olefins and alkanes). ROAV results showed that isovaleraldehyde, 1-octen-3-ol, 1-octen-3-one, and dimethyl sulfide were the characteristic flavor components of fresh truffles, and hexanal, octanal, (E)-2-octenal, nonanal, and decanal potentially modified the flavor of truffles. The total relative content of volatile components that contributed to the flavor of truffles (ROAV > 0.1) changed significantly after 14 days of storage, which became the inflection point of the flavor change. Among them, 3-methylthiopropanal and (E,E)-2,4-nonadienal were the key volatile substances that caused the odor of truffles. The PCA results showed that ketones, aldehydes, and ethers had the greatest effect on the flavor of truffles during storage and increases in the levels of these compounds can be used as an indicator of spoilage in the quality of truffles. The evaluation results of aroma characteristics showed that the aroma characteristics of truffles changed significantly during storage. On day 0 of storage, the truffles mainly produced a mushroom

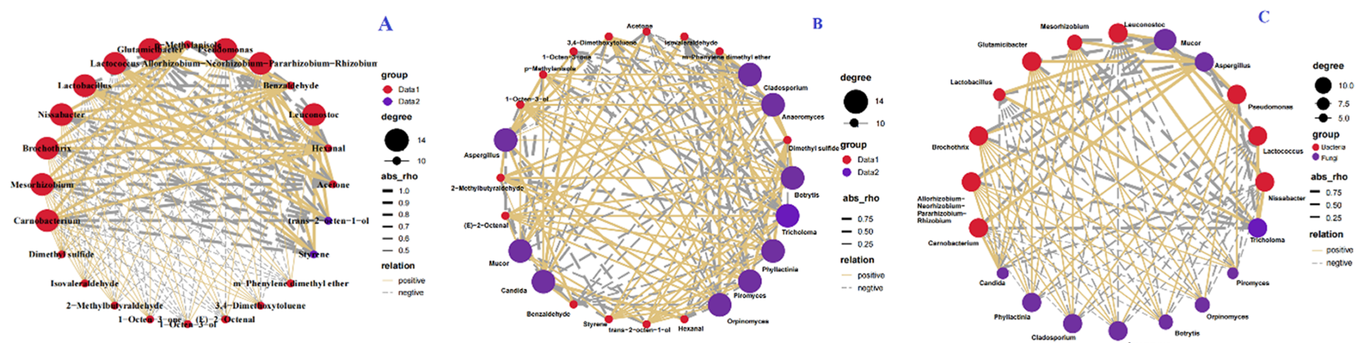


Figure 8. Network diagram showing the results from the analysis of the correlation between the microbiota and volatile flavor compounds (A), between fungal flora and volatile flavor compounds (B), and between fungal flora and bacterial flora (C) based on the Pearson correlation coefficients.

aroma; after 14 days of storage, they mainly had the aroma of soil; and after 28 days of storage, they mainly produced the smell of cooking.

4.2. Analysis of the Truffle Microbiota during AMAP Storage. *Pseudomonas* is an obligate aerobic Gram-negative bacillus that promotes the growth and development of truffles before harvest; it is the main pathogenic bacterial species detected in fruits, vegetables, and edible fungi.[49] It secretes cell wall-degrading enzymes, thereby destroying cell tissues, which cause the degradation of lettuce, tomato, and other fruit and vegetable tissues, and the surface is sticky and odorous.[50] The abundance of *Pseudomonas* at 14 days of storage was significantly lower than that observed at the other two periods. At the same time, two spoilage pathogens, *Cyclostomyces* and *Clostridium*, were among the top ten species in abundance throughout the storage period. They are not common in edible fungi and often appear in poultry meat.[51] *Brochothrix* is a facultative anaerobic bacterium.[52] When the O_2 content in the package decreases, this bacterium can first use glucose, mannose, and other sugars as growth substrates for rapid growth, and thus, its abundance increases to the highest value after 28 days of storage. *S. thermophila*, a member of this genus, has a strong ability to decompose proteins and fats, which can cause spoilage and odor.[53] *Clostridium* is also a facultative anaerobic bacterium, and many members of this genus can grow at a low temperature of 0 °C.[54] This bacterium can produce volatile substances with an aroma similar to a butter odor, which exerts a substantial effect on food spoilage.[55] Its abundance also showed an increasing trend and reached its highest level at 28 days of storage. However, the abundance of these two genera reached the lowest level on the 14th day of storage, indicating that 40% O_2 + 60% CO_2 controlled atmosphere storage at 4 °C effectively inhibited the growth of spoilage-causing bacteria.

Leuconostoc has a wide range of sources and has been detected in edible fungi,[56] fermented vegetable products,[57] tropical fruits,[58] milk samples,[59] soil,[60] and even animal intestinal feces (79), and some bacteria can survive under facultative anaerobic conditions. We speculated that *Leuconostoc* became an absolutely dominant genus after 14 days of storage or was related to this characteristic. At the same time, *Leuconostoc* can convert polysaccharides into mannitol and glucan, providing a carbon source for the growth of *Lactococcus* and *Lactobacillus* bacteria,[61] while the genera *Lactococcus* and *Lactobacillus* exhibit strong acid resistance and survive well in the storage environment, which explains why the abundances of *Lactococcus* and *Lactobacillus* increased to the maximum values after storage for 14 days, and these bacteria became two of the dominant genera. In addition, *Lactococcus* and *Lactobacillus* also produce acid or synthesize bacteriocins, which inhibit the growth of Gram-positive bacteria, thus competing to participate in the nutritive decomposition of truffles. *Lactobacillus* species also inhibit the growth of microorganisms that breakdown proteins. However, *Lactococcus* has been described as a ubiquitous microorganism associated with postharvest spoilage of truffles, has the ability to produce amines, and should be further controlled. The low temperature of 4 °C can also inhibit the growth of most microorganisms, and with the progression of truffle respiration, a large amount of CO_2 dissolved in water is generated in the package with 40% O_2 + 60% CO_2 controlled atmosphere storage at 4 °C. The acidic environment also helped to inhibit the growth of acid-labile bacteria, making *Leuconostoc* the dominant genus after storage for 14 days. After 28 days of storage, the proportions of the above-mentioned genera decreased to the lowest levels, which may be related to the negative feedback caused by the accumulation of acidic metabolites and the excessively low pH environment formed by the high concentration of CO_2 dissolved in water or because the nutrients on the surface of the truffles were exhausted. When the nutrients are exhausted, the above-mentioned bacteria enter a period of decline, the inhibitory effect on other flora is weakened, and the proportion of some spoilage-causing pathogens also increases. Other genera present at higher abundances may have less of an effect on truffle quality, such as *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Brachyrhizobium*, which have the ability to fix nitrogen[62] and may be related to truffle growth and development.

4.3. Correlation Analysis between the Bacterial Community and Truffle Volatile Components. An assessment of the potential relationship between microbial communities and flavor compounds provides a template for screening functional microorganisms. An analysis of the relationship between microorganisms and flavor compounds is beneficial to identifying potential flavor-functional flora and controlling food flavor characteristics. A correlation analysis was conducted between volatile components exhibiting significant changes during different storage periods and core microorganisms. IBM SPSS software was used to analyze the correlations between 14 significantly different volatile flavor substances and the top ten core microorganisms in terms of abundance during storage. Figure 8A was generated using the OmicStudio tools; the nodes represent volatile compounds and

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

microorganism categories, and the lines between the nodes represent the correlation coefficients.

4.3.1. Linking Volatile Components to the Bacterial Community Structure through Pearson's Correlation Analysis. The proportions of *Lactococcus* and *Lactobacillus* increased first and then decreased during storage. The abundances of *Lactococcus* and *Lactobacillus* were related to acetone, 2-octenal, and styrene. The abundance of *Pseudomonas* increased to its highest level at 28 days of storage, and the relationship between its abundance and volatile flavor substances was opposite to that of *Lactococcus* and *Lactobacillus*. *Lactococcus* and *Lactobacillus* were significantly positively correlated with acetone, while *Pseudomonas* and *Glutamicibacter* exhibited negative correlations. *Lactococcus* and *Lactobacillus* are the major flora present in dairy products, plant products, etc.[63] *Lactococcus* produces lactic acid and can use alkanes as a carbon source. Lactic acid bacteria convert carbohydrates into ethanol to produce a series of acids, esters, and higher alcohols.[31] *Pseudomonas* is one of the main pathogenic bacteria detected in fruits, vegetables, and edible fungi. It secretes cell wall-degrading enzymes, thereby destroying cell tissues, causing the surface of truffles in the later storage period to be sticky and slippery and produce a peculiar smell. It also causes soft rot and brown disease in edible fungi.[50] The results showed that the contributions of *Lactococcus* and *Lactobacillus* to the same flavor may be consistent but opposite to the contributions of *Pseudomonas* and *Glutamicibacter* to the same flavor. Various results indicated that the interaction between microorganisms affected the changes in flavor compounds in truffles during storage, and thus multiple genera in the bacterial communities may be related to flavor compounds.[64]

4.3.2. Correlation Analysis between the Fungal Community and Truffle Volatile Components. Figure 8B shows the correlation network between fungi and volatile flavor substances during truffle storage. The correlations between 10 fungi and 14 volatile flavor compounds were described. *Aspergillus* exhibited a significant positive correlation with three aldehydes, one ketone, and one ether, including 2-methylbutyraldehyde, hexanal, benzaldehyde, 1-octen-3-one, and *p*-methylanisole, indicating that *Aspergillus* may participate in the synthesis of these substances and in the decomposition of trans-2-octen-1-ol. *Aspergillus* secretes glucose oxidase, saccharifying enzymes, proteases, and lipases. Enzymes affect the biological activity of fresh truffles, resulting in degradation of their nutrients and deterioration. *Aspergillus* is also very important for the industrial production of ester alcohols and other substances.[65] *Candida* displayed a significant negative correlation with styrene, 1-octen-3-ol, and acetone, indicating that *Candida* may be involved in the degradation of these substances. *Candida* virulence is closely related to its adhesion to body tissues. The bacteria secrete adenosine to block the production and release of oxygen free radicals by neutrophils; they also produce aspartic proteases to degrade the extracellular matrix and cause tissue damage.[66]

4.3.3. Correlation Analysis of Bacteria and Fungi on the Surface of Truffle Fruiting Bodies. Based on the genus level, the top ten bacterial and fungal genera in terms of relative abundance during storage were selected for the correlation analysis, as shown in Figure 8C, and the relationship between bacterial and fungal genera was revealed based on the Pearson correlation coefficient. The study found that *Mucor* was significantly positively correlated with *Lactobacillus* and *Lactococcus* and significantly negatively correlated with *Brochothrix*. *Mucor* is an important pathogenic fungus that seriously harms the peanut and soybean industries, and its metabolites are widely present in phytopathogenic fungi as phytotoxins.[67] *Lactococcus* produces lactic acid and can use alkanes as a carbon source. Lactic acid bacteria convert carbohydrates into ethanol to produce a series of acids, esters, and higher alcohols,[68] indicating that the interaction between *Mucor* and *Lactobacillus* spp.and *Lactococcus* led to the spoilage of truffles during storage. By consulting relevant data, the core flora that cause the spoilage of fresh truffles are all aerobic bacteria.[69] Therefore, the active spontaneous atmosphere used in this study effectively inhibits the life activities of spoilage-causing flora and prolongs the storage time of truffles.

5. CONCLUSIONS

We explored the trends for the changes in volatile components during the storage of truffles at 4 °C with a 40% O_2 + 60% CO_2-modified atmosphere. The results showed that 3-methylthiopropanal and (E,E)-2,4-nonadienal, which are aldehydes, played an important role in the generation of truffle odor, which may be used as indicators of truffle spoilage characteristics. According to the evaluation of aroma characteristics, the aroma characteristics detected during storage changed from the initial mushroom aroma to an earthy aroma and finally to a cooking smell. The dynamic changes of the bacterial community on the body surface of truffles during storage at 4 °C with a 40% O_2 + 60% CO_2 controlled atmosphere were revealed. Proteobacteria and Firmicutes were the common dominant phyla detected during storage. *Leuconostoc* was the absolute dominant bacterial genus detected in truffles stored for 14 days, accounting for 35.76%. On the 28th day of storage, *Pseudomonas* became one of the dominant genera, and the abundance of *Cyclostomyces* and *Clostridium* also increased to their highest levels during this period. The core flora that leads to the spoilage of fresh truffles are all aerobic bacteria, and *Mucor* is a thermophilic fungus, which may also prove that the active spontaneous atmosphere used in this study effectively inhibits the life activities of spoilage-causing flora to prolong the storage time of truffles. This study provides a reference for the green preservation of truffles.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c08375.

Principal component eigenvalues, observed valid sequences, and alpha diversity index (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Xiang Li − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China;* orcid.org/0000-0002-9696-0940; Phone: +86-028-84616653; Email: lixiang@cdu.edu.cn

Authors

Qiang Li − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*

Haiyang Hu − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347

Xingyi Tan − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*
Jianhui Wang − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*
Ruhuai Mei − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*
Fangguo Jiang − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*
Yunkun Ling − *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan 610106, China*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.3c08375

Author Contributions

Q.L.: Writing−original draft, Writing−review and editing. H.H.: Conceptualization, Methodology, Software, Visualization, Writing - original draft. X.T.: Data curation. J.W.: Methodology. R.M.: Resources. F.J.: Writing, Visualization, Editing. Y.L.: Formal analysis, Resources. X.L.: Investigation, Conceptualization, Supervision, Writing - review and editing.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported Key R & D Plan of Sichuan Provincial Science and Technology Department (No.2022YFG0030, 24GJHZ0041) funds for the financial support.

REFERENCES

(1) Bonito, G. M.; Gryganskyi, A. P.; Trappe, J. M.; Vilgalys, R. A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular ecology* **2010**, *19* (22), 4994−5008.

(2) Le Tacon, F.; Rubini, A.; Murat, C.; Riccioni, C.; Robin, C.; Belfiori, B.; Zeller, B.; De la Varga, H.; Akroume, E.; Deveau, A.; et al. Certainties and uncertainties about the life cycle of the Périgord black truffle (Tuber melanosporum Vittad.). *Annals of Forest Science* **2016**, *73* (1), 105−117.

(3) Yun, W.; Pei-Gui, L. J. Verification of Chinese Names of Truffles and Their Conservation in Natural Habitats. *Plant Divers.* **2011**, *33* (6), 625−642, DOI: 10.3724/SP.J.1143.2011.11059.

(4) Bellesia, F.; Pinetti, A.; And, B. T.; Bianchi, A. J. Temperature-dependent evolution of volatile organic compounds in Tuber borchii from Italy. *Flavour Fragr. J.* **2001**, *16*, 1−6, DOI: 10.1002/1099-1026(200101/02)16:1<1::AID-FFJ936>3.0.CO;2-Y.

(5) Strojnik, L.; Grebenc, T.; Ogrinc, N. Species and geographic variability in truffle aromas. *Food Chem. Toxicol.* **2020**, *142*, No. 111434, DOI: 10.1016/j.fct.2020.111434.

(6) Feng, T.; Shui, M. Z.; Song, S. Q.; Yu, Y. X.; Sun, M.; Xu, Z. M. Comparative Analysis of Volatile Flavor Compounds of Fresh Truffles and Dried Truffles in Yunnan. *Fine Chem.* **2019**, *36* (2), 262−270.

(7) Culleré, L.; Ferreira, V.; Venturini, M. E.; Marco, P.; Blanco, D. Chemical and sensory effects of the freezing process on the aroma profile of black truffles (Tuber melanosporum). *Food Chem.* **2013**, *136* (2), 518−525, DOI: 10.1016/j.foodchem.2012.08.030.

(8) Trappe, J. M.; Claridge, A. W. The hidden life of truffles. *Sci. Am.* **2010**, *302* (4), 78−82.

(9) Hajjar, S. E.; Massantini, R.; Botondi, R.; Kefalas, P.; Mencarelli, F. Influence of high carbon dioxide and low oxygen on the postharvest physiology of fresh truffles. *Postharvest Biol. Technol.* **2010**, *58* (1), 36−41, DOI: 10.1016/j.postharvbio.2010.04.008.

(10) Reale, A.; Sorrentino, E.; Iacumin, L.; Tremonte, P.; Manzano, M.; Maiuro, L.; Comi, G.; Coppola, R.; Succi, M. Irradiation Treatments to Improve the Shelf Life of Fresh Black Truffles (Truffles Preservation by Gamma-Rays). *J. Food Sci.* **2009**, *74* (4), M196−200, DOI: 10.1111/j.1750-3841.2009.01142.x.

(11) Rivera, C. S.; Venturini, M. E.; Oria, R.; Blanco, D. Selection of a decontamination treatment for fresh Tuber aestivum and Tuber melanosporum truffles packaged in modified atmospheres. *Food Control* **2011**, *22* (3−4), 626−632, DOI: 10.1016/j.foodcont.2010.10.015.

(12) Rivera, C. S.; Venturini, M. E.; Marco, P.; Oria, R.; Blanco, D. Effects of electron-beam and gamma irradiation treatments on the microbial populations, respiratory activity and sensory characteristics of Tuber melanosporum truffles packaged under modified atmospheres. *Food Microbiol.* **2011**, *28* (7), 1252−1260, DOI: 10.1016/j.fm.2011.05.002.

(13) Guo, Y.; Gao, Z.; Li, L.; Wang, Y.; Zhao, H.; Hu, M.; Li, M.; Zhang, Z. Effect of controlled atmospheres with varying O2/CO2 levels on the postharvest senescence and quality of broccoli (Brassica oleracea Lvar. italica) florets. *Eur. Food Res. Technol.* **2013**, *237* (6), 943−950.

(14) Shasha, L.; Yuxiao, Z.; Liang, W.; Liping, Y.; Yong, C.; Xinhua, Z.; Yan-yin, G. Effect of active modified atmosphere on the inhibition of Gilbertella persicaria. *Food Sci.* **2020**, *41*, 186−192, DOI: 10.7506/spkx1002-6630-20190217-080.

(15) Jiang, F. G.; Hu, H. Y.; Gong, X. Y.; Liu, J.; Li, X. Effects of O2/CO2 active modified atmosphere packging on storage quality and microstructure of postharvest truffles. *Food Mach.* **2021**, *38* (2), 123−129.

(16) Gryndler, M.; Soukupová, L.; Hršelová, H.; Gryndlerová, H.; Borovička, J.; Streiblová, E.; Jansa, J. A quest for indigenous truffle helper prokaryotes. *Environmental microbiology reports* **2013**, *5* (3), 346−352.

(17) Li, Q.; Yan, L.; Ye, L.; Zhou, J.; Zhang, B.; Peng, W.; Zhang, X.; Li, X. Chinese Black Truffle (Tuber indicum) Alters the Ectomycorrhizosphere and Endoectomycosphere Microbiome and Metabolic Profiles of the Host Tree Quercus aliena. *Front Microbiol* **2018**, *9*, 2202.

(18) Li, Q.; Zhao, J.; Xiong, C.; Li, X.; Chen, Z.; Li, P.; Huang, W. Tuber indicum shapes the microbial communities of ectomycorhizosphere soil and ectomycorrhizae of an indigenous tree (Pinus armandii). *PLoS One* **2017**, *12* (4), No. e0175720.

(19) Li, X.; Ye, L.; Zhang, X.; Tan, H.; Li, Q. Root-tip cutting and uniconazole treatment improve the colonization rate of Tuber indicum on Pinus armandii seedlings in the greenhouse. *Microbial biotechnology* **2020**, *13* (2), 535−547.

(20) Fu, Y.; Li, X.; Li, Q.; Wu, H.; Xiong, C.; Geng, Q.; Sun, H.; Sun, Q. Soil microbial communities of three major Chinese truffles in southwest China. *Can. J. Microbiol.* **2016**, *62* (11), 970−979.

(21) Barbieri, E.; Ceccaroli, P.; Agostini, D.; Zeppa, S. D.; Gioacchini, A. M.; Stocchi, V.. *Truffle-Associated Bacteria: Extrapolation from Diversity to Function*; Springer: Cham, 2016.

(22) Vahdatzadeh, M.; Deveau, A.; Splivallo, R. Are bacteria responsible for aroma deterioration upon storage of the black truffle Tuber aestivum: A microbiome and volatilome study. *Food microbiology* **2019**, *84*, No. 103251.

(23) Rivera, C. S.; Venturini, M. E.; Oria, R.; Blanco, D. Selection of a decontamination treatment for fresh Tuber aestivum and Tuber melanosporum truffles packaged in modified atmospheres. *Food Control* **2011**, *22*, 626−632, DOI: 10.1016/j.foodcont.2010.10.015.

(24) Xinglian, X. ″ROAV″ Method: A New Method for Determining Key Odor Compounds of Rugao Ham. *Food Sci.* **2008**, *29*, 370−374.

(25) Wang, Z. H.; Yue, C. N.; Tong, H. R. Analysis of taste characteristics and identification of key chemical components of fifteen Chinese yellow tea samples. *Journal of food science and technology* **2021**, *58* (4), 1378−1388.

(26) Liu, C.; Yao, M.; Stegen, J. C.; Rui, J.; Li, J.; Li, X. Long-term nitrogen addition affects the phylogenetic turnover of soil microbial community responding to moisture pulse. *Sci. Rep.* **2017**, *7*, 17492 DOI: 10.1038/s41598-017-17736-w.

(27) Li, Q.; Xiong, Z.; Xiang, P.; Zhou, L.; Zhang, T.; Wu, Q.; Zhao, C. Effects of uranium mining on soil bacterial communities and

functions in the Qinghai-Tibet plateau. *Chemosphere* **2024**, *347*, No. 140715.
(28) Xiang, P.; Liao, W.; Xiong, Z.; Xiao, W.; Luo, Y.; Peng, L.; Zou, L.; Zhao, C.; Li, Q. Effects of polystyrene microplastics on the agronomic traits and rhizosphere soil microbial community of highland barley. *Sci. Total Environ.* **2024**, *907*, No. 167986.
(29) Xiang, P.; Zhang, Y.; Zhang, T.; Wu, Q.; Zhao, C.; Li, Q. A novel bacterial combination for efficient degradation of polystyrene microplastics. *J. Hazard Mater.* **2023**, *458*, No. 131856.
(30) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids Res.* **2013**, *41* ((Database issue)), D590−D596.
(31) Fan, G.; Fu, Z. L.; Chao, T. C.; Wu, Q. H.; Liu, P. X.; Yang, R.; Minhazul, K. A. H. M; Li, X. T. Comprehensive analysis of different grades of roasted-sesame like flavored Daqu. *Int. J. Food Prop.* **2019**, *22*, 1205−1222.
(32) Andrewes, P.; Bullock, S.; Turnbull, R.; Coolbear, T. Chemical instrumental analysis versus human evaluation to measure sensory properties of dairy products: What is fit for purpose? *Int. Dairy J.* **2021**, *121*, No. 105098, DOI: 10.1016/j.idairyj.2021.105098.
(33) An, H. B.; Deng, B.; Chang, M. C.; Meng, J. L.; Liu, J. Y.; Feng, C. P. Study on Surface Microflora Structure and Dominant Spoilage Bacteria of Agaricus Bisporus During Post-Harvest Storage. *Food Sci. Technol.* **2020**, *45*, 23−30.
(34) Gang, Y. X.; Yang, Y. E.; Yuan, Z. J.; Jie, X.; Yan, J.; Hang, X. Y.; Guo, W. Z.; Yu, Z. X.; University, C. J. C. T.. Fungal community diversity changes of Lianzhifan solution during fermentation process by high-throughput sequence technology. *Chin. Tradit. Herb. Drugs* **2018**, No. 24, 4259−4268.
(35) Louca, S.; Jacques, S. M. S.; Pires, A. P. F.; Leal, J. S.; Srivastava, D. S.; Parfrey, L. W.; Farjalla, V. F.; Doebeli, M. High taxonomic variability despite stable functional structure across microbial communities. *Nature ecology & evolution* **2017**, *1* (1), 15.
(36) Zuo, Y.; Xie, W.; Pang, Y.; Li, T.; Li, Q.; Li, Y. Bacterial community composition in the gut content of Lampetra japonica revealed by 16S rRNA gene pyrosequencing. *PloS one* **2017**, *12* (12), No. e0188919.
(37) Pinela, J.; Barreira, J. C.; Barros, L.; Verde, S. C.; Antonio, A. L.; Carvalho, A. M.; Oliveira, M. B.; Ferreira, I. C. Suitability of gamma irradiation for preserving fresh-cut watercress quality during cold storage. *Food chemistry* **2016**, *206*, 50−58.
(38) Olesen, P. T.; Stahnke, L. H.; Talon, R. Effect of ascorbate, nitrate and nitrite on the amount of flavour compounds produced from leucine by Staphylococcus xylosus and Staphylococcus carnosus. *Meat science* **2004**, *68* (2), 193−200.
(39) Pérez-Palacios, T.; Ruiz, J.; Martín, D.; Grau, R.; Antequera, T. Influence of pre-cure freezing on the profile of volatile compounds during the processing of Iberian hams. *Journal of the science of food and agriculture* **2010**, *90* (5), 882−890.
(40) Xu, N.; Lu, H.; Feng, L. G.; Huang, X. H. Volatile components analysed by HS-SPME-GC-MS in different parts of fruiting bodies and nutritional composition of Oudemansiella raphanipes. *Mycosystema* **2020**, *39* (10), 1933−1947.
(41) Pham, A. J.; Schilling, M. W.; Mikel, W. B.; Williams, J. B.; Martin, J. M.; Coggins, P. C. Relationships between sensory descriptors, consumer acceptability and volatile flavor compounds of American dry-cured ham. *Meat science* **2008**, *80* (3), 728−737.
(42) Lin, H. B.; Bi, X. P.; Fang, J. X.; Liu, Y.; Liu, P.; Ding, W. W.; Che, Z. M.; He, Q. Pattern of Variations in Volatile Substances and Formation Mechanism of Characteristic Aroma Substances in Pixian Broad-Bean Paste. *Food Sci.* **2020**, *41*, 259−266.
(43) Splivallo, R.; Novero, M.; Bertea, C. M.; Bossi, S.; Bonfante, P. Truffle volatiles inhibit growth and induce an oxidative burst in Arabidopsis thaliana. *New phytologist* **2007**, *175* (3), 417−424.
(44) Yan, R. X.; Chen, C. K.; Li, C. Y.; Ren, Z. H. Effect of 1-MCP and ClO2 on aromatic compounds of shiitake. *Food Sci. Technol.* **2010**, *35*, 44−47.
(45) Misharina, T. A.; Mukhutdinova, S. M.; Zharikova, G. G.; Terenina, M. B.; Krikunova, N. I.; Medvedeva, I. B. Formation of flavor of dry champignons (Agaricus bisporus). *Prikl. Biokhim. Mikrobiol.* **2010**, *46* (1), 119−124.
(46) Niimi, J.; Deveau, A.; Splivallo, R. Aroma and bacterial communities dramatically change with storage of fresh white truffle Tuber magnatum. *LWT* **2021**, *151*, No. 112125, DOI: 10.1016/j.lwt.2021.112125.
(47) Culleré, L.; Ferreira, V.; Venturini, M. E.; Marco, P.; Blanco, D. Chemical and sensory effects of the freezing process on the aroma profile of black truffles (Tuber melanosporum). *Food chemistry* **2013**, *136* (2), 518−525.
(48) Piloni, M.; Tat, L.; Tonizzo, A.; Batistutta, F. Aroma characterisation of white truffle by GC-MS and GC-O. *Ital. J. Food Sci.* **2005**, *17*, 463−468.
(49) Zhang, T.; Qi, M.; Wu, Q.; Xiang, P.; Tang, D.; Li, Q. Recent research progress on the synthesis and biological effects of selenium nanoparticles. *Frontiers in nutrition* **2023**, *10*, No. 1183487.
(50) Paillart, M. J. M.; van der Vossen, J. M. B. M.; Levin, E.; Lommen, E.; Otma, E. C.; Snels, J. C. M. A.; Woltering, E. J. Bacterial population dynamics and sensorial quality loss in modified atmosphere packed fresh-cut iceberg lettuce. *Postharvest Biol. Technol.* **2017**, *124*, 91−99, DOI: 10.1016/j.postharvbio.2016.10.008.
(51) Yang, J.; Yang, X.; Liang, R.; Zhu, L.; Mao, Y.; Dong, P.; Hopkins, D. L.; Luo, X.; Zhang, Y. The response of bacterial communities to carbon dioxide in high-oxygen modified atmosphere packaged beef steaks during chilled storage. *Food research international (Ottawa, Ont.)* **2022**, *151*, No. 110872.
(52) Xiang, P.; Zhang, T.; Wu, Q.; Li, Q. Systematic Review of Degradation Processes for Microplastics: Progress and Prospects. *Sustainability* **2023**, *15* (17), 12698.
(53) Nowak, A.; Piotrowska, M. Biochemical activities of Brochothrix thermosphacta. *Meat science* **2012**, *90* (2), 410−413.
(54) Wu, Q.; Li, L.; Xiang, P.; Zhang, T.; Peng, L.; Zou, L.; Li, Q. Phages in Fermented Foods: Interactions and Applications. *Fermentation* **2023**, *9* (3), 201.
(55) Joffraud, J. J.; Leroi, F.; Roy, C.; Berdagué, J. L. Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *International journal of food microbiology* **2001**, *66* (3), 175−184.
(56) Pan, X. Y.; Yan, J.; Shi, L. L.; Cai, Z. Y.; Li, R. X.; Cheng, B. Z.; Jiang, Y. J. Bacterial Microflora on Exterior of Flammulina velutipes Fruiting Bodies during Chilled Storage. *Acta Edulis Fungi* **2020**, *27*, 29−35.
(57) Zhang, J.; Song, H. S.; Zhang, C.; Kim, Y. B.; Roh, S. W.; Liu, D. Culture-independent analysis of the bacterial community in Chinese fermented vegetables and genomic analysis of lactic acid bacteria. *Archives of microbiology* **2021**, *203* (7), 4693−4703.
(58) Fessard, A.; Bourdon, E.; Payet, B.; Remize, F. Identification, stress tolerance, and antioxidant activity of lactic acid bacteria isolated from tropically grown fruits and leaves. *Canadian journal of microbiology* **2016**, *62* (7), 550−561.
(59) Soleymanzadeh, N.; Mirdamadi, S.; Kianirad, M. Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy Sci. Technol.* **2016**, *96* (4), 443−457, DOI: 10.1007/s13594-016-0278-1.
(60) Woo, C.; Jung, S.; Fugaban, J. I. I.; Bucheli, J. E. V.; Holzapfel, W. H.; Todorov, S. D. Bacteriocin production by Leuconostoc citreum ST110LD isolated from organic farm soil, a promising biopreservative. *Journal of applied microbiology* **2021**, *131* (3), 1226−1239.
(61) Zhuo, N.; Yi, L.; Hao, S.; Jirimu, T. Application of 16S rRNA high-throughput sequencing for comparative study of the microbial diversity of traditional fermented bactrian camel milk from Alxa bactrian camel and Sonid bactrian camel. *Acta Microbiol. Sin.* **2019**, *59*, 1948−1959.
(62) Purahong, W.; Sadubsarn, D.; Tanunchai, B.; Wahdan, S. F. M.; Sansupa, C.; Noll, M.; Wu, Y. T.; Buscot, F. First Insights into the Microbiome of a Mangrove Tree Reveal Significant Differences in

Taxonomic and Functional Composition among Plant and Soil Compartments. *Microorganisms* **2019**, *7* (12), 585.
(63) Kim, T. W.; Lee, J. H.; Kim, S. E.; Park, M. H.; Chang, H. C.; Kim, H. Y. Analysis of microbial communities in doenjang, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *International journal of food microbiology* **2009**, *131* (2−3), 265−271.
(64) Splivallo, R.; Deveau, A.; Valdez, N.; Kirchhoff, N.; Frey-Klett, P.; Karlovsky, P. Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. *Environmental microbiology* **2015**, *17* (8), 2647−2660.
(65) Gray, S. R.; Rawsthorne, H.; Dirks, B.; Phister, T. G. Detection and enumeration of Dekkera anomala in beer, cola, and cider using real-time PCR. *Letters in applied microbiology* **2011**, *52* (4), 352−359.
(66) Chaves-López, C.; Serio, A.; Martuscelli, M.; Paparella, A.; Osorio-Cadavid, E.; Suzzi, G. Microbiological characteristics of kumis, a traditional fermented Colombian milk, with particular emphasis on enterococci population. *Food microbiology* **2011**, *28* (5), 1041−1047.
(67) Du, H.; Wang, X.; Zhang, Y.; Xu, Y. Exploring the impacts of raw materials and environments on the microbiota in Chinese Daqu starter. *International journal of food microbiology* **2019**, *297*, 32−40.
(68) McAuliffe, O.; Kilcawley, K.; Stefanovic, E. Symposium review: Genomic investigations of flavor formation by dairy microbiota. *Journal of dairy science* **2019**, *102* (1), 909−922.
(69) Niimi, J.; Deveau, A.; Splivallo, R. Aroma and bacterial communities dramatically change with storage of fresh white truffle *Tuber magnatum*. *LWT-Food Sci. Technol.* **2021**, *151*, No. 112125.

https://doi.org/10.1021/acsomega.3c08375
ACS Omega 2024, 9, 9331−9347