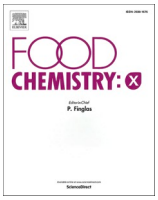
Food Chemistry: X 22 (2024) 101478

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



The potential correlation between the succession of microflora and volatile flavor compounds during the production of Zhenba bacon

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ARTICLE INFO

Chemical compounds studied in this article:
Pyrrole (PubChem CID: 8027)
2-Pentanol (PubChem CID: 22386)
Methyl isobutyl ketone (PubChem CID: 7909)
Ethyl acetate (PubChem CID: 8857)
Hexanal (PubChem CID: 6184)
Guaiacol (PubChem CID: 460)

Keywords:
Zhenba bacon
Volatile flavor compounds
Microbial communities
High-throughput sequencing
Correlation analysis

ABSTRACT

Microbial composition plays an important role in the quality and flavor of bacon. The aims of this study were to detect bacterial community succession using high-throughput sequencing (HTS) and volatile flavor compound changes using gas chromatography-ion mobility spectrometry (GC-IMS) during the production of Zhenba bacon. The results showed that a total of 70 volatile compounds were detected. Among them, ketones, hydrocarbons, aldehydes, esters and alcohols were the main substances in the curing and smoking stages. In addition, the fungal abundance was greater than the bacterial abundance, and there was obvious succession of the microbial community with changes in fermentation time and processing technology. The main functional bacterial genera in the curing and smoking stages were *Staphylococcus*, *Psychrobacter* and *Latilactobacillus*, and the main fungal genera were *Fusarium* and *Debaryomyces*. Through correlation analysis, we found that pyrrole, 2-pentanol, methyl isobutyl ketone (MIBK) and ethyl acetate (EA) were significantly correlated with *Staphylococcus*, *Psychrobacter*, *Pseudomonas* and *Myroides* ($p < 0.01$), and it is speculated that they contribute significantly to flavor formation. The results of this study are helpful for understanding the microbial dynamics and characteristic volatile flavor compounds in Zhenba bacon, and provide new insights into the relationship between microorganisms and flavor through potential correlations.

1. Introduction

Bacon is a traditional fermented meat product with a unique flavor and rich nutrition that is deeply loved by people. It is well known that proteins and fats in raw meat are decomposed by microbial or enzyme fermentation, resulting in the special flavor, color, and texture of bacon (Wang et al., 2022). The microbiota is a key factor affecting its flavor, taste and nutritional value during fermentation (Kim, Lee, Kim, & Oh, 2022). Bacon has obvious regional characteristics and flavor, which may be caused by differences in microbial populations during processing (Zhang et al., 2023). Different processing methods can lead to differences in key volatile flavor substances and microbial communities in the same region (Yang, Li, Wu, Su, & He, 2023). In addition, there were significant differences among different tissue types of microbial communities fermented by the same bacon (Gong, Zhu, Shi, Zhang, & Wen, 2021).

Zhenba bacon is a special cured and smoked food in Zhenba, Hanzhong, China. Zhenba County, Shaanxi Province, China, has a northern subtropical monsoon climate, with rich species and an excellent natural environment, and has a long history of bacon production and mature production technology (Xi, Zhang, Wu, Wang, & Ding, 2021). Therefore, Zhenba bacon has formed its own unique flavor and taste, which is deeply loved by local people. It was protected by Chinese National Geographical Indication Products in 2010. The traditional processing method for the production of Zhenba bacon has been adopted. No starter is added in the production process, and it depends on the natural fermentation process. The formation of its flavor mainly depends on many biochemical reactions of raw meat with the participation of microorganisms during the fermentation process (Ashaolu, Khalifa, Mesak, Lorenzo, & Farag, 2023). Therefore, microbial diversity and population

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https://doi.org/10.1016/j.fochx.2024.101478
Received 8 January 2024; Received in revised form 13 May 2024; Accepted 13 May 2024

structure are very important for the formation of the flavor of Zhenba bacon.

At present, the detection technologies for volatile flavor compounds in bacon mainly include gas chromatography-ion mobility spectrometry (GC-IMS), electronic nose, high-performance liquid chromatography-mass spectrometry (HPLC-MS) and gas chromatography-olfactometry-mass spectrometry (GC-O-MS) (Qin et al., 2017). With the rapid development of sequencing technology, high-throughput sequencing (HTS) and metagenomics have been widely used in food microbiology research, which is helpful for studying the mechanism of fermented food flavor formation by analyzing microbial communities (Li et al., 2021). However, the relationship between microbial communities and volatile flavor compounds at fermentation stages of Zhenba bacon has not been revealed. Therefore, it is necessary to explore the effect of microbial composition on bacon flavor substances.

In this study, four stages (raw meat, mid-curing, mid-smoking and end-smoking) were selected to investigate the microbial community succession and its correlation with the formation of volatile flavor substance fermentation in Zhenba bacon. GC-IMS and HTS were used to analyze the changes in volatile flavor substances and the microbial community during the fermentation of Zhenba bacon. At the same time, the correlation between the bacterial community and flavor substances was analyzed based on the O2PLS model. This study provides data support for optimizing the fermentation process of Zhenba bacon, and provides a foundation for the innovation of Zhenba bacon products and the development of related industries.

2. Materials and methods

2.1. Preparation of the bacon samples

Meat and bacon were obtained from Shaanxi Zhen Hong Shu Le Food Science and Technology Development Co., Ltd. (Hanzhong, Shaanxi, China). The production of Zhenba bacon mainly includes pretreatment, curing and smoking. The pretreatment of raw meat mainly divides the raw materials into meat blanks of the corresponding sizes, washes away blood stains and oil slicks on the meat surface, and drains the surface moisture. Then, the edible coarse salt was evenly spread on the surface, put in a camphor barrel and cured at room temperature for 8–9 days. The samples were transferred to the fumigation room after curing, and the temperature of the fumigation room was maintained at 25–45 °C with smoke for 30–32 days. According to the production process of Zhenba bacon, samples of raw meat (trimmed, M), end-curing (cured for 8 d, S8D), mid-smoking (smoked for 16 d, F16D) and end-smoking (smoked for 32 d, F32D) were selected. Surface meat samples (approximately 0.3–0.5 cm) were cut, and 3 parallel samples were collected from each group, labeled M1-M3, S8D1-S8D3, F16D1-F16D3 and F32D1-F32D3. The samples were placed in 50 mL sterile centrifuge tubes, stored at low temperature, quickly sent back to the laboratory and stored in the refrigerator at −80 °C until analysis. To eliminate the influence of external bacteria in the sampling process, the whole sampling process was carried out on an ultraclean sterile bench.

2.2. Sensory evaluation and physicochemical analysis

The sensory properties, including flavor, acceptability and color and lustre, were assessed by referring to Wang et al. (2024) and some modifications were made. Prior to the sensory evaluation, the panel consisted of 10 people (5 males and 5 females) who had attended professional sensory training courses. The following 10-point scale was used as the standard for evaluation: acceptability, 1 = unacceptable and 10 = very favorite; color and lustre, 1 = pale and 10 = dark red; flavor, 1 = nondetectable and 10 = intense. During testing, the samples were cut into thick slices (approximately 1–2 cm thick) and kept in petri dishes at room temperature. The results are presented as average values of 10 replicates.

The total fat of Zhenba Bacon was extracted to measuring the peroxide value (POV) and acid value (AV). Take 5 g samples of Zhenba bacon from different periods (M, S8D, F16D and F32D), homogenize the tissue with a 2:1 chloroform-methanol mixture, and extracts were washed with 0.2 volumes of water or the appropriate salt solution to it. The resulting solution was mixed by a vortex mixer (Coroic, Shanghai, China) for 30 s and then centrifuged at 2500 rpm for 10 min at 4 °C (Centrifuge 5702 R, Eppendorf, Germany). The resulting mixture underwent layering, with the lower layer being the total pure lipid extract.

The AV was measured according to the GB/T CN (2008) standard method. The POV was measured according to the method of Grunwald and Richards (2006), and the results are expressed in g/100 g. The pH and moisture were quantified according to the ISO recommended standards 2917: 1999 and 1442: 2023. Each experiment was conducted three times. The data are presented as the means ± SDs of three independent replicates.

2.3. GC-IMS analysis

The FlavorSpec® flavor analyzer (G.A.S, Dortmund, Germany) was used to detect volatile flavor substances in this study. Bacon samples (2 g) were placed in a 20 mL headspace (HS) bottle and sealed with a magnetic cap before analysis. Then, the samples were incubated at 60 °C for 15 min at 500 rpm. Subsequently, the head-space (500 μL) was automatically injected into the injector by a heated syringe (85 °C). The samples were transferred to an MXT-5 (15 m × 0.53 mm) capillary column using nitrogen (99.99%) as the carrier gas. The flow rates were programmed as follows: initially 2.0 mL/min, followed by 2 mL for 2 min, and finally 100 mL for 20 min. The ionized analyte ions were directed into a drift tube maintained at a constant temperature of 45 °C and the drift gas (nitrogen, 99.99% purity) was set at a flow rate of 150 mL/min. The final result was obtained as an average of three replicates.

2.4. Extraction and sequencing of microbial DNA

The bacon samples (5 g) were cut to a size of approximately 1 cm, 10 mL of sterilized normal saline was added, vortex and vibrate for 3–5 min, repeat twice, and collect the supernatant. After centrifugation at 6000 r/min for 10 min, the supernatant was discarded, and the bacteria were collected. The Genomic DNA of microorganisms was extracted with a genomic DNA extraction kit (TransGen Biotech, Beijing, China), and the quality and concentration of DNA were detected and stored at −20 °C for later use.

The treated samples were sent to Beijing Qingke Biotechnology for DNA extraction. The 16 S rDNA genes (V3-V4) of the bacterium were amplified by primer 5′- ACTCCTACGGGAGGCAGCA −3′ and primer 5′- GGACTACHVGGGTWTCTAAT −3′. The ITS1 region of fungi was amplified by the primers 5′- CTTGGTCATTTAGAGGAAGTAA −3′ and premier 5′- GCTGCGTTCTTCATCGATGC -3′. The following PCR amplification system was used: 5 μL of 10× PCR buffer, 35 μL of dNTPs (10 mmol/L), 10 ng of template DNA, 2 μL of forward and reverse primers (10 μmol/L), 0.5 U of Taq DNA polymerase, and dd H_2O was added to complete the system to 50 μL. All the PCR reactions were carried out in 2× *Exp Taq* Master Mix (Accurate Biology). The following PCR conditions were used: initial denaturation at 95 °C (5 min), 25 cycles of denaturation, annealing at 52 °C (30 s), extension at 72 °C (1 min), and a final extension at 72 °C (5 min). After the target DNA was purified, sequencing was completed based on the Illumina NovaSeq sequencing platform of the company.

2.5. Statistical analysis

All the results were processed using the SPSS (v 20.0) statistical package for Windows. Three biological replicates were performed for each group of samples, and a P value<0.05 was considered statistically significant. Analysis of variance (Duncan method) was performed on the

volatile flavor data, and the results are expressed as the mean ± standard deviation (SD). The graphs were generated using Origin 2021 (OriginLab Corporation, Northampton, MA, USA).

After the DNA samples passed the quality inspection, based on the Illumina NovaSeq 6000 platform, the original sequence was obtained by double-ended sequencing and base recognition. Trimmomatic v0.33 and Cutadapt v1.9.1 were used to filter the original sequences, after which the primer sequences were identified and removed. The sequence was spliced by using Usearch v10 software, and the length of the spliced data was filtered according to the length range of different regions. Finally, QIIME2 software was used to denoise and remove the chimera sequences, and the final effective sequences were obtained. To explore the relationship between microbial species and bacon flavor fermentation. We divided the operational taxonomic units (OTUs) of the samples and calculated the ACE, Chao 1, Simpson and Shannon indices of the samples to evaluate the α diversity of the samples. Based on the Silva database, the feature sequence is annotated by a naive Bayesian classifier, and the horizontal distribution histogram of microorganism phyla and genera is drawn. *t*-test and line discriminant analysis effect size (LEfSe) were used to analyze the differences between sample groups.

3. Results and discussion

3.1. Sensory evaluation and physicochemical analysis

Sensory analysis was conducted on Zhenba bacon during the raw meat stage and three stages (S8D, F16D and F32D) of production to explore the quality changes during the production and processing. Compared with the raw meat period (M), there was no significant change in the quality of the bacon during the curing period (S8D). As the smoking progresses, the fat tissue of Zhenba bacon gradually decreases and the color gradually deepens (Fig. 1A). With increasing processing time, the color, flavor and acceptability of the product gradually improved (Fig. 1B). These results indicated that there were no significant differences ($p > 0.05$) in flavor and acceptability scores between M and S8D, but there were significant differences ($p < 0.05$) in color scores.

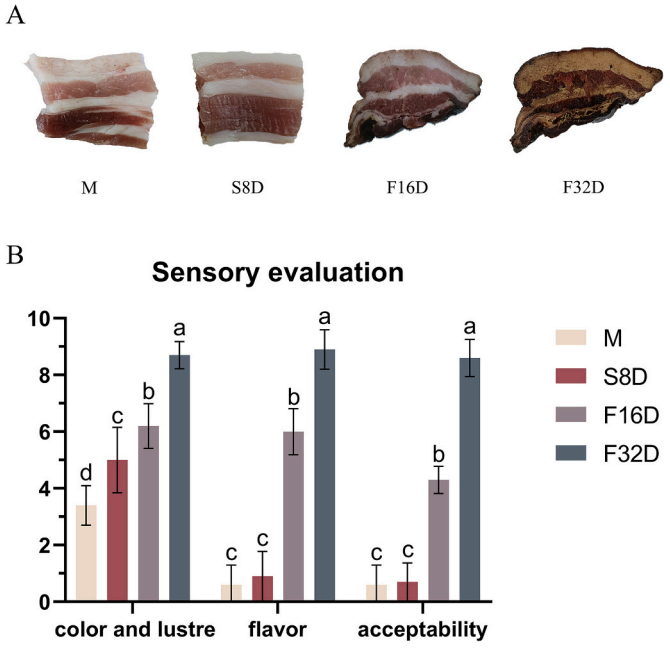

Fig. 1. Samples of Zhenba bacon in different processing periods (A). Effects of different processing periods of Zhenba bacon on sensory evaluation (B). Different lowercase letters (a-d) indicate significant differences between different periods of sensory evaluation ($p < 0.05$). (M: raw samples; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d).

Smoking is the most important period for improving the overall quality of bacon, and there were significant differences ($p < 0.05$) in scores between groups. This may be due to the succession of microbial communities and the occurrence of different biochemical reactions during different periods of bacon. The quality of bacon is closely related to microbial activity.

The sensory properties of bacon are affected by the raw materials and processing conditions. The color, texture and flavor are very important quality characteristics of bacon (Zhou et al., 2019). We analyzed the moisture content, peroxide value (POV), pH, and acid value (AV) of Zhenba Bacon (Table 1). This study revealed that with the progression of curing and smoking, the moisture content of the sample decreased to approximately twice that of M. The moisture content of the samples varied significantly ($p < 0.05$) during the different periods, and a lower moisture content improved the preservation of the bacon. The decrease in pH in S8D and F16D may be caused by the accumulation of lactic acid caused by carbohydrate decomposition, but the difference was not significant ($p > 0.05$). However, the pH of the bacon samples increased to 6.03 at F32D. This may be caused by the decomposition of proteins to produce alkaline substances. Bosse Née Danz et al. (2018) reported that microorganisms were more suitable for colonization of dry-cured ham when pH > 6.0. We speculate that microorganisms in the bacon samples after F32D may be more likely to function, thereby affecting the quality of bacon. The acid value (AV) is an important indicator of fat hydrolysis and represents the content of free fatty acids (FFAs) in fat. With the production processing, AV continued to increase and reached a maximum value in F32D. Xia et al. (2021) indicated that the characteristic aroma flavor of sauced-ducks is significantly associated with specific free fatty acids (FFAs). Therefore, the FFAs content in bacon reached a maximum at F32D, resulting in an increase in flavor substances and an improvement in quality. The peroxide value (POV) is closely related to the quality of fat in the bacon and represents the degree of fat oxidation. During Zhenba bacon processing, AV and POV continued to increase and reached the highest in F32D, with significant differences in each period ($p < 0.05$). Possibly because microbial metabolism promotes lipid oxidation, and flavor compounds (ketones, hydrocarbons, aldehydes, esters, and alcohols, etc.) accumulate during this period (Ji et al., 2024).

3.2. Volatile compound analysis

GC-IMS can quickly and accurately detect the composition of flavor substances and sample quality (Wang, Chen, & Sun, 2020). The volatile components of Zhenba bacon were identified by GC-IMS at different stages of curing and smoking. A total of 70 volatile flavor compounds were detected (Table 2), including 14 ketones, 15 aldehydes, 9 alcohols, 4 esters, 5 aromatic hydrocarbons, 9 alkenes, 2 alkanes and 12 others (including 1 ether, 1 pyrrole, 1 pyrazine 1 furan and 8 unidentified). As shown in Fig. 2A, the main volatile flavor compounds were ketones

Table 1
Physicochemical properties in different processing periods of Zhenba bacon.

Detection indicators	M	S8D	F16D	F32D
AV (acid value)	0.62 ± 0.04c	0.96 ± 0.03b	0.97 ± 0.03b	1.20 ± 0.03a
POV (peroxide value)	0.052 ± 0.003c	0.082 ± 0.003b	0.087 ± 0.002ab	0.095 ± 0.007a
pH	6.01 ± 0.03a	5.93 ± 0.03ab	5.83 ± 0.04b	6.03 ± 0.08a
Moisture content	44.1 ± 1.02a	35.9 ± 1.00b	21.20 ± 0.30c	16.00 ± 0.32d

Each data was the mean ± standard deviation ($n = 3$).
Different letters (a-d) in the same row indicate significant difference at a significant level ($p < 0.05$).
M: raw sample; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d.

Table 2
Relative abundance of volatile substances in Zhenba bacon at different stages.

Compounds	CAS	Formula	RI	M	S8D	F16D	F32D	Odor
Ketones								
5-Methyl-3-heptanone	541,855	C8H16O	957	26.25 ± 1.88c	60.66 ± 2.88c	203.29 ± 21.61b	330.75 ± 46.22a	fruity
Methyl-5-hepten-2-one	110,930	C8H14O	990	18.94 ± 0.99c	177.83 ± 9.84a	90.13 ± 7.32b	75.28 ± 5.08b	fresh, lemon
Acetophenone	98,862	C8H8O	1060.9	29.33 ± 1.02c	40.54 ± 3.36c	201.97 ± 11.74a	126.84 ± 15.54b	almond, fruity
2-Heptanone	110,430	C7H14O	889.7	12.24 ± 0.45b	195.54 ± 14.23a	24.15 ± 6.13b	20.65 ± 1.37b	pear, cheese
Methyl isobutyl ketone	108,101	C6H12O	100.2	14.78 ± 2.25d	671.98 ± 3.44a	82.28 ± 7.31b	52.59 ± 10.72c	earthy, vegetable
Cyclopentanone	120,923	C5H8O	785.9	286.11 ± 11.33c	224.15 ± 4.83c	2241.19 ± 47.81a	1189.8 ± 76.95b	mint
2-Pentanone-M	107,879	C5H10O	683.6	166.10 ± 13.44b	267.13 ± 31.06a	112.84 ± 13.26c	79.95 ± 4.24c	alcohol, fruity
2-Pentanone-D	107,879	C5H10O	678.5	74.66 ± 4.41d	876.71 ± 6.53a	728.30 ± 23.07b	460.07 ± 18.61c	
Acetone	67,641	C3H6O	486.9	1610.91 ± 39.76c	6497.05 ± 11.79a	4561.5 ± 416.77b	3983.6 ± 118.04b	mint, sweet
2-Butanone	78,933	C4H8O	571	92.88 ± 4.88d	1317.30 ± 47.34c	2740.9 ± 123.33a	1704.9 ± 118.95b	ether
3-Hydroxybutan-2-one	513,860	C4H8O2	712.8	1189.8 ± 155.36b	1247.34 ± 27.23b	1745.95 ± 101a	584.74 ± 109.48c	buttery, green
2,3-Dihydrofuran-M	96,480	C4H6O2	915.7	30.39 ± 0.87c	118.45 ± 5.38b	474.51 ± 44.65a	450.85 ± 42.56a	coconut, almond
2,3-Dihydrofuran-D	96,480	C4H6O2	915.7	36.89 ± 0.92b	82.38 ± 5.19b	422.45 ± 117.09a	515.08 ± 84.06a	
2,3-Pentanedione	600,146	C5H8O2	682.8	139.41 ± 4.08b	218.74 ± 7.08ab	293.93 ± 57.65a	234.32 ± 22.44a	caramel, nutty
Aldehydes								
(E, Z)-2,6-Nonadienal	557,482	C9H14O	1241.9	55.13 ± 2.13c	97.35 ± 2.68bc	230.48 ± 115.9b	407.06 ± 30.70a	violet, cucumber
Nonanal	124,196	C9H18O	1109.7	330.4 ± 158.6ab	424.31 ± 38.89a	306.23 ± 9.68ab	207.91 ± 18.79b	rose, citrus
Octanal	124,130	C8H16O	1005	78.89 ± 13.11c	184.64 ± 21.90a	138.51 ± 11.07b	85.55 ± 17.87c	fruity
(*E*)-Hept-2-enal	18,829,555	C7H12O	953.6	88.19 ± 5.66b	381.38 ± 130.16a	74.61 ± 14.72b	136.23 ± 30.14b	green
5-Methylfurfural	620,020	C6H6O2	962	25.07 ± 3.04b	17.27 ± 3.01b	128.43 ± 27.64a	107.61 ± 1.27a	caramel, fragrant
Benzaldehyde	100,527	C7H6O	956.8	20.67 ± 4.93b	62.74 ± 5.88b	205.86 ± 33.65a	168.35 ± 4.96a	almond, sweet
Heptanal	111,717	C7H14O	900.2	95.01 ± 12.12b	266.84 ± 75.02a	121.45 ± 7.39b	74.74 ± 17.50b	fruity, pungent
Furfural-M	98,011	C5H4O2	825.8	13.88 ± 1.73d	60.80 ± 1.79c	272.73 ± 11.63a	219.68 ± 4.88b	almond, baked
Furfural-D	98,011	C5H4O2	824.3	33.74 ± 5.91c	58.73 ± 6.58c	1110.4 ± 102.32b	1292.65 ± 63.79a	
Hexanal- M	66,251	C6H12O	790.6	905.69 ± 22.94a	605.74 ± 52.78b	172.76 ± 19.08c	163.82 ± 6.96c	apple, fresh
Hexanal- D	66,251	C6H12O	791.8	1751.19 ± 84.87a	1201.9 ± 478.5b	208.39 ± 25.16c	352.62 ± 49.83c	
2,4-Hexadienal	80,466,348	C6H8O	906.8	41.30 ± 3.48c	65.87 ± 6.84c	468.61 ± 76.92a	319.70 ± 20.69b	green
3-Methylbutanal	590,863	C5H10O	650.5	17.08 ± 2.22c	1279.1 ± 100.2a	1058.5 ± 18.25ab	947.19 ± 19.01b	cheese, malt
Pentanal	110,623	C5H10O	694	252.80 ± 20.07a	141.40 ± 47.00b	79.34 ± 5.50c	47.68 ± 3.97c	almond, pungent
Butanal	123,728	C4H8O	550.1	19.96 ± 4.13b	43.86 ± 5.42b	260.09 ± 29.87a	131.97 ± 15.21b	banana, pungent
Alcohols								
1-Menthol	2,216,515	C10H20O	1246.6	76.97 ± 3.56d	442.24 ± 8.63a	214.91 ± 17.82b	164.42 ± 4.78c	mint, cool
2-Octanol	123,966	C8H18O	1003.9	59.27 ± 3.22b	74.47 ± 8.09b	156.51 ± 22.19a	140.25 ± 7.20a	fat, mushroom
Linalool	78,706	C10H18O	1101.2	63.65 ± 9.55d	1519.96 ± 48.36a	558.91 ± 60.98b	380.02 ± 57.35c	floral, woody
Oct-1-en-3-ol	3,391,864	C8H16O	980.9	28.04 ± 1.86c	199.78 ± 6.50a	53.94 ± 3.58b	55.85 ± 6.47b	earthy, mushroom
3-Octanol	589,980	C8H18O	986.6	82.69 ± 7.06c	314.59 ± 21.90a	231.40 ± 33.81b	193.87 ± 33.16b	nutty, mushroom
Ethanol	64,175	C2H6O	444.7	609.22 ± 25.16b	698.02 ± 16.69b	273.62 ± 30.74c	927.47 ± 95.04a	sweet
3-Methylbutan-1-ol	123,513	C5H12O	730.5	8.42 ± 0.43c	416.22 ± 4.55a	20.23 ± 1.22b	21.72 ± 3.92b	floral, malt
1-Propanethiol	107,039	C3H8S	621.9	10.57 ± 2.12d	390.00 ± 6.52a	71.37 ± 3.13c	98.16 ± 4.95b	cabbage, onion
2-Pentanol	6,032,297	C5H12O	704.6	20.09 ± 1.36c	39.17 ± 3.80c	308.65 ± 44.20b	1384.96 ± 165.2a	fusel, green
Esters								
Linalyl acetate	115,957	C12H20O2	1397	79.11 ± 2.79d	952.90 ± 66.81a	455.51 ± 35.32b	295.33 ± 55.55c	fruity
Ethyl butyrate	105,544	C6H12O2	785.5	101.76 ± 2.43a	46.42 ± 3.33c	74.81 ± 7.57b	102.10 ± 14.03a	fruity, sweet
Ethyl acetate	141,786	C4H8O2	595.2	80.90 ± 4.73d	900.88 ± 64.56c	2757.1 ± 142.51b	5477.75 ± 244.8a	brandy, grape
Methyl acetate	79,209	C3H6O2	525	47.53 ± 2.89c	115.34 ± 11.99c	1842.1 ± 170.14a	1532.57 ± 26.73b	green
Aromatic hydrocarbons								
Anethol-M	104,461	C10H12O	1450.7	205.42 ± 18.44d	9210.9 ± 618.73a	4625.0 ± 728.98b	1519.1 ± 299.19c	anise
Anethol-D	104,461	C10H12O	1451.2	85.99 ± 9.19b	576.88 ± 70.47a	165.91 ± 40.01b	79.66 ± 4.38b	
Ortho-Guaiacol-M	90,051	C7H8O2	1086.4	42.08 ± 10.49b	31.71 ± 1.52b	876.59 ± 110.95a	663.04 ± 91.06a	burnt, wood
Ortho-Guaiacol-D	90,051	C7H8O2	1087.4	21.00 ± 0.46b	56.46 ± 6.31b	247.10 ± 33.10a	195.42 ± 16.70a	
O-Cresol	95,487	C7H8O	1058.5	38.30 ± 3.04b	36.46 ± 5.43b	432.44 ± 58.49a	353.65 ± 40.91a	phenolic, sweet
Alkenes								
α-Pinene	80,568	C10H16	929.3	6.48 ± 1.78c	74.58 ± 18.43a	31.14 ± 4.67b	23.81 ± 7.51bc	pine, turpentine
β-Pinene	127,913	C10H16	969.8	17.62 ± 0.73b	1001.6 ± 136.58a	135.32 ± 10.67b	95.48 ± 5.29b	pine, wood
α-Terpinene	99,865	C10H16	1012.7	8.83 ± 2.72c	101.29 ± 10.76a	36.70 ± 1.59b	33.76 ± 2.70b	lemon
γ -Terpinene	99,854	C10H16	1062.4	29.97 ± 8.85d	363.64 ± 13.23a	166.32 ± 32.15b	98.50 ± 10.71c	bitter, citrus
β-Ocimene	13,877,913	C10H16	1050.9	29.62 ± 2.24c	356.84 ± 35.27a	147.21 ± 21.26b	74.06 ± 6.61c	floral

(*continued on next page*)

Table 2 (*continued*)

Compounds	CAS	Formula	RI	M	S8D	F16D	F32D	Odor
Limonene	138,863	C10H16	1022.2	29.39 ± 1.37d	529.18 ± 63.81a	203.13 ± 24.21b	116.32 ± 10.38c	sweet, citrus
β-Pyronene	514,965	C10H16	994	14.17 ± 1.46c	197.88 ± 334.13a	71.68 ± 1.84b	67.81 ± 1.97b	–
2-Methyl-2-propenal	78,853	C4H6O	550.8	69.41 ± 5.04c	149.02 ± 7.31c	405.49 ± 1.77b	576.21 ± 12.64a	floral
α-Thujene	2,867,052	C10H16	922.9	5.48 ± 0.38c	46.43 ± 14.99a	16.74 ± 1.48bc	16.72 ± 6.49bc	pine, fresh
Alkanes								
1,8-Cineole-M	470,826	C10H18O	1026.1	43.79 ± 9.19d	2156.50 ± 33.93a	710.30 ± 123.09b	422.76 ± 39.40c	camphor, cool, mint
1,8-Cineole-D	470,826	C10H18O	1025.2	39.38 ± 2.91c	276.59 ± 2.91a	72.44 ± 16.33b	44.10 ± 1.50c	
Others								
Pyrrole	109,977	C4H5N	748.9	22.38 ± 1.08bc	16.54 ± 1.78c	35.73 ± 10.12b	144.05 ± 6.63a	nutty, pungent
2-Methyl-3-methylthiopyrazine	2,882,204	C6H8N2S	1243.4	74.27 ± 6.68b	74.26 ± 7.80b	514.85 ± 241.6a	576.14 ± 47.83a	vegetable, nutty
Dimethyl disulfide	624,920	C2H6S2	734.6	168.06 ± 28.21c	77.48 ± 5.23d	250.66 ± 20.71b	358.51 ± 9.26a	cabbage, onion
2,5-Dimethylfuran	625,865	C6H8O	706.4	39.25 ± 8.05a	16.24 ± 0.80b	31.85 ± 6.23a	26.69 ± 3.12ab	savory, meaty
1	–	–	1106.8	125.16 ± 27.60b	136.47 ± 12.50b	259.43 ± 23.15a	330.61 ± 22.64a	–
2	–	–	833.7	16.90 ± 2.63c	45.01 ± 1.55c	1138.39 ± 119.6a	847.83 ± 160.64b	–
3	–	–	857.4	49.78 ± 3.19c	293.31 ± 8.50c	1970.51 ± 355.0a	991.23 ± 313.15b	–
4	–	–	906.2	16.32 ± 1.84c	28.95 ± 2.20c	192.39 ± 36.62b	304.51 ± 37.17a	–
5	–	–	785.1	62.46 ± 3.69c	90.13 ± 5.60c	272.86 ± 11.81b	516.35 ± 43.00a	–
6	–	–	646	7.12 ± 0.84c	88.51 ± 8.65b	292.02 ± 50.67a	277.23 ± 3.66a	–
7	–	–	559.8	181.27 ± 3.70d	424.61 ± 12.57c	792.91 ± 90.41b	1163.73 ± 67.54a	–
8	–	–	495.4	451.67 ± 1.91b	420.24 ± 4.86b	427.64 ± 34.80b	753.52 ± 93.48a	–

Each data was the mean ± standard deviation (n = 3).
Note: "RI", retention index. "-", unidentified. "CAS": Chemical Abstract Service.
Different letters (a-d) in the same row indicate significant difference at a significant level ($p < 0.05$).
M: raw sample; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d.

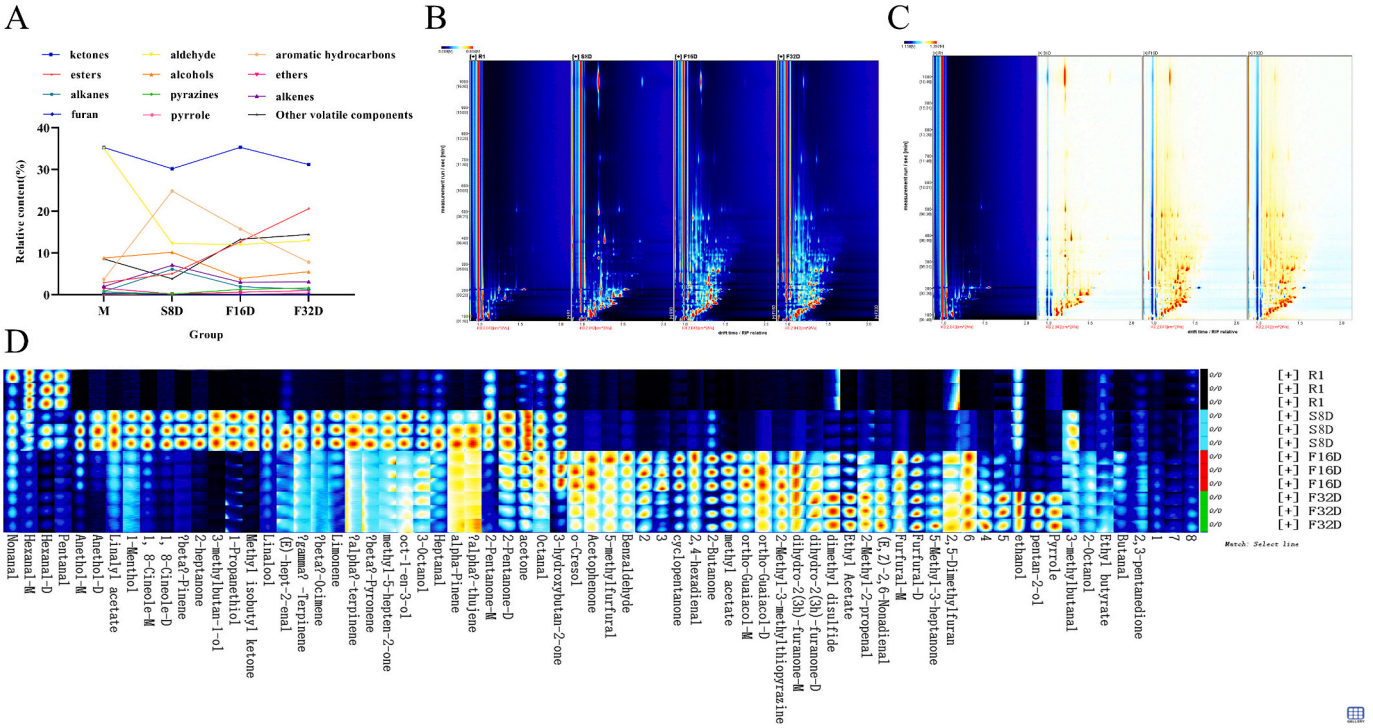

Fig. 2. Analysis of volatile components in Zhenba bacon at different stages. (A) Relative content of volatile compounds in Zhenba bacon at different processing stages. (B) Spectrum of VOCs in the samples (top view). (C) Comparative difference spectrum of VOCs in samples. (D) Fingerprints of VOCs isolated from different stages using GC–IMS. (M/R1: raw samples; S8D: cured samples; F16D: mid-smoking samples; F32D: end-smoking samples; VOCs: volatile organic compounds).

(35.27%) and aldehydes (35.09%) in the M samples. The relative content of ketones decreased gradually from the M to S8D samples, and gradually increased to the maximum (35.31%) in the F16D samples. It has been reported that ketones are abundant in meat and emit a special smell that contributes to the flavor of meat products (Peña-Saldarriaga, Fernández-López, & Pérez-Alvarez, 2020). The relative aldehyde content decreased rapidly during the curing stage, but increased slowly during smoking. Aldehydes are important components of meat flavor, mainly derived from lipid oxidative degradation and Strecker degradation reactions. The main aldehyde in pork is hexanal, which has a

refreshing grassy smell and is mainly derived from ω-6 unsaturated fatty acids (Myers, Scramlin, Dilger, Souza, & Killefer, 2008). Hydrocarbons occupy the largest proportion (38.03%) in the curing stage. The relative content of aromatic hydrocarbons was lower (3.7%) in the M samples, and increased rapidly to 24.86% in the curing stage. As fermentation progressed, it decreased gradually during smoking. Hydrocarbons generally have a high aroma threshold and can form important intermediates of heterocyclic compounds, which play an important role in improving the overall flavor of meat products (Wang, Song, Zhang, Tang, & Yu, 2016). The relative content of esters was lower in the M samples and increased gradually with curing and smoking stage. It then reaches a maximum value in the F32D sample. Esters are mainly produced by the esterification of acids and alcohols in meat products and usually have a typical sweet and fruity taste. The two processing methods of pickling and smoking will produce different flavors in the bacon. Fat oxidation is inhibited to help maintain the original flavor of the meat through curing, and organic compounds are generated to form a unique smoky flavor through smoking. These results indicated that the volatile flavor compounds in the curing and smoking stages of Zhenba bacon were mainly ketones, hydrocarbons, aldehydes, esters and alcohols.

To further clarify the components of volatile flavor substances, gas phase ion mobility spectra and fingerprints (Gallery Plot) were analyzed. The results showed that the VOCs of the samples were well separated from each other in the curing and smoking stages, and the VOCs showed obvious differences on the topographic map in different fermentation stages (Fig. 2B, C). The contents of nonanal, hexanal and pentanal were greater in the R1 samples (Fig. 2D). Most aldehyde compounds are generated by the oxidative degradation of fatty acids. Hexanal is a reliable indicator for evaluating the oxidation status and flavor of meat, which helps to increase sweetness and grass aroma (Biller, Boselli, Obiedziński, Karpiński, & Waszkiewicz-Robak, 2016). For example, the contents of α-thujene, methyl isobutyl ketone, limonene, α-terpinene, β-pinene, 1-cineole and linalool were greater in the S8D samples. Terpenes have an insignificant impact on odor due to their high threshold. Higher contents of guaiacol, 2-pentanol, cresol, benzaldehyde, ethyl acetate, pyrrole, furfural, acetophenone and 2-dimethylfuran were found during the smoking stage (F16D and F32D). These compounds provide the woody, smoky, fruity, nutty, and toasted aromas of bacon and play an important role in overall flavor formation. The results showed that the type and content of VOCs increased with increasing of fermentation time. This finding is similar to that previously reported that fermentation can increase VOCs (Wu et al., 2022).

3.3. Microbial richness and diversity

Illumina MiSeq sequencing was used to analyze the microbial abundance, diversity and community structure in Zhenba bacon samples. There were significant differences in the microbial abundance of Zhenba bacon at different fermentation stages of curing and smoking (Table 3). Overall, fungal abundance was greater than bacterial abundance, while fungal OTUs decreased continuously during processing. A total of 395 bacterial OTUs and 1626 fungal OTUs were detected in the samples at different stages, which reflects the abundance of microorganisms in the samples.

Table 3
The operational taxonomic units (OTUs) of microorganisms in Zhenba bacon samples.

Sample	Bacteria (OTUS)	Fungi (OTUS)
M	141	718
S8D	172	705
F16D	199	578
F32D	162	561

M: raw samples; S8D: cured for 8 d; F16D: smoked for 16 d; F32D: smoked for 32 d.

Cluster analysis was performed on the microbial OTUs in the four stages of bacon fermentation, and a Venn diagram was drawn. The number of common and unique features can be clearly displayed by using Venn diagrams in the samples, and to evaluate the similarities and differences in OTU composition and distribution among different samples. There were 10 bacterial OTUs and 75 fungal OTUs common to all stages of the Zhenba bacon fermentation process. The unique bacterial OTUs of samples M, S8D, F16D, and F32D were 57, 70, 37, and 12, respectively, and the unique fungal OTUs were 379, 362, 113, and 109, respectively. There were 141 bacterial OTUs and 718 fungal OTUs in the M samples, 172 bacterial OTUs and 705 fungal OTUs in the S8D samples, 199 bacterial OTUs and 578 fungal OTUs in the F16D samples, and 162 bacterial OTUs and 561 fungal OTUs in the F32D sample (Fig. 3). The results showed that in the fermentation process of Zhenba bacon, the bacterial abundance was the lowest, while the fungal abundance was the highest in the M samples; in contrast, the bacterial abundance was the highest and the fungal abundance was the lowest in the F16D and F32D samples. The microbial composition of samples from different production stages varied. Fungal OTUs fluctuated greatly with changes in processing technology and time, indicating that the fungal abundance was greatly affected in the Zhenba bacon samples.

The microbial alpha diversities were detected of samples in different stages, including the Shannon, Simpson, Chao1 and ACE indices. Alpha diversity reflects the species abundance and diversity of a single sample. The Chao1 and ACE indices show the abundance of the microbial community and reflect the number of species. Shannon and Simpson indices are used to measure species diversity and are proportional to the species diversity of samples (Li, Xiong, Zhou, Xu, & Sun, 2021). In addition, coverage was counted, and it is worth noting that coverage was 100% in both bacterial and fungal samples. This shows that all the microorganisms in the samples were detected.

Compared with the M samples, there was no significant difference in the Shannon index of bacteria in the F16D and F32D samples, and the highest value was reached in the F16D samples (Fig. 4A_1). Moreover, the Simpson index was the lowest in the M samples and the highest in the F32D samples (Fig. 4A_2). The results showed that bacterial species diversity was the lowest in the raw meat stage and the highest in the smoking stage. Bacterial diversity is positively affected by curing and smoking during bacon fermentation. The Chao1 and ACE indices were the lowest in the M samples and the highest in the F16D samples, indicating that the species richness of bacteria was the highest in the middle stage of smoking (F16D) (Fig. 4A_3, A_4). The Shannon and Simpson indices of fungi were high level in the M and S8D samples, and decreased during the smoking stage (Fig. 4B_1, B_2). This suggests that smoking has a negative impact on fungal diversity in bacon samples. The Chao1 and ACE indices were the lowest in the S8D samples and increased significantly during the smoking stage (F16D and F32D) (Fig. 4B_3, B_4). In summary, curing and smoking are important fermentation steps in the production process of Zhenba bacon, which has a great influence on the composition of microorganisms in bacon samples.

3.4. Composition of microbial communities

As shown in Fig. 5, *Proteobacteria* and *Firmicutes* were the main dominant bacteria, while *Ascomycota* and *Basidiomycota* were the main components of the fungal community according to taxonomic annotation, statistics of characteristic sequences, and cluster analysis. This finding is consistent with previous reports (Hu et al., 2020). The most significant result at the bacterial level was that *Proteobacteria* was absolutely dominant in the M samples, with a relative abundance of 91%. However, the relative abundance decreased significantly to 34% after curing, possibly because due to the fact that some *Proteobacteria* are aerobic. Finally, the relative abundance rebounded to approximately 50% with the extension of curing time and smoking processing. The relative abundance of *Firmicutes* also fluctuated and increased with

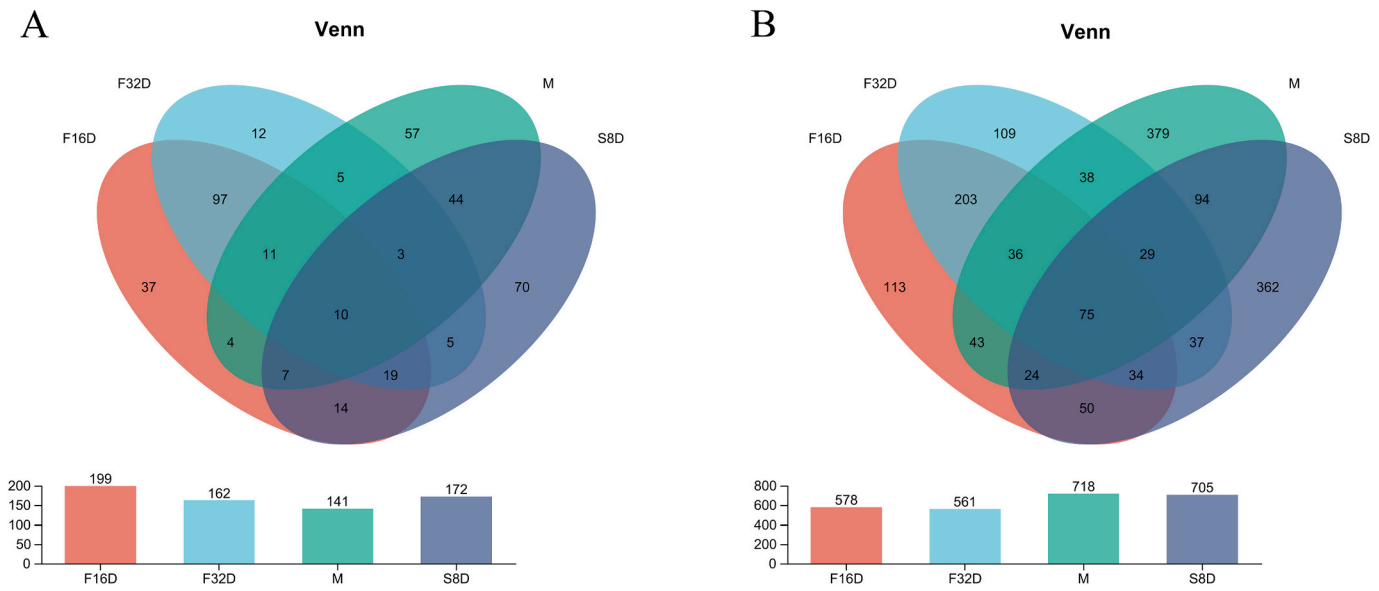

Fig. 3. Cluster analysis of microbial community diversity by bacteria (A) and fungi (B). (M: raw samples; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d).

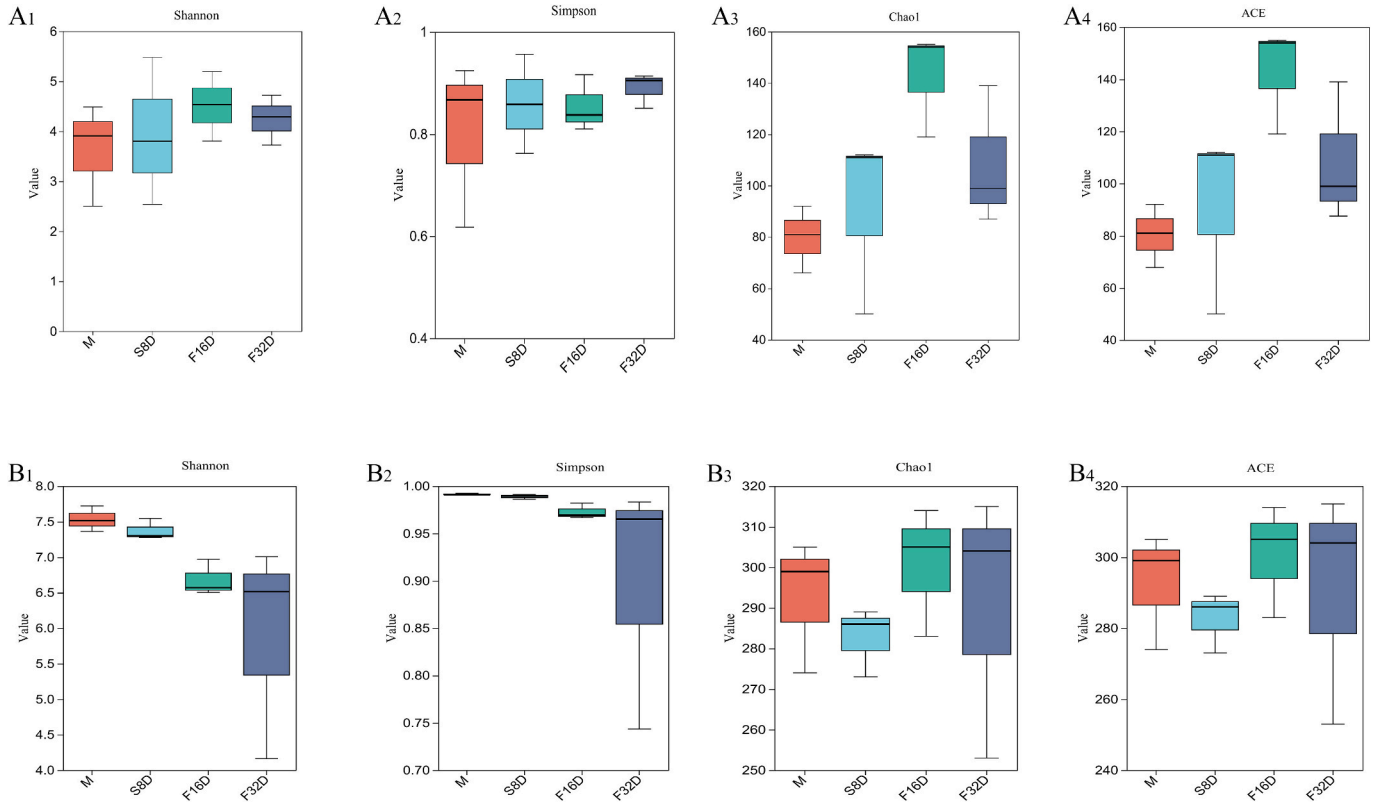

Fig. 4. Alpha diversity indices of bacteria (A) and fungi (B) in bacon samples of Zhenba at different stages. (M: raw sample; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d).

increasing curing time and technological changes in the bacon samples, and finally stabilized at approximately 50% (Fig. 5A). Significantly, the relative abundance of *Ascomycetes* remained above 50% during the whole fermentation process, which was the most dominant fungal group in the bacon samples (Fig. 5B).

Cluster analysis at the genus classification level was performed to explore changes in the microbial composition of Zhenba bacon produced at different stages (Fig. 5C, D). The highest proportion of bacteria was *Escherichia-Shigella* in the M samples, which had a relative abundance of 37%. After fermentation treatment, the relative abundance decreased <10%. The relative abundances of *Staphylococcus*, *Macrococcus* and *Psychrobacter* increased continuously in the curing stage, and the relative abundances of *Staphylococcus* and *Psychrobacter* bacteria increased to 12% at the end of curing (S8D). *Staphylococcus* is the dominant bacterium in the step with a higher salt concentration of the product; thus, it can be used as an indicator to control the curing process and play an

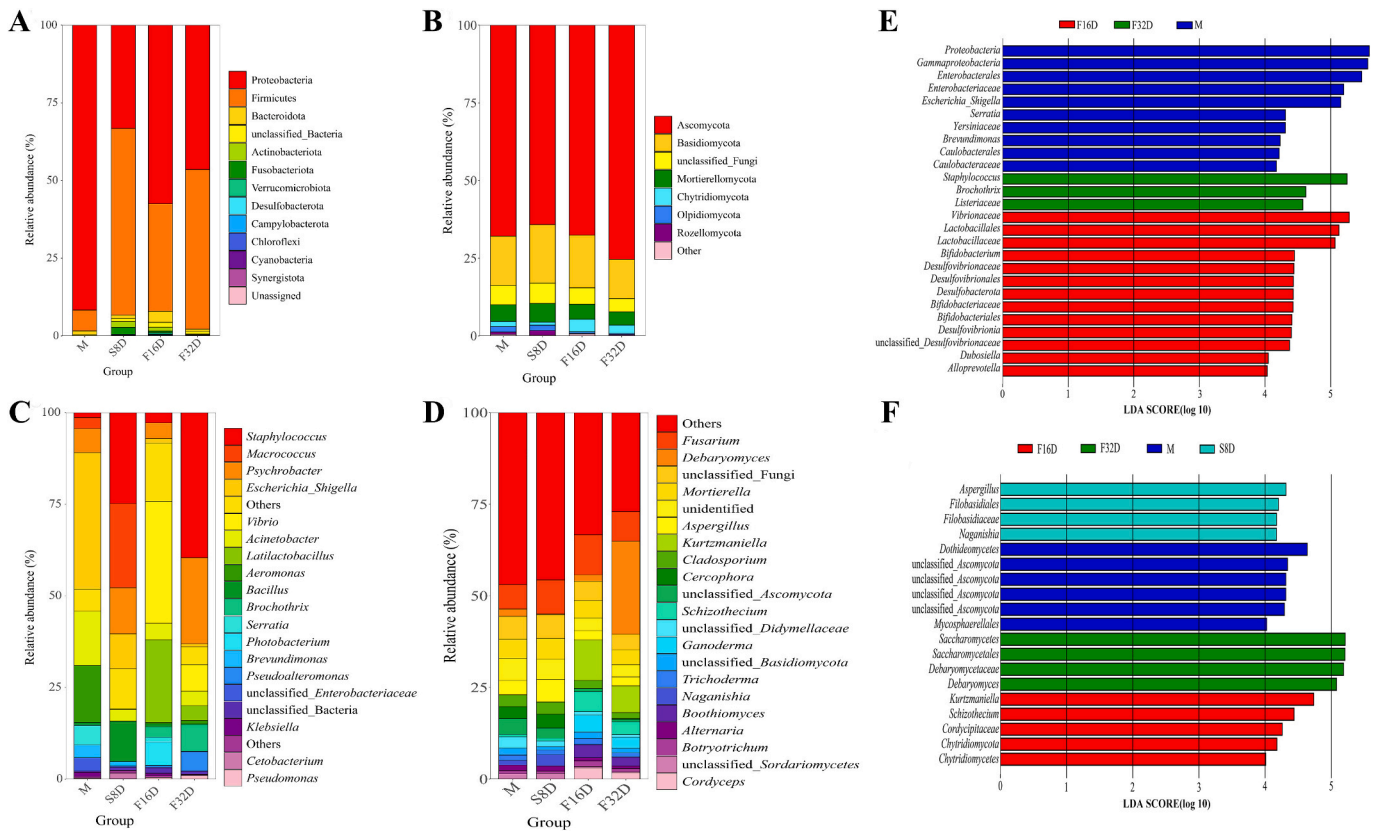

Fig. 5. Relative abundance and composition of microorganisms in different bacon samples at the phylum level and genus level: (A) Bacterial phylum level (B) Fungal phylum level (C) Bacterial genus level (D) Fungal genus level. LEfSe analysis of bacteria (E) and fungi (F) in Zhenba bacon (LDA score > 4.0) (M: raw samples; S8D: cured for 8d; F16D: smoked for 16d; F32D: smoked for 32d).

important role in the development of the final flavor of the product (Dias, Chambel, Tenreiro, Nunes, & Loureiro, 2021). The main bacteria in the sample were *Vibrio* and *Latilactobacillus*, with relative abundances of 35% and 19%, respectively, in the F16D samples. The relative abundance of *Staphylococcus* and *Psychrobacter* bacteria increased to 39% and 23% at the end of smoking (F32D) and became the dominant flora in this stage (Fig. 5C). *Vibrio* and *Staphylococcus* are both salt-tolerant bacteria and have a strong ability to produce enzymes. The secreted extracellular enzymes promote lipid oxidation and protein degradation in bacon, which play important roles in the formation of bacon quality and flavor. Fungal communities are more complex and diverse than bacterial communities. The relative abundance of *Fusarium* was approximately 10% in each fermentation stage, and reached a maximum (11%) in the middle stage of smoking (F16D). Meanwhile, the relative abundance of *Mortierella* was stable at approximately 5% in each fermentation stage. It is worth mentioning that the relative abundances of *Kurtzmaniella* and *Debaryomyces* increased and became the dominant fungi in the smoking stages (F16D and F32D) (Fig. 5D). This indicates that the relative content of beneficial bacteria increases with the progression of fermentation, and these species are associated with the production of key metabolites (food aroma formation) and different enzymes.

In summary, the results showed that the microbial composition was constantly changing and that the dominant bacteria had obvious succession during the fermentation process of Zhenba bacon. For example, *Escherichia-Shigella*, *Acinetobacter,* and *Aeromonas* are the main bacteria in untreated raw meat samples. These kinds of bacteria are widely distributed in the external environment, such as water and soil (Majeed, De Silva, Kumarage, & Heo, 2023). These results may be due to the attachment of microorganisms to the sample surface during the storage of the raw meat and the processing period. However, the relative abundance decreased during curing and smoking. *Staphylococcus* and other nondominant bacteria in the raw meat stage become the main components of the fermentation process. *Latilactobacillus* and other bacteria became the dominant bacteria in the later stage of fermentation. This shows that there is a succession phenomenon not only in the dominant flora, but also in the nondominant flora. In addition, it has become the key factor for bacon quality and flavor formation.

3.5. LDA analysis of differential microorganisms

The importance of microbial species in Zhenba bacon samples at different stages was analyzed and evaluated by using LDA effect size (LEfSe). We set the filter condition as LDA > 4.0, and the length of the bar chart represents the influence of different species. A total of 26 bacterial biomarkers and 19 fungal biomarkers were identified (Fig. 5E, F). The microbial structure of the processed samples was significantly different from that of the raw meat. Most of the bacterial communities were harmful bacteria in the M samples, which were transformed into *Staphylococcus* and other bacteria after curing. *Latilactobacillus* appeared in the early stage of smoking (F16D), but *Staphylococcus* still occupied the main position in the samples in the later stage of smoking (F32D) (Fig. 5E). The fungal community succession was obvious in each fermentation stage, and the structural composition changed greatly. We found that *Saccharomycetes* and *Debaryomyces* were the main bacteria in the F32D samples, and their LDA scores were the highest. The results showed that *Saccharomycetes* and *Debaryomyces* played important roles in the late fermentation stage to the finished product stage of Zhenba bacon (Fig. 5F). Overall, these findings indicated that microflora succession clearly occurred during the fermentation process of Zhenba bacon, and the microbial components that played an important role in each stage changed greatly. Moreover, the fermentation process of

Zhenba bacon involves the joint action of many kinds of microorganisms. The LDA scores of *Staphylococcus*, *Saccharomycetes* and *Proteobacteria* were greater than those of other microorganisms, indicating that they play an important role in the fermentation of Zhenba bacon.

3.6. Analysis of the correlation between microorganisms and volatile substances

The O2PLS (two-way orthogonal PLS) model was used for integration analysis between two sets of data. By using the O2PLS model for correlation analysis, not only can correlation coefficients be obtained, but also the weights of variables can be obtained in the model, thereby more accurately identifying the key points of the study (Bylesjö, Eriksson, Kusano, Moritz, & Trygg, 2007). At present, the O2PLS model analysis has been widely used to study the correlation between microorganisms and volatile compounds in fermented foods. To date, there have been many studies on the correlation between microorganisms and flavor in all kinds of traditional Chinese bacon. The dynamic changes in flavor substances and microbial communities at different stages during the production process of Zhenba bacon are crucial to quality generation. Therefore, we focused on the interactions and potential correlations of microorganisms with volatile flavor compounds. In this study, the correlation between bacteria and key volatile flavor compounds during the production process of Zhenba bacon was analyzed by the O2PLS model and heatmap (Fig. 6 and S1). We found that there are 11 main types of alcohols ($p < 0.01$), including pentan-2-ol, 3-methylbutan-1-ol, oct-1-en-3-ol, 1-propanethiol, 1,8-cineole-D, linalool, 1,8-cineole-M, 1-menthol, 2,4-hexadienal, 2-octanol, and ethanol. There are 10 main types of ketones ($p < 0.01$), namely methyl isobutyl ketone, 2-heptanone, cyclopentanone, 2-pentanone-M, 5-methyl-3-heptanone, methyl-5-hepten-2-one, 2,3-dihydrofuran, 2,3-pentanedione, and 3-hydroxybutan-2-one. The main aldehydes ($p < 0.01$) found were (E, Z)-2,6-nonadienal, 5-methylfurfural, butanal, furfural-D, 2-methyl-2-propenal, pentanal, benzaldehyde, 3-methylbutanal, furfural-M, nonanal, heptanal and (*E*)-hept-2-enal for a total of 12 types. The least is that there are mainly 3 kinds of esters ($p < 0.01$), which are ethyl acetate, methyl acetate and linalyl acetate.

The results showed that 70 kinds of volatile compounds were significantly correlated with *Psychrobacter*, *Pseudomonas* and *Myroides* ($p < 0.05$). *Psychrobacter* and *Pseudomonas* are common dominant bacteria in fermented meat products. *Psychrobacter* can break down lipids and hydrolyze proteins to produce special odors (Broekaert, Noseda,

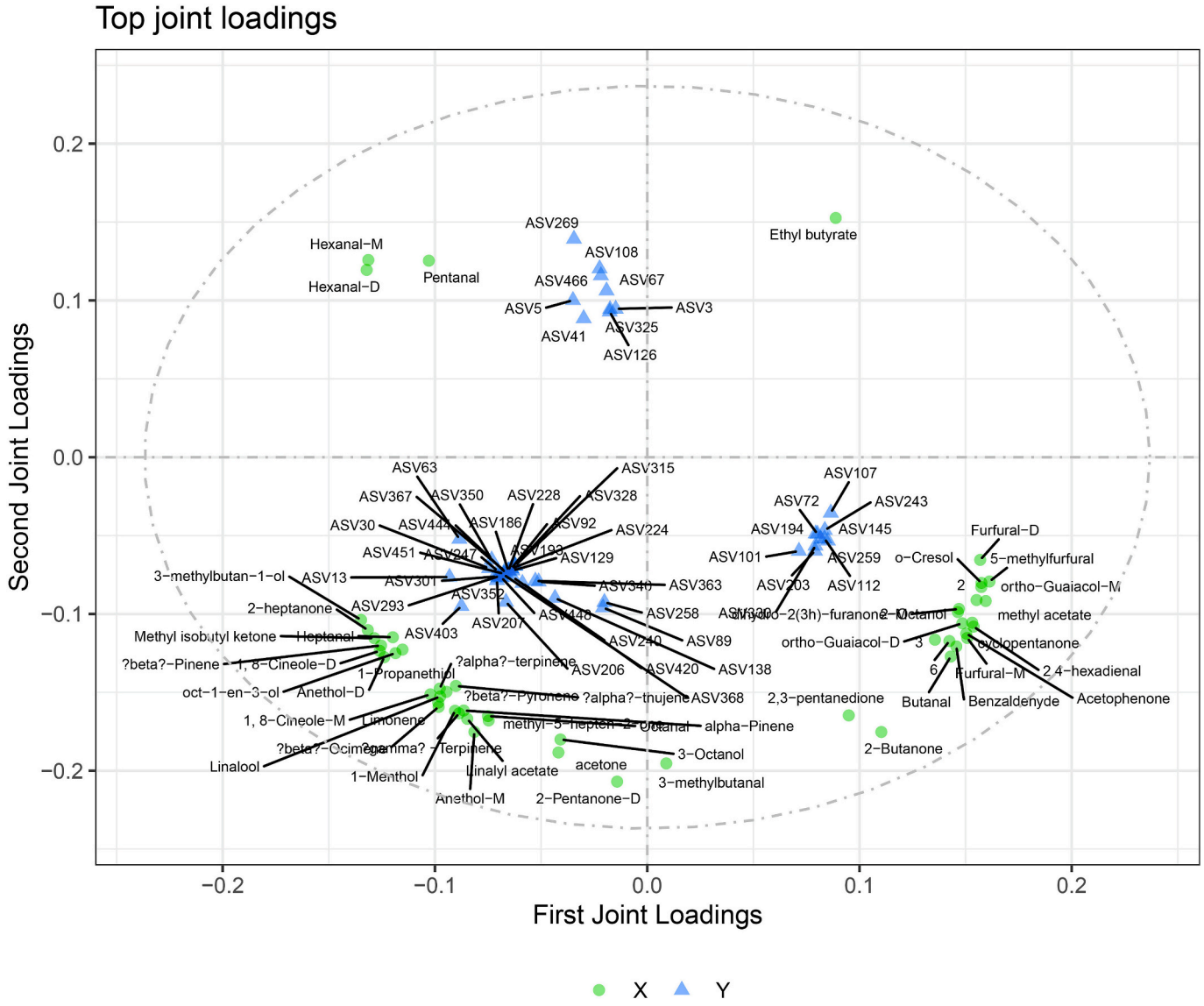

Fig. 6. Correlation between microorganisms and volatile compounds during the production of Zhenba bacon. (X: Volatile compounds; Y: "ASV + numbers" represents microbial species).

Heyndrickx, Vlaemynck, & Devlieghere, 2013). *Pseudomonas* belongs to aerobic Gram-negative bacteria. *Pseudomonas* proliferates rapidly and has strong ammonia production in aerobic environment. Therefore, a large number of *Pseudomonas* exist in the early and middle stages of fermentation to decompose proteins and fats through a variety of metabolic pathways in fermented meat products, and producing flavor precursors through metabolism (Lessie & Phibbs Jr, 1984). With the decrease of oxygen content and the increase of acid value, the abundance decreased gradually. Further analysis found that pyrrole was significantly correlated with *Psychrobacter*, *Pseudomonas* and *Brochothrix* ($p < 0.01$) during the fermentation of Zhenba bacon. Pyrroles are known to be volatile flavor compounds that produce distinctive sweet and slightly burnt smells (Toci & Farah, 2014). Microbial metabolism promotes the Maillard reaction and caramelization reaction, and further promotes the formation of volatile flavor compounds (Keawkim & Na Jom, 2022). Therefore, we speculate that *Psychrobacter*, *Pseudomonas* and *Brochothrix* induce the production of pyrrole through metabolism and interaction, thus endowing Zhenba bacon with a unique sweetness and slight burnt aroma. 2-Pentanol is a colourless liquid with winey-ethereal and is often used as an edible spice (Api et al., 2020). Hu, Qiu, Dai, Tian, and Wei (2022) found that 2-pentanol could smell mint, grease and pungent notes through sensory evaluation. 2-Pentanol was significantly correlated with *Psychrobacter*, *Pseudomonas*, *Staphylococcus* and *Shewanella* in the process of bacon production ($p < 0.01$). Previous studies have shown that *Shewanella* can accelerate the oxidation of protein in meat products, change the tertiary structure of protein and reduce moisture content. *Shewanella* can degrade nitrogenous substances into amine, sulfides, and organic acids, producing unpleasant flavors and odors (Feng, Bi, Chen, Zhu, & Liu, 2021). In this study, we found that 2-pentanol mainly came from F32D. The results showed that *Psychrobacter*, *Pseudomonas* and *Shewanella* played an important role in the formation of flavor in the later stage of smoking. However, it needs to be further verified whether the metabolites of *Shewanella* will have adverse factors on the quality of bacon. Methyl isobutyl ketone (MIBK) an aliphatic ketone, is a widely used volatile organic compound (VOC) with a pleasant smell (Johnson Jr., 2004). In this study, we found that MIBK was significantly correlated with *Pseudomonas* and *Bacteroides* ($p < 0.01$). *Bacteroides* is a kind of gram-negative rod-shaped bacteria with a large number of glycosyl hydrolases that can decompose sugars to produce acetic acid and succinic acid. This caused an increase in AV. It is worth noting that MIKB is mainly exists in S8D. The analysis of physicochemical shows that there was a significant difference ($p < 0.05$) in AV between M and S8D. We speculate that *Bacteroides* played an important role in the curing period. Current research has found that *Bacteroides* can produce γ-aminobutyric acid (GABA) and a variety of short-chain fatty acids (Shin et al., 2024; Strandwitz et al., 2019). Therefore, *Bacteroides* played an active role in the formation of bacon quality and improved the nutritional value of Zhenba bacon. Ethyl acetate (EA) has a fruit flavor, which can make fermented food produce a unique flavor and fragrance. Commonly used as food flavors and industrial solvents (Löser, Urit, & Bley, 2014; Sumby, Grbin, & Jiranek, 2010). EA was significantly correlated with *Psychrobacter*, *Pseudomonas* and *Acinetobacter* ($p < 0.01$). *Psychrobacter*, *Pseudomonas* and *Acinetobacter* are the dominant genera for refrigerated storage, which can be more involved in fat hydrolysis and protein oxidation at low temperature. Volatile compound analysis showed that EA reached the maximum at late smoking. Therefore, we speculate that *Psychrobacter*, *Pseudomonas* and *Acinetobacter* are the key bacteria for the formation and maintenance of bacon flavor during late smoking to low temperature storage, and affect the quality changes of bacon during low temperature storage.

The volatile flavor compounds produced are closely related to microbial activities during the fermentation of bacon (Shen et al., 2021; Wang et al., 2021). Combined with the analysis results of the main volatile compounds, we screened out the microflora of *Psychrobacter*, *Pseudomonas* and *Myroides* which were significantly related to pyrrole, 2-pentanol, MIBK and EA. This can represent the main microorganisms involved in the flavor formation of Zhenba bacon.

4. Conclusion

In this study, the changes in microbial communities and volatile flavor compounds during the fermentation process were analyzed. GC-IMS was used to identify 70 critical volatile compounds, among which ketones, hydrocarbons, aldehydes, esters and alcohols were the most important volatile compounds in the curing and smoking stages. The composition of the microbial community of Zhenba bacon changed greatly during the fermentation process. The dominant bacteria were *Staphylococcus*, *Psychrobacter* and *Latilactobacillus* in the curing and smoking stages, and the dominant fungi were *Fusarium* and *Debaryomyces*. Correlation analysis based on O2PLS indicated that the bacteria *Staphylococcus*, *Psychrobacter*, *Latilactobacillus* and *Myroides* were important in the formation of the volatile compounds. Nonetheless, it is necessary to further combine metabolomics and metagenomics methods to verify the microbial distribution of different metabolic pathways and screen out the role of key enzymes and metabolites in the production of flavor substances. These findings can reveal the relationship between microorganisms and flavor development during the fermentation process of Zhenba bacon, and help in screening starter cultures for improving bacon quality and flavor characteristics.

CRediT authorship contribution statement

Bo Ning: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Yao Zuo:** Writing – review & editing, Validation, Investigation, Data curation. **Ling Wang:** Writing – review & editing, Supervision, Formal analysis. **Lianxu Zhu:** Resources, Investigation. **Hongqiang Ren:** Resources, Investigation. **Shanshan Wang:** Investigation, Formal analysis. **Wenxian Zeng:** Investigation, Formal analysis. **Hongzhao Lu:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Tao Zhang:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study was supported by Research Project of Shaanxi Science and Technology Department (2024NC-YBXM-101 and 2024NC-YBXM-164).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101478.

References

Api, A. M., Belmonte, F., Belsito, D., Biserta, S., Botelho, D., Bruze, M., … Tsang, S. (2020). RIFM fragrance ingredient safety assessment, 2-pentanol, CAS registry number 6032-29-7. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 138*(Suppl. 1), Article 111175. https://doi.org/10.1016/j.fct.2020.111175

Ashaolu, T. J., Khalifa, I., Mesak, M. A., Lorenzo, J. M., & Farag, M. A. (2023). A comprehensive review of the role of microorganisms on texture change, flavor and biogenic amines formation in fermented meat with their action mechanisms and

safety. *Critical Reviews in Food Science and Nutrition, 63*(19), 3538–3555. https://doi.org/10.1080/10408398.2021.1929059
Biller, E., Boselli, E., Obiedziński, M., Karpiński, P., & Waszkiewicz-Robak, B. (2016). The profile of volatile compounds in the outer and inner parts of broiled pork neck is strongly influenced by the acetic-acid marination conditions. *Meat Science, 121*, 292–301. https://doi.org/10.1016/j.meatsci.2016.06.029
Bosse Née Danz, R., Müller, A., Gibis, M., Weiss, A., Schmidt, H., & Weiss, J. (2018). Recent advances in cured raw ham manufacture. *Critical Reviews in Food Science and Nutrition, 58*(4), 610–630. https://doi.org/10.1080/10408398.2016.1208634
Broekaert, K., Noseda, B., Heyndrickx, M., Vlaemynck, G., & Devlieghere, F. (2013). Volatile compounds associated with Psychrobacter spp. and Pseudoalteromonas spp., the dominant microbiota of brown shrimp (*Crangon crangon*) during aerobic storage. *International Journal of Food Microbiology, 166*(3), 487–493. https://doi.org/10.1016/j.ijfoodmicro.2013.08.013
Bylesjö, M., Eriksson, D., Kusano, M., Moritz, T., & Trygg, J. (2007). Data integration in plant biology: The O2PLS method for combined modeling of transcript and metabolite data. *The Plant Journal: for Cell and Molecular Biology, 52*(6), 1181–1191. https://doi.org/10.1111/j.1365-313X.2007.03293.x
Dias, S., Chambel, L., Tenreiro, R., Nunes, L., & Loureiro, V. (2021). Microbial characterization of yellow curing process of codfish. *International Journal of Food Science, 2021*, 6072731. https://doi.org/10.1155/2021/6072731
Feng, L., Bi, W., Chen, S., Zhu, J., & Liu, X. (2021). Regulatory function of sigma factors RpoS/RpoN in adaptation and spoilage potential of Shewanella baltica. *Food Microbiology, 97*, Article 103755. https://doi.org/10.1016/j.fm.2021.103755
Gong, W., Zhu, Y., Shi, X., Zhang, W., & Wen, P. (2021). Influence of tissue type on the bacterial diversity and Community in Pork Bacon. *Frontiers in Microbiology, 12*, Article 799332. https://doi.org/10.3389/fmicb.2021.799332
Grunwald, E. W., & Richards, M. P. (2006). Studies with myoglobin variants indicate that released hemin is the primary promoter of lipid oxidation in washed fish muscle. *Journal of Agricultural and Food Chemistry, 54*(12), 4452–4460. https://doi.org/10.1021/jf0603228
Hu, L., Qiu, S., Dai, Y., Tian, L., & Wei, C. (2022). Determination of 2-Pentanol enantiomers via chiral GC-MS and its sensory evaluation in Baijiu. *Foods (Basel, Switzerland), 11*(17). https://doi.org/10.3390/foods11172584
Hu, Y., Zhang, L., Liu, Q., Wang, Y., Chen, Q., & Kong, B. (2020). The potential correlation between bacterial diversity and the characteristic volatile flavour of traditional dry sausages from Northeast China. *Food Microbiology, 91*, Article 103505. https://doi.org/10.1016/j.fm.2020.103505
Ji, L., Wang, S., Zhou, Y., Nie, Q., Zhou, C., Ning, J., … Zhang, J. (2024). Effects of Saccharomyces cerevisiae and Kluyveromyces marxianus on the physicochemical, microbial, and flavor changes of sauce meat during storage. *Foods (Basel, Switzerland), 13*(3), 396. https://doi.org/10.3390/foods13030396
Johnson, W., Jr. (2004). Safety assessment of MIBK (methyl isobutyl ketone). *International Journal of Toxicology, 23*(Suppl. 1), 29–57. https://doi.org/10.1080/10915810490274298
Keawkim, K., & Na Jom, K. (2022). Metabolomics and flavoromics analysis of chemical constituent changes during roasting of germinated Sacha inchi (*Plukenetia volubilis* L.). *Food Chemistry: X, 15*, Article 100399. https://doi.org/10.1016/j.fochx.2022.100399
Kim, J. H., Lee, E. S., Kim, B. M., & Oh, M. H. (2022). Potential correlation between microbial diversity and volatile flavor compounds in different types of Korean dry-fermented sausages. *Foods (Basel, Switzerland), 11*(20), 3182. https://doi.org/10.3390/foods11203182
Lessie, T. G., & Phibbs, P. V., Jr. (1984). Alternative pathways of carbohydrate utilization in pseudomonads. *Annual Review of Microbiology, 38*, 359–388. https://doi.org/10.1146/annurev.mi.38.100184.002043
Li, X., Xiong, Q., Xu, B., Wang, H., Zhou, H., & Sun, Y. (2021). Bacterial community dynamics during different stages of processing of smoked bacon using the 16S rRNA gene amplicon analysis. *International Journal of Food Microbiology, 351*, Article 109076. https://doi.org/10.1016/j.ijfoodmicro.2021.109076
Li, X., Xiong, Q., Zhou, H., Xu, B., & Sun, Y. (2021). Analysis of microbial diversity and dynamics during bacon storage inoculated with potential spoilage Bacteria by high-throughput sequencing. *Frontiers in Microbiology, 12*, Article 713513. https://doi.org/10.3389/fmicb.2021.713513
Löser, C., Urit, T., & Bley, T. (2014). Perspectives for the biotechnological production of ethyl acetate by yeasts. *Applied Microbiology and Biotechnology, 98*(12), 5397–5415. https://doi.org/10.1007/s00253-014-5765-9
Majeed, S., De Silva, L., Kumarage, P. M., & Heo, G. J. (2023). Occurrence of potential virulence determinants in Aeromonas spp. isolated from different aquatic environments. *Journal of Applied Microbiology, 134*(3). https://doi.org/10.1093/jambio/lxad031
Myers, A. J., Scramlin, S. M., Dilger, A. C., Souza, C. M., & Killefer, J. (2008). Contribution of lean, fat, muscle color and degree of doneness to pork and beef species flavor. *Meat Science, 82*(1), 59–63. https://doi.org/10.1016/j.meatsci.2008.12.004
Peña-Saldarriaga, L. M., Fernández-López, J., & Pérez-Alvarez, J. A. (2020). Quality of chicken fat by-products: Lipid profile and colour properties. *Foods (Basel, Switzerland), 9*(8), 1046. https://doi.org/10.3390/foods9081046
Qin, L., Zhang, Y. Y., Xu, X. B., Wang, X. S., Liu, H. W., Zhou, D. Y., … Thornton, M. (2017). Isotope dilution HPLC-MS/MS for simultaneous quantification of acrylamide and 5-hydroxymethylfurfural (HMF) in thermally processed seafood. *Food Chemistry, 232*, 633–638. https://doi.org/10.1016/j.foodchem.2017.04.069
Shen, Y., Wu, Y., Wang, Y., Li, L., Li, C., Zhao, Y., & Yang, S. (2021). Contribution of autochthonous microbiota succession to flavor formation during Chinese fermented mandarin fish (*Siniperca chuatsi*). *Food Chemistry, 348*, Article 129107. https://doi.org/10.1016/j.foodchem.2021.129107
Shin, J. H., Tillotson, G., MacKenzie, T. N., Warren, C. A., Wexler, H. M., & Goldstein, E. J. C. (2024). Bacteroides and related species: The keystone taxa of the human gut microbiota. *Anaerobe, 85*, Article 102819. https://doi.org/10.1016/j.anaerobe.2024.102819
Strandwitz, P., Kim, K. H., Terekhova, D., Liu, J. K., Sharma, A., Levering, J., … Lewis, K. (2019). GABA-modulating bacteria of the human gut microbiota. *Nature Microbiology, 4*(3), 396–403. https://doi.org/10.1038/s41564-018-0307-3
Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*(1), 1–16. https://doi.org/10.1016/j.foodchem.2009.12.004
Toci, A. T., & Farah, A. (2014). Volatile fingerprint of Brazilian defective coffee seeds: Corroboration of potential marker compounds and identification of new low quality indicators. *Food Chemistry, 153*, 298–314. https://doi.org/10.1016/j.foodchem.2013.12.040
Wang, H., Yin, X., Zhang, L., Wang, X., Zhang, J., Wen, R., & Cao, J. (2024). Insight into the relationship between the causes of off-odour and microorganism communities in Xuanwei ham. *Foods (Basel, Switzerland), 13*(5), 776. https://doi.org/10.3390/foods13050776
Wang, S., Chen, H., & Sun, B. (2020). Recent progress in food flavor analysis using gas chromatography-ion mobility spectrometry (GC-IMS). *Food Chemistry, 315*, Article 126158. https://doi.org/10.1016/j.foodchem.2019.126158
Wang, Y., Li, F., Chen, J., Sun, Z., Wang, F., Wang, C., & Fu, L. (2021). High-throughput sequencing-based characterization of the predominant microbial community associated with characteristic flavor formation in Jinhua ham. *Food Microbiology, 94*, Article 103643. https://doi.org/10.1016/j.fm.2020.103643
Wang, Y., Song, H. L., Zhang, Y., Tang, J. N., & Yu, D. H. (2016). Determination of aroma compounds in pork broth produced by different processing methods. *Flavour and Fragrance Journal, 31*(4), 319–328. https://doi.org/10.1002/ffj.3320
Wang, Y., Wang, Z., Han, Q., Xie, Y., Zhou, H., Zhou, K., Li, X., & Xu, B. (2022). Comprehensive insights into the evolution of microbiological and metabolic characteristics of the fat portion during the processing of traditional Chinese bacon. *Food Research International (Ottawa, Ont.), 155*, Article 110987. https://doi.org/10.1016/j.foodres.2022.110987
Wu, D., Xia, Q., Cheng, H., Zhang, Q., Wang, Y., & Ye, X. (2022). Changes of volatile flavor compounds in sea buckthorn juice during fermentation based on gas chromatography-ion mobility spectrometry. *Foods (Basel, Switzerland), 11*(21), 3471. https://doi.org/10.3390/foods11213471
Xi, L., Zhang, J., Wu, R., Wang, T., & Ding, W. (2021). Characterization of the volatile compounds of Zhenba bacon at different process stages using GC-MS and GC-IMS. *Foods (Basel, Switzerland), 10*(11), 2869. https://doi.org/10.3390/foods10112869
Xia, C., He, Y., Cheng, S., He, J., Pan, D., Cao, J., & Sun, Y. (2021). Free fatty acids responsible for characteristic aroma in various sauced-ducks. *Food Chemistry, 343*, Article 128493. https://doi.org/10.1016/j.foodchem.2020.128493
Yang, L., Li, H., Wu, H., Su, C., & He, Z. (2023). Quality relationship between smoked and air-dried bacon of Sichuan-Chongqing in China: Free amino acids, volatile compounds, and microbial diversity. *Food Research International (Ottawa, Ont.), 164*, Article 112274. https://doi.org/10.1016/j.foodres.2022.112274
Zhang, J., Zhao, K., Li, H., Li, S., Xu, W., Chen, L., Xie, J., & Tang, H. (2023). Physicochemical property, volatile flavor quality, and microbial community composition of Jinhua fatty ham and lean ham: A comparative study. *Frontiers in Microbiology, 14*, 1124770. https://doi.org/10.3389/fmicb.2023.1124770
Zhou, C. Y., Wang, C., Tang, C. B., Dai, C., Bai, Y., Yu, X. B., … Cao, J. X. (2019). Label-free proteomics reveals the mechanism of bitterness and adhesiveness in Jinhua ham. *Food Chemistry, 297*, Article 125012. https://doi.org/10.1016/j.foodchem.2019.125012