Journal of Biotech Research [ISSN: 1944-3285] 2026; 25:86-98

RESEARCH ARTICLE

Microbial ecology and chemical trajectories in vacuum-fermented Weifang radish: A one-radish, one-bag approach

Meng Li[1, 2, 3], Xiaoqing Zan[1], Ning Yang[4], Jing Xu[1, 2, 3], Xiaoyan Shen[5], Man Lyu[1, 2, 3, *]

[1]Department of Biological and Chemical Engineering, Shandong Vocational College of Science and Technology, Weifang, Shandong, China. [2]Research and Development Center for New Technology of Green Pharmaceutical Production of Shandong Universities, Weifang, Shandong, China. [3]Bioprocessing Technology Inheritance and Innovation Platform of Shandong Vocational Education, Weifang, Shandong, China.[4]Weifang Haituo Medical Laboratory Co., Ltd., Weifang, Shandong, China. [5]Weifang High-Tech Zone Xinsheng Biotechnology Development Co., Ltd., Weifang, Shandong, China.

Received: December 25, 2025; **accepted:** April 18, 2026.

Vacuum fermentation is widely used for radish processing. However, the coupled trajectories of key chemical parameters and microbial succession as well as their unit-to-unit variability remain insufficiently resolved. This research applied a one-radish–one-bag vacuum-fermentation design to track chemical dynamics and microbial ecology during 28 days of Weifang radish fermentation. Brine pH, titratable acidity (TA), and nitrite were monitored at nine time points (biological triplicates). Bacterial (16S rDNA) and fungal (ITS1) communities were characterized by amplicon sequencing. The results demonstrated that fermentation showed rapid, significant acidification with pH decreasing from 6.67 ± 0.01 (Day 0) to 4.14 ± 0.06 (Day 4) and remaining at low values thereafter accompanied by a marked increase in TA ($P < 0.001$). Nitrite exhibited a pronounced early peak at 26.39 ± 5.48 mg/kg at Day 2 ($P < 0.001$) followed by rapid depletion to 1.76 ± 0.69 mg/kg by Day 4 and < 1 mg/kg thereafter, indicating a narrow safety-critical window within the first 48 h. Microbial communities shifted significantly over time with early facultative taxa being replaced by lactic acid bacteria such as *Weissella*, *Leuconostoc*, *Lactiplantibacillus* and concurrent changes in several fungal genera ($P < 0.05$). Correlation analysis linked lactic acid bacteria to acidification with pH *vs*. *Lactiplantibacillus* demonstrating $r = -0.73$ ($P < 0.001$), whereas nitrite was positively associated with *Enterobacter* ($r = 0.38$, $p = 0.044$) and *Fusarium* ($r = 0.45$, $p = 0.021$). Notably, substantial unit-to-unit variability was observed for TA, nitrite, and microbial abundances despite standardized conditions, highlighting intrinsic raw-material heterogeneity. The results integrated microbial ecology with chemical trajectories in vacuum-fermented radish and provided actionable evidence for improving early-stage process control and product consistency.

Keywords: radish fermentation; lactic acid bacteria; microbial succession; nitrite safety; fungal community; inter-sample variability.

[*]**Corresponding author:** Man Lyu, Department of Biological and Chemical Engineering, Shandong Vocational College of Science and Technology, Weifang, Shandong 261021, China. Email: lilydunn689@hotmail.com.

Introduction

Fermentation, a time-honored technique for food transformation and preservation, has played an indispensable role across cultures and continents [1]. Among fermented foods, vegetables such as cabbage, cucumber, and radish (*Raphanus sativus* L.) have garnered increasing attention for their health-promoting properties [2], extended shelf life, and sensory appeal [3]. This biological process, driven primarily by indigenous or inoculated microorganisms, yields not only acidified and safe foods but also dynamic ecosystems where microbial interactions and metabolite transitions shape the final product's nutritional, safety, and flavor profile [4]. Radish is widely consumed and fermented in many East Asian countries with regionally distinct varieties contributing to the diversity of pickled products [5]. In China, preparations such as Sichuan Paocai and Suancai often incorporate vegetables including radish as a core substrate, and fermentation is typically spontaneous, relying on ambient microbes present on the plant surface or in the environment [6]. Among various radish cultivars, the Weifang fruit radish, a regionally protected cultivar from Shandong, China, exhibits distinct green skin and emerald flesh and enjoys high consumer acceptance owing to its crisp texture and mild sweetness.

Current research emphasizes that the microbial consortia involved in spontaneous vegetable fermentation are highly dynamic, strongly influenced by substrate characteristics such as sugar and nitrate levels, environmental parameters of temperature, oxygen, humidity, initial microbial load, and types of containers [7]. These factors govern critical quality and safety outcomes, especially pH decline, nitrite accumulation and clearance, organic acid production, and the development or inhibition of spoilage organisms [8]. Radish is an ideal model for studying fermentation microbiota because of its relatively simple structure, low sugar content, and rapid softening behavior during pickling. It also serves as a significant source of nitrates. Under bacterial reduction, these nitrates are converted into nitrites, thereby presenting a challenge that encompasses both functional and safety dimensions. Several studies have shown that nitrate-reducing bacteria including members of *Enterobacteriaceae* are present on raw vegetables and active during the initial days of fermentation, contributing to nitrite accumulation unless suppressed by acidification [9]. Spontaneous fermentations, especially those using only raw vegetables and salt, are increasingly favored by consumers for their "clean label" attributes. Therefore, resolving their variability and safety issues without introducing synthetic additives or heat treatments remains a major research imperative [10]. The scientific characterization of microbial succession during radish fermentation has lagged that of other model systems like kimchi or sauerkraut [11]. In many spontaneous fermentation systems, microbial succession is unpredictable and highly variable between batches, often leading to inconsistent flavor, texture, or even product safety [12]. This variability arises from several interlinked causes including raw material heterogeneity in microbial colonization or sugar and nitrate content [13], a lack of inoculum standardization [14], minor differences in fermentation vessel microenvironments [15], and significant safety concerns regarding nitrate-to-nitrite conversion [16]. Although prior studies have explored the dynamics of microbial communities in fermented vegetables, most have focused on mixed vegetable systems or industrial-scale fermentations, analyzed pooled samples that obscure intra-batch heterogeneity, and prioritized bacterial community shifts while neglecting fungal community dynamics. Crucially, to date, no published study has investigated the fermentation process of individually vacuum-sealed radishes at high temporal resolution while simultaneously assessing both chemical parameters and the two major microbial domains. Furthermore, few studies have contextualized the nitrite hazard window, typically the first 48 hours, as a process-critical phase that could benefit from targeted microbial or physical interventions.

To address the above knowledge gaps, this study aimed to elucidate microbial succession dynamics, identify safety-critical points in spontaneous fermentation systems, and advance the understanding of how initial epiphytic microbiota were filtered by environmental constraints over time by designing and implementing a single-radish, vacuum-sealed fermentation system that allowed for independent monitoring of microbial and chemical transformations in 40 individual Weifang radishes over a 28-day period to standardize brine concentration, fermentation temperature, and eliminate cross-contamination to create a controlled but naturalistic setting. By high-throughput sequencing of bacterial 16S rDNA (V4 region) and the fungal ITS1 region alongside detailed chemical analyses, a comprehensive timeline of radish fermentation under minimally controlled conditions was constructed. The results of this research provided vital insights into the micro-ecological mechanisms governing vacuum-fermented radishes. By resolving these variability and safety issues without introducing synthetic additives or heat treatments, this study offered a robust scientific basis for optimizing industrial parameters and ensuring the safe, standardized production of fermented vegetables.

Materials and methods

Brine preparation

Brine solution was prepared at a concentration of 6% (w/w) by using non-iodized, food-grade sodium chloride (Shandong Luyan Group Co., Ltd., Jinan, Shandong, China) dissolved in sterile distilled water and stored in sealed plastic bags. Prior to use, the sterility of the brine was confirmed by incubation on plate count agar at 37°C for 72 hours to verify absence of microbial growth.

Raw material selection and pretreatment

A total of 40 Weifang fruit radishes (Weicheng Beiguan Huanzheng Agricultural Products Store, Weifang, Shandong, China) were selected for fermentation. All radishes were from the same harvest batch and grown under similar conditions with a growth period of approximately 90 days. Radishes were chosen based on the criteria of cylindrical shape, maximum diameter of 6.0 ± 0.5 cm, average individual weight of approximately 600 g, and absence of visible lesions, damage, or puncture wounds. Radishes were cleaned thoroughly with surface root hairs and stems being removed followed by washing under tap water and three sequential rinses with sterile distilled water to minimize residual surface microorganisms and impurities prior to fermentation.

Fermentation system setup

Each radish was transversely sliced into 0.8 ± 0.1 cm thick disks. For each fermentation unit, 500 ± 5 g of sliced radish tissue preferentially including sections from both the stem and root ends was weighed and transferred into a sterile vacuum-sealable 30 × 30 cm PET+PE composite plastic bag (Deli Group Co., Ltd., Ningbo, Zhejiang, China). An equal mass (500 ± 5 g) of sterile 6% NaCl brine was added to the bag. Air was removed by using a Deli 14198 vacuum sealer (Deli Group Co., Ltd., Ningbo, Zhejiang, China) to ensure anaerobic conditions. Care was taken to confirm vacuum integrity and absence of leakage across all bags. The sealed fermentation units were incubated in darkness at 20 ± 0.5°C in a Putian SHP-250 biochemical incubator (Putian Instrument Manufacturing Co., Ltd., Changzhou, Jiangsu, China) for up to 28 days.

Sampling process

Sampling was conducted at nine predetermined time points including days 0, 2, 4, 8, 12, 16, 20, 24, and 28. At each time point, three fermentation bags were randomly selected for analysis. Samples collected on Day 0 were exclusively designated for initial chemical parameter analysis including pH, titratable acidity (TA), and nitrite content to establish baseline conditions before fermentation commenced. Microbial community analysis was performed on samples collected from Day 2 onwards, encompassing the fermentation progression. Bag surfaces were disinfected with 75% ethanol before opening. Under sterile conditions in a SCW-CJ-1F laminar flow hood (Horizon Purification Technology Co., Ltd., Suzhou, Jiangsu, China), the brine was transferred into sterile beakers and thoroughly mixed. 1 mL aliquot of well-mixed brine from each bag was collected in a sterile microcentrifuge tube and immediately flash-frozen in liquid nitrogen for subsequent microbial analysis. Remaining brine samples were used for chemical measurements.

Chemical analysis

The pH of brine sample was measured by using a INASE PHS-3E calibrated digital pH meter (INASE Scientific Instrument Co., Ltd., Shanghai, China). Nitrite concentration was determined spectrophotometrically in accordance with the Chinese National Food Safety Standard: Determination of nitrate and nitrite in food (GB 5009.33-2016). Brine samples were centrifuged at 8,000 rpm for 20 minutes at 4°C before the supernatants being analyzed. The results were expressed in mg/kg. TA was measured *via* acid-base titration using standard NaOH solution following Chinese National Food Safety Standard: Determination of total acid in foods (GB 12456-2021). Values were expressed as g of lactic acid equivalent per liter of brine (g/L).

Microbial community analysis

Total microbial DNA was extracted from 1 mL of brine using the cetyltrimethylammonium bromide (CTAB) (Solarbio Biotech Co., Ltd, Shanghai, China) method. After verifying DNA integrity and concentration using agarose gel electrophoresis and spectrophotometry, all DNA samples were diluted to 1 ng/μL with sterile water. The V4 region of the bacterial 16S rDNA region was amplified by using primers 515F (5'-GTG YCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3'). The fungal ITS1 region was amplified by using primers ITS5-1737F (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS2-2043R (5'-GCT GCG TTC TTC ATC GAT GC-3'). Polymerase chain reaction (PCR) was conducted by using Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, Frankfurt am Main, Germany) following manufacturer's instructions with the cycling program of 98°C for 1 min followed by 30 cycles of 98°C for 10 s, 50°C for 30s, 72°C for 30 s, and a final 5 min extension at 72°C. PCR products were purified by using the TIANgel Purification Kit (TIANGEN BIOTECH, Beijing, China) following manufacturer's instructions. Sequencing libraries were constructed by using the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs, Frankfurt am Main, Germany) and sequenced on an Illumina NovaSeq6000 platform (Illumina Inc., San Diego, CA, USA). Raw sequencing data were subjected to quality filtering using fastp (v0.20.0) (https://github.com/OpenGene/fastp) and merged with FLASH (v1.2.11) (https://ccb.jhu.edu/software/FLASH). Chimeric sequences were removed with Vsearch (v2.15.0) (https://github.com/torognes/vsearch). DADA2 in the QIIME2 (v202006) (https://qiime2.org) pipeline was employed for denoising and feature table construction [17]. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity by using the UPARSE (https://www.drive5.com/usearch/index.html). Taxonomic classification was performed by using the SILVA database (v138) (https://www.arb-silva.de) for bacteria and UNITE database (v8.2) (https://unite.ut.ee) for fungi.

Statistical analysis

Statistical analysis and data visualization were performed by using R (v4.4.0) with packages of tidyverse, rstatix, PMCMRplus, DescTools, vegan (https://www.r-project.org). The chemical parameters of pH, TA, and nitrite concentration across fermentation time points were assessed by using one-way analysis of variance (ANOVA). Normality was checked prior to ANOVA using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. Tukey's Honestly Significant Difference (HSD) post-hoc test was performed for pairwise comparisons. For microbial relative abundance data, the non-parametric Kruskal-Wallis H test was used to determine overall significant differences across time points. Dunn's test was subsequently performed for pairwise comparisons between time points. *P* values were adjusted by using the Benjamini-Hochberg (BH) method to control the false discovery rate. To quantify inter-sample variability, the mean, standard deviation (SD),

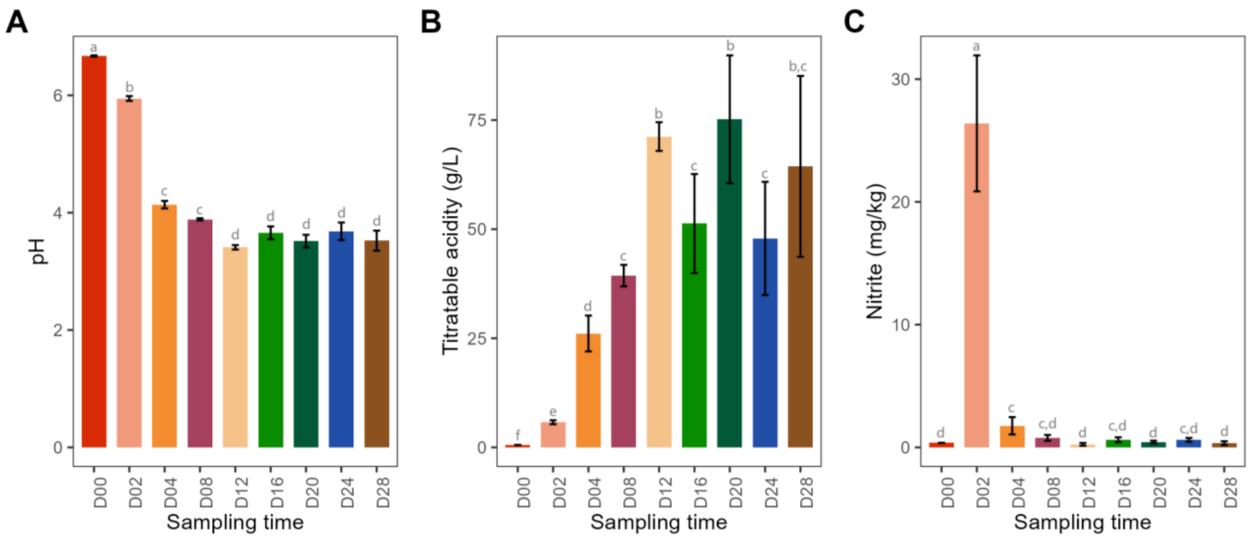

Figure 1. Dynamic changes of chemical parameters (mean ± se) during fermentation of radish. Different lowercase letters indicated statistically significant differences ($P < 0.05$) between time points.

and coefficient of variation (CV = SD / Mean × 100%) were calculated for all measured parameters and microbial taxa at each time point. Spearman's rank correlation analysis was performed to assess the relationships between chemical parameters and the relative abundances of key bacterial and fungal taxa. Correlation coefficients (r) and corresponding P values were calculated. P values less than 0.05 were defined as statistically significant differences.

Results

Chemical dynamics during fermentation

The fermentation of single-unit Weifang radishes under anaerobic conditions exhibited rapid and statistically significant stage-dependent shifts in key chemical parameters. The pH of the fermentation brine demonstrated a swift and highly significant decline throughout the process ($P < 0.001$). Initially, the brine presented a neutral pH of 6.67 ± 0.01 on Day 0. This value significantly decreased to 5.95 ± 0.04 by Day 2 and further dropped to 4.14 ± 0.06 on Day 4. The pH then stabilized, maintaining values within the range of 3.41 to 3.89 from Day 12 onwards with no significant differences observed among Days 12, 16, 20, 24, and 28 (Figure 1A). This pattern highlighted a rapid initial acidification followed by a prolonged stable acidic phase. Concurrently, TA exhibited a highly significant and consistent increase, mirroring the pH decline ($P < 0.001$). Beginning at a low 0.54 ± 0.01 g/L on Day 0, TA rose significantly to 5.76 ± 0.44 g/L by Day 2 and reached 26.37 ± 4.09 g/L on Day 4. Accumulation continued with TA values peaking significantly at 71.22 ± 3.25 g/L on Day 12 and 75.18 ± 9.87 g/L on Day 20. Although fluctuations were observed in the late stages with Day 28 reaching 64.38 ± 20.89 g/L, this value was not significantly different from Day 12 and Day 20 (Figure 1B). Notably, individual samples exhibited a wide range of TA, reaching as high as 104.76 g/L in a Day 28 sample, indicating considerable inter-sample variability in acid accumulation (Table 1). The concentration of nitrite in the fermentation brine also underwent a sharp and statistically significant transient peak in the early stage followed by rapid and significant depletion ($P < 0.001$). From an initial low of 0.37 ± 0.00 mg/kg on Day 0, nitrite surged dramatically to a peak of 26.39 ± 5.48 mg/kg on Day 2, which was significantly higher than all other time points and concerningly exceeded the Chinese National Food Safety Limit at 20 mg/kg. However, by Day 4, nitrite levels had significantly declined to 1.76

Table 1. Variability of chemical parameters and key bacterial/fungal genera across fermentation time points.

Parameters/genera	Day 0	Day 2	Day 4	Day 8	Day 12	Day 16	Day 20	Day 24	Day 28
pH	0.30	1.12	2.65	0.81	1.83	5.48	5.00	7.02	8.35
Titratable_acidity	3.21	13.43	26.50	10.66	7.91	37.94	33.38	45.13	56.43
Nitrite	0.00	36.30	66.49	54.12	75.28	55.07	43.68	43.83	79.28
Weissella	-	141.67	92.41	47.18	34.84	86.77	73.66	83.21	92.51
Lactiplantibacillus	-	82.21	52.47	59.75	19.66	146.17	79.76	121.73	88.08
Pantoea	-	54.12	103.44	20.41	38.30	65.44	152.01	40.87	32.55
Leuconostoc	-	25.53	122.43	15.27	91.58	60.61	74.07	143.15	61.27
Enterobacter	-	111.37	104.78	147.77	37.70	58.79	174.31	38.59	141.06
Lactococcus	-	154.47	77.38	97.11	11.74	95.04	67.55	139.44	63.79
Morchella	-	146.28	98.87	127.00	114.73	167.68	116.69	105.31	144.90
Aspergillus	-	171.05	170.09	200.00	215.23	170.97	173.3	200.00	154.88
Candida	-	200.00	139.03	175.54	62.73	105.27	150.00	173.20	64.15
Fusarium	-	163.93	176.49	170.16	167.31	158.63	100.19	139.06	169.90
Cladosporium	-	96.29	27.06	64.71	150.08	40.35	93.85	142.84	87.98
Ceratobasidium	-	200.00	169.93	114.68	106.17	173.21	145.52	121.82	79.48

Note: "-" indicated missing data.

± 0.69 mg/kg and remained consistently below 1.00 mg/kg for the remainder of the fermentation with Days 12, 20, and 28 values statistically indistinguishable from Day 0 (Figure 1C). This dynamic change highlighted a critical, albeit narrow, safety window during the initial 48 hours of fermentation.

Bacterial community succession

High throughput 16S rDNA amplicon sequencing revealed a clear and statistically significant temporal succession in the bacterial community, predominantly by *Firmicutes* and *Proteobacteria* (Figure 2). Kruskal-Wallis tests indicated statistically significant differences across time points for *Weissella* ($P < 0.05$), *Lactiplantibacillus* ($P < 0.01$), *Pantoea* ($P < 0.01$), *Leuconostoc* ($P < 0.05$), and *Lactococcus* ($P < 0.05$). *Enterobacter*, however, did not show a statistically significant change in its overall abundance across time points. Early in fermentation, *Pantoea* from the *Erwiniaceae* family was highly abundant, accounting for an average of 47.78% of bacterial reads on Day 2, which was significantly higher than its levels from Day 12 onwards ($P < 0.01$), indicating its dominance in the initial phase. Similarly, *Enterobacter* was prominent in early samples, contributing to the initial microbial landscape. As fermentation progressed, *Leuconostocaceae* became dominant with *Leuconostoc* showing significant increases from Day 2 to Day 4 and Day 12 ($P < 0.01$), peaking in abundance during the mid-fermentation stage (mean 44.61%) (Figure 2D). *Weissella* also rapidly increased in early samples with its abundance on Day 8 being significantly higher than that on Day 2 ($P < 0.01$), remaining relatively abundant thereafter. In the mid-to-late stages, *Lactobacillaceae* gradually increased from 1.31% on Day 2 to 42.12% on Day 28. *Lactiplantibacillus* was at low levels in the first 8 days but expanded significantly in mid- and late-stage samples (Day 12-20) with the mean value of 23.16%. Post-hoc analysis confirmed that *Lactiplantibacillus* abundance on Day 2 was significantly lower than that on Days 12, 20, and 28 ($P < 0.01$). *Streptococcaceae*, specifically *Lactococcus*, also contributed significantly to mid-fermentation with Day 2 abundance being significantly lower than that on Day 4 and Day 12 ($P < 0.01$) before declining in later samples. This succession indicated a shift from early facultative anaerobes to acid-tolerant lactic acid bacteria (LAB).

Fungal community composition

ITS1 amplicon sequencing revealed that *Ascomycota* consistently dominated the fungal community across the entire fermentation

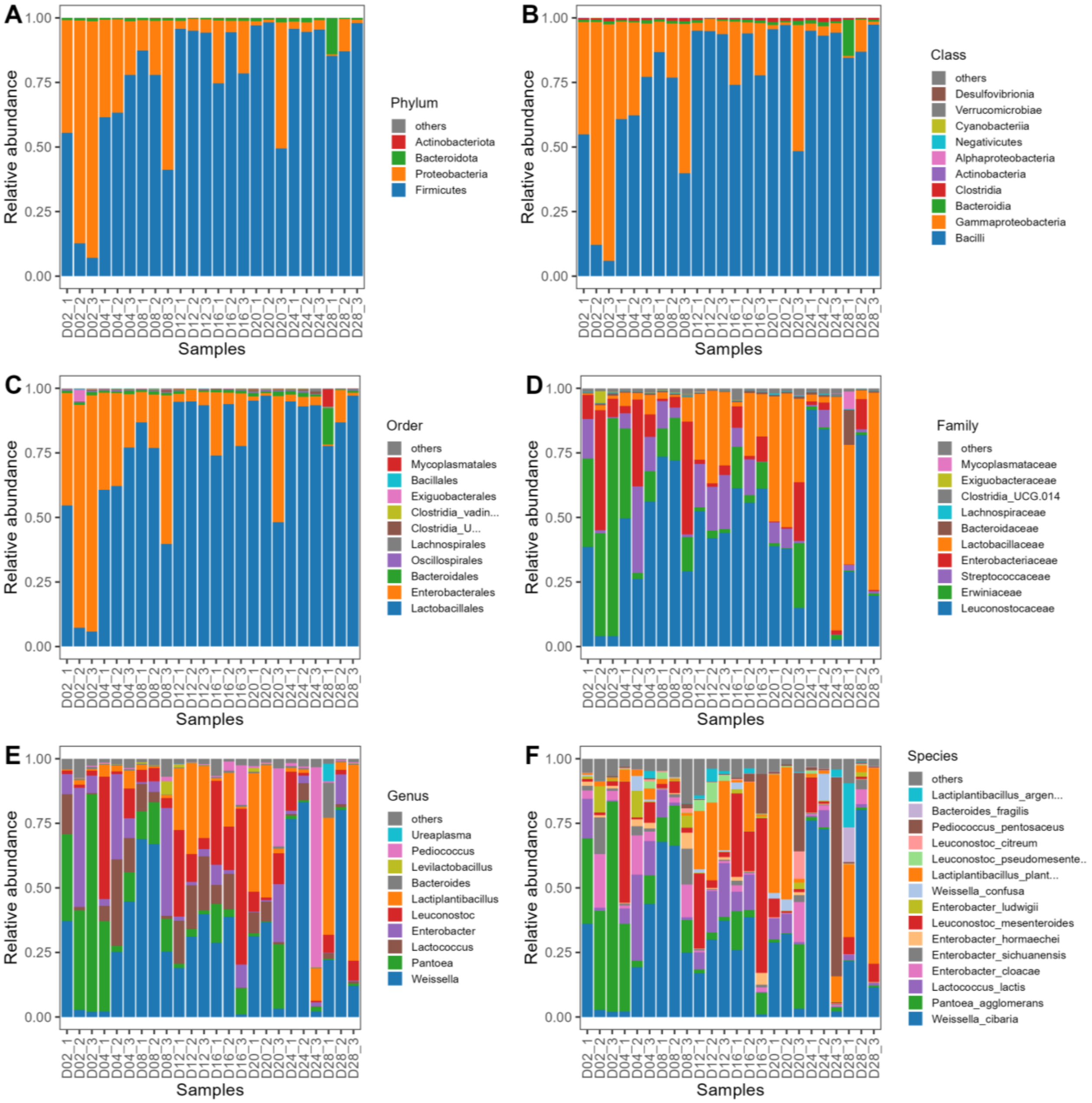

Figure 2. Bacterial community dynamics in fermented radish samples based on 16S rDNA amplicon sequencing. **A.** Phylum-level bacterial composition. **B.** Class-level bacterial composition. **C.** Order-level bacterial composition. **D.** Family-level bacterial composition. **E.** Genus-level bacterial composition. **F.** Species-level bacterial composition.

period with the mean value of 77.75% followed by *Basidiomycota* with the mean value of 12.06%. At the genus level, Kruskal-Wallis tests indicated statistically significant temporal changes for *Morchella*, *Fusarium*, and *Ceratobasidium* ($P < 0.05$). Conversely, *Aspergillus*, *Candida*, and *Cladosporium* did not show statistically significant changes in their overall abundance across time points. In the early and mid-fermentation stages up to Day 20, *Fusarium* abundance, though initially low, significantly increased with Day 2 levels being significantly lower than that on Days 4, 8, 12, and 20 ($P < 0.01$). *Ceratobasidium*, initially

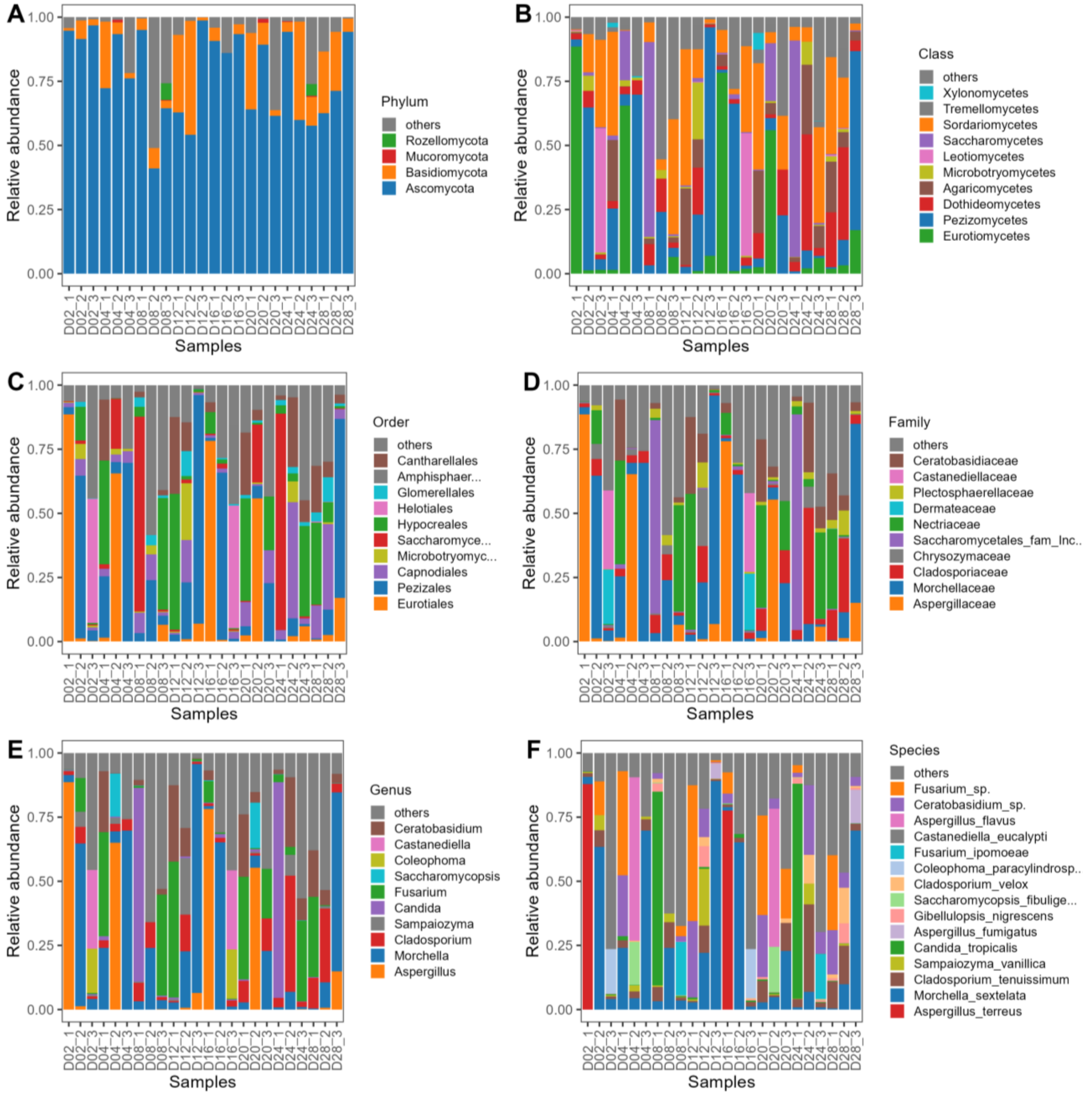

Figure 3. Fungal community dynamics in fermented radish samples based on ITS1 amplicon sequencing. **A.** Phylum-level fungal composition. **B.** Class-level fungal composition. **C.** Order-level fungal composition. **D.** Family-level fungal composition. **E.** Genus-level fungal composition. **F.** Species-level fungal composition.

undetectable on Day 2, also significantly increased its presence by Days 4, 12, and 20 ($P < 0.01$). Later in the fermentation from Day 12 onwards, *Morchella* abundance on Day 2 was significantly lower than that on Day 12 and Day 28 ($P < 0.01$), indicating its late-stage proliferation (Figure 3). The emergence of various yeast-like fungi (e.g., *Candida tropicalis*) and filamentous fungi (e.g., *Aspergillus flavus* and *Fusarium* spp.) in later stages suggested a shift in fungal ecology as bacterial activity stabilized and environmental conditions evolved.

Sample variability and system stability

Despite all fermentation units being prepared under identical conditions, quantifiable inter-sample variability was observed across

Table 2. Spearman correlations between chemical parameters and key bacterial/fungal genera (Day 2 to Day 28).

Genus	pH	Titratable acidity (TA)	Nitrite
Weissella	*r* = -0.52 (*p* = 0.007)	*r* = 0.47 (*p* = 0.016)	*r* = -0.16 (*p* = 0.407)
Lactiplantibacillus	*r* = -0.73 (*p* < 0.001)	*r* = 0.69 (*p* < 0.001)	*r* = -0.17 (*p* = 0.380)
Pantoea	*r* = 0.32 (*p* = 0.106)	*r* = -0.29 (*p* = 0.139)	*r* = 0.12 (*p* = 0.548)
Leuconostoc	*r* = -0.59 (*p* = 0.002)	*r* = 0.56 (*p* = 0.003)	*r* = -0.07 (*p* = 0.740)
Enterobacter	*r* = 0.10 (*p* = 0.613)	*r* = -0.06 (*p* = 0.764)	*r* = 0.38 (*p* = 0.044)
Lactococcus	*r* = -0.24 (*p* = 0.230)	*r* = 0.21 (*p* = 0.298)	*r* = -0.03 (*p* = 0.884)
Morchella	*r* = -0.36 (*p* = 0.053)	*r* = 0.33 (*p* = 0.083)	*r* = 0.18 (*p* = 0.352)
Aspergillus	*r* = 0.06 (*p* = 0.760)	*r* = -0.05 (*p* = 0.807)	*r* = 0.09 (*p* = 0.640)
Candida	*r* = -0.14 (*p* = 0.463)	*r* = 0.12 (*p* = 0.536)	*r* = 0.05 (*p* = 0.801)
Fusarium	*r* = -0.51 (*p* = 0.008)	*r* = 0.50 (*p* = 0.010)	*r* = 0.45 (*p* = 0.021)
Cladosporium	*r* = -0.20 (*p* = 0.308)	*r* = 0.18 (*p* = 0.360)	*r* = 0.07 (*p* = 0.723)
Ceratobasidium	*r* = -0.22 (*p* = 0.270)	*r* = 0.20 (*p* = 0.316)	*r* = 0.11 (*p* = 0.578)

fermentation batches for various parameters and microbial taxa. The pH values consistently exhibited low coefficients of variation across all time points, indicating highly consistent acidification and suggesting that the fundamental process of acidification was robust and reproducible. However, TA and nitrite concentrations showed moderate to high CV% values with TA varied from 7.91% to 56.43% during fermentation and its highest variability observed in the mid-to-late stages. Nitrite concentrations displayed even higher variability, ranging from 0.36% to a striking 77.29% on Day 12, particularly during the crucial early and intermediate phases, which indicated that, while the overall trajectory of acid accumulation and nitrite degradation was predictable, the quantitative extent of these changes could differ significantly between individual radish units. Microbial communities consistently exhibited high to extremely high CV% values, often exceeding 100% at most time points (Table 1). *Lactiplantibacillus* showed a CV% of 121.73% on Day 24. *Pantoea* reached 152.01% on Day 20. Among fungi, *Aspergillus* was up to 230.13% on Day 12. *Candida* was up to 188.75% on Day 20. *Fusarium* was up to 169.60% on Day 28. The results displayed substantial inter-sample variability. These high CVs underscored significant differences in microbial composition and relative abundance among individual radish units, despite their standardized processing. Such heterogeneity highlighted the stochastic nature of microbial colonization and succession within individual fermentation systems.

Correlation analysis of chemical parameters and microbial taxa

Spearman's rank correlation analysis was performed to investigate the relationships between chemical parameters (pH, titratable acidity, nitrite) and the relative abundances of key bacterial and fungal taxa across fermentation time points. The results revealed strong and significant negative correlations between pH and the key LAB including *Lactiplantibacillus* (r = -0.73) ($P < 0.001$), *Leuconostoc* (r = -0.59) ($P < 0.01$), and *Weissella* (r = -0.52) ($P < 0.01$). Conversely, TA showed strong positive correlations with these same LAB genera where the r numbers for *Lactiplantibacillus*, *Leuconostoc*, and *Weissella* were 0.69 ($P < 0.001$), 0.56 ($P < 0.01$), and 0.47 ($P < 0.05$). These findings robustly supported the role of these LAB in driving the acidification process and the accumulation of organic acids during radish fermentation. Interestingly, the fungal genus *Fusarium* also exhibited significant negative correlation with pH (r = -0.51) ($P < 0.01$) and positive correlation with TA (r = 0.50) ($P < 0.05$), suggesting its potential involvement in acid production or its strong adaptation to acidic environments. Furthermore, nitrite concentration displayed a significant positive correlation with *Fusarium* (r = 0.45) ($P < 0.05$) and a weaker but significant positive correlation with *Enterobacter* (r = 0.38) ($P < 0.05$) (Table 2). These correlations suggested that these microbial taxa might play a role in the dynamics of nitrite, potentially through nitrate reduction or nitrite consumption pathways.

Discussion

Rapid acidification and nitrite dynamics: A critical safety consideration

The observed rapid and statistically significant acidification kinetics were a cornerstone of safe vegetable fermentation. Within the first four days, the pH significantly dropped from a neutral 6.67 to 4.14 accompanied by a substantial and significant increase in titratable acidity. This rapid acid production was driven by LAB, as strongly evidenced by Spearman correlation analysis, which revealed significant negative correlations between pH and *Lactiplantibacillus*, *Leuconostoc*, and *Weissella*, along with corresponding positive correlations with TA. These findings were consistent with previous studies that highlighted LAB as the primary drivers of acidification and organic acid accumulation in fermented plant matrices [18, 19]. This rapid acidification was crucial for mitigating the nitrite hazard. The results explicitly demonstrated a statistically significant transient nitrite peak on Day 2, which exceeded the Chinese National Food Safety Limit. However, as acidification intensified and pH dropped below 4.5 by Day 4, nitrite levels significantly declined and remained low thereafter. This transient nature of the nitrite peak underscored the critical importance of a rapid and robust acidification process in spontaneous fermentations [20, 21]. The rapid dominance of acid-producing LAB appeared essential to suppress nitrate-reducing facultative anaerobes before nitrite reached prolonged hazardous concentrations. The positive correlation between nitrite and *Enterobacter* suggested its role in initial nitrite accumulation from nitrate, a common pathway in early fermentation stages [9]. Thus, a well-controlled, rapid acidification trajectory, supported by a vigorous LAB community, was the primary lever for ensuring the microbiological safety of nitrite-prone fermented vegetables.

Microbial community ecology and functional succession

The bacterial succession observed in this study followed a canonical trajectory for traditional fermented vegetables, transitioning from early dominance by *Proteobacteria* to a stable community dominated by *Firmicutes* (LAB) [22 - 24]. Early in fermentation, genera like *Pantoea* and *Enterobacter* were prevalent. The statistically significant early abundance of *Pantoea* and the positive correlation of *Enterobacter* with nitrite suggested an initial community adapted to relatively neutral pH and potentially involved in nitrate reduction. As the environment acidified, heterofermentative LAB including *Weissella* and *Leuconostoc* significantly increased their abundance, becoming dominant in the early-to-mid fermentation stage. These genera were renowned for their rapid sugar metabolism, contributing to initial acidification and CO_2 production [25, 26]. Subsequently, in the mid-to-late stages, *Lactiplantibacillus* expanded markedly and significantly, stabilizing the low pH environment and driving the community toward a LAB-dominated plateau, a hallmark of mature fermentation ecosystems. This robust succession among various LAB groups along with the transient contribution of *Lactococcus* in mid-fermentation highlighted metabolic niche partitioning over time, collectively contributing to robust acid generation and pathogen suppression. The fungal community, generally considered less dominant than bacteria, also exhibited significant temporal changes. *Morchella*, *Fusarium*, and *Ceratobasidium* showed statistically significant shifts across time points. Notably, *Fusarium* exhibited a significant negative correlation with pH and a positive correlation with TA, suggesting its potential acid tolerance or even contribution to acid production. The significant positive correlation between nitrite and *Fusarium* was a novel and intriguing finding, indicating a possible, yet previously less explored, role for this fungal genus in nitrite dynamics. The late-stage proliferation of yeasts like *Morchella* and other filamentous fungi observed in some samples could be attributed to their ability to exploit residual sugars and tolerate acidic conditions, possibly under microaerophilic conditions in imperfectly sealed bags, which might pose a spoilage risk [13].

Unveiling inter-batch variability: Implications for process control

A distinctive contribution of this study was the quantitative assessment of inter-sample variability using a single-radish, one-bag fermentation model. Despite stringent standardization of external conditions of brine, temperature, anaerobic sealing, statistically significant batch-to-batch differences were observed across various parameters and microbial taxa as evidenced by the high coefficients of variation (CV%). While pH stability was remarkably consistent with low CV%, TA and nitrite concentrations displayed moderate to high CV% values, particularly in the later stages, suggesting that the quantitative outcomes of these metabolic processes were more susceptible to inherent variability. More strikingly, both bacterial and fungal communities consistently exhibited high to extremely high CV% values, often exceeding 100% at most time points. This substantial heterogeneity underscored that even seemingly identical fermentation units could follow divergent microbial trajectories. Several factors might contribute to this inherent variability, which included individual raw radish units likely possessed intrinsic biological differences such as variations in initial sugar content, nitrate levels, or moisture gradients, which could significantly influence microbial growth and metabolism. Physical characteristics including micro-topographical differences like surface cracks, root-end damage or variations in cut surface exposure could create distinct micro-environments affecting oxygen ingress or microbial attachment. Subtle differences in packing geometry within sealed bags might influence osmotic pressure, gas distribution, and the development of micro-anaerobic niches. These factors acting synergistically contributed to the stochastic nature of microbial colonization and succession within individual fermentation units. The observed unit-to-unit variability was rarely quantified in traditional fermentation studies that typically relied on bulk or pooled samples, potentially masking critical insights into process robustness. The results of this study offered a compelling argument for incorporating unit-level variance into future microbiome studies of fermented foods, especially for understanding quality control in small-batch or artisan production. From a production standpoint, these insights suggested that pre-fermentation standardization including uniform slicing, brine saturation, cut surface area exposure, and packing density could reduce batch-to-batch variation. In higher-volume operations, inoculation with functionally verified LAB starters might help override this stochastic variability and yield more consistent fermentation outcomes.

Future perspectives

This study employed a novel single-radish, one-bag anaerobic fermentation model to elucidate the intricate and statistically significant dynamic interplay between microbial communities and chemical parameters in radish (*Raphanus sativus* L.) fermentation. A clear three-stage microbial succession was identified from early facultative anaerobes (*Pantoea, Enterobacter*) to heterofermentative LAB (*Weissella*, *Leuconostoc*) and culminating in *Lactiplantibacillus*-dominated stabilization. These microbial shifts were tightly coupled with the chemical environment, showing strong and statistically significant correlations between pH reduction, titratable acid accumulation, nitrite depletion, and specific microbial genera. Importantly, *Lactiplantibacillus*, *Leuconostoc*, and *Weissella* were robustly identified as key drivers of acidification, while *Fusarium* and *Enterobacter* showed significant positive correlations with nitrite, suggesting their roles in nitrite dynamics. Critically, the transient nitrite peak observed in the early fermentation stage reinforced the necessity for rapid and consistent acidification within the first 48 hours to mitigate food safety risks effectively. Furthermore, the study quantitatively revealed substantial sample-to-sample variability in microbial composition and fermentation outcomes as evidenced by high coefficients of variation, even under standardized external conditions. This variability pointed to intrinsic differences among individual radishes as key modulators of fermentation trajectories, a crucial insight often overlooked in bulk sampling. This research highlighted the value of unit-scale fermentation systems for studying host-microbe interactions and microbial ecology in food systems and practically suggested that enhancing raw material uniformity, optimizing pre-treatment, ensuring packaging consistency, and potentially utilizing starter cultures could significantly improve the safety and consistency of naturally fermented vegetable products. Future research could build upon these insights by integrating metabolomic profiling and targeted strain isolation to develop more tailored and robust fermentation strategies.

Acknowledgements

This study was funded by Science and Technology Support Plan for Youth Innovation of Colleges and Universities of Shandong Province of China (Grant No. 2024KJG065), Shandong Provincial Natural Science Foundation Project (Grant No. ZR2021QC201 and ZR2021QC232), Weifang Science and Technology Plan Project (Grant No. 2022GX045).

References

1. Sharma S, Singh A, Sharma S, Kant A, Sevda S, Taherzadeh MJ, *et al*. 2021. Functional foods as a formulation ingredient in beverages: Technological advancements and constraints. Bioengineered. 12(2):11055-11075.
2. Tan X, Cui F, Wang D, Lv X, Li X, Li J. 2023. Fermented vegetables: Health benefits, defects, and current technological solutions. Foods. 13(1):38.
3. Sawant SS, Park HY, Sim EY, Kim HS, Choi HS. 2025. Microbial fermentation in food: Impact on functional properties and nutritional enhancement—A review of recent developments. Fermentation. 11(1):15.
4. Siddiqui SA, Erol Z, Rugji J, Taşçı F, Kahraman HA, Toppi V, *et al*. 2023. An overview of fermentation in the food industry - looking back from a new perspective. Bioresour Bioprocess. 10(1):85.
5. Hu Y, Liu X, Wu X, Zhang Z, Wu D, Chen C, *et al*. 2022. Several natural phytochemicals from Chinese traditional fermented food-pickled *Raphanus sativus* L.: Purification and characterization. Food Chem X. 15:100390.
6. Rao Y, Tao Y, Chen X, She X, Qian Y, Li Y, *et al*. 2020. The characteristics and correlation of the microbial communities and flavors in traditionally pickled radishes. LWT. 118:108804.
7. Zheng Z, Zhou Q, Li D, Wu Y, Zhong K, Gao H. 2024. Effects of enhanced fermentation with *Lactiplantibacillus plantarum* WWPC on physicochemical characteristics and flavor profiles of radish paocai and dried-fermented radish. Food Biosci. 59:103941.
8. Lu Y, Wu J, Huang J, Zheng M, Hong Z, Manzoor MF, *et al*. 2024. Fermented vegetables in China: Overview, novel processes, influencing factors, lactic acid bacteria and industrialization status. Int J Food Sci Technol. 59(7):4420-4436.
9. Yang X, Hu W, Xiu Z, Jiang A, Yang X, Saren G, *et al*. 2020. Microbial community dynamics and metabolome changes during spontaneous fermentation of Northeast Sauerkraut from different households. Front Microbiol. 11:1878.
10. Inguglia ES, Song Z, Kerry JP, O'Sullivan MG, Hamill RM. 2023. Addressing clean label trends in commercial meat processing: Strategies, challenges and insights from consumer perspectives. Foods. 12(10):2062.
11. Choi HW, Park SE, Kim EJ, Seo SH, Woong Whon T, Son HS. 2023. Effects of ingredient size on microbial communities and metabolites of radish kimchi. Food Chem X. 20:100950.
12. Yee CS, Zahia-Azizan NA, Abd Rahim MH, Mohd Zaini NA, Raja-Razali RB, Ushidee-Radzi MA, *et al*. 2025. Smart fermentation technologies: Microbial process control in traditional fermented foods. Fermentation. 11(6):323.
13. Zheng Z, Zhou Q, Wu Y, Zhang X, Zhang F, Jia Z, *et al*. 2023. Effects of assorted radishes on the flavor development and bacterial community succession of radish paocai during fermentation. LWT. 187:115377.
14. Zhao N, Huang Y, Lai H, Wang Y, Mei Y, Zeng X, *et al*. 2024. The role of abiotic and biotic factors of aged brine in directing microbial assembly and volatile profiles of Paocai during fermentation. Food Biosci. 57:103592.
15. Huang TT, Wu ZY, Zhang WX. 2020. Effects of garlic addition on bacterial communities and the conversions of nitrate and nitrite in a simulated pickle fermentation system. Food Control. 113:107215.
16. Seyyedsalehi MS, Mohebbi E, Tourang F, Sasanfar B, Boffetta P, Zendehdel K. 2023. Association of dietary nitrate, nitrite, and N-nitroso compounds intake and gastrointestinal cancers: A systematic review and meta-analysis. Toxics. 11(2):190.

17. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 13(7):581-583.
18. Shi J, Wang S, Song X, Tan M. 2025. From microbial transformation to machine learning optimization: Health benefits and technological advances in fermented fruits and vegetables. Food Rev Int. 2025:1-26.
19. Wei L, Beeck WV, Hanlon M, DiCaprio E, Marco ML. 2025. Lacto-fermented fruits and vegetables: Bioactive components and effects on human health. Annu Rev Food Sci Technol. 16:289-314.
20. Cardinali F, Milanović V, Osimani A, Aquilanti L, Taccari M, Garofalo C, *et al*. 2018. Microbial dynamics of model Fabriano-like fermented sausages as affected by starter cultures, nitrates and nitrites. Int J Food Microbiol. 278:61-72.
21. Joshi VK, Chauhan A, Devi S, Kumar V. 2014. Application of response surface methodology in optimization of lactic acid fermentation of radish: Effect of addition of salt, additives and growth stimulators. J Food Sci Technol. 52(8):4935-4944.
22. Wang Y, Zhang C, Liu F, Jin Z, Xia X. 2023. Ecological succession and functional characteristics of lactic acid bacteria in traditional fermented foods. Crit Rev Food Sci Nutr. 63(22):5841-5855.
23. Jeong SH, Jung JY, Lee SH, Jin HM, Jeon CO. 2013. Microbial succession and metabolite changes during fermentation of dongchimi, traditional Korean watery kimchi. Int J Food Microbiol. 164(1):46-53.
24. Li Y, He W, Liu S, Hu X, He Y, Song X, *et al*. 2024. Innovative omics strategies in fermented fruits and vegetables: Unveiling nutritional profiles, microbial diversity, and future prospects. Compr Rev Food Sci Food Saf. 23(6):e70030.
25. Chen YS, Yanagida F, Hsu JS. 2006. Isolation and characterization of lactic acid bacteria from suan-tsai (fermented mustard), a traditional fermented food in Taiwan. J Appl Microbiol. 101(1):125-130.
26. Lee M, Song JH, Lee SH, Jung MY, Chang JY. 2018. Effect of seasonal production on bacterial communities in Korean industrial kimchi fermentation. Food Control. 91:381-389.