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Differences in the bacterial profiles and physicochemical between natural and inoculated fermentation of vegetables from Shanxi Province

Zhidi Chen[1†], Jianyi Kang[1,2†], Yao Zhang[1†], Xinxin Yi[1], Xiaona Pang[1], Hongmei Li-Byarlay[3] and Xiuzhi Gao[1*]

Abstract

Purpose: Fermented vegetables can be divided into two types, natural fermented and artificially inoculated fermented. By detecting and identifying the changes of bacterial diversity using physical and chemical indicators during natural and inoculation fermentation, we analyzed and determined the dominant bacteria in the fermentation process and revealed the relationship between bacteria and volatile substances.

Methods: We used the Illumina Miseq to sequence the bacteria in fermented vegetable samples at different fermentation periods, and calculated the total number of mesophilic microorganisms and lactic acid bacteria. We used the pH and nitrite to monitor the acidification process. GC-MS was used to determine volatile flavor compounds. Finally, we analyzed the correlation between volatile flavor compounds and bacteria.

Results: Total mesophilic microorganisms and the number of lactic acid bacteria in the inoculated fermentation were higher than the natural fermentation. The bacterial diversity Shannon and Simpson indexes of the natural fermentation, higher than those of inoculated fermentation in 0~7 days, were between 55~71% and 36~45%, respectively. On the 7th day, the proportion of *Lactobacillus* in the natural fermentation and inoculated fermentation were 53.4% and 90.2%, respectively, which were significantly different. *Lactobacillus* was the dominant genus in the fermented vegetables and an important genus to promote the formation of volatile flavors. *Lactobacillus* was negatively correlated with two volatile substances (4-[2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl]-3-Buten-2-one (K4) and a-Phellandrene (X1)) and played a leading role in the fermentation process.

Conclusions: Results demonstrated that the total number of mesophilic microorganisms and lactic acid bacteria in inoculated fermentation were more than those in natural fermentation. Inoculated fermentation can shorten the fermentation cycle and reduce the content of nitrite. Lactic acid bacteria were the dominant bacteria in fermented vegetables.

Keywords: Natural fermentation, Inoculated fermentation, High-throughput sequencing, Bacterial diversity, Correlation Analysis

* Correspondence: gxz@bua.edu.cn
†Zhidi Chen, Jianyi Kang, and Yao Zhang contributed equally to this work.
[1]Beijing Laboratory of Food Quality and Safety, Beijing Key Laboratory of Agricultural Product Detection and Control of Spoilage Organisms and Pesticide Residue, Beijing Engineering Laboratory of Probiotics Key Technology Development, Beijing University of Agriculture, Beijing 102206, China
Full list of author information is available at the end of the article

Introduction

Fermentation has been applied in food processing for millennia. Fermented foods constitute an integral part of the human diet all over the world (Tamang et al. 2016). Fermented vegetables, which are made of vegetables such as cabbage, radish, beet, celery, or cucumber with various seasonings, are generally fermented from a few days to several months.

Similar to other fermented foods such as fermented soy, yogurt, and cheese, fermented vegetables have a unique aroma, taste, and great nutritional value, which are entirely dependent upon the fermentation conditions (Mcfeeters 2004). Many clinical and animal studies have demonstrated that fermented vegetables significantly reduce the risks of obesity (Kim et al. 2011; Kwak et al. 2012), diabetes (Zhang et al. 2018), oxidation (Kim et al. 2017a), hypercholesterolemia (Jung et al. 2014a), cancer (Yeh and Yen 2005), and help to stimulate the immune system (Caggianiello et al. 2016). Fermented vegetables have been considered as one of the most nutritional and health-beneficial foods in the world (Kim et al. 2012).

The most important factor associated with its health benefits is probiotic lactic acid bacteria (LAB), including Lactobacillus strains (Xiong et al. 2013). Different LAB strains have been used in fermented vegetables, such as *Lactococcus lactis* WiKim0098 and *Leuconostoc citreum* WiKim0096 (Kim et al. 2019), *Lactiplantibacillus plantarum* D-103 (Lee et al. 2016), *Leuconostoc mesenteroides* strain B1 (Jung et al. 2012), *Weissella cibaria* WC018, and *Lactiplantibacillus plantarum* LP067 (Xiang et al. 2020).

Microbial community composition at the initial fermentation process may be related to different types of raw materials. Given that the majority of fermented vegetables include several raw ingredients (Chen et al. 2014b; Yu et al. 2012), due to the natural transfer of the various indigenous microbiota, a competitive or cooperative microbial relationships may be established during initial fermentation, and the growth of dominant microbes during late fermentation. Fermentation failure, defined as a dysbiotic microbial community in foods, may cause food spoilage followed by the outgrowth of toxic or food poisoning microbes (Wei et al. 2020). Unfavorable microbial communities might result from the decreased resistance of desirable fermentative microbes to invasion caused by undesirable microbes. In most fermented vegetables, colonization resistance is mainly conferred by the outgrowth of LAB (Jung et al. 2018). Therefore, the LAB starter was added during initial vegetable preparation can avoid fermentation failure. However, many traditional household fermented foods still rely upon the natural transfer of fermentative microbes from the raw ingredients (Jung et al. 2014b; Lee et al. 2015a; Lee et al. 2015b).

Taxonomic studies based on microbial culture-dependent and independent approaches (e.g., bacterial 16S rRNA gene amplicon sequencing) showed that fermented vegetables possess a distinct microbial community (Tamang et al. 2016). LAB, including *Weissella*, *Lactobacillus*, and *Leuconostoc* species, are the dominant microbes in the vegetable fermentation (Jung et al. 2011; Lee et al. 2017; Park et al. 2012). The vast majority of fermented vegetable consumption in China is household fermented vegetables (Yang et al. 2020). Raw ingredient-derived fermentative microbes are the main bacteria in household fermented vegetables. The direct origin sources of the fermentative microbes and their causative role(s) on microbial community assembly patterns and processes during fermented vegetable fermentation are scarcely known.

Fresh vegetables have a short storage time at room temperature, which may lead to decomposition during transportation. Fermented vegetables not only have high nutritional value but also can be stored at room temperature for a long time. This study compared the number of microorganisms, the bacterial diversity, and physicochemical indexes of Shanxi local fermented vegetables during natural fermentation (NF) and inoculated fermentation (IF). Further analysis revealed the correlation between bacterial and flavors in the fermented vegetables. LAB isolated from naturally fermented vegetables (LV02 and LV73) are suggested to be used to inoculate naturally fermented vegetables. Inoculated fermentation can improve the stability of fermented vegetable products and shorten the fermentation cycle. This study provides a foundation of knowledge for the standardized industrialization of local fermented vegetables.

Materials and methods

Bacterial cultures

Probiotic LV02 (*Lactiplantibacillus plantarum* V02 MH885507) and LV73 (*Lentilactobacillus diolivorans* V73 MH885506) isolated and identified from traditional fermented vegetables was obtained from Shanxi Province local fermented vegetables. LV02 and LV73 were deposited in the China General Microbiological Culture Collection Center (CGMCC) on November 12, 2018; the deposit number of LV02 is CGMCC No. 16727, and LV73 is CGMCC No. 16728. Strains were stored in 30% (v/v) glycerol at − 80 °C. They were inoculated into MRS (Man Rogasa Sharpe) (Land bridge, Beijing) broth and grown at 37 °C for 12 h to obtain a cell count of 8–9 lg CFU/mL, then used for inoculation at 0.5% (w/v) in vegetables to prepare the starter for fermentation. All vegetables were purchased from the local supermarket.

Preparation of fermented vegetables

The fermented vegetables were prepared following the methods in Jung et al. (2016), with slight modification. We mixed sliced cabbages (8 kg), celeries (1 kg), and carrots (1 kg) with 500 mL of 4% brine, then divided them into two equal portion groups: (1) the control fermented vegetables as natural fermentation (NF), and (2) treated group inoculated with 0.5% (w/v) of probiotic LV02 and LV73 to prepare probiotic fermented vegetables (IF). The seasoned vegetables were fermented at 25 °C for 7 days in the jar. We collected 500 g of samples (both liquid and vegetable) each day in the polyethylene bags. The NF and IF products (n = 3 per product group) were stored under − 80 °C until analysis. The chemical and microbiological analyses of the two groups were determined immediately (day 0) and after every day until 7 days of fermentation.

Enumeration of viable bacteria

The fermented vegetable samples were cultured in PCA agar (Land bridge, Beijing) at 37 °C for 48 h to determine the total mesophilic microorganisms, and in MRS agar (Land bridge, Beijing) at 37 °C for 24 h to determine the number of LAB (Jampaphaeng et al. 2018). Viable counts were expressed as colony-forming unit (CFU) per gram of the fermented vegetable samples (Han et al. 2004). Ten times echelon dilution was used in this experiment. All the experiments were performed in triplicate and recorded as mean ± standard deviation (SD).

Extraction of microbial genome

According to the specification of E.Z. N.A. ®soil kit (Omega Bio-tek, Norcross, GA, USA), the total DNA (Axygen Biosciences, Union City, CA, USA) was extracted, the concentration and purity of DNA were detected, and the quality of DNA was detected by 1% agarose gel electrophoresis. The variable region of V3–V4 was amplified by PCR with 338F (5′ undefined ACTCCTACGGAGGAGCAGCAGCAGCAG 3′ undefined) primers and 806 R (5′ undefined GGACTA CHVGGGTCTAAT 3′ undefined) primers. Primers were purchased from Sangon Biotech Co., Ltd., Beijing, China.

The amplification procedure was as follows: predenaturation at 95 °C for 3 min, 27 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s), and extension of 10 min at 72 °C.

The amplification system was 20 μL, 4 μL 5 × FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL primer (5 μM), 0.4 μL FastPfu polymerase, 10 ng DNA template.

High-throughput sequencing

PCR products were recovered by 2% agarose gel, purified, eluted by Tris-HCl, and detected by 2% agarose gel electrophoresis. According to Illumina MiSeq Miseq PE300 Illumina, San Diego, USA, platform for sequencing (Shanghai Meiji Biopharmaceutical Technology Co., Ltd.). The original data has been uploaded to the NCBI database.

Bioinformatics analysis

Original sequencing sequence used Trimmomatic software quality control, used FLASH software for splicing. The UPARSE software was used to cluster the sequences with OTU according to 97% similarity, and removed the single sequence and chimera in the process of clustering, and annotated the species classification of each sequence. The Silva database (SSU123) was compared and the threshold value of the ratio was set to 70%.

Acidification process monitoring

Production of total acid in the fermentation process of the samples was expressed by measuring changes of pH; the pH level of the samples was determined with a pH meter (pH-250L, ISTEK, Seoul, Korea) (Rao et al. 2020).

Determination of nitrite content was according to the method by Khan et al. (2018), with some modifications. Then, 12.5 mL of 50 g/L saturated borax solution was added to a beaker with 5 g (accurate to 0.01 g) uniform samples; the contents were diluted with water. Heated in boiling water bath for 15 min, removed and cooled to room temperature. Then 5.0 mL of 106 g/L potassium ferrocyanide and 5.0 mL of 220 g/L zinc acetate solution were added and the solution was shaken thoroughly and the contents were diluted to the mark (500 mL) with distilled water, mixed well, and left for 30 min. Further, 40 mL of extracted and filtered sample solutions was removed into a 50 mL colorimetric tube with stopper; 2.0 mL of 4 g/L paminobenzenesulfonic acid solution was added, mixed well after, and left for 3–5 min; and 1.0 mL of 2 g/L naphthalene ethylenediamine hydrochloride solution was added and the contents were diluted to the mark with distilled water, mixed well, and left for 15 min; blank reagent was used as a control. Absorbance was measured at wavelength of maximum absorption (538 nm) against the corresponding reagent blank using single beam UV-Visible spectrophotometer. The amount of nitrite present in the unknown solution was computed from the calibration graph.

SPME–GC/MS profiling

Extraction of volatile flavor substances was examined according to the method described by He et al. (2020), with slight modifications. Weigh accurately 5 g of the cut vegetables from the fermentation process into a 20

mL headspace vial and cap it. Pre-equilibrate the headspace bottle containing the sample in a thermostatic water bath at 85 °C for 5 min, and then insert the aged 50/30 μm diethylbenzene/carbon molecular sieve/polydimethy lsiloxane (DVB/CAR/PDMS) After the extraction head continued to equilibrate and enrich for volatile matter for 30 min, the fiber head was retracted, the extraction head was pulled out, and the extraction head was reinserted into the GC-MS (7980A-5975C, US, Agilent Technologies Inc.) manual inlet for 5 min at 250 °C.

GC-MS analysis

Column DB-5MS (30 m × 0.32 mm × 0.25 μm); splitless injection mode; carrier gas: He; carrier gas flow: 1 mL/min; inlet temperature: 250 °C; column temperature program temperature rise: 40 °C for 3 min, 5 °C/min linearly increased to 150 °C, 10 °C/min linearly increased to 250 °C, and then maintained at 250 °C for 5 min. MS conditions: electron ion source (EI), electron energy 70 eV, ion source temperature 220 °C, transmission line temperature 280 °C; use full-scan mode to acquire signals, scanning range 50–1000 amu; ionization voltage is 70 eV.

Data analysis

The data were statistically analyzed with Excel 2007, SPSS 18 software, and compared with Duncan undefined method. The data obtained by GC-MS was compared using the NIST08 library (matching degree > 80%); the obtained data was imported into SIMCA-P 14.0 for correlation analysis using PLS-DA. All the experiments were repeated three times, and the results were expressed by mean ± standard deviation.

Results

Evolution of inoculum

Change of bacterial viable counts in the samples of NF and IF throughout the 7 days of storage at 25 °C was shown in the Fig. 1. The total mesophilic microorganisms and the number of LAB both showed an increasing trend in the early stage of both fermentations. The initial number of total mesophilic microorganisms in NF and IF were 4.76 lg CFU/g and 5.38 lg CFU/g, respectively, and the LAB contents were 4.22 lg CFU/g and 5.31 lg CFU/g, respectively. The number of total mesophilic microorganisms and LAB in the IF vegetables was slightly higher than that of NF due to the difference in fermentation methods and individual vegetables. On the second day, the number of total mesophilic microorganisms reached the highest were 8.21 lg CFU/g and 8.96 lg CFU/g, respectively. At the end of stages, the total mesophilic microorganisms and the number of LAB gradually became stable. The total mesophilic microorganisms in NF and IF were 8.02 lg CFU/g and 8.53 lg CFU/g, respectively, and the number of LAB was 8.01 lg CFU/g and 8.45 lg CFU/g, respectively. It can be seen from the above results that the overall change trend of the total mesophilic microorganisms and the number of LAB in the two fermentation modes was similar. In addition, inoculation of LAB can increase the content of LAB in the fermented vegetables, accelerate the growth and reproduction rate, and increase the number of total mesophilic microorganisms in the fermented vegetables.

Monitoring of the acidification process

As shown in Fig. 2, the two fermentation modes had a pH of about 6.4 at the start of fermentation. In the initial stage of fermentation, the pH value decreased rapidly. In the subsequent fermentation process, with the prolongation of fermentation time, the nutrients in the fermented vegetables gradually decreased, the microbial metabolites gradually accumulated, and the pH decreased gradually. In the fermentation process of fermented vegetables, the total acid content of the fermented vegetables continuously increases with the prolongation of fermentation time. On the 4th day, for NF, the pH reached 3.71, the total acid content was 0.88 g/kg, and then slowly decreased to the pH was 3.44, and the total acid content was 1.09 g/kg at the end of the stage. For the IF, the

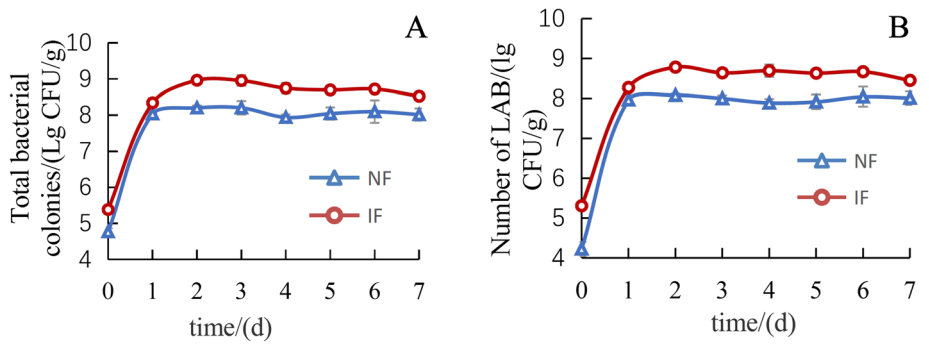

Fig. 1 Changes in microbial populations during vegetable fermentation. **a** Total mesophilic microorganisms counts. **b** Number of LAB. *NF* natural fermentation, *IF* inoculated fermentation

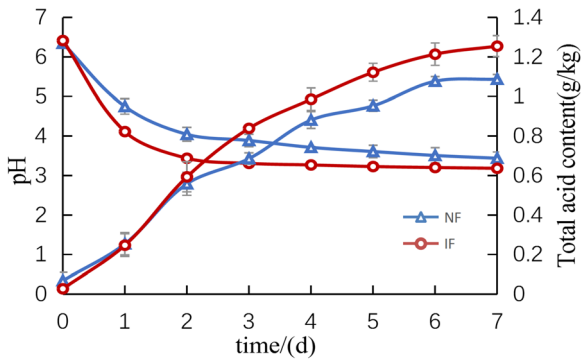

Fig. 2 Changes in pH and total acid content during vegetable fermentation. *NF* natural fermentation, *IF* inoculated fermentation

total acid content was 1.25 g/kg, and pH was 3.18 at the end of the stage. Studies had shown that the pH value of fermented vegetables was about 3.50~3.80 (Zhang et al. 2016), the IF has reached the pH of fermentation on the 2nd day, and the fermentation cycle of IF was significantly shorter than that of NF. It was consistent with previous research.

Nitrite content changes in NF and IF

In these fermented vegetables, nitrite levels were below 2.0 mg/100 g (calculated as sodium nitrite), a safe level was below the World Health Organization's Acceptable Daily Intake for a healthy adult (0.06 mg/kg body weight per day ~ 3.6 mg per day for a 60 kg person) (Ding et al. 2018). The production of nitrite in fermented vegetables was mainly due to the action of enzymes in the raw materials to reduce nitrates to nitrites. The nitrite content in the NF and the IF were increased and then decreased and the nitrite content of the IF was significantly lower than that of the NF (Fig. 3). A small "Nitrite peak" appeared on the first day of NF. The nitrite content reached 2.18 mg/kg, which gradually decreased in the subsequent fermentation process, and then stabilized. For the IF, the content also reached the maximum value

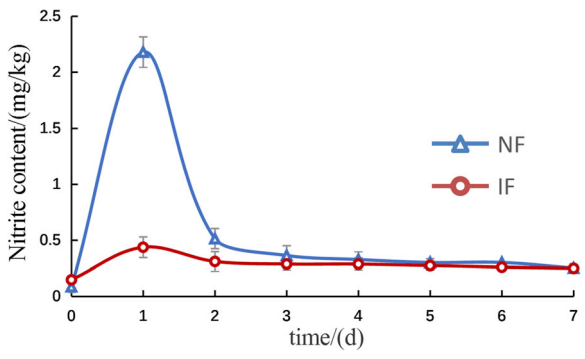

Fig. 3 Changes in Nitrate content during vegetable fermentation. *NF* natural fermentation, *IF* inoculated fermentation

on the first day, which was 0.44 mg/kg, and then gradually decreased, and it became stable at the end of fermentation. It was reported that in the early stage of NF, the content of LAB was less, and there were more other bacteria. The reductase produced by the bacteria can reduce the nitrate to nitrite, thus producing a "nitrite peak" (Guan et al. 2020). LAB can decompose nitrite and reduce the nitrite content in fermented vegetables, so the peak value of nitrite oxide in IF was significantly lower than that of NF.

Volatile metabolite profiles determined by headspace SPME-GC/MS

To understand the physical and chemical characteristics of fermented vegetables, SPME-GC/MS was used to analyze the volatile flavor substances in the products of NF and IF vegetables, including esters, acids, alcohols, alkenes, aldehydes, phenols, ketones, and benzene. There were 49 kinds of volatile flavor substances in NF vegetables and 63 kinds of volatile flavor substances in IF vegetables. In detail, twelve esters, seven alcohols, seven ketones, four benzenes, five alkenes, two phenols, eight aldehydes, and four acids were identified on the 7th day of NF; and thirteen esters, eleven alcohols, eight ketones, six benzenes, twelve alkenes, three phenols, six aldehydes, and four acids were identified on the 7th day of IF. Most of the volatile substances in the two fermentation methods were similar; among them, alcohols gave products a fresh smell, and were also the main source of esters. The relative content of alcohols in IF was 17.46%, which was significantly higher than 14.29% of NF.

Variable importance in projection (VIP) is an index to measure the importance of independent variables in the O2PLS model. The VIP of 14 microorganism were demonstrated in Fig. 4. The increased VIP value indicated the more significant effect of microorganisms on volatile flavor substances. *Lactobacillus* was an important genus because it can use carbohydrates in vegetables to produce flavor substances. The final product of heterogeneous lactic acid fermentation was more abundant, which had a more significant impact on the flavor of fermented vegetables.

Analysis of bacterial diversity NF and IF

High-throughput 16S rDNA sequencing was performed on 28 samples, and 3,627,007 original sequences were obtained. After quality control, 3,202,325 sequences were obtained. The average coverage of the sample was 56,543. The sample sequence length was mostly concentrated on 430~450 bp. According to the sequence similarity level of 97%, the samples were classified into operable classification units (OTUs). As shown in Table 1, the coverage of all samples was greater than 0.999, indicating that the data obtained by this sequencing can truly reflect the actual

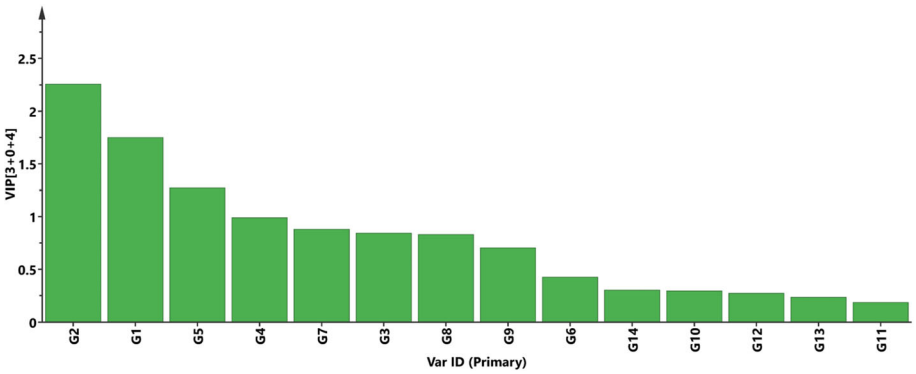

Fig. 4 *VIP* plot of the important microbiota correlated with flavors. G1: Cyanobacteria (norank); G2: *Lactobacillus*; G3: *Leuconostoc*; G4: *Weissella*; G5: *Lactococcus*; G6: Lactobacillales (unclassified); G7: *Enterobacter*; G8: *Klebsiella*; G9 *Pectobacterium*; G10: Enterobacteriaceae (unclassified); G11: *Aeromonas*; G12: *Pantoea*; G13: *Acinetobacter*; G14: *Pseudomonas*

situation of the samples. The amount of OTUs throughout the NF fermentation process was higher than that of IF. The OTUs and ACE indices were the highest at 0 day for both fermentation methods. Shannon and Simpson indicates the approximate number of species in the sample and the uniformity of its distribution, which shown opposite trends during the two fermentations. During the fermentation period of 0~7 days, the Shannon and Simpson indices of NF and IF were between 55~71% and 36~45%, respectively. The bacterial diversity and richness of NF were higher than that of IF.

Analysis of bacterial community composition in NF and IF

The number of OTUs in four samples NF0, IF0, NF7 and IF7 was analyzed by Venn plot to evaluate the distribution of bacterial in the two different fermentation modes before and after fermentation. As shown in Fig. 5a, the bacterial diversity of NF vegetables was higher than that of IF. The relative abundance at the level of bacteria was shown in Fig. 5b. In the early stage of fermentation, due to the attachment of some bacteria to the surface of the vegetables, the species of the genus was more complicated, and the cyanobacteria account for a large proportion. The dominant genus in the NF process were *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterobacter*, *Klebsiella*, *Pectobacterium*, *Leuconostoc*. *Lactococcus*, and *Weissella* were rapidly increased to 28.4% and 23.7% on the first day of fermentation, but gradually decreased during the fermentation. With the prolongation of fermentation time, *Lactobacillus* gradually became the dominant genus in NF vegetables, accounting for 53.4% on the 7th day of fermentation, and 15.7% and 7.9% for *Lactococcus* and *Weissella*, respectively. During the IF process, the dominant genus was *Lactobacillus*. At the beginning of fermentation, *Lactobacillus* accounted for 18.3%. On the 2nd day, the growth rate of *Lactobacillus* reached the maximum, and the proportion continued to increase in the subsequent fermentation process. The proportion reached 90.2% on the 7th day. The results showed that between the two fermentation methods, the *Lactobacillus* was the dominant one for the fermentation process. *Lactobacillus* can play an important role in the glycolysis pathway of the fermentation process, and the dominant species of NF was significantly more than the IF, indicating that IF can inhibit the growth

Table 1 Sequence abundance and microbial diversity in samples

Sample	Average tags		OTUs		Shannon		Simpson		ACE		Coverage	
	NF	IF	NF	IF	NF	IF	NF	IF	NF	IF	NF	IF
0 day	51,209	60,529	178	166	2.55	1.69	0.17	0.36	194.98	191.47	0.9995	0.9995
1 days	51,388	52,995	85	76	2.22	1.08	0.18	0.62	161.25	116.17	0.9995	0.9996
2 days	57,548	55,692	86	81	2.36	1.08	0.14	0.63	115.73	161.00	0.9997	0.9996
3 days	60,000	54,983	86	83	2.49	1.28	0.13	0.53	111.54	124.88	0.9996	0.9996
4 days	70,047	48,912	92	77	2.36	0.97	0.16	0.66	154.68	112.21	0.9997	0.9995
5 days	64,134	53,700	89	69	2.20	0.70	0.21	0.75	133.21	122.78	0.9997	0.9996
6 days	60,016	50,783	86	69	2.19	0.67	0.21	0.75	126.76	130.21	0.9996	0.9995
7 days	63,976	48,773	84	69	1.82	0.62	0.32	0.78	112.73	112.81	0.9997	0.9995

NF natural fermentation, *IF* inoculated fermentation

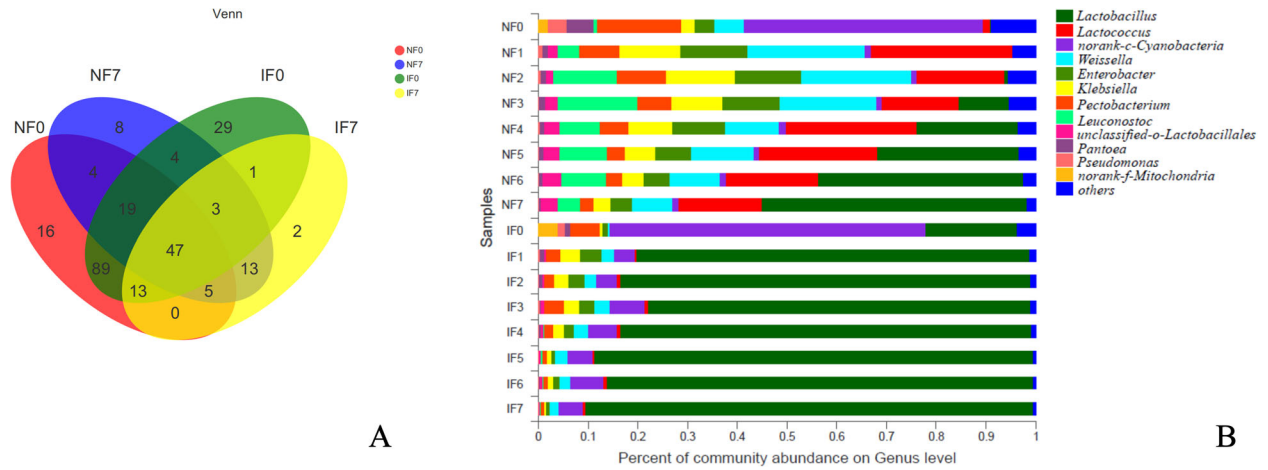

Fig. 5 Comparative analysis of bacterial community composition. **a** Analysis of flora structure of Veen. **b** Bacterial community composition. *NF* natural fermentation, *IF* inoculated fermentation; 0~7: days of fermentation

and reproduction of other microorganisms, so that fermented vegetables bacterial species were becoming more singular (Perez-Cataluna et al. 2018).

Heat map analysis of NF and IF

Four samples of NF0, IF0, NF7, and IF7 were analyzed by means of a heat map. The color gradients can be used to visually display the community differences of the samples and analyze the similarity of the samples. It can be seen from Fig. 6 that after the fermentation of the two fermentation modes, the genus of the abundance in the sample mainly included *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterobacter*, *Klebsiella*, *Pectobacterium*, *Leuconostoc*, and *Pseudomonas*, etc. The distribution of bacteria in NF0 and IF0 was similar. The dominant genus of NF0 were *Lactococcus* and *Pectobacterium*. *Lactobacillus* was highly abundant in IF0 due to inoculation of LAB. The most abundant genus of NF7 were *Lactobacillus* and *Lactococcus*, and the dominant genus in IF7 was *Lactobacillus*.

It can be seen from the change of color depth that the content of bacteria in the IF was lower than that of NF. The initial, *Stenotrophomonas*, *Sphingobacterium*, *Rhizobium*, *Flavobacterium*, *Saccharibacillus*, *Halomonas*, *Rheinheimera*, *Methylobacterium*, etc. were mainly derived from soil and water. With the increase of fermentation time, the richness gradually was decreased in the IF samples, indicating that the IF can inhibit the growth and reproduction of some spoiled bacteria, retain only the dominant bacteria, increase the stability of the fermentation process, and improve the quality of fermented vegetables.

Principal component analysis of NF and IF

Principal component analysis (PCA) used the evolutionary information between sample sequences to calculate the sample distance. The closer the sample distribution points in the PCA diagram, the closer the bacterial diversity in the sample (Dubois et al. 2010). It can be observed from Fig. 7 that the first principal component (PC1) and the second principal component (PC2) can explain 96.6% of the original variable information. To a large extent, it can reflect the difference of bacterial diversity caused by the change of fermentation time under two different fermentation methods.

The NF process was divided into four clusters NF0; NF1, NF2, NF3; NF4; NF5, NF6, NF7. There were three advances, including two rapid shifts and one slower shift. The first rapid shift occurred in the first cluster to the second cluster of PC2, and the bacteria attached to the vegetables themselves gradually changed with the start of fermentation; the second rapid shift occurred in the negative correlation region of PC1, the second cluster to the third cluster, due to the gradual decrease of metabolite production during this fermentation, and the bacterial changes were also slowed down; the third slower shift occurred in the third cluster to the fourth cluster, the negative correlation region transfer to the positive correlation area indicated that the bacteria change tended to enter the late fermentation stage and the fermentation was basically completed.

IF can be divided into three clusters IF0; IF1, IF2, IF3; IF4, IF5, IF6, IF7. A total of two developments, including a rapid shift of PC1 and PC2, resulted in large changes due to the mutual influence of the bacteria carried by the vegetables and the inoculated LAB. The other progress was a slower displacement, which was manifested in the positive correlation region of PC1, and the fermentation entered the late stage until the end of the fermentation process.

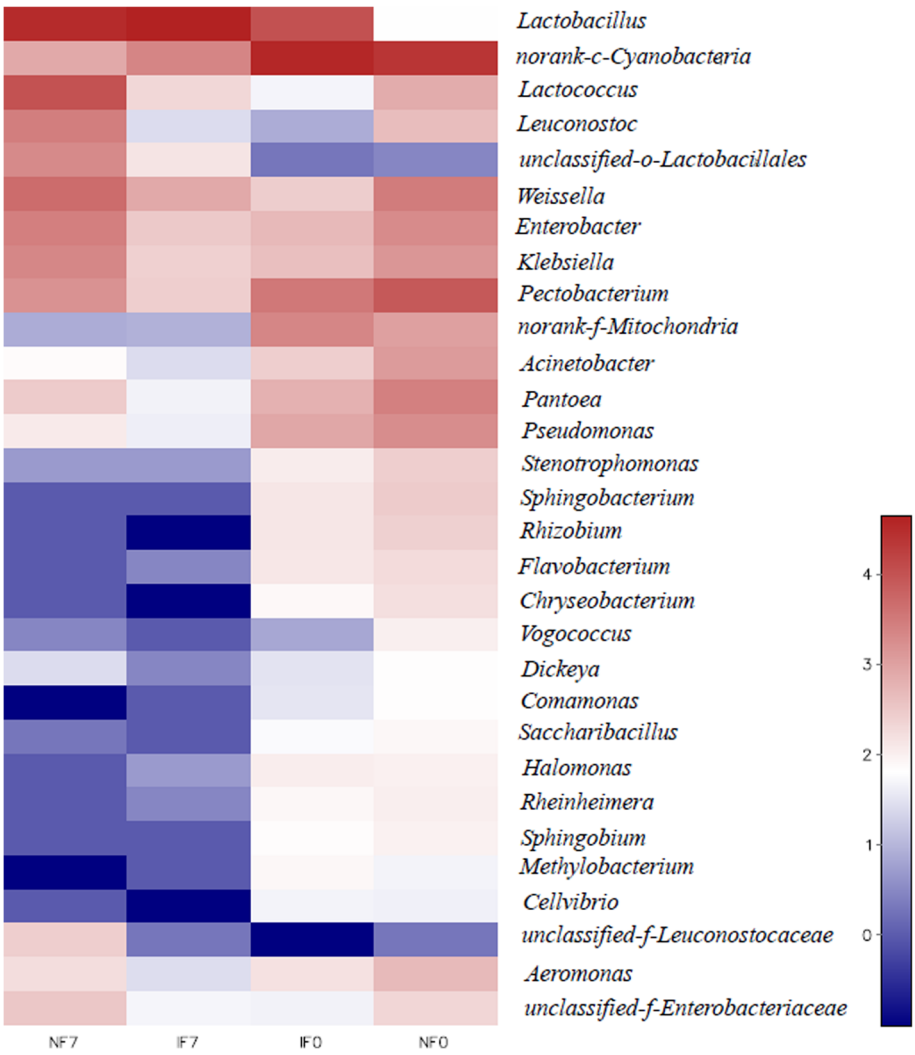

Fig. 6 Heat map of clustering during vegetable fermentation. *NF0* natural fermentation day 0, *NF7* natural fermentation day 7, *IF0* inoculated fermentation day 0, *IF7* inoculated fermentation day 7

Correlation analysis of NF and IF

The association between bacterial communities and pH, total acid, nitrite

At the bacterial genus level, the results of correlation analysis of pH, total acid (TA), nitrite content (N), and bacterial community of NF and IF vegetables were shown in the Fig. 8. RDA analysis explained a total of 46.43% of community changes, RDA1 explained 39.43%, and RDA2 explained 7.00%. pH (P = 0.001) was positively correlated with Norank-c-Cyanobacteria and *Pectobacterium*, and negatively correlated with *Lactococcus* and *Lactobacillus*. Total acid (P = 0.001) was positively correlated with *Lactococcus* and *Lactobacillus*; nitrite content (P = 0.008) with Norank-c-Cyanobacteria, *Pectobacterium*, and *Lactococcus* were positively correlated, and negatively correlated with *Lactobacillus*. It was obtained that *Lactobacillus* gradually increased to become a dominant genus in the vegetable fermentation process, which could significantly reduce pH and nitrite content.

The association between bacterial communities and flavors

Bidirectional orthogonal partial least squares (O2PLS) (Bylesjö et al. 2007) was used to analyze the correlation between the bacterial and volatile flavor substances in the vegetable fermentation process. It included 14 bacterial and 64 volatile flavor substances, after modeled with SIMCA-P software; its R^2X = 0.908 > 0.5 showed that the model had a better interpretation. According to Pearson's method, the relevant indexes of 0.6–0.8 was considered to indicate correlation (Rao et al. 2020). The results of the plot were shown in Fig. 9; a total of 11 volatile flavor substances showed correlation with LAB, where G1, G5, and G7 were correlated with one volatile flavor substance, respectively; G2, G3, G4, G8, and G10 showed correlation

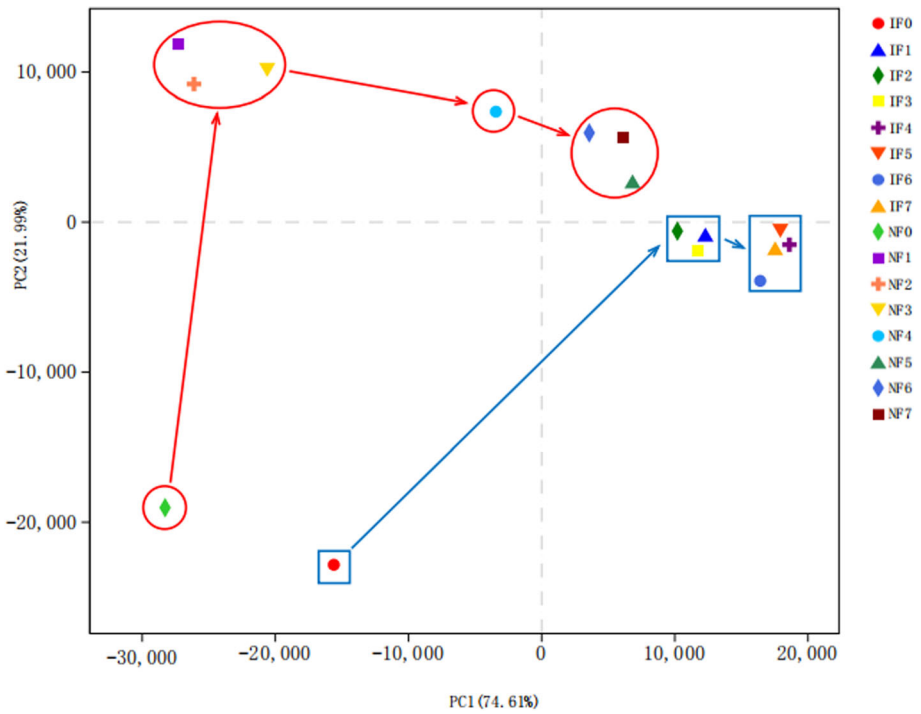

Fig. 7 Principal component analysis of bacterial community structure during vegetable fermentation. PC1 and PC2 explain 74.61% and21.99% of variation, respectively. The statistics were performed based on Bray-Curtis distance. *NF* natural fermentation, *IF* inoculated fermentation; 0~7: days of fermentation

with two kinds of volatile flavor substances, respectively; G6 and G11 were correlated with three volatile flavor substances. Esters were one of the main characteristic flavors of fermented vegetables. Esters were positively correlated with bacteria, among which ethyl palmitate (E9) was positively correlated with four bacteria, namely G3, G4, G5, and G6 (Fig. 9). Our results showed that LAB played a vital role in the formation of fermented vegetable flavors.

Discussion

To determine whether there is a difference of the microbe biodiversity between NF and IF and understand

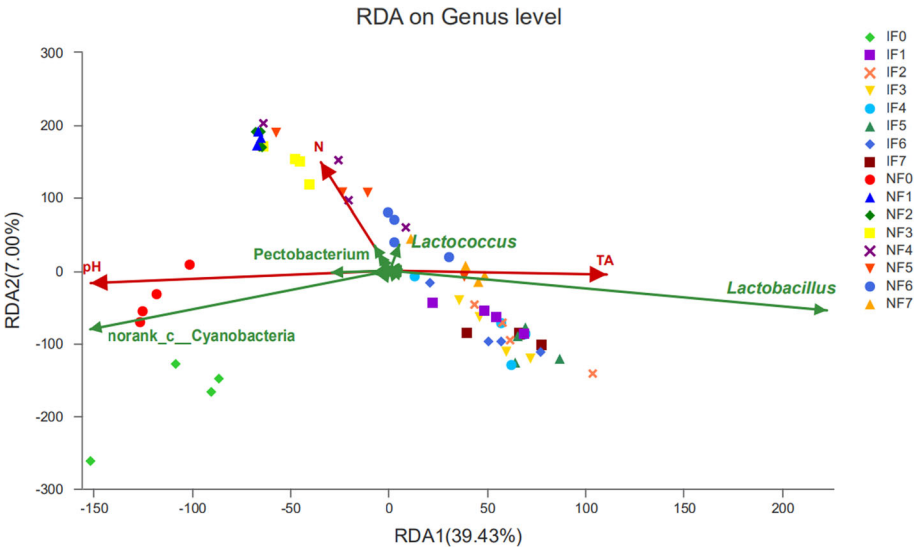

Fig. 8 Redundancy analysis of vegetable fermentation process. *TA* total acid, *N* nitrite, *NF* natural fermentation, *IF* inoculated fermentation; 0~7: days of fermentation

the fermentation process better, our study shows dramatic differences in the number of microorganisms, the bacterial diversity, and physicochemical indexes of Shanxi local fermented vegetables between NF and IF. We found changes in pH, total acid content, and nitrate content during the fermentation process. The correlation analysis of pH, total acid (TA), nitrite content (N), and bacterial community was revealed by the redundancy analysis. There is a negative correlation between the LAB bacteria and flavors in the fermented vegetables.

Total mesophilic microorganisms and the number of LAB increased in the early stage and kept stable in the later stage. We detected that LAB to be dominant in the NF and IF. The number of LAB in IF was higher than that of NF. For the results of the bacterial community composition, we found that the diversity of the NF and IF gradually decreased with the extension of fermentation time, and the IF decreased more significantly.

As expected, in NF, the dominant bacterial genera were *Lactobacillus* and *Weissella* in the early stage of fermentation. Many studies showed that *Lactobacillus*, *Lactococcus*, and *Weissella* dominate during the fermentation of vegetables (Jeong et al. 2013, Young et al. 2014). Satoru et al. (2020) determined the bacterial community structure in 29 naturally fermented sunki samples through metagenomics analysis targeted by 16S rRNA genes. The results showed that *Lactobacillus* was dominant in all samples and various bacterial species. Yang et al. (2018) revealed that the predominant bacteria in Chongqing radish kimchi brine were *Lactobacillus*, *Lactococcus*, and *Weberella*. These studies were consistent with the results of this study.

The bacterial diversity of fermented vegetables of the NF and IF were characterized using Illumina MiSeq sequencing. The result was similar to the change of the LAB count. In both fermented vegetables, the dominant genus was LAB in the late fermentation period, consistent with Xiong et al. (2015). Also, previous research showed that the *Lacticaseibacillus casei*, *Levilactobacillus brevis*, and *Lactiplantibacillus plantarum* were detected in fermented vegetables (Yu et al. 2012). Through the Venn diagram, PCA, and heat map cluster analysis, we identified the differences in the bacterial flora structure between two fermentation methods.

The changes in pH and total acid as measures of the maturation time of fermented vegetables were as expected. During the fermentation process, the growth of microorganisms with lower acid and salt tolerance was gradually suppressed (Vethachai et al. 2007). The LAB in the IF quickly became the dominant bacterial group. This trend is consistent with previous reports in bacterial count and bacterial diversity analysis (Xiong et al. 2016; Wu et al. 2013). The OTU and Shannon index of bacterial communities in fermented vegetables correlated with the change of pH and total acid. There was no "nitrite peak" during the IF fermentation. IF fermentation was safer and more controlled than that of NF. It is known that LAB could reduce nitrite and improve food safety (Chen et al. 2014a; Tripathi and Giri 2014). IF inhibited the growth of mixed bacteria and decreased the content of reductase and the peak value of nitrite

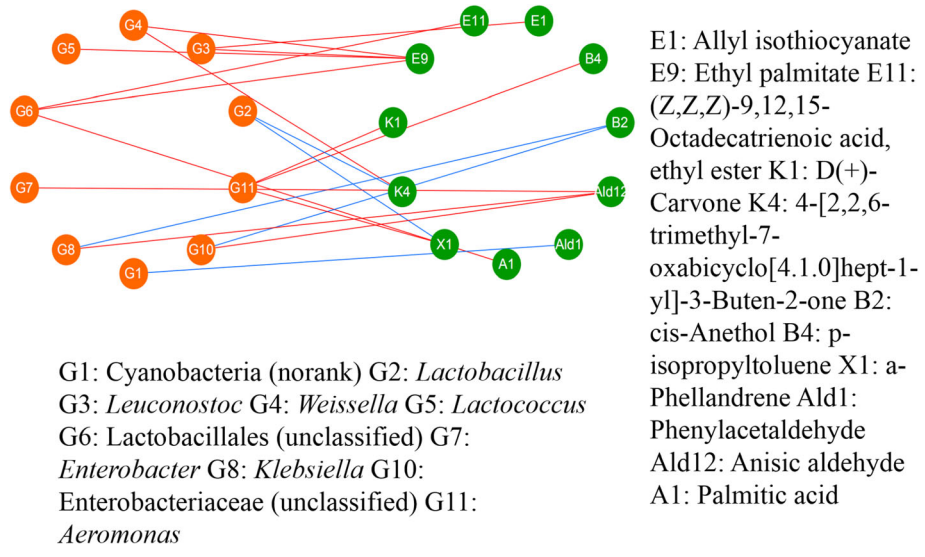

Fig. 9 Correlation network between the bacterial and flavors of fermented vegetables during the 0~7 days. |ρ| > 0.6. The circle on the left represents bacteria that are generally associated with flavor. The circle on the right represents the different flavors associated with the bacterial community. The long red line connecting the circles indicates a positive correlation, and the blue indicates a negative correlation between bacterial community and flavor

(Guan et al. 2020). Kim et al. (2017b) found that LAB can improve the nitrite removal ability of kimchi. The fermentation process tended to stabilize with the extension of the fermentation time by the data of total mesophilic microorganisms, LAB, changes in pH, and total acid.

There is a correlation between the bacterial and volatile flavor substances in the vegetable fermentation process based on our results. Volatile substances in fermented vegetables are mainly produced by the metabolism of raw and auxiliary materials and microorganisms of fermented vegetables. LAB played an important role in the fermentation process, especially *Lactobacillus*, *Lactococcus*, and *Weissella* were the dominant bacteria in flavor production. *Lactococcus* was positively correlated with ethyl palmitate (E9), whereas *Weissella* was positively correlated with ethyl palmitate (E9) and 4-[2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl]-3-Buten-2-one (K4). *Lactobacillus* had a negative correlation with 4-[2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl]-3-Buten-2-one (K4) and a-Phellandrene (X1). However, Xiao et al. (2018) analyzed the correlation between bacterial and flavor substances in fermented pickles; *Lactobacillus* was correlated with 28 flavors, of which 13 flavors are negatively correlated. There was a difference between the current correlation between bacterial and flavor with his research results. This may be related to the selected raw materials and fermentation methods. Notably, some associations were revealed based on the statistical correlations.

In summary, this study shows higher biodiversity of bacteria in the natural fermentation process than the inoculated fermentation process. It was interesting to see the difference of nitrite change between two fermentation methods, indicating different dynamics of metabolism between two groups of microbiomes. Based on changes of pH, total acid, and nitrite, the inoculated fermentation seems to be safer and the product quality is more stable. LAB were the dominant strain in the inoculated fermentation process as expected. Profiles of volatile flavor substances compared between NF and IF were revealed. Overall, we have provided the novel scientific knowledge in the bacteria biodiversity and how they affect the taste and flavor, which is the foundation for food processing industry to form a standardization for the vegetable fermentation process.

Conclusions

The diversity of bacteria in naturally fermented vegetables was more enriched than that in inoculated fermentation. The total number of mesophilic microorganisms and lactic acid bacteria in inoculated fermentation was dominant. However, inoculation fermentation can shorten the time of the fermentation cycle and reduce nitrite content, which can provide a theoretical basis for the standardized production of traditional fermented vegetables.

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Authors' contributions

ZC conducted research on correlation analysis and drafted a manuscript. JK designed the research and carried out the experiments. YZ participated in the experiments. XP participated in the data analysis. XY participated in its design and coordination. HL-B improved the writing of the manuscript. XG designed the experimental scheme, and carried out the overall planning and improvement of the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interest.

Author details

[1]Beijing Laboratory of Food Quality and Safety, Beijing Key Laboratory of Agricultural Product Detection and Control of Spoilage Organisms and Pesticide Residue, Beijing Engineering Laboratory of Probiotics Key Technology Development, Beijing University of Agriculture, Beijing 102206, China. [2]Beijing Dongcheng District Youth Lake Primary School, Beijing 100011, China. [3]Agricultural Research and Development Program, Central State University, Wilberforce, OH 45305, USA.

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