The effects of Lactiplantibacillus plantarum 3-19 and Pediococcus pentosaceus 18-1 on preventing the accumulation of biogenic amines and promoting the production of volatile organic compounds during sour meat fermentation

The effects of *Lactiplantibacillus plantarum* 3-19 and *Pediococcus pentosaceus* 18-1 on preventing the accumulation of biogenic amines and promoting the production of volatile organic compounds during sour meat fermentation

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Abstract

Lactic acid bacteria (LAB) are frequently used in meat fermentation, and mixed stater cultures are reported to perform better than single ones. *Lactiplantibacillus plantarum* 3-19 and *Pediococcus pentosaceus* 18-1 were chosen from 28 sour-meat-origin strains to examine the effects of single and combined inoculation on sour meat quality. Natural fermentation was used as a control to investigate changes in pH, water activity (a_w), amino acid nitrogen (AN), texture, microbial diversity, and volatile organic compounds (VOCs) during fermentation. The pH and a_w of each inoculation group were significantly decreased, and AN content was significantly increased. The inoculation of *P. pentosaceus* 18-1 significantly reduced putrescine, cadaverine, and tryptamine content ($p < 0.05$), while the inoculation of *Lpb. plantarum* 3-19 significantly reduced cadaverine amounts ($p < 0.05$). At the fermentation endpoint, the total biogenic amines content in the C group was 992.96±14.07, which was 1.65, 2.57, and 3.07 times higher than that in the Lp, Pe, and M groups, respectively. The mixed inoculation group combined the advantages of both strains and decreased total biogenic amines most significantly. At the end of fermentation, the VOCs in C, Lp, Pe, and M groups were 10.11, 11.56, 12.45, and 13.39 times higher than those at the beginning of fermentation. Inoculation promoted the production of key VOCs (OAV > 2000) such as heptanal, octanal, and (E)-2-nonanal. The mixed inoculation group had the highest variety and content of VOCs and the highest content of the above key VOCs, significantly enhancing its fruity, floral, ester, and other aromas. Sensory evaluation indicated that the M group had the best overall acceptability. Finally, it was suggested that a combination of *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 is a novel and efficient starter culture for processing sour meat since they lower the amounts of biogenic

47 amines in the meat and promote the production of VOCs.

48 **Keywords:** sour meat, *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*; biogenic

49 amines; volatile organic compounds;

1. Introduction

Fermentation is a traditional meat processing method that can provide beneficial properties to the product, such as microbial community succession, improved safety, and extended food shelf life. This process significantly improves the texture and taste of meat products while imparting unique flavor characteristics. There are two types of fermented meats: cubed meat, such as sour meat, and minced meat products, such as fermented sausages.

Sour meat, a popular naturally fermented meat product in Southwest China, is made from fresh pork mixed with rice flour, salt, and other ingredients. The mixture is thoroughly blended and subjected to anaerobic fermentation for one month. However, a variety of variables, including the process and surroundings, can impact fermentation, resulting in significant variances in the final product's quality and an extended fermentation period. As a result, the industrial production of sour meat is limited.

The addition of starter cultures to meat products has been shown to improve texture, flavor, color, food safety, and shelf life by slowing unwanted bacterial growth (Lorenzo et al., 2014). The most common starter culture in fermented meats, LAB contributes to the color, texture, and flavor of fermented meat products (Sun et al., 2016). LAB can also improve food safety by generating organic acids, primarily lactic and acetic acid, as well as other antimicrobial substances that restrict the formation of unwanted microorganisms (Candogan et al., 2009).

The effect of *Lpb. plantarum* LPL-1 on the bacterial community and physicochemical properties of fermented sausages was evaluated by Zhang et al. (2020), and they found *Lpb. plantarum* LPL-1 could enhance the safety of fermented sausages by restricting the growth of unwanted bacteria such as *Pseudomonas*, *Listeria monocytogenes*, and *Enterobacteriaceae.*

72 The production of hydrolyzing myofibrillar and sarcoplasmic protein-hydrolyzing proteases by
73 *P. pentosaceus* R1 has been shown to stimulate protein hydrolysis in meat products and improve
74 the characteristic organoleptic properties of Harbin dry sausages (Sun et al., 2019).
75 Fermentation with a single strain commonly forms a single colony dominance and has
76 limitations. Liu et al. (2023) reported that inoculation with *Lpb. plantarum* effectively increased
77 the number of LAB in fermented lamb sausage and inhibited the production of putrescine,
78 histamine, cadaverine, and tyramine but did not have significant inhibition on the production
79 of spermidine. Zhao et al. (2022) showed that the concentration of ketones, acids, and esters
80 was significantly higher in fermented sausages with *P. pentosaceus* alone, with a lower
81 concentration of aldehydes and alcohols compared to the C group, and alcohol concentrations
82 were increased by fermented sausages that solely inoculated *Staphylococcus carnosus*. Notably,
83 mixed fermentation of *P. pentosaceus* and *S. carnosus* resulted in significantly higher
84 concentrations of aldehydes, ketones, acids, esters, and alcohol. In general, mixed inoculation
85 outperforms a single inoculation. For example, Sun et al. (2016) showed that Harbin dry
86 sausages fermented with a mixed inoculum (*Staphylococcus xylosus, Lpb. plantarum*) inhibited
87 various biogenic amines (cadaverine, putrescine, tryptamine, 2-phenylethylamine, histamine,
88 and tyramine) significantly better than the results of a single bacterial inoculation.
89 Thus, we intend to employ mixed inoculation to improve the quality of sour meat. The pH
90 of sour meat decreases rapidly during fermentation, and eventually drop to around 4.5. We
91 concentrated on assessing strain acid tolerance in the hopes of screening strains with strong
92 acid tolerance for use in sour meat fermentation. So, 28 strains from sour meat were tested for
93 their acid tolerance ability and we selected *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 as

starter culture. The effects of mixed and single inoculations on sour meat were investigated by studying the physicochemical properties, bacterial diversity, biogenic amine, and VOCs during sour meat fermentation, as well as the correlation analysis between the microflora structure and other indexes (safety indexes and flavor indexes). This research aims to provide a starter culture for sour meat fermentation and to establish the groundwork for its industrialization.

2. Material and methods

2.1 Starter culture and sour meat preparation

As shown in Table S1, we looked for LAB isolated from sour pork and found 28 strains in the laboratory, and these two strains are included on the Chinese list of food-grade microorganism. The 28 strains were subjected to acid tolerance characterization by incubating them for 60 hours at medium pH of 3, 4, and 5 and their growth was determined as shown in Figure S1. Among them, *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 were found to have better acid tolerance characteristics. Therefore, we selected *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 as starter culture for the fermentation of sour meat.

Lpb. plantarum 3-19 and *P. pentosaceus* 18-1 were incubated in MRS broth at 37 ℃ for 24 h. Then, the cell pellet was harvested by centrifuge at 10,000 *g* for 10 min at 4 °C. They were then washed with sterile saline (0.9 % NaCl), resuspended thoroughly in 5 mL of sterile saline, and adjusted to a cell concentration of 7-9 log CFU/mL.

The pork used in making sour meat is fresh fat and lean pork belly, purchased from Sam's store on the day the sour pork is made, and rice flour and salt were purchased from a local market (Dalian, China) and before using the rice flour, stir-fried them until they are golden brown. Four batches of sour meat were manufactured: (1) C group, a control batch without

starter cultures; (2) Lp group, sour meat inoculated with *Lbp. plantarum* 3-19; (3) Pe group, sour meat inoculated with *P. pentosaceus* 18-1 and (4) M group, sour meat inoculated with a mixture of bacterial strains (*Lpb. plantarum* 3-19:*P. pentosaceus* 18-1 = 1:1). The preparation of sour meat refers to the method of Lv et al. (2023). The pork belly was rinsed with tap water and cut into pieces. The sliced meat was combined with rice flour (20 % w/w) and salt (4.5 %). The bacterial inoculum for the inoculated groups (Lp, Pe, M) was 10^7 CFU/g of meat. Twelve jars of samples were prepared for the C, Lp and M groups, respectively. Samples were fermented at 25 ℃ in sealed jars and separately taken out from the center of three parallel jars under sterile circumstances at 0, 7, 14 and 28 days, and they were subsequently stored at -20 °C.

2.2 Determination of pH value, a_w, and amino nitrogen

Samples were analyzed for physicochemical indicators in triplicate. For pH evaluation，2 g samples were mixed with 20 mL of water, homogenized, and centrifuged, and the supernatant was collected. The acidity of the supernatant was determined using a pH meter (FE28, Mettler Toledo, Switzerland).

A sample of sour meat (5 g) was used to determine a_w with a water activity meter (Aqualab TDL, Decagon Devices, USA).

The concentrations of amino nitrogen were analyzed according to Zhang's method (Zhang et al., 2020) with slight modifications. First, 1 g of sour meat was homogenized with 20 mL of deionized water. After centrifugation (8,000 *g*, 10 min, 4 ℃), 10 mL of the supernatant was transferred to a volumetric flask (50 mL) and adjusted to 50 mL. Then, the above solution was transferred to a beaker (100 mL) and 0.1 M sodium hydroxide was used to adjust the pH to 8.2. Next, 5 mL of the diluted solution was transferred to a beaker (100 mL), along with 30 mL of

deionized water and 10 mL of formaldehyde solution. Finally, 0.1 M sodium hydroxide was used to titrate the solution until pH 9.20 was reached.

Amino nitrogen content (mg/100 g) = $V_a \cdot N \cdot 0.014 / [\, m\ (1/50)] \times 100$

Where V_a is the consumption volume of 0.1 mol/L NaOH standard solution used to adjust the solution pH from 8.2 to 9.2, mL; N is the concentration of NaOH standard solution, mol/L; m is the sample weight, g; 0.014 is the milliequivalent of nitrogen.

2.3 Microbiological analyses

Sour meat samples were collected aseptically and stored at -80 °C for DNA collection. Genomic DNA was extracted using a DNA extraction kit (MO BIO Laboratories, USA) and then analyzed by agarose gel electrophoresis. DNA concentration was measured using a NanoDrop2000. The V3-V4 region of the bacterial 16S rRNA gene was amplified with 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3') primers.

The raw data was processed and analyzed according to Lv's method (Lv et al., 2019) with some modifications. PCR products were purified by VAHTS™ DNA Clean Beads and sequenced using the Illumina HiSeq sequencing platform (HiSeq 2500, Illumina, USA). Raw reads were merged with FLASH 1.2.11, filtered with Trimmomatic 4.2, and chimeras were removed with UCHIME 4.2 for valid tags. The suitable markers were then clustered using USEARCH 10.0 to group those with a parameter of sequence similarity greater than or equal to 97% into one OUT unit. The clustering was based on the above level of similarity, i.e., greater than 97%, by using the Ribosomal Database Project (RDP) classifier (version 2.2, http://sourceforge.net/projects/rdpclassifier/) in the Greengenes database. The raw data has been uploaded to NCBI with SRP478532.

160 **2.4 Biogenic amines**

161 Biogenic amines were extracted from samples according to the method Liu et al. (2018).
162 Sample (5 g) were homogenized with 20 mL perchloric acid (0.4 mol/L) for 1 min at 4 °C in
163 an ice bath and centrifuged at 4 °C and 3,000 *g* for 10 min. Then, the aqueous phase was
164 collected using a volumetric flask and the residue was extracted once again using the same
165 procedure. Twice extracts were combined into the same volumetric flask and added with 0.4
166 mol/L perchloric acid to 50 mL.

167 The detection conditions of biogenic amines were performed according to Zhang et al.
168 (2019). The analysis of biogenic amine was carried out via ultrahigh-performance liquid
169 chromatography coupled (UPLC; Nexera LC-30A system, Shimaduz, Kyoto, Japan) with triple
170 quadrupole mass spectrometer (Qtrap R 5500 mass spectrometer, AB Sciex, Toronto, Canada).
171 The biogenic amines were separated using a Luna 3 μm HILIC 200A column (150 × 3.0 mm;
172 Phenomenex). Mobile phase A was 5 mmol/L ammonium acetate with a flow rate of 0.32
173 mL/min. Phase B was pure methanol flowed with a rate of 0.08 mL/min at 35 °C. The injection
174 volume was 5 μL. All experiments were performed in triplicate.

175 **2.5 Textural analysis**

176 Texture analysis was performed with TA-XT plus texture analyzer (Stable Micro Systems
177 Ltd., Surrey, UK) equipped with a P 50 probe. The following parameters were used: pre-test
178 speed 3.0 mm/s, test speed 2.0 mm/s, post-test speed 2.0 mm/s, trigger force 5 g, compression
179 of the center of the sourdough meat slices to 40 % of their previous height, and a time of 5 s
180 between contractions. All experiments were performed in six repetitions.

181 **2.6 Determination of VOCs**

After being steam-cooked for 20 minutes, sour meat was ground in a blender. Two grams of the sample were placed in a headspace extraction vial (20 mL, 18 mm). After adding 20 μL of the internal standard, 50 mg/L cyclohexanone, each sample was left in a water bath at 60 °C for 30 minutes. After that, it was extracted using solid-phase microextraction (SPME) for 40 minutes. The GC oven was held at an initial temperature of 35 °C for 5 min, raised to 50 °C at 3 °C/min and maintained for 3 min, then heated to 150 °C at 5 °C /min, and finally to 250 °C at 20 °C/min and kept for 5 min. The retention index (RI) was calculated using the same GC conditions on 0.2 μL of C_7-C_{30} saturated alkane, and NIST 11 was used for VOCs' identification. A semi-quantitative analysis of VOCs was performed based on the peak area of the internal standard substance. Then, the odor activity value (OAV) was calculated based on OAV = C/OT, where C is the concentration of the volatile organic compounds (VOCs) and OT is its odor threshold. All experiments were performed in triplicate.

2.7 Sensory evaluation

The sensory evaluation panel of sour meat comprised 25 faculty members and graduate students from our lab. After fermentation, the sour meat samples were cut into 5 mm thick pieces and steamed for 20 minutes in a home steaming pot (⌀28 × 19 cm) on an induction burner. The sensory examination was then completed at an ambient temperature. The evaluation criteria were referenced to Lv et al. (2023) 9-point linear scale (a 9-point linear scale) in which a score of 1 equals immensity dislike, a score of 5 is similar to neither like nor dislike, and a score of 9 is equivalent to remarkably like. These samples were randomly coded using 1-4, and the assessment members evaluated the cooked pieces. The items assessed were color, texture, flavor, taste, and overall acceptability.

2.8. Data analysis

SPSS 23.0 (International Business Machines Corp., IL, USA) software was used for data analysis. Origin 8.5 (OriginLab Corp., MA, USA) software was used to draw line plots, histograms, and radar plots. Orthogonal partial least squares discriminant analysis (OPLS-DA) of multivariate analysis was performed using SIMCA 14.1 (Umetrics, Sweden). Correlation network plot was rendered using Cytoscape 3.9.1 software

3. Results and discussion

3.1 Analysis of pH value, a_w, and amino nitrogen

As shown in Fig. 1a, the pH values of the four groups of sour meat were obviously lower during the early fermentation phase (0-7 days), and then the pH values tended to stabilize. The pH values of the inoculated groups were lower than those of the C group at the end of fermentation. This result is comparable to a study conducted by Zhang et al. (2020), which discovered that the pH values of the sour meat inoculated with *Latilactobacillus curvatus* and *P. pentosaceus* were significantly lower than the control group following fermentation. The growth of unwanted bacteria in sour meat can be inhibited by other bacteria, such as *Lactiplantibacillus*, which can metabolize carbohydrates to create organic acids like lactic acid (Noda et al., 2019). These bacteria multiply in an environment conducive to their growth and produce organic acids, which could explain the quick pH drop at the early fermentation phase. The pH value of the M group was significantly lower than that of the C group at the end of fermentation. This implies that sour meat is fermented using both *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 significantly increased the acidity value of sour meat. This process effectively halted the formation of unwanted bacteria, significantly enhancing its safety.

As shown in Fig.1b, a_w decreased with fermentation time. The a_w value dropped sharply during the early fermentation period (0-7 d). The decrease in a_w helped maintain fermented food's microbiological and quality stability (Laranjo et al., 2015). Similar trends were observed in other fermented products, such as fermented sausages (Wang et al., 2013) and fermented fish (Gao et al., 2016). It has been reported that a decrease in pH induces a change in protein conformation, leading to a reduction in the water-holding capacity of the sample (Gómez and Lorenzo, 2013), and the trend of a_w in our experiment is consistent with pH. In the end, there was no significant difference in the a_w of the four samples, indicating that the a_w of the sour meat would not be considerably impacted by the addition of *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1.

All four groups of samples' AN tended to rise during fermentation, as seen in Fig. 1c and peaked at the end of fermentation (28 d). The concentrations of AN were considerably greater in the Lp, Pe, and M groups than in the C group during the middle and late stages of fermentation (14–28 d). Hu et al. (2020) found that LAB in traditional dry sausages from the Northeast region exhibited high protease activity. These enzymes helped break down proteins into peptides, degrading them into oligopeptides. At the same time, LAB used small molecule peptides such as oligopeptides to generate free amino acids or peptide derivatives, providing essential precursors for the VOCs of the product (Wang et al., 2022). In our research, *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 may have similar effects, such as protease and peptidase secretion, enhancing the release of AN from sour meat.

3.2 Microbial community analysis

247 Fig. 2 shows the bacterial succession (top 20 relative abundance) throughout the
248 fermentation of sour meat. *Acinetobacter* (11.24%) and *Brochothrix* (8.48%) were the two most
249 common bacteria in the sour meat at day 0. This is consistent with the research results reported
250 by Lv et al.(2023). Acinetobacter is commonly found in various environmental sources like
251 water and soil (Casaburi et al., 2014), while *Brochothrix* is typically recognized as spoilage-
252 causing microorganisms in meat products (Zhao and Eun, 2020). These microbes primarily
253 originate from the environment and raw materials before fermentation. During the fermentation
254 process, *Lactococcus garvieae*, *Lpb. plantarum*, *Latilactobacillus sakei*, and *Weissella* gradually
255 became the prevailing organisms in the C group. By the end of fermentation, their proportions
256 were 25.26%, 23.94%, 11.85%, and 5.07%, respectively, alongside minor quantities of *Serratia*,
257 *Macrococcus*, and other bacteria.

258 After fermentation, the dominant bacteria in the Lp and Pe groups were *Lpb. plantarum*
259 and *Pediococcus*, respectively. *Lpb. plantarum* and *Pediococcus* were the dominant bacteria in
260 M group, with relative abundances of 49.79% and 33.20% at the end of fermentation. It
261 demonstrated that *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1, or both, were co-inoculated,
262 they could quickly become the dominant bacterium. Furthermore, the mixed inoculation group
263 showed no discernible inhibition between the two species.

264 During the fermentation process of the Pe group, *Pediococcus* was found to be the
265 dominant bacterium, with *Enterococcus* present at a lower relative abundance (2.17-8.52%).
266 *Enterococcus* remained at a relative abundance of 5.42% at the end of fermentation. This
267 suggests that even though *Pediococcus* was the dominant bacterium, it may not have effectively
268 inhibited the growth of *Enterococcus*. *Lpb. plantarum* are the dominant bacteria in Lp and M

groups. During fermentation, the Lp and M groups experienced a significant decrease in *Enterococcus* content (0.03-0.75%), resulting in a final relative abundance of 0.09% and 0.19%. *Lpb. plantarum* may effectively prevent the growth of *Enterococcus*. Jung et al. (2019) found that *Lpb. plantarum* could inhibit *Enterococcus* biofilm formation and disrupt the preformed biofilm. Thus, *Lpb. plantarum* can inhibit the growth of unwanted bacteria and benefit the safety of the product.

3.3 Analysis of biogenic amines

Biogenic amines are the product of the amination of aldehydes and ketones and the decarboxylation of amino acids (Gao et al., 2023), which is commonly found in fermented food. As shown in Table 1, six biogenic amines were detected in different sour meat samples. In C group, the total biogenic amines content was 992.96 ± 14.07 mg/kg. The total biogenic amine content in the Lp, Pe, and M groups was 1.65, 2.57, and 3.02 times lower than in the C group. The results showed that the inhibition of biogenic amines had a more pronounced effect in the group inoculated with the mixed starter culture compared to the other groups. Sara et al. (2000) investigated the effect of single and mixed fermentation on biogenic amines in dry sausages, and the results showed that mixed fermentation significantly reduced the accumulation of biogenic amines. This may be due to *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 can compete with other bacteria containing amino acid decarboxylase, and mixed fermentation provides advantages for both strains, further reducing the accumulation of biogenic amines (Sun et al., 2016).

Current research and legislation do not contain precise requirements for the total amount of biogenic amines in meat products. Requirements for specific biogenic amine levels vary

291 from country to country. In the United States, the histamine content in aquatic products should
292 not exceed 50 mg/kg (Hazards, 2011), while in the European Union, the histamine content in
293 food products should be kept below 100 mg/kg and tyramine content between 100-800 mg/kg
294 (Shalaby, 1996). In our country, high-histamine fish should contain less than 400 mg/kg of
295 histamine, while other marine fish should contain less than 200 mg/kg of histamine (GB 2733-
296 2015). In our study, the biogenic amine content complied with these regulations. Sun et al.
297 (2016) observed increasing levels of biogenic amines in all experimental groups during
298 fermentation, and the total biogenic amine content at the end of the fermentation was above
299 250 mg/kg. Similarly, Komprda et al. (2009) noted that the total biogenic amine content in
300 sausages at the end of fermentation was more than 550 mg/kg and putrescine more than 150
301 mg/kg.

302 Before fermentation, the samples contained 3.55 ± 0.04 mg/kg histamine; by the time
303 fermentation was finished, the histamine content in the inoculation group was significantly
304 lower than that in the control group ($p < 0.05$). It suggests that both strains can block histamine
305 more effectively. Inoculated starter could compete with other bacteria containing histamine
306 decarboxylase, decreasing the accumulation of histamine (Lee et al., 2016). On the other hand,
307 *Lpb. plantarum* and *P. pentosaceus* may contain histamine oxidase, which degrades histamine
308 formed during fermentation (Li et al., 2021). Also, the acidity of the meat matrix is another
309 factor that could hinder biogenic amine formation (Maijala and Eerola, 1993).

310 Following fermentation, the putrescine content experienced a notable elevation of 287.22
311 mg/kg within the C group. Despite increases observed in the other groups, these increments
312 were comparatively lesser than the observed rise in the C group. This implies a distinct

inhibitory effect of *P. pentosaceus* 18-1 on putrescine levels in sour meat. The formation of putrescine is usually associated with the activity of decarboxylase-positive contaminating microorganisms (*Enterobacteriaceae*). *P. pentosaceus* considerably reduced the buildup of putrescine in tilapia sausages by preventing the growth of *Citrobacter*, *Streptococcus*, and *Enterobacteriaceae*, according to Li et al. (2021). Therefore, *P. pentosaceus* may have a similar effect in inhibiting putrescine in this experiment. However, at the end of fermentation, the Pe group's tyramine content was significantly higher ($p < 0.05$) than the remaining groups. The main reason for tyramine production is the presence of tyrosine decarboxylase-producing microorganisms (Kim and Hur, 2018). According to the results above, *P. pentosaceus* 18-1 may be selective and capable of inhibiting putrescine formation but not tyramine generation. However, compared to the Pe group, the M group inhibited biogenic amines more significantly, such as tyramine, cadaverine and phenethylamine. This suggests that the combined inoculation may have a compound inhibitory impact on the accumulation of biogenic amines. Sun et al. (2016) investigated the effects of single versus dual bacterial fermentation (*S. xylosus* and *Lpb. plantarum*) on biogenic amines in Harbin dry sausages. This study showed that mixed bacterial fermentation produced better results than single bacterial fermentation.

3.4 Texture analysis

Table 2 displays the textural characteristics of sour meat from several additive groups. Every group's hardness trended to rise and eventually level off with time. The early fermentation phase showed a considerable increase ($p < 0.05$) in the hardness of sour meat. El Adab et al. (2015) suggested that the increase in hardness was mainly due to protein coagulation at low pH and reduced a_w. After combining the data for pH and a_w, it was possible to see that

during the early fermentation phase, both indexes exhibited a sharply declining pattern before stabilizing, which was in line with the rising trend of sour meat hardness values. The springiness of sour meat showed a decreasing trend with the fermentation time. Zhao et al. (2022) discovered that certain muscle fibers can attach to lipids through the action of bacteria and their enzymes, which causes protein hydrolysis. In fermented meat, this process results in tissue relaxation and a loss of elasticity. At the end of fermentation, the difference in cohesiveness between each inoculated group and the C group was insignificant ($p > 0.05$), indicating that the addition of bacteria did not significantly affect the hardness of sour meat. Chewiness can comprehensively show the sensory quality of meat. As shown in Table 2, the chewiness tended to increase and decrease with the increase in fermentation time. At the end of fermentation, the Pe group had the highest chewiness, followed by the Lp group, significantly different from group C ($p < 0.05$). In conclusion, fermentation can make sour meat hardness increase and springiness decrease, while adding LAB can help improve the chewiness of sour meat.

3.5 Analysis of VOCs

A total of 45 VOCs, including 7 acids, 3 ketones, 12 esters, 6 alcohols, and 17 aldehydes were detected during the fermentation process (Table S2). At the beginning of fermentation, the VOCs in the sour meat were 913.32 ± 307.64 μg/100 g. All groups' total volatile compound content showed an increasing trend with fermentation. At the end of fermentation, the VOCs in C, Lp, Pe, and M groups were 10.11, 11.56, 12.45, and 13.39 times higher than those at the beginning of fermentation. Aldehydes, produced mainly by the oxidative breakdown of lipids or further reacted by creating chemicals to generate other compounds, are characterized by high volatility and low threshold values (Zhao et al., 2016). Alcohols and acids are esterified, mainly

357 resulting in esters, and making food fruity (Montanari et al., 2016). Alcohols are often produced
358 in fermented meat and are involved in various metabolic pathways such as amino acid
359 metabolism, lipid oxidation, and methyl ketone reduction, so they are essential in aroma
360 formation (Sidira et al., 2015). These three compounds constitute the main components of
361 VOCs in sour meat.

362 Alcohols are one of the main components of VOCs in sour meat. Only two alcohols were
363 detected in the sample at 0 d with a total content of 25.88 ± 1.17 μg/100 g. At the end of
364 fermentation, the total amount of alcohols in C, Lp, Pe, and M were 11.74, 16.26, 28.63, and
365 19.45 times more than that of the 0 d, respectively. Thus, it can be seen that alcohol accumulated
366 in the sour meat after fermentation, and the total amount of alcohol in the inoculated groups
367 was higher than that in the control group.

368 Aldehydes are another significant component of VOCs in sour meat. Only four aldehydes
369 were detected in the 0 d sample, including hexanal, (E)-2-decanal, (E, E)-2,4-decadienal, and
370 hexadecane were detected in the fermentation samples, suggesting that other aldehydes may
371 have been produced by fermentation. After fermentation, the Lp, Pe, and M groups had higher
372 hexanal, heptanal, and octanal content than the C group. M group had the highest hexanal,
373 heptanal, and octanal content, reaching 271.77±171.45, 223.16±95.96, and 542.83±510.12
374 μg/100 g, respectively. Hexanal can indicate oxidation level in fermented meat and imparts a
375 green grassy odor. At the same time, straight-chain aldehydes, such as heptanal and octanal,
376 with floral and fruity flavors, contribute to the flavors of the food (Latorre-Moratalla et al.,
377 2011). The higher content of aldehydes in M group samples may be due to the beneficial
378 synergistic effect of *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 on forming aldehydes.

Esters were the second largest category of VOCs after sour meat fermentation. Following fermentation, the samples had more than 10 esters, compared to only five in the 0 d sample. This suggests that fermentation can increase the variety of esters in sour meat. Gao et al. (2016) reported that sour fish samples inoculated with *Lactiplantibacillus* had a more extensive ester content than the control group. This suggests that *Lactiplantibacillus* possesses esterification activity, which encourages the formation of ester in fermented meat. Pe and M groups had ester contents that were 1.65 and 1.12 times higher than the C group. Medium-chain fatty acid esters (MCFA), such as ethyl caprylate, have been widely reported in many fermented foods due to their low threshold value and essential contribution to product flavor (Hu et al., 2018). Ethyl caprylate is the characteristic volatile compound of sour meat, providing a pleasant aroma (fruity and floral) to the product (Giri et al., 2010). It is indicated that adding *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 will promote the production of fruity volatiles in sour meats.

OAV values were calculated for each compound to determine the main contributing compounds in the flavor of sour meat (Table 3). The samples contained 45 VOCs, with 7, 17, 19, 24, and 21 VOCs in the sour meat samples of groups 0 d, C, Lp, Pe, and M, respectively. These results suggested that the inoculation with *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1 might benefit the production of VOCs in sour meat. Among them, heptanal, octanal, phenylacetaldehyde, nonanal, (E)-2-nonanal, decanal, and (E, E)-2,4-decadienal had larger OAV values (>20,000), and these seven substances may contribute more to the flavor of sour meat.

3.6 Correlation analysis

Fig. 3a shows the relationship between microorganisms and flavor under the OPLS-DA

mode. The R^2 and Q^2 of the model were 0.667 and 0.747, indicating that the model-fitting degree was satisfied for data analysis. The top 20 microorganisms in sour meat in terms of abundance and essential VOCs (OAV > 1) were9 selected for variable importance in projection (VIP) analysis. Bacteria with higher VIP scores were *Lpb. plantarum, Enterococcus, Latilactobacillus, and Pediococcus,* indicating that they are the critical bacteria that affect the flavors of sour meat.

Correlation analysis was performed for critical bacteria (VIP scores > 1) and biogenic amines affecting the VOCs of sour meat as shown in Fig. 3b. The previous study reported fermented sausages inoculated with *Pediococcus* had a higher content of ketones, acids, and esters (Zhao et al., 2022). *Pediococcus*, as a dominant bacterium in Pe and M groups, showed a significant positive correlation with (E)-2-octen-1-ol (r = 0.997), oleic acid (r = 0.739), ethyl caprylate (r = 0.767) and negatively correlated with tryptamine (r = -0.822). *Pediococcus* promoted the formation of characteristic flavor substances in sour meat while inhibiting tryptamine production. It has been reported that in addition to improving flavor by *P. pentosaceus*, it could also promote the maturation of meat products and speed up the production process (Corral et al., 2016). *Lpb. plantarum*, as a dominant bacterium in Lp group, showed a significant negative correlation with tryptamine (r = -0.822) and phenylethylamine (r = -0.771); this phenomenon was similar to that reported by Sun et al. (2016), *Lpb. plantarum* was positively correlated with octanoic acid (r = 0.969), (E)-2-nonenal (r = 0.873). This indicates that *Lpb. plantarum* significantly promotes the accumulation of octanoic acid and (E)-2-nonenal in sour meat.

In conclusion, the dominant bacteria (*Lpb. plantarum* and *P. pentosaceus*) had a key

influence on the excellent quality features of sour meat, mainly reflected in promoting the formation of VOCs and reducing the accumulation of biogenic amines.

3.7 Sensory evaluation

Figure 4 shows the sensory evaluation results for sour meat in terms of color, texture, flavor, taste, and overall acceptability. In terms of color, the Lp group scored the highest, followed by the M and Pe groups. The Lp, Pe, and M groups had higher texture scores than the C group, which had lower scores ($p < 0.05$). The M group had a taste score of 7.9, outperforming the others. Overall acceptability ratings for the C, Lp, Pe, and M groups were 7.2, 7.4, 7.4, and 7.7, with the M group receiving the highest grade. Thus, the inoculation with LAB strains improved the sensory rating of sour meat, with group M having the best overall acceptability.

4. Conclusion

This study investigates the impact of *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1, isolated from traditional sour meat, on the quality of sour meat. During fermentation, the pH and a_w of all four sample groups showed a decreasing trend, while the content of AN significantly increased. The addition of the two strains inhibited the accumulation of biogenic amines; the total amount of biogenic amines in the M group was 328.92±19.43, which was lower than that of the other groups at the fermentation endpoint. GC-MS analysis revealed that adding strains facilitated the generation of VOCs in sour meat, notably observing the richest VOCs in the M group at the end of fermentation. Furthermore, the M group exhibited a higher overall acceptance level in sensory evaluation and received the highest total score. Therefore, collectively, the mixed fermentation agents containing *Lpb. plantarum* 3-19 and *P. pentosaceus*

18-1 could be an ideal choice to enhance the quality of sour meat.

CRediT authorship contribution statement

Hao Shang: Formal analysis, Investigation, Data curation, Writing-Original Draft, Writing-Reviewing and Editing. Ying Yue: Data curation, Methodology. Bingrui Guo: Data curation. Chaofan Ji: Writing-review, Methodology. Sufang Zhang: Writing-review. Liang Dong: Investigation, Resources. Ilario Ferrocino: Methodology, Resources. Luca Simone Cocolin: Methodology, Resources. Xinping Lin: Conceptualization, Supervision, Project administration.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (32372363), Project of Liaoning Provincial Department of Education (JYTMS20230426).

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Table 1

Biogenic amine contents of sour meat samples with or without starter culture.

Biogenic amine (mg/kg)	0 d	28 d			
		C	Lp	Pe	M
Histamine	3.55±0.04^{b}	25.40±0.73^{a}	3.62±0.05^{b}	3.51±0.01^{b}	3.54±0.02^{b}
Putrescine	36.28±0.10^{c}	323.50±11.61^{a}	295.35±39.30^{a}	54.86±7.45^{c}	120.54±17.06^{b}
Tyramine	4.61±0.23^{c}	158.13±18.58^{b}	156.47±13.90^{b}	252.91±25.41^{a}	169.65±1.31^{b}
Cadaverine	8.75±2.01^{cd}	391.28±5.91^{a}	58.35±7.07^{b}	22.59±1.70^{c}	5.83±0.64^{d}
Tryptamine	24.10±0.02^{b}	89.00±3.63^{a}	81.68±8.12^{a}	25.00±0.07^{b}	25.94±0.13^{b}
Phenethylamine	0.10±0.02^{c}	4.79±0.05^{b}	5.98±4.16^{b}	26.98±4.16^{a}	3.42±0.27^{bc}
Total	77.39±2.32^{d}	992.96±14.07^{a}	601.45±72.60^{b}	385.85±38.8^{c}	328.92±19.43^{c}

$^{a-d}$ Values in a line with different lowercase letters are significantly different ($p < 0.05$). For abbreviations, see Figure 1.

Table 2

Texture parameters of sour meat with or without starter culture during the fermentation.

Fermentation days	Group	Hardness/g	Springiness	Chewiness/g	Resilience	Cohesiveness /g. s
0 d	C	544.67±57.12^{Ab}	0.87±0.05^{Aa}	271.85±63.38^{Ac}	0.37±0.02^{Aa}	0.77±0.02^{Aa}
	Lp	531.16±39.96^{Ab}	0.89±0.08^{Aa}	277.28±104.40^{Ab}	0.23±0.01^{Ba}	0.73±0.02^{Ba}
	Pe	540.02±22.89^{Ab}	0.78±0.04^{Aa}	254.43±49.47^{Ab}	0.30±0.06^{Aa}	0.74±0.01^{Aa}
	M	541.57±56.42^{Aa}	0.78±0.04^{Aa}	291.54±64.30^{Ab}	0.29±0.01^{Ba}	0.67±0.01^{Cb}
7 d	C	1084.69±54.66^{Aa}	0.67±0.12^{Aab}	565.69±58.34^{Aa}	0.21±0.02^{Abc}	0.68±0.07^{Aa}
	Lp	722.33±79.12^{Aab}	0.73±0.04^{Aa}	666.11±172.10^{Aa}	0.23±0.01^{Aa}	0.74±0.04^{Aa}
	Pe	924.54±229.64^{Aa}	0.75±0.04^{Aa}	727.87±98.53^{Aa}	0.27±0.09^{Aa}	0.73±0.02^{Aa}
	M	786.80±331.67^{Aa}	0.74±0.02^{Aa}	557.96±51.28^{Aa}	0.22±0.02^{Ab}	0.73±0.03^{Aa}
14 d	C	1163.03±48.36^{Aa}	0.69±0.05^{Ab}	621.73±42.27^{Aa}	0.22±0.00^{Ab}	0.72±0.06^{Aa}
	Lp	822.46±61.33^{Aa}	0.71±0.04^{Aa}	605.26±89.78^{Aa}	0.21±0.01^{Aa}	0.75±0.02^{Aa}
	Pe	777.57±87.86^{Ab}	0.70±0.06^{Aa}	762.02±116.56^{Aa}	0.22±0.04^{Aa}	0.71±0.04^{Aa}
	M	739.80±261.52^{Aa}	0.79±0.05^{Aa}	659.85±116.52^{Aa}	0.22±0.01^{Ab}	0.76±0.00^{Aa}
28 d	C	1070.85±83.51^{Aa}	0.65±0.06^{Ab}	443.85±35.10^{Cb}	0.18±0.01^{Bc}	0.72±0.02^{Aa}
	Lp	776.12±218.26^{Aab}	0.68±0.04^{Aa}	588.23±90.99^{Ba}	0.20±0.00^{ABa}	0.74±0.01^{Aa}
	Pe	749.00±224.78^{Aab}	0.69±0.03^{Aa}	711.58±6.26^{Aa}	0.22±0.02^{Aa}	0.70±0.05^{Aa}
	M	721.25±19.38^{Aa}	0.74±0.05^{Aa}	453.85±20.96^{Cb}	0.21±0.01^{ABb}	0.74±0.01^{Aa}

$^{a-c}$ Values in the same sample group are significantly different ($p < 0.05$). $^{A-C}$ Values in the different sample groups are significantly different at the same fermentation period ($p < 0.05$). For abbreviations, see Figure 1.

Table 3

The OAV value of the key volatile compounds (OAV > 1) in sour meat with and without starter culture during the fermentation.

Entry	Volatile compounds	Threshold value (μg/100 g)	OAV				
			0d	C	Lp	Pe	M
1	1-Octen-3-ol	0.03	0.00	5551.11	0.00	0.00	0.00
2	(E)-2-Octen-1-ol	0.20	0.00	0.00	185.60	1410.55	442.65
3	Phenylethyl Alcohol	1.60	4.25	51.36	60.64	67.53	69.69
4	2-Nonen-1-ol	1.30	0.00	0.00	0.00	90.82	130.20
5	Hexanal	2.50	7.83	11.51	32.43	45.22	108.71
6	Heptanal	0.01	0.00	5508.89	5828.89	7297.78	24795.56
7	(Z)-2-Heptenal	2.20	0.00	0.00	0.00	26.20	0.00
8	Benzaldehyde	0.85	0.00	142.02	356.42	167.74	28.87
9	(E)-2-Heptenal	28.00	0.00	0.00	0.00	3.25	0.00
10	Octanal	0.00	0.00	0.00	22425.00	39177.50	135707.50
11	Phenylacetaldehyde	0.02	0.00	39154.12	9427.65	12401.76	2085.29
12	(E)-2-Octenal	0.03	0.00	3380.00	4608.52	13698.15	1344.07
13	Nonanal	0.03	0.00	106013.46	11660.00	5464.62	3829.62
14	(E)-2-Nonenal	0.00	0.00	33522.22	3537011.11	35588.89	4450766.67
15	Decanal	0.03	0.00	4914.62	1821.54	10167.69	3561.15
16	(E)-2-Decenal	0.03	821.11	7170.00	717.04	3300.00	17487.78
17	(E,E)-2,4-Decadienal	0.02	4275.65	19941.30	32634.78	64013.04	15280.00
18	2,4-Decadienal	21.50	0.00	0.00	0.00	3.03	0.00
19	Butanoic acid	0.20	51.15	206.30	1003.10	1139.20	1798.15
20	Hexanoic acid	0.40	0.00	121.05	225.30	267.13	56.68
21	Octanoic acid	0.05	0.00	1825.49	5872.16	155.49	3181.96
22	Dodecanoic acid	1.00	3.49	169.98	42.07	539.68	626.39
23	Oleic Acid	440.00	0.04	0.00	0.05	0.49	0.58
24	Ethyl hexadecanoate	1.00	437.05	611.86	26.04	100.77	252.90
25	Ethyl caprylate	40.00	0.00	0.00	2.53	3.55	1.66

Figure Captions

Fig. 1. Dynamic changes of pH (a), a_w (b) and amino nitrogen (AN) (c) of sour meat with or without starter culture during the fermentation process. C: control, naturally fermented; Lp: sample inoculated *Lactiplantibacillus plantarum* 3-19; Pe: sample inoculated *Pediococcus pentosaceus* 18-1; M: sample inoculated both *Lpb. plantarum* 3-19 and *P. pentosaceus* 18-1.

Fig. 2. The bacterial succession (relative abundance of TOP 20) in sour meats during the fermentation with or without starter culture. For abbreviations, see Figure 1.

Fig. 3. (a) VIP plot of microbial community (defined as X matrix) vs. flavor (defined as Y matrix). (b) A co-occurrence network chart showing the positive correlates (solid lines) and negative correlates (dotted lines).

Fig. 4. Sensory evaluation results of sour meat with or without starter culture. For abbreviations, see Figure 1.

Fig. 1.

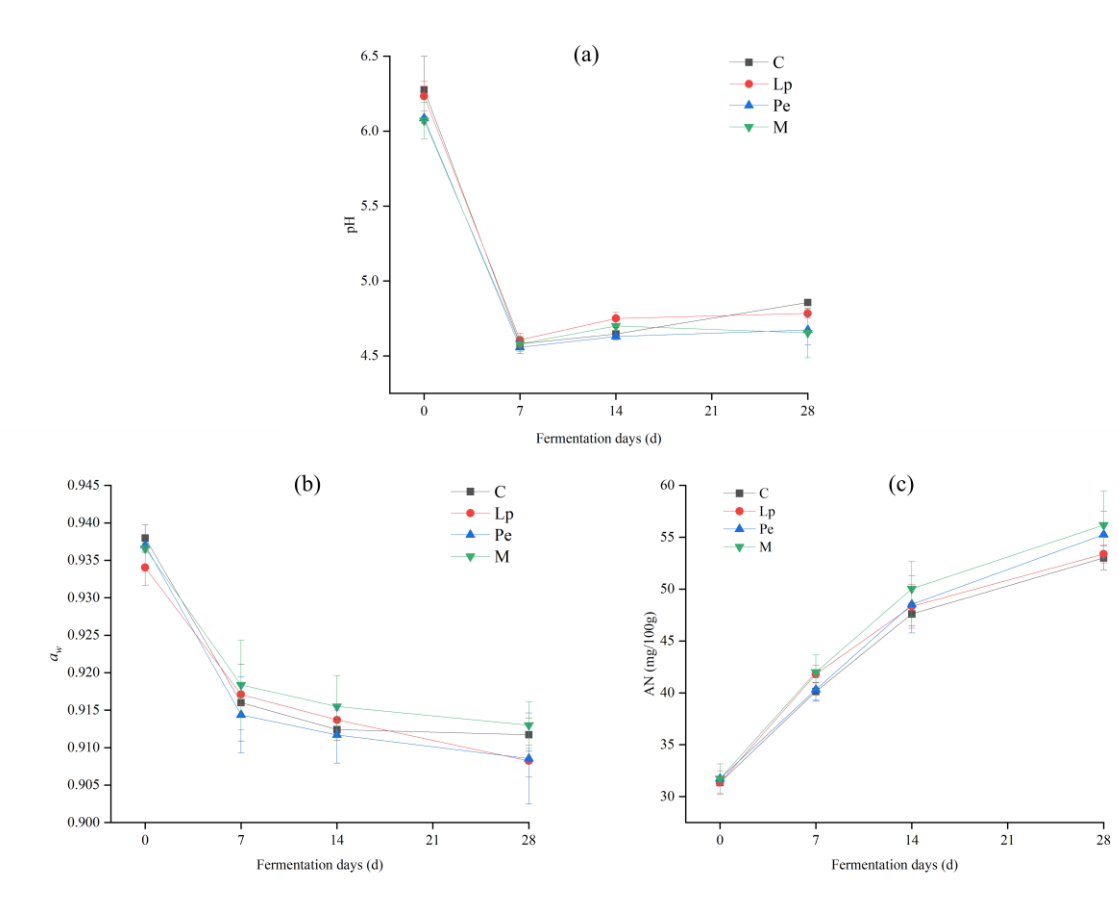

(a)
C
Lp
Pe
M
pH
6.5
6.0
5.5
5.0
4.5
0
7
14
21
28
Fermentation days (d)
(b)
C
Lp
Pe
M
a_w
0.945
0.940
0.935
0.930
0.925
0.920
0.915
0.910
0.905
0.900
0
7
14
21
28
Fermentation days (d)
(c)
C
Lp
Pe
M
AN (mg/100g)
60
55
50
45
40
35
30
0
7
14
21
28
Fermentation days (d)

Fig. 2.

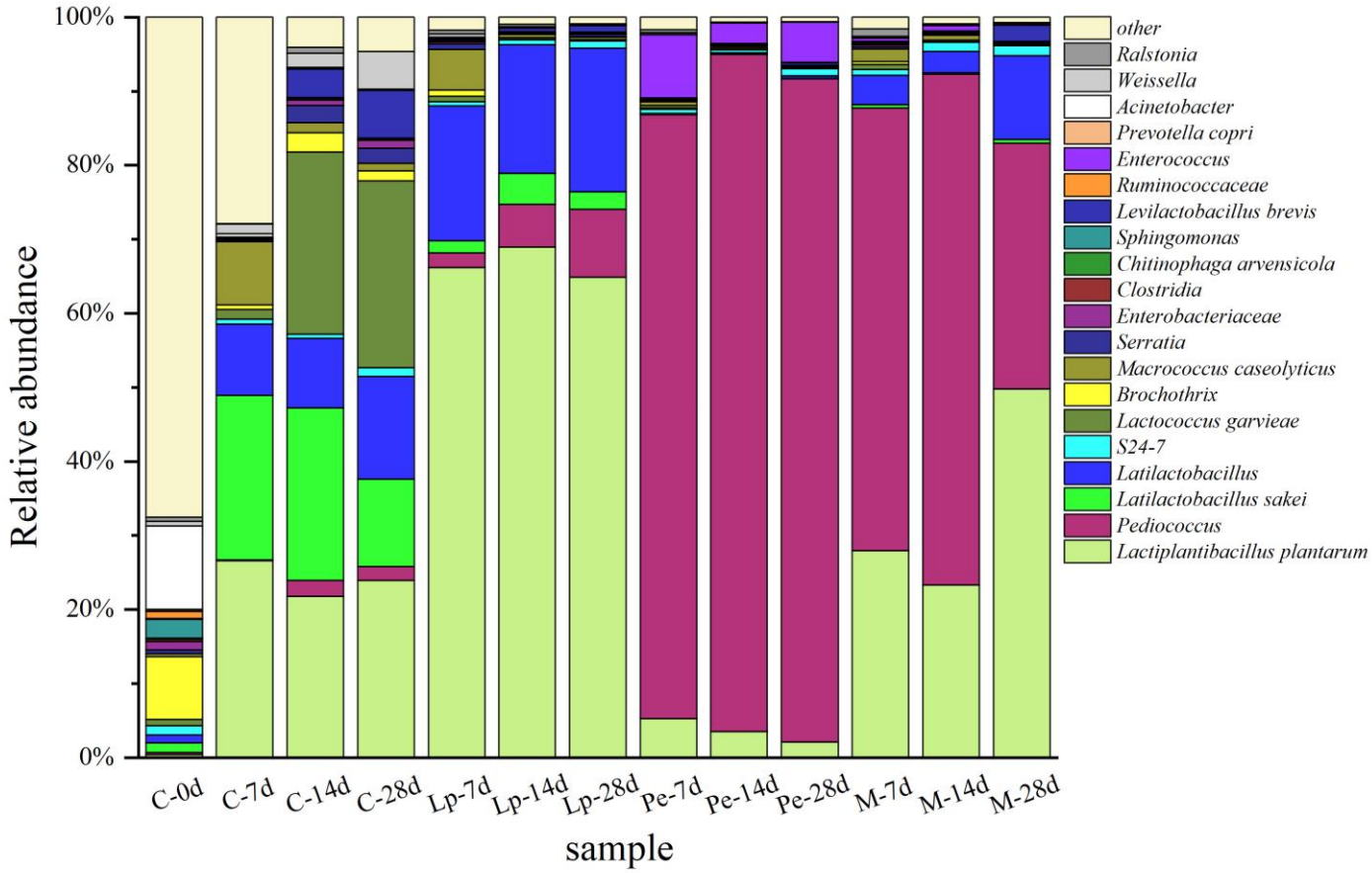

100%
80%
60%
40%
20%
0%
Relative abundance
C-0d
C-7d
C-14d
C-28d
Lp-7d
Lp-14d
Lp-28d
Pe-7d
Pe-14d
Pe-28d
M-7d
M-14d
M-28d
sample
other
Ralstonia
Weissella
Acinetobacter
Prevotella copri
Enterococcus
Ruminococcaceae
Levilactobacillus brevis
Sphingomonas
Chitinophaga arvensicola
Clostridia
Enterobacteriaceae
Serratia
Macrococcus caseolyticus
Brochothrix
Lactococcus garvieae
S24-7
Latilactobacillus
Latilactobacillus sakei
Pediococcus
Lactiplantibacillus plantarum

Fig. 3.

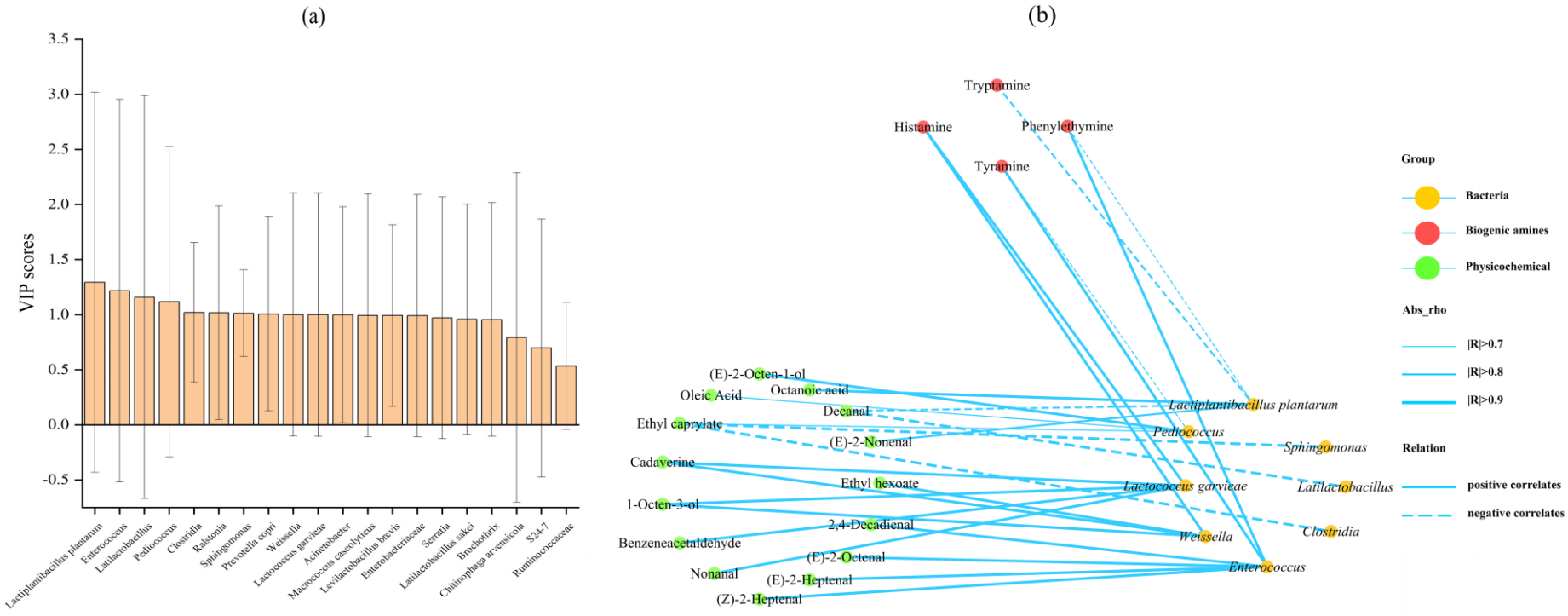
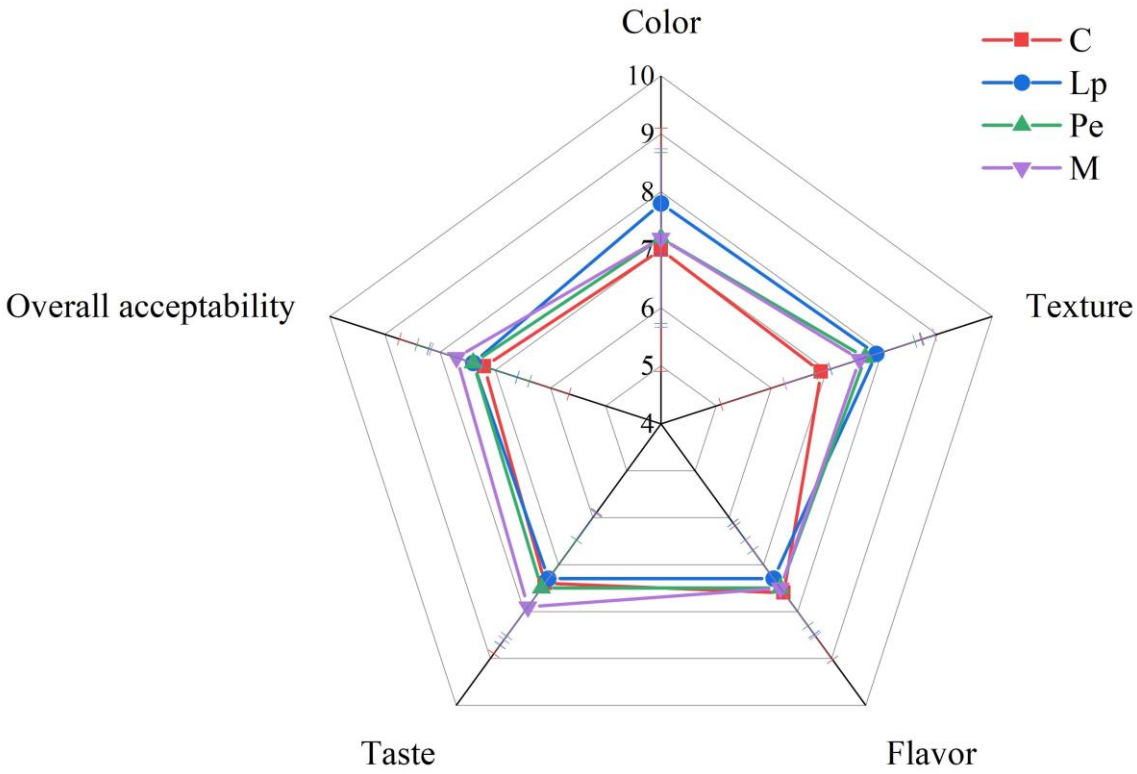

(a)
VIP scores
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Lactiplantibacillus plantarum
Enterococcus
Latilactobacillus
Pediococcus
Clostridia
Ralstonia
Sphingomonas
Prevotella copri
Weissella
Lactococcus garvieae
Acinetobacter
Macrococcus caseolyticus
Levilactobacillus brevis
Enterobacteriaceae
Serratia
Latilactobacillus sakei
Brochothrix
Chitinophaga arvensicola
S24-7
Ruminococcaceae
(b)
Tryptamine
Histamine
Phenylethymine
Tyramine
(E)-2-Octen-1-ol
Oleic Acid
Octanoic acid
Decanal
Ethyl caprylate
(E)-2-Nonenal
Cadaverine
Ethyl hexoate
1-Octen-3-ol
2,4-Decadienal
Benzeneacetaldehyde
(E)-2-Octenal
Nonanal
(E)-2-Heptenal
(Z)-2-Heptenal
Laetiplantibacillus plantarum
Pediococcus
Sphingomonas
Lactococcus garvieae
Latilactobacillus
Clostridia
Weissella
Enterococcus
Group
Bacteria
Biogenic amines
Physicochemical
Abs_rho
|R|>0.7
|R|>0.8
|R|>0.9
Relation
positive correlates
negative correlates

Fig. 4.


Color
Texture
Flavor
Taste
Overall acceptability
10
9
8
7
6
5
4
C
Lp
Pe
M

Supplementary data

The effects of *Lactiplantibacillus plantarum* 3-19 and *Pediococcus pentosaceus* 18-1 on preventing the accumulation of biogenic amines and promoting the production of volatile organic compounds during sour meat fermentation

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Table S1 Strain information

Serial Number	Strain	source
1	*Lactiplantibacillus plantarum* 18-1	Sour meat
2	*Lactiplantibacillus plantarum* 3-10	Sour meat
3	*Lactiplantibacillus plantarum* 2-29	Sour meat
4	*Lactiplantibacillus plantarum* 1-24	Sour meat
5	*Lactiplantibacillus plantarum* 2-7	Sour meat
6	*Lactiplantibacillus plantarum* 3-19	Sour meat
7	*Lactiplantibacillus plantarum* 3-10	Sour meat
8	*Pediococcus pentosaceus* 18-1	Sour meat
9	*Lactiplantibacillus plantarum* 2-5	Sour meat
10	*Lactiplantibacillus plantarum* 3-22	Sour meat
11	*Lactiplantibacillus plantarum* 2-22	Sour meat
12	*Pediococcus pentosaceus* 1-26	Sour meat
13	*Lactiplantibacillus plantarum* 3-14	Sour meat
14	*Lactiplantibacillus plantarum* 3-6	Sour meat
15	*Pediococcus pentosaceus* 15-1	Sour meat
16	*Pediococcus pentosaceus* 14-1	Sour meat
17	*Pediococcus pentosaceus* 13-1	Sour meat
18	*Pediococcus pentosaceus* 16-1	Sour meat
19	*Pediococcus pentosaceus* 19-1	Sour meat
20	*Pediococcus pentosaceus* 11-1	Sour meat
21	*Pediococcus pentosaceus* 5-1	Sour meat
22	*Pediococcus pentosaceus* 6-1	Sour meat
23	*Pediococcus pentosaceus* 7-1	Sour meat
24	*Pediococcus pentosaceus* 8-1	Sour meat
25	*Pediococcus pentosaceus* 12-1	Sour meat
26	*Pediococcus pentosaceus* 2-1	Sour meat
27	*Pediococcus pentosaceus* 3-1	Sour meat
28	*Pediococcus pentosaceus* 4-1	Sour meat

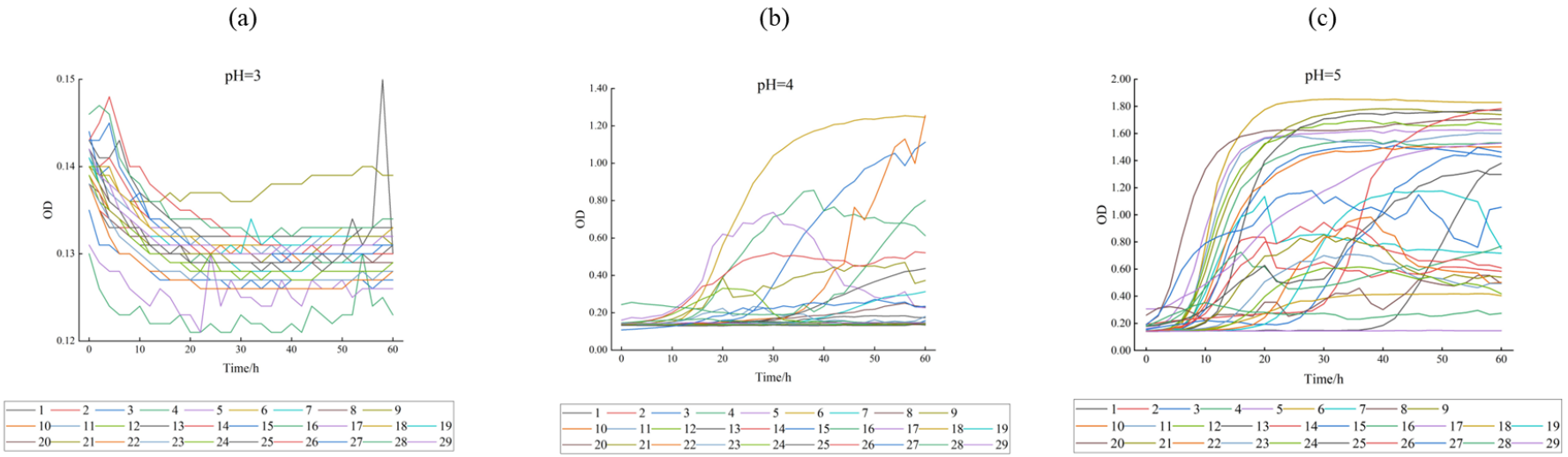

Figure S1 Growth curves of 28 strains at pH 3(a), 4 (b)and 5(c). For strain number in the legend, see Table S1.

Table S2 The content of VOCs in sour meats during the fermentation.

VOCs (μg/100g)	0d	C	Lp	Pe	M
1-Octen-3-ol	N	149.88±81.35	N	N	N
2-Ethylcyclohexanol	N	N	137.97±36.68	157.64±96.70	N
(E)-2-Octen-1-ol	N	N	37.12±20.04	282.11±94.94	88.53±20.33
Phenylethyl alcohol	6.80±1.17	82.17±25.26	97.03±50.96	108.05±66.54	111.51±42.87
2-Nonen-1-ol	N	N	N	118.07±106.27	169.26±10.13
2-Heptanol	19.08±9.99	71.87±9.95	148.76±64.10	75.19±18.08	134.09±83.51
Alcohols total	175.72±87.12	4911.38±2234.08	6657.4±1294.4	3337.14±1828.94	6247.4±3121.49
Hexanal	19.57±7.77	28.77±17.78	81.07±15.94	113.04±62.08	271.77±171.45
Heptanal	N	49.58±31.2	52.46±36.08	65.68±44.32	223.16±95.96
3-Methyl-hexanal,	N	29.42±25.89	31.17±25.72	53.02±33.54	38.38±22.33
(Z)-2-Heptenal	N	N	N	57.63±30.45	N
Benzaldehyde	N	120.72±66.71	302.96±215.05	142.58±48.78	24.54±6.65
4-Methylcyclohex-3-enecarbaldehyde	N	37.27±29.18	N	N	N
(E)-2-Heptenal	N	N	N	90.97±39.95	N
Octanal	N	N	89.70±30.58	156.71±54.04	542.83±510.12
Phenylacetaldehyde	N	665.62±94.34	160.27±34.58	210.83±69.57	35.45±25.19
2-Octenal	N	91.26±46.27	124.43±68.92	369.85±242.25	36.29±19.08
Nonanal	N	2756.35±1360.65	303.16±133.81	142.08±91.28	99.57±13.77

2-Nonenal,	N	30.17±22.75	3183.31±361.25	32.03±7.82	4005.69±1427.92
Decanal	N	127.78±103.28	47.36±27.43	264.36±229.16	92.59±82.07
(E)-2-Decenal	22.17±12.36	193.59±45.12	19.36±7.66	89.10±41.35	472.17±460.14
(E,E)-2,4-Decadienal	98.34±49.39	458.65±167.03	750.60±121.79	1472.30±786.80	351.44±289.86
2,4-Decadienal	N	N	N	65.07±43.82	N
Hexadecane	35.64±17.60	322.20±223.88	1511.55±215.59	11.89±3.73	53.52±16.95
Aldehydes total	175.72±87.12	4911.38±2234.08	6657.4±1294.4	3337.14±1828.94	6247.4±3121.49
Hexanoic acid	N	48.42±32.19	90.12±31.81	106.85±12.90	22.67±5.66
Octanoic acid	N	93.10±58.30	299.48±18.31	7.93±7.93	162.28±139.54
2-Decenoic acid	N	53.55±36.74	87.88±40.75	118.74±92.80	130.41±72.69
Dodecanoic acid	3.49±1.89	169.98±42.73	42.07±18.65	539.68±276.7	626.39±562.92
Tetradecanoic acid	12.37±5.30	122.56±90.99	48.02±34.48	107.62±34.79	219.27±135.78
Hexadecanoic acid	56.77±28.40	N	109.28±18.55	75.58±45.91	128.72±67.58
Oleic Acid	17.25±2.14	N	20.21±1.25	217.30±196.85	256.48±196.64
Acids total	89.88±37.73	487.61±260.95	697.06±163.8	1173.7±667.88	1546.22±1180.81
3-Octen-2-one	N	806.69±240.77	235.92±96.96	264.67±50.97	19.73±0.29
2-Pentanone	42.27±24.26	166.42±96.16	64.75±34.94	264.47±129.61	107.80±47.98
2-Hexanone	N	N	635.45±432.25	45.75±38.46	315.12±266.24
Ketones total	42.27±24.26	806.69±336.93	936.12±564.15	574.89±219.04	442.65±314.51
Ethyl 3-methylpentanoate	N	72.49±48.79	341.81±312.07	350.22±290.87	12.49±2.86

Ethyl butanoate	437.05±34.46	611.86±381.75	26.04±18.83	100.77±7.23	252.90±216.89
Ethyl 2-methyl-2-butenoate	N	18.76±10.28	38.16±16.79	355.86±170.31	58.43±1.07
Ethyl 2-methylbutyrate	123.14±117.65	12.35±1.39	495.76±14.48	564.96±145.60	328.9±112.00
Ethyl pentanoate	N	N	101.17±19.51	142.14±36.69	66.47±29.43
Propyl octanoate	N	192.66±78.80	276.69±269.35	2788.16±1084.39	1369.61±65.95
Ethyl caprate	N	777.13±396.57	23.20±2.89	107.80±59.5	237.38±113
Ethyl octanoate	N	N	101.17±19.51	142.14±36.69	66.47±29.43
Ethenyl decanoate	N	507.52±396.72	66.94±51.04	64.91±51.72	125.89±64.09
Pentyl decanoate	N	N	58.57±12.12	77.96±17.36	34.04±13.15
Ethyl myristate	13.67±4.47	159.62±100.95	107.4±22.4	593.96±364.88	831.64±771.81
Isobutyl acetate	11.10±0.77	209.87±90.87	89.63±8.92	245.37±181.88	89.36±34.91
Ethyl hexadecanoate	13.69±0.01	166.07±73.37	217.51±42.51	228.1±193.89	80.37±54.88
Esters total	598.65±157.36	2728.33±1579.49	1944.05±800.42	5684.39±2641.01	3553.95±1489.47
Total	932.4±307.64	9237.93±4528.05	10655.51±3004.55	11511.18±5702.71	12293.51±6252.99

Declaration of Interest Statement

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.