



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript	
Original Citation:	
Availability:	
This version is available http://hdl.handle.net/2318/2031349	since 2024-11-20T16:15:20Z
Published version:	
DOI:10.1016/j.ijfoodmicro.2024.110806	
Terms of use:	
Open Access	
Anyone can freely access the full text of works made available as under a Creative Commons license can be used according to the of all other works requires consent of the right holder (author or protection by the applicable law.	terms and conditions of said license. Use

(Article begins on next page)

1	The effects of <i>Lactiplantibacillus plantarum</i> 3-19 and
2	Pediococcus pentosaceus 18-1 on preventing the
3	accumulation of biogenic amines and promoting the
4	production of volatile organic compounds during sour meat
5	fermentation
6	Hao Shang ^a , Ying Yue ^a , Bingrui Guo ^a , Chaofan Ji ^a , Sufang Zhang ^a , Liang Dong ^a , Ilario
7	Ferrocino ^b , Luca Simone Cocolin ^b , Xinping Lin ^{a, *}
8	^a SKL of Marine Food Processing & Safety Control, National Engineering Research Center of
9	Seafood, Collaborative Innovation Center of Provincial and Ministerial Co-construction for
10	Deep Processing, Collaborative Innovation Center of Seafood Deep Processing, School of Food
11	Science and Technology, Dalian Polytechnic University, Dalian, Liaoning, 116034, China
12	^b Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy
13	*Corresponding author: Xinping Lin a,*
14	Tel: +8615898160352
15	E-mail: yingchaer@163.com
16	Hao Shang: 18340804606@163.com
17	Ying Yue: nicoleyue1996@163.com
18	Bingrui Guo: bingruig@163.com
19	Chaofan Ji: jichaofan@outlook.com
20	Sufang Zhang: 18163080@qq.com
21	Liang Dong: dongzhongxiang@126.com
22	Ilario Ferrocino: ilario.ferrocino@unito.it
23 24	Luca Simone Cocolin: lucasimone.cocolin@unito.it

Abstract

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

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

- 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*.

The production of hydrolyzing myofibrillar and sarcoplasmic protein-hydrolyzing proteases by P. pentosaceus R1 has been shown to stimulate protein hydrolysis in meat products and improve the characteristic organoleptic properties of Harbin dry sausages (Sun et al., 2019). Fermentation with a single strain commonly forms a single colony dominance and has limitations. Liu et al. (2023) reported that inoculation with Lpb. plantarum effectively increased the number of LAB in fermented lamb sausage and inhibited the production of putrescine, histamine, cadaverine, and tyramine but did not have significant inhibition on the production of spermidine. Zhao et al. (2022) showed that the concentration of ketones, acids, and esters was significantly higher in fermented sausages with P. pentosaceus alone, with a lower concentration of aldehydes and alcohols compared to the C group, and alcohol concentrations were increased by fermented sausages that solely inoculated *Staphylococcus carnosus*. Notably, mixed fermentation of P. pentosaceus and S. carnosus resulted in significantly higher concentrations of aldehydes, ketones, acids, esters, and alcohol. In general, mixed inoculation outperforms a single inoculation. For example, Sun et al. (2016) showed that Harbin dry sausages fermented with a mixed inoculum (Staphylococcus xylosus, Lpb. plantarum) inhibited various biogenic amines (cadaverine, putrescine, tryptamine, 2-phenylethylamine, histamine, and tyramine) significantly better than the results of a single bacterial inoculation.

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

93

Thus, we intend to employ mixed inoculation to improve the quality of sour meat. The pH of sour meat decreases rapidly during fermentation, and eventually drop to around 4.5. We concentrated on assessing strain acid tolerance in the hopes of screening strains with strong acid tolerance for use in sour meat fermentation. So, 28 strains from sour meat were tested for 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 °C 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 10⁷ CFU/g of meat. Twelve jars of samples were prepared for the C, Lp and M groups, respectively. Samples were fermented at 25 °C 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 °C), 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.

2.4 Biogenic amines

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

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

2.5 Textural analysis

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

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 μ L 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 μ L of C₇-C₃₀ 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 (\$\noting{9}28 \times 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

204

205

206

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

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

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

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

During the fermentation process of the Pe group, *Pediococcus* was found to be the dominant bacterium, with *Enterococcus* present at a lower relative abundance (2.17-8.52%). *Enterococcus* remained at a relative abundance of 5.42% at the end of fermentation. This suggests that even though *Pediococcus* was the dominant bacterium, it may not have effectively 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

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

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

Following fermentation, the putrescine content experienced a notable elevation of 287.22 mg/kg within the C group. Despite increases observed in the other groups, these increments 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

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

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 $913.32 \pm 307.64 \,\mu\text{g}/100 \,\text{g}$. 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

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

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

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

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) were 9 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.

401

402

403

404

405

406

407

408

409

410

411

412

413

414

415

416

417

418

419

420

421

422

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)-2nonenal 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.

446

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).

References

458

- Candogan, K., Wardlaw, F.B., Acton, J., 2009. Effect of starter culture on proteolytic changes during
- processing of fermented beef sausages. Food Chem. 116, 731-737.
- 461 Casaburi, A., De Filippis, F., Villani, F., Ercolini, D., 2014. Activities of strains of *Brochothrix*
- 462 *thermosphacta* in vitro and in meat. Food Res. Int. 62, 366-374.
- 463 Corral, S., Salvador, A., Flores, M., 2016. Effect of the use of entire male fat in the production of reduced
- salt fermented sausages. Meat Sci. 116, 140-150.
- 465 El Adab, S., Essid, I., 2015. Microbiological, biochemical and textural characteristics of a Tunisian dry
- 466 fermented poultry meat sausage inoculated with selected starter cultures. J. Food Saf. 35, 75-85.
- 467 Gao, P., Wang, W.X., Jiang, Q.X., Xu, Y.S., Xia, W.S., 2016. Effect of autochthonous starter cultures on
- the volatile flavour compounds of Chinese traditional fermented fish (Suan yu). Int. J. Food Sci.
- 469 Tech. 51, 1630-1637.
- 470 Gao, X.L., Zhao, X., Hu, F., Fu, J.Y., Zhang, Z.K., Liu, Z., Wang, B., He, R.H., Ma, H.L., Ho, C.T., 2023.
- 471 The latest advances on soy sauce research in the past decade: Emphasis on the advances in China.
- 472 Food Res. Int. 173, 113407.
- 473 Giri, A., Osako, K., Ohshima, T., 2010. Identification and characterisation of headspace volatiles of fish
- 474 miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species
- 475 and meat washing. Food Chem. 120, 621-631.
- Gómez, M., Lorenzo, J.M., 2013. Effect of fat level on physicochemical, volatile compounds and sensory
- characteristics of dry-ripened "*chorizo*" from Celta pig breed. Meat Sci. 95, 658-666.
- Hazards, E.P.o.B., 2011. Scientific opinion on risk based control of biogenic amine formation in
- fermented foods. Efsa J. 9, 2393.

- 480 Hu, K., Jin, G.J., Mei, W.C., Li, T., Tao, Y.S., 2018. Increase of medium-chain fatty acid ethyl ester
- content in mixed *H.uvarum/S. cerevisiae* fermentation leads to wine fruity aroma enhancement.
- 482 Food Chem. 239, 495-501.
- Hu, Y.Y., Zhang, L., Liu, Q., Wang, Y., Chen, Q., Kong, B.H., 2020. The potential correlation between
- 484 bacterial diversity and the characteristic volatile flavour of traditional dry sausages from Northeast
- 485 China. Food Microbiol. 91, 103505.
- 486 Jung, S., Park, O.J., Kim, A.R., Ahn, K.B., Lee, D., Kum, K.Y., Yun, C.H., Han, S.H.J., 2019. Lipoteichoic
- 487 acids of *lactobacilli* inhibit *Enterococcus* faecalis biofilm formation and disrupt the preformed
- 488 biofilm. J Microbiol. 57: 310-315.
- Komprda, T., Sládková, P., Dohnal, V., 2009. Biogenic amine content in dry fermented sausages as
- influenced by a producer, spice mix, starter culture, sausage diameter and time of ripening. Meat
- 491 Sci. 83, 534-542.
- Kim, H.S., Hur, S.J., 2018. Effect of in vitro human digestion on biogenic amine (tyramine) formation
- in various fermented sausages. J. Food Protect. 81, 365-368.
- Laranjo, M., Agulheiro-Santos, A.C., Potes, M.E., Cabrita, M.J., Garcia, R., Fraqueza, M.J., Elias, M.,
- 495 2015. Effects of genotype, salt content and calibre on quality of traditional dry-fermented sausages.
- 496 Food Control 56, 119-127.
- Latorre-Moratalla, M.L., Bosch-Fusté, J., Bover-Cid, S., Aymerich, T., Vidal-Carou, M.C., 2011.
- Contribution of enterococci to the volatile profile of slightly-fermented sausages. LWT Food Sci.
- 499 Technol. 44, 145-152.
- Lee, Y.C., Kung, H.F., Huang, C.Y., Huang, T.C., Tsai, Y.H., 2016. Reduction of histamine and biogenic
- amines during salted fish fermentation by *Bacillus polymyxa* as a starter culture. J. Food Drug Anal.

- 502 24, 157-163.
- Li, C.S., Zhao, Y., Wang, Y.Q., Li, L.H., Yang, X.Q., Chen, S.J., Zhao, Y.Q., Zhou, W.G., 2021. Microbial
- 504 community changes induced by *Pediococcus pentosaceus* improve the physicochemical properties
- and safety in fermented tilapia sausage. Food Res. Int. 147, 110476.
- Liu, S.J., Xu, J.J., Ma, C.L., Guo, C.F., 2018. A comparative analysis of derivatization strategies for the
- determination of biogenic amines in sausage and cheese by HPLC. Food Chem. 266, 275-283.
- Liu, T., Zhang, T.W., Zhai, Y.J., Sun, L.N., Zhai, M.Q., Kang, L.T., Zhao, X., Wang, B.H., Duan, Y., Jin,
- Y., 2023. Effect of Lactiplantibacillus plantarum X22-2 on biogenic amine formation and quality
- of fermented lamb sausage during storage. Fermentation-Basel 9, 883.
- 511 Lorenzo, J.M., Gómez, M., Fonseca, S., 2014. Effect of commercial starter cultures on physicochemical
- characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. Food
- 513 Control 46, 382-389.
- 514 Lv, J., Lin, X.P., Liu, M.Y., Yan, X., Liang, H.P., Ji, C.F., Li, S.J., Zhang, S.F., Chen, Y.X., Zhu, B.W.,
- 515 2023. Effect of Saccharomyces cerevisiae LXPSC1 on microorganisms and metabolites of sour
- meat during the fermentation. Food Chem. 402, 134213.
- 517 Lv, J., Yang, Z., Xu, W., Li, S., Liang, H., Ji, C., Yu, C., Zhu, B., Lin, X.J.I.j.o.f.m., 2019. Relationships
- between bacterial community and metabolites of sour meat at different temperature during the
- fermentation. Int. J. Food Microbiol. 307, 108286.
- 520 Maijala, R., Eerola, S., 1993. Contaminant lactic acid bacteria of dry sausages produce histamine and
- 521 tyramine. Meat Sci. 35, 387-395.
- 522 Montanari, C., Bargossi, E., Gardini, A., Lanciotti, R., Magnani, R., Gardini, F., Tabanelli, G., 2016.
- 523 Correlation between volatile profiles of Italian fermented sausages and their size and starter culture.

- 524 Food Chem. 192, 736-744.
- Noda, M., Danshiitsoodol, N., Inoue, Y., Okamoto, T., Sultana, N., Sugiyama, M., 2019. Antibiotic
- 526 susceptibility of plant-derived lactic acid bacteria conferring health benefits to human. J. Antibiot.
- 527 72, 834-842.
- 528 Bover-Cid S., Izquierdo-Pulido M., Vidal-Carou M C., 2000. Mixed starter cultures to control biogenic
- amine production in dry fermented sausages. J. Food Protect. 63, 1556-1562.
- 530 Shalaby, A.R. (1996). Significance of biogenic amines to food safety and human health. Food Res. Int.
- 531 29, 675–690.
- 532 Sidira, M., Kandylis, P., Kanellaki, M., Kourkoutas, Y., 2015. Effect of immobilized *Lactobacillus casei*
- on the evolution of flavor compounds in probiotic dry-fermented sausages during ripening. Meat
- 534 Sci. 100, 41-51.
- 535 Sun, F.D., Hu, Y.Y., Chen, Q., Kong, B.H., Liu, Q., 2019. Purification and biochemical characteristics of
- 536 the extracellular protease from *Pediococcus pentosaceus* isolated from Harbin dry sausages. Meat
- 537 Sci. 156, 156-165.
- 538 Sun, Q.X., Chen, Q., Li, F.F., Zheng, D.M., Kong, B.H., 2016. Biogenic amine inhibition and quality
- 539 protection of Harbin dry sausages by inoculation with Staphylococcus xylosus and Lactobacillus
- 540 plantarum. Food Control 68, 358-366.
- Wang, X.H., Ren, H.Y., Liu, D.Y., Zhu, W.Y., Wang, W., 2013. Effects of inoculating Lactobacillus sakei
- starter cultures on the microbiological quality and nitrite depletion of Chinese fermented sausages.
- 543 Food Control 32, 591-596.
- Wang, Y., Han, J., Wang, D., Gao, F., Zhang, K., Tian, J., Jin, Y., 2022. Research update on the impact of
- lactic acid bacteria on the substance metabolism, flavor, and quality characteristics of fermented

- meat products. Foods 11, 2090.
- 547 Zeng, X.F., Xia, W.S., Jiang, Q.X., Yang, F., 2013. Chemical and microbial properties of Chinese
- 548 traditional low-salt fermented whole fish product *Suan yu*. Food Control 30, 590-595.
- 549 Zhang, J.B., Hu, J., Wang, S., Lin, X.P., Liang, H.P., Li, S.J., Yu, C.X., Dong, X.P., Ji, C.F., 2019.
- Developing and validating a UPLC-MS method with a stagetip-based extraction for the biogenic
- amines analysis in fish. J. Food Sci. 84, 1138-1144.
- 552 Zhang, Y., Qin, Y.X., Wang, Y., Huang, Y.X., Li, P.F., Li, P.L., 2020. Lactobacillus plantarum LPL-1, a
- bacteriocin producing strain, changed the bacterial community composition and improved the safety
- of low-salt fermented sausages. LWT Food Sci. Technol. 128, 109030.
- Zhang, Y.L., Hu, P., Xie, Y.Y., Wang, X.Y., 2020. Co-fermentation with *Lactobacillus curvatus* LAB26
- and *Pediococcus pentosaceus* SWU73571 for improving quality and safety of sour meat. Meat Sci.
- 557 170, 108240.
- 558 Zhao, C.C., Eun, J.B., 2020. Shotgun metagenomics approach reveals the bacterial community and
- 559 metabolic pathways in commercial *hongeo* product, a traditional Korean fermented skate product.
- 560 Food Res. Int. 131, 109030.
- 561 Zhao, C.Q., Zhao, X.X., Lu, Z.Y., Huang, J., He, S., Tan, H., Wang, G., Li, Y.B., Liu, D.Y., 2016.
- Production of fermented pork jerky using *Lactobacillus bulgaricus*. LWT Food Sci. Technol. 72,
- 563 377-382.
- Zhao, X.X., Li, S.L., Liu, Y.H., He, Y.G., Hu, R., Yang, J., Wang, Q., 2022. Production of fermented
- spicy rabbit meat using *Lactobacillus paracasei*. Bioproc. Biosyst. Eng. 45, 87-95.
- Zhao, Y.F., Zhou, C.Y., Ning, J.W., Wang, S., Nie, Q., Wang, W., Zhang, J.M., Ji, L.L., 2022. Effect of
- fermentation by *Pediococcus pentosaceus* and *Staphylococcus carnosus* on the metabolite profile

1 Table 1

2

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

Biogenic amine		28 d				
(mg/kg)	v a	С	Lp	Pe	М	
Histamine	3.55±0.04b	25.40±0.73ª	3.62±0.05 ^b	3.51±0.01 ^b	3.54±0.02 ^b	
Putrescine	36.28±0.10°	323.50±11.61 ^a	295.35±39.30 ^a	54.86±7.45°	120.54±17.06 ^b	
Tyramine	4.61±0.23°	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±5.91ª	58.35±7.07 ^b	22.59±1.70°	5.83±0.64 ^d	
Tryptamine	24.10±0.02 ^b	89.00±3.63ª	81.68±8.12a	25.00±0.07 ^b	25.94±0.13b	
Phenethylamine	0.10±0.02°	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°	328.92±19.43°	

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

9

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
	С	544.67±57.12 ^{Ab}	0.87 ± 0.05^{Aa}	271.85±63.38 ^{Ac}	0.37±0.02 ^{Aa}	$0.77{\pm}0.02^{\mathrm{Aa}}$
0.1	Lp	531.16±39.96 ^{Ab}	0.89 ± 0.08^{Aa}	$277.28{\pm}104.40^{Ab}$	$0.23{\pm}0.01^{Ba}$	$0.73{\pm}0.02^{\rm Ba}$
0 d	Pe	$540.02{\pm}22.89^{Ab}$	$0.78{\pm}0.04^{\rm Aa}$	$254.43{\pm}49.47^{Ab}$	0.30 ± 0.06^{Aa}	$0.74{\pm}0.01^{Aa}$
	M	$541.57{\pm}56.42^{Aa}$	$0.78{\pm}0.04^{\rm Aa}$	$291.54{\pm}64.30^{Ab}$	$0.29{\pm}0.01^{\rm Ba}$	0.67 ± 0.01^{Cb}
	C	$1084.69{\pm}54.66^{\mathrm{Aa}}$	$0.67{\pm}0.12^{Aab}$	$565.69{\pm}58.34^{\mathrm{Aa}}$	$0.21{\pm}0.02^{Abc}$	$0.68{\pm}0.07^{\mathrm{Aa}}$
7.1	Lp	$722.33{\pm}79.12^{Aab}$	$0.73{\pm}0.04^{\mathrm{Aa}}$	$666.11{\pm}172.10^{Aa}$	$0.23{\pm}0.01^{Aa}$	$0.74{\pm}0.04^{\mathrm{Aa}}$
7 d	Pe	$924.54{\pm}229.64^{\mathrm{Aa}}$	0.75 ± 0.04^{Aa}	$727.87 {\pm} 98.53^{\mathrm{Aa}}$	0.27 ± 0.09^{Aa}	$0.73{\pm}0.02^{\mathrm{Aa}}$
	M	786.80 ± 331.67^{Aa}	$0.74{\pm}0.02^{Aa}$	$557.96{\pm}51.28^{Aa}$	0.22 ± 0.02^{Ab}	$0.73{\pm}0.03^{\mathrm{Aa}}$
	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}
14.1	Lp	822.46 ± 61.33^{Aa}	0.71 ± 0.04^{Aa}	$605.26{\pm}89.78^{\mathrm{Aa}}$	0.21 ± 0.01^{Aa}	$0.75{\pm}0.02^{\mathrm{Aa}}$
14 d	Pe	$777.57 {\pm} 87.86^{Ab}$	0.70 ± 0.06^{Aa}	$762.02{\pm}116.56^{Aa}$	0.22 ± 0.04^{Aa}	0.71 ± 0.04^{Aa}
	M	$739.80 {\pm} 261.52^{\mathrm{Aa}}$	0.79 ± 0.05^{Aa}	659.85±116.52 ^{Aa}	0.22 ± 0.01^{Ab}	$0.76 \pm 0.00^{\mathrm{Aa}}$
	C	1070.85 ± 83.51^{Aa}	0.65 ± 0.06^{Ab}	443.85 ± 35.10^{Cb}	$0.18\pm0.01^{\mathrm{Bc}}$	$0.72 \pm 0.02^{\mathrm{Aa}}$
28 d	Lp	$776.12 {\pm} 218.26^{Aab}$	0.68 ± 0.04^{Aa}	$588.23{\pm}90.99^{Ba}$	$0.20{\pm}0.00^{ABa}$	$0.74{\pm}0.01^{\mathrm{Aa}}$
	Pe	$749.00 {\pm} 224.78^{Aab}$	0.69 ± 0.03^{Aa}	711.58±6.26 ^{Aa}	0.22 ± 0.02^{Aa}	$0.70{\pm}0.05^{\rm Aa}$
	M	$721.25{\pm}19.38^{Aa}$	$0.74{\pm}0.05^{Aa}$	$453.85{\pm}20.96^{Cb}$	0.21 ± 0.01^{ABb}	$0.74{\pm}0.01^{Aa}$

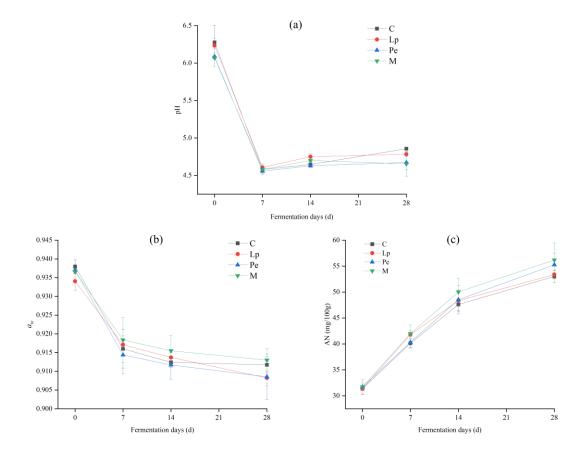
^{a-c} Values in the same sample group are significantly different (p < 0.05). ^{A-C} Values in the different sample groups are significantly different at the same fermentation period (p < 0.05). For abbreviations, see Figure 1.

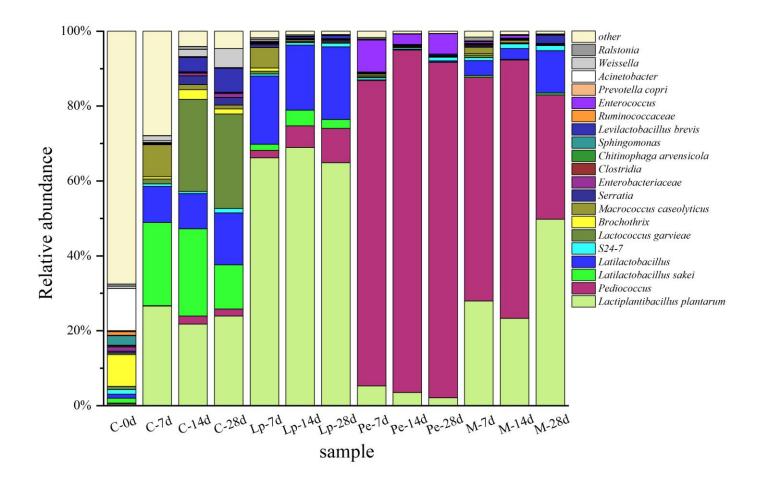
Table 3
 The OAV value of the key volatile compounds (OAV > 1) in sour meat with and without starter
 culture during the fermentation.

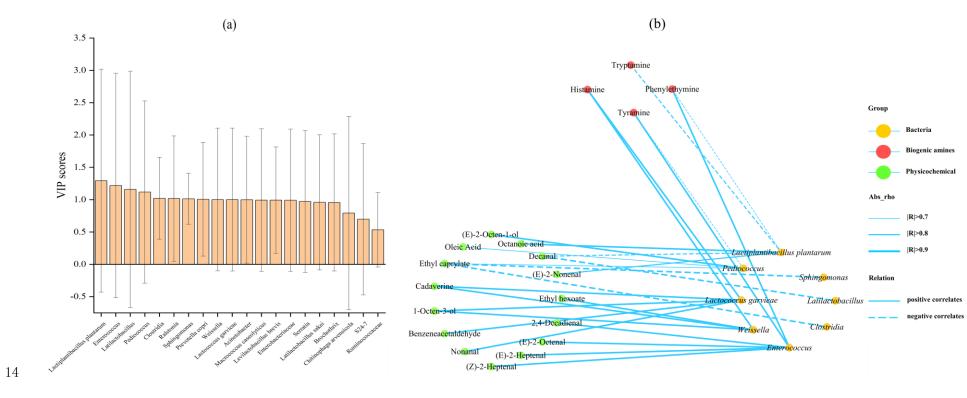
Entry Volatile compounds		Threshold value					
Entry	voiathe compounds	$(\mu g/100 g)$	0d	С	Lp	Pe	M
1	1-Octen-3-ol	0.03	0.00	5551.11	0.00	0.00	0.00
2	(E)-2-Octen-1-ol	0.20	0.00	0.00	185.60	1410.55	442.65
3	Phenylethyl Alcohol	1.60	4.25	51.36	60.64	67.53	69.69
4	2-Nonen-1-ol	1.30	0.00	0.00	0.00	90.82	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

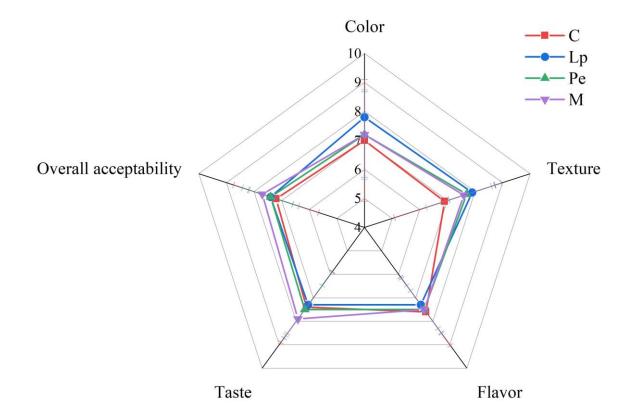
1 Figure Captions

- 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.
- 4 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
- 7 (solid lines) and negative correlates (dotted lines).
- 8 **Fig. 4.** Sensory evaluation results of sour meat with or without starter culture. For abbreviations, see Figure 1.









- 1 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
- 4 meat fermentation
- Hao Shang^a, Ying Yue^a, Bingrui Guo^a, Chaofan Ji ^a, Sufang Zhang ^a, Liang Dong ^a, Ilario Ferrocino^b, Luca Simone Cocolin ^b, Xinping Lin ^{a,*}
- 6 a SKL of Marine Food Processing & Safety Control, National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic
- 7 University, Dalian, 116034, China
- 8 b Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy

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

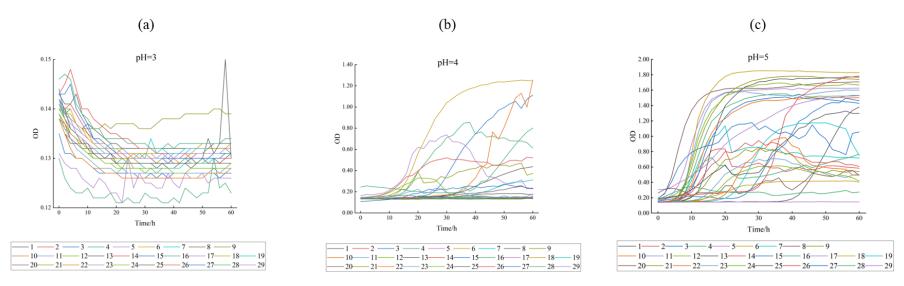


Figure S1 Growth curves of 28 strains at pH 3(a), 4 (b)and 5(c). For strain number in the legend, see Table S1.

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

VOCs (μg/100g)	0d	С	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	000 4 1 207 5	0007 00 4500 05	$10655.51\pm$	11511 19 4 5702 71	$12293.51 \pm$
Total	932.4 ± 307.64 9237.93 ± 4528.05		3004.55	11511.18 ± 5702.71	6252.99

Declaration of Interest Statement

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