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Relationships between bacterial community and metabolites of sour meat at different temperature during the fermentation

Jing Lv[a], Zhaoxia Yang[a], Wenhuan Xu[a], Shengjie Li[a], Huipeng Liang[a], Chaofan Ji[a], Chenxu Yu[b], Beiwei Zhu[a,*], Xinping Lin[a,*]

[a] National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China
[b] Department of Agricultural and Biosystems Engineering, Iowa State University, IA 50011, USA

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ABSTRACT

This study was designed to explore the temperature effects on bacterial communities and metabolites, as well as their relationships during the fermentation of sour meat, a traditional fermented meat product in the ethnic minority regions of China. Results showed that reduction of pH and increase of lactic acid and free amino acid contents occurred ($p < 0.05$) as the fermentation temperature and time increased, and the tendency was more apparent at higher temperature. During the fermentation, *Lactobacillus* gradually replaced other genera, and higher the temperature, more rapid was the process. Both the number and amount of volatile organic compounds increased at higher temperatures. Hexanal, benzaldehyde, nonanal, (E,E)-2,4-decadienal, 1-octen-3-ol and octanal were identified as the key volatile organic compounds produced by *Lactobacillus* in sour meat as main contributors to odor as confirmed by variable importance in the projection analysis. Redundancy analysis and Pearson correlation showed positive correlation between *Lactobacillus* and desired product characteristics, such as higher content of lactic acid, free amino acids, volatile organic compounds, and lower pH and water activity values, which may represent a better quality and longer shelf life after fermentation at higher temperature. Therefore fermentation at 20 °C and 25 °C are proposed as optimum temperatures for sour meat production.

1. Introduction

Fermented meats are unique for pleasant flavor and taste and are important sources of functional nutrients (Kumar et al., 2017). Various fermented meats products, such as fermented sausage (Chen et al., 2017a), fermented ham (Tu et al., 2010) and fermented meat sauce (Ohata et al., 2016), are produced around the world following region-specific starting materials, formulations and processing techniques. Sour meat (Nanx Wudl), a traditional artisanal fermented meat product popular in Dong, Miao and Tujia nationalities of China, is made by mixing pork belly, salt, rice and other seasonings, and then naturally fermented in sealed jars. Owning to the beneficial characteristics of sour meat, such as unique flavor, richness in nutrients and long shelf-life, research has been carried out on the identification and purification of bacteriocin from *Lactobacillus* strains from sour meats (Chen et al., 2014; Chen et al., 2016; Hu et al., 2017). However, studies evaluating the temperature effect on the formation of the special flavor during the fermentation are still rare.

As a fermented meat product, sour meat has a typical but complex flavor due to non-volatile and volatile organic compounds (VOCs) which result from the activity of meat and microbial enzymes (Fonseca et al., 2013). In addition to the function of endogenous meat enzymes, microorganisms are believed to have great impact on the formation of flavor, by lipid hydrolysis and autoxidation, proteolysis and metabolism of amino acids and carbohydrates (Sidira et al., 2015). It was reported that lactic acid bacteria (LAB) could produce lactic acid and other aroma compounds by carbohydrate fermentation; coagulase negative staphylococci are essential for color and flavor development in fermented products by degradation of branched-chain amino acids and fatty acids (Flores et al., 2017). Therefore, microbial community and its evolution during fermentation, including both cultivable and non-cultivable microorganisms, are crucial for the evaluation of products quality, especially the formation of flavor.

Sour meat can be traditionally manufactured year-round, but differences exist in the sour and fatty flavors among the products, mainly because of differences in the temperature. It is known that temperature is closely related to microbiological activities, and consequently influences food safety and shelf life. Due to the lack of strict temperature

* Corresponding authors at: School of Food Science and Technology, Dalian Polytechnic University, National Engineering Research Center of Seafood, Dalian 116034, PR China.
E-mail addresses: chenxuyu@iastate.edu (C. Yu), zhubeiwei@163.com (B. Zhu), yingchaer@163.com (X. Lin).

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control, the quality of sour meat is often inconsistent, and the effect of temperature on microorganisms and physicochemical features of the sour meat during fermentation is not clear. Therefore, evolution of microbial community and metabolite changes during sour meat fermentation at different temperatures (10 °C, 15 °C, 20 °C, and 25 °C) were monitored in this study, aiming to establish the critical correlations between microbial community and VOCs, and to identify the optimum temperature(s) for sour meat production.

2. Material and methods

2.1. Sour meat-making procedure and sampling

Pork belly, rice and salt were purchased from a local Walmart store (Dalian, China). The production process of sour meat was carried out according to the method of previous report (Lv et al., 2019). The pork belly was sliced into strips (5 cm × 3 cm × 1 cm). Rice was stir-fried until turned golden and pulverized into flour (Sieve Mesh No. 20 m). The ingredients included 10% rice flour and 4.5% salt, which were computed with the proportions to the pork belly (mass based). Then mixtures of pork belly and ingredients were compressively filled into jars (net mass of 300 g mixtures each jar). Jars were held under constant temperature at 10 °C, 15 °C, 20 °C and 25 °C, respectively in triplicate for fermentation (50% relative humidity). Samples were taken from the center of the jars at 0, 7, 14, 21 and 28 d for analysis.

2.2. Determination of pH, water activity and salinity

Two grams of minced samples were placed in a 50-mL test tube and homogenized (3 × 15 s at 3000 rpm) with 20 mL distilled water using a homogenizer (T25 digital ULTRA TURRAX®, IKA, Germany), and the homogenate solutions were used for pH determination with a pH meter (Five Easy Plus FE28, Mettler Toledo, China). Water activity (a_w) was detected at 25 °C using a water activity meter (Aqualab TDL, Decagon Devices, USA).

2.3. Determination of lactic acid content

Sample was cut into small pieces and minced thoroughly, and then 0.1 g minced sample was used for testing. 10 mL ultrapure water was added, and the sample was subjected to ultrasonic extraction for 10 min. Following that, the sample was centrifuged at 10,000*g* for 15 min. Then, the supernatant was filtered with a 0.45 μm membrane filter, and allowed to flow through a Cleanert SC18 SPE column. The filtrate was then diluted × 20 times, and its lactic acid content was determined with high-performance ion chromatograph (Dionex ICS-5000 + DC, Thermo Scientific, USA). The chromatographic condition was selected according to Ramos's method (Ramos et al., 2012). Briefly, the chromatographic column and guard column was IonPac AS23 (250 mm × 4 mm) and IonPac AG23 (50 mm × 4 mm), respectively. The conductivity detector was equipped with a suppressor. The eluent was a mixture of 0.8 mM $NaHCO_3$/4.5 mM Na_2CO_3 using an isocratic flowrate at 1 mL/min and 30 °C.

2.4. Microbial succession by high throughput sequencing

Sour meat was aseptically sampled from the ferment jar and stored at −80 °C before DNA extraction. The genomic DNA was extracted from 0.25 g sample by using PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA). The V3-V4 region of the bacterial 16S rRNA gene was PCR-amplified with the universal primers 338F (5′-ACTCCTACGG GAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), PCR procedure was performed according to Zang's method (Zang et al., 2018). Then PCR products were purified using MinElute® PCR Purification Kit (QIAGEN, Germany) and VAHTS™ DNA Clean Beads (Vazyme Biotech Co., Ltd., China). The amplified products were recovered after 1.8% agarose gel electrophoresis. Following that, the 16S rRNA gene amplicons were sequenced by sequencing system (HiSeq 2500, Illumina, USA) at Biomarker Bioinformatics Technology Co., Ltd., Beijing, China, and the sequencing reads were normalized for the subsequent analysis.

2.5. Analysis of free amino acid

The amino acid profiles of sour meat samples were analyzed according to the method of Sun et al., 2017 with some modifications. All sample solutions were subjected to derivatization with 2,4-dinitrofluorobenzene. The sample solutions were then filtered by 0.45 μm membrane filter, and Elite-AAK Amino acid analysis system (Elite Analytical Instruments Co., Ltd., China) was used for amino acid profiling. 10 μL sample was eventually separated through Elite-AAK C18 reversed phase chromatography (250 mm × 4.6 mm). The analysis conditions were: column temperature 27 °C, flow rate 1.2 mL/min and detection wavelength 360 nm.

2.6. Analysis of volatile organic compounds

Analysis of VOCs was conducted according to the method of Wang et al. (2014) with some modifications. Sour meat was steamed for 20 min and pulverized in a blender. 2 g minced sample was taken into the headspace extraction vial (20 mL, 18 mm), and 20 μL 50 mg/L cyclohexanone (Aladdin, American) internal standard was added to each sample. These vials were incubated at 60 °C water bath for 30 min prior to extraction. Subsequently, a solid-phase microextraction (SPME) fiber was inserted into headspace of each vial and was kept there for 40 min for VOC extraction. Then, the fiber was withdrawn and inserted into GC–MS 7890A/5975C (Agilent Technologies Inc., American) for 10 min to ensure complete VOC transport. Agilent 19091S-433HP-5MS (30 m × 250 μm × 0.25 μm) was used for analysis and gradient program was applied as follows: oven temperature was kept at 35 °C for 5 min, then heated to 50 °C for 3 min at a rate of 3 °C/min, raised to 150 °C at 5 °C/min and then heated to 250 °C for 5 min at 20 °C/min immediately. The injector was operated in splitless mode and flow rate was 1 mL/min. In addition, 0.2 μL C7 - C30 Saturated Alkanes (1000 μg/mL, Sigma-Aldrich, American) were analyzed under the same GC conditions to calculate the retention index (RI), the equation was from Cheok's (Cheok et al., 2017) method.

The identification of VOCs was performed by comparing the RI and matching mass spectra fragment with the NIST 11.L MS library. The concentration of the VOC in sour meat was obtained using semi-quantitative analysis method involving the use of internal standard. Then, odor activity value (OAV) was calculated according to OAV = C / OT, where C was the concentration of VOC and OT was its odor threshold, and odor thresholds were obtained from literature (van Gemert, 2011).

2.7. Bioinformatics and statistical analysis

For the data of high-throughput sequencing, analysis was performed with the Quantitative Insights Into Microbial Ecology (QIIME) software package. The paired-end reads were merged into raw tags using FLASH version 1.2.11 and the clean tags were obtained by quality filtered using Trimmomatic version 0.33. After further processing to remove chimeras using UCHIME version 4.2, the effective tags were obtained. Then, the effective tags were clustered using USEARCH version 10.0, thereby getting the operational taxonomic units (OTUs) for bacteria. In addition, the clustering was based on a 97% sequence similarity level, and 0.005% of total effective tags as a threshold for filtering OUT. The taxonomic classification was assigned to OTUs by searching against the Silva databases (Release128, http://www.arb-silva.de) using the Ribosomal Database Project (RDP) classifier (version 2.2, http://sourceforge.net/projects/rdpclassifier/). Alpha diversity (Ace richness

and Chao1 richness estimators, Simpson and Shannon diversity indices) was evaluated using the Mothur version 1.30 program. The rawdata had been uploaded to the NCBI website, with the accession number of SRP201061 (Table S2).

For the results of physicochemical analyses, samples were analyzed in triplicate. Interaction analysis and significant differences were conducted with two-way ANOVA of General Linear Model (GLM) and one-way ANOVA in SPSS 20.0 (International Business Machines Corp., USA), respectively. The line graph and histogram were created using Origin 8.5 (OriginLab Corp., USA). The heatmaps were generated by using a program tool (TBtools, version 0.6652). Bidirectional Partial Least Square (O2PLS) modeling was used to estimate the relationship between microbiota and VOCs in this article, which consisted of simultaneous projection of both the X and Y matrices on low dimensional hyper planes (Wang et al., 2016). The large R^2 (close to 1) and Q^2 (> 0.5) are necessary conditions for a good model, which indicate a good predictive ability. O2PLS of multivariate analysis was performed using SIMCA 14.1 (Umetrics, Sweden). Redundancy analysis (RDA) was performed using Canoco for Windows 4.5 (Microcomputer Power, USA). The visualized network planning of Pearson correlation coefficient was conducted using Cytoscape 3.7.1.

3. Results and discussion

3.1. pH, lactic acid content and water activity analysis

The pH and lactic acid content of the sour meat samples during the fermentation process are shown in Table 1. The pH value significantly decreased ($p < 0.05$) with time and as temperature used for fermentation was higher. Reduction in pH was due to the utilization of carbohydrates by bacteria and subsequent production of organic acids (Sun et al., 2016). The lowest pH of ~4.10 at 28 d was obtained at higher temperature groups of 20 °C and 25 °C, respectively. Fermentation temperature supposedly had important influences on microbial growth and enzymatic activities, as Lee et al. (2014) reported higher temperature resulted in rapid growth of bacteria in saeu-jeot, a traditional Korean fermented shrimp, and was account for the greater acidification degree compared to lower temperature groups.

Lactic acid content showed a conversely increasing tendency ($p < 0.05$) with the rise of fermentation time and temperature in the sour meat. Acid-producing bacteria, such as LAB, could metabolize nutrients and consequently produce lactic acid, which was consistent with the reduction of pH (Swetwiwathana and Visessanguan, 2015). Lactic acid was generated rapidly in the initial fermentation stage in 20 °C and 25 °C groups, it reached 8624.39 ± 2273.97 mg/kg at 7 d in the 25 °C group. However, it was not detected at 7 d and 14 d in the groups of 10 °C and 15 °C, respectively, although pH decreased in these groups at those relevant time points ($p < 0.05$), which may be due to other organic acids such as acetic acid, citric acid and benzoic acid being produced in the fermenting meat (Ferrocino et al., 2018; Mologni et al., 2018). Therefore, food safety considering pathogenic and spoilage bacteria in the samples fermented at 20 °C and 25 °C, which reached lower pH values and higher lactic acid amounts, might be superior compared to that of lower temperature batches.

The value of a_w dropped from 0.99 to 0.93 over the course of the sour meat fermentation process, with a sharp decline within 7 d and a relative plateau to the end. Similar trend on a_w was observed in other fermented products such as fermented sausage (Wang et al., 2013) and fermented fish (Gao et al., 2016). The reduction of a_w contributes to maintain microbial and quality stability (Laranjo et al., 2015). Meanwhile, this change in a_w was also related to the declining pH that led to acidic denaturation of muscle proteins which may promote gelation and lead to the weakening of the water holding capacity of proteins (Sun et al., 2017b).

Overall, the variation tendency above occurred more obvious when fermented at higher temperature, and the similar phenomenon was also

Table 1
pH value, a_w and lactic acid content of sour meat with different fermentation temperature and time.

	Temperature	Fermentation time (d)					*p* value		
		0	7	14	21	28	Temperature	Time	Temperature * time
pH	10 °C	6.07 ± 0.01^{Aa}	5.85 ± 0.01^{Ba}	5.70 ± 0.01^{Ca}	5.53 ± 0.01^{Da}	5.32 ± 0.03^{Ea}	< 0.001	< 0.001	< 0.001
	15 °C	6.07 ± 0.01^{Aa}	5.89 ± 0.02^{Ba}	4.62 ± 0.02^{Eb}	5.32 ± 0.04^{Cb}	5.06 ± 0.04^{Db}			
	20 °C	6.07 ± 0.01^{Aa}	5.39 ± 0.07^{Bb}	4.42 ± 0.01^{Cc}	4.25 ± 0.02^{Dd}	4.15 ± 0.01^{Ec}			
	25 °C	6.07 ± 0.01^{Aa}	4.41 ± 0.02^{Bc}	4.30 ± 0.03^{Cd}	4.29 ± 0.01^{Cc}	4.11 ± 0.03^{Dc}			
a_w	10 °C	0.991 ± 0.000^{Aa}	0.935 ± 0.001^{BCc}	0.941 ± 0.001^{Ba}	0.934 ± 0.007^{Cab}	0.934 ± 0.000^{Ca}	< 0.001	< 0.001	< 0.001
	15 °C	0.991 ± 0.000^{Aa}	0.948 ± 0.000^{Ba}	0.942 ± 0.001^{Ca}	0.942 ± 0.005^{Ca}	0.932 ± 0.001^{Db}			
	20 °C	0.991 ± 0.000^{Aa}	0.930 ± 0.002^{Dd}	0.933 ± 0.001^{Bc}	0.930 ± 0.002^{Db}	0.931 ± 0.001^{BCb}			
	25 °C	0.991 ± 0.000^{Aa}	0.941 ± 0.003^{Bb}	0.936 ± 0.001^{Cb}	0.934 ± 0.001^{CDab}	0.932 ± 0.001^{Db}			
Lactic acid (mg/kg)	10 °C	0^{Ca}	0^{Cb}	0^{Cb}	5678.05 ± 692.89^{Bc}	7001.24 ± 35.85^{Ac}	< 0.001	< 0.001	< 0.001
	15 °C	0^{Ba}	0^{Bb}	0^{Bb}	6926.83 ± 1951.44^{Ac}	7082.09 ± 57.44^{Ac}			
	20 °C	0Da	656.91 ± 109.66^{Db}	9847.15 ± 1167.57^{Ca}	15,710.57 ± 1904.07^{Bb}	20,380.49 ± 946.94^{Ab}			
	25 °C	0^{Ca}	8240.65 ± 1502.58^{Ba}	9918.70 ± 1729.67^{Ba}	22,256.91 ± 1298.34^{Aa}	23,801.63 ± 200.26^{Aa}			

Values are represented as mean ± standard deviation.
$^{a-d}$Values in a column with different lowercase letters are significantly different ($p < 0.05$).
$^{A-E}$Values in a row with different uppercase letters are significantly different ($p < 0.05$).

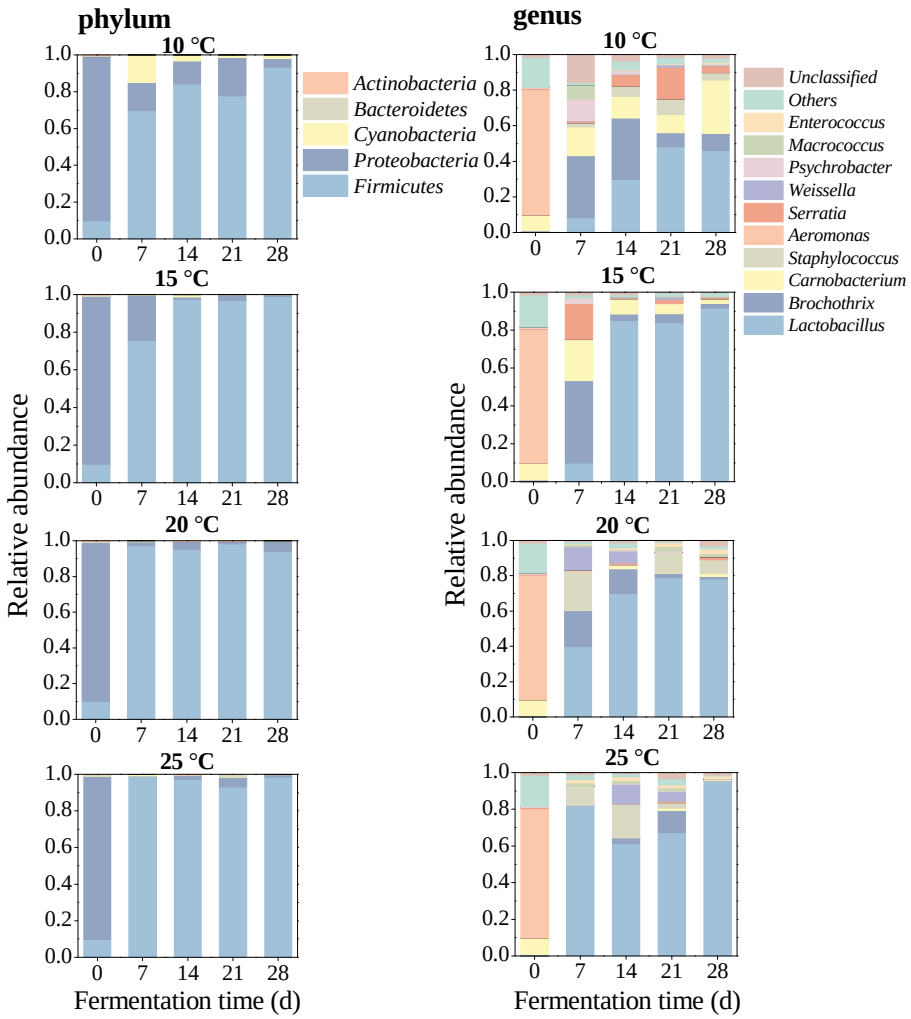

Fig. 1. The bacterial taxonomic compositions (relative abundance of top 10) showing microbial succession at the level of phylum and genus in sour meats during entire fermentation period.

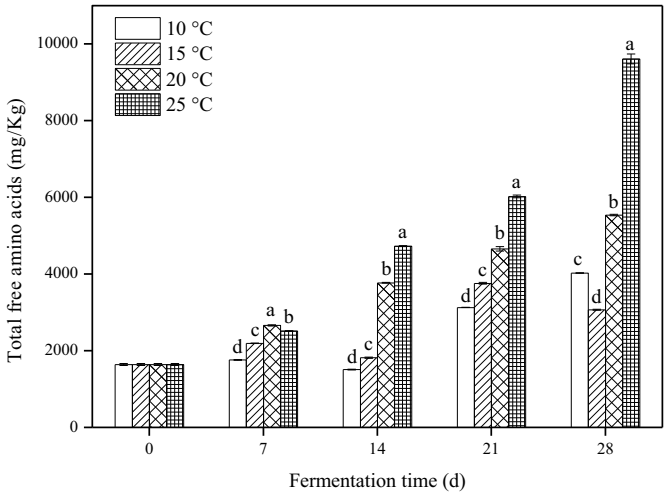

Fig. 2. The content of total free amino acid in sour meats incubated at 10 °C, 15 °C, 20 °C and 25 °C during fermentation period. The lowercase letters (a–d) indicate significant ($p < 0.05$) among different temperatures at every fermentation time.

found in other fermented products such as fish sauce (Jung et al., 2016), yogurt (Lopes et al., 2019), in which higher temperature resulted in an increased content of metabolites due to the faster fermentation process. Moreover, interactions between fermentation temperature and time for pH, lactic acid and a_w in the sour meat were significant ($p < 0.001$), indicating a better growth of LAB and a rapid fermentation process could be achieved through controlling the temperature.

3.2. Microbial succession

A total of 1,729,799 pairs of raw reads were obtained from 17 samples by Illumina Hiseq sequencing, and 1,419,263 clean tags were generated after quality control processing, including paired-reads assembly and raw reads filtration. Each sample produced > 40,375 effective tags and covered with an average of 79,463 effective tags (Table S1). A total of 87 OTUs were obtained, and Good's coverage were all above 99.90% (Table S1), suggesting that sufficient bacterial diversity was obtained from the sampling regime.

The relative abundance of different bacterial microorganisms during the course of the fermentation of sour meats is shown in Fig. 1. Five phyla were identified in all samples, including *Firmicutes*, *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes* and *Actinobacteria*. Obviously, *Proteobacteria* was dominant (89.19% of the OTUs) at day 0 and was replaced by *Firmicutes* which reached about 95% of the OTUs at the end of fermentation whatever the temperature incubation.

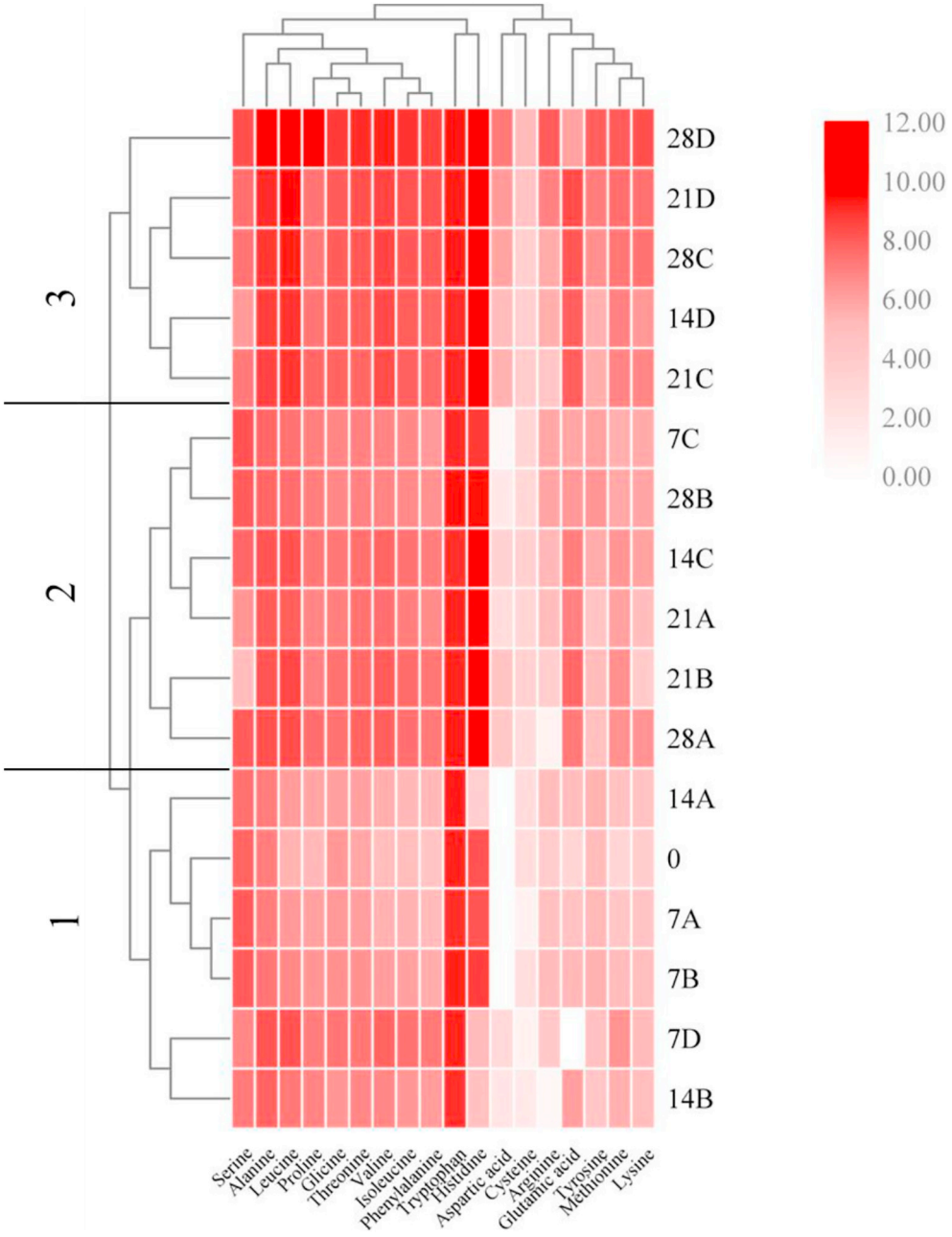

Fig. 3. The content distribution of free amino acids during the fermentation in sour meats. The clustering was performed with Euclidean distance and complete method. The colors corresponded to normalized mean levels from low (white) to high (red). The color scale was shown at the top. 0, 7, 14, 21 and 28 represented fermentation time (day). A, B, C and D represented samples incubated at 10 °C, 15 °C, 20 °C and 25 °C, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Aeromonas (70.62%) and *Carnobacterium* (8.80%) were the dominant genera at the beginning of the process while *Lactobacillus* genus appeared at day 7 and dominated batches incubated at 20 °C and 25 °C with relative abundance of 39.93% and 82.00%, respectively. However, *Lactobacillus* remained at lower levels in samples fermented at 10 °C (8.45%) and 15 °C (9.96%) which were dominated by *Brochothrix* genus. Meanwhile, *Staphylococcus* emerged at day 7, especially in the 20 °C and 25 °C groups. Several *Staphylococcus* species such as *S. equorum* or *S. xylosus* are known to exist in fermented meat products (Angeliki et al., 2018; Leroy et al., 2009) and are used as starter for their role in aroma production of fermented meat products (Ojha et al., 2015). Except for samples incubated at 10 °C which were still dominated by *Brochothrix* genus at day 14, *Lactobacillus* was dominant in all samples where it replaced *Carnobacterium*, *Staphylococcus*, *Brochothrix*, *Serratia* and *Weissella* until the end of fermentation. *Lactobacillus* is more acid tolerant and capable of adapting to ecological factors better than other bacterial species (Chen et al., 2017b; Fraqueza, 2015), which explains its increasing dominance throughout the fermentation process. It is also in line with the reduction of pH value and increase of lactic acid content (Table 1).

In short, *Lactobacillus* was dominant in all sour meats at the end of fermentation regardless of incubation temperature, and other microorganisms (e.g., species with poor acid resistance, spoilage microbes) were inhibited during the fermentation process. Furthermore, the changes in bacterial diversity occurred faster and were more extensive in the “higher temperature” groups.

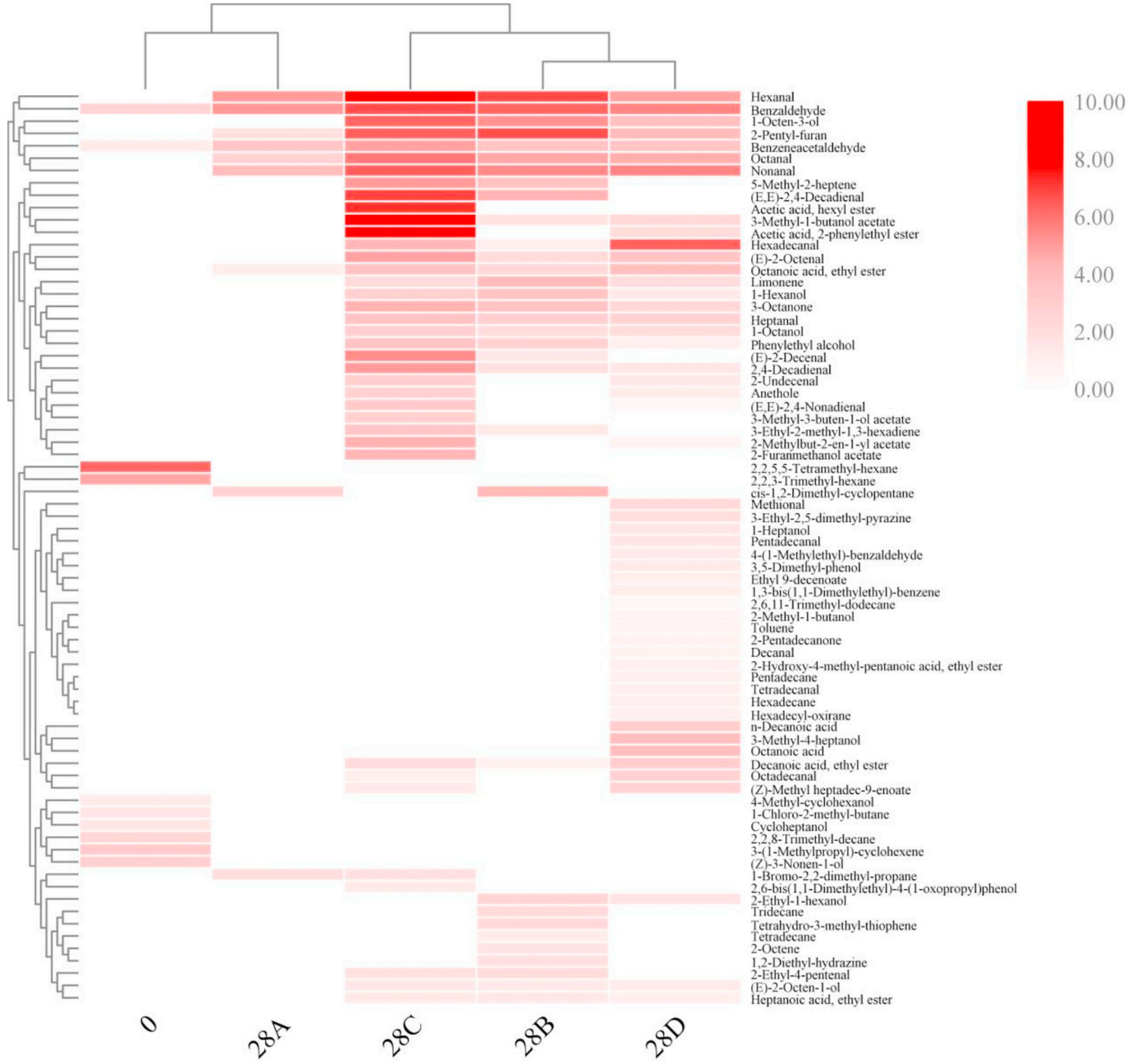

Fig. 4. The content distribution of the VOCs in sour meats. The clustering was performed with Euclidean distance and Average linkage. The colors corresponded to normalized mean levels from low (white) to high (red). The color scale was shown at the top. 0 and 28 represented fermentation time (day). A, B, C and D represented samples incubated at 10 °C, 15 °C, 20 °C and 25 °C, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Analysis of free amino acid profile

Free amino acid (FAA) content increased during fermentation in all batches (Fig. 2) with a significant difference ($p < 0.05$) between temperatures used. The total content reached a maximum value of 9610.89 ± 128.24 mg/kg in the 25 °C group at 28 d, which was 4.86 times higher than that of the initial sample (0 d). Moreover, both of the essential and nonessential amino acids for human nutrition were increased and shown in Tables S3 and S4, indicating the fermentation enhanced the nutritional value of sour meat for human consumption. These results indicated that both the temperature and time had effects on proteolysis, consistent with previous reports in fermented meat, such as fermented sausages (Tang et al., 2018). Some endogenous and microbial enzymes were involved in the production of smaller peptides and FAAs through hydrolyzing proteins and polypeptides, which eventually provided a potential source of VOCs and tastes compounds (Toldrá et al., 2000; Herranz et al., 2006).

The FAAs were clustered and further divided into three groups based on their concentrations and trends across the fermentation process (Fig. 3). It could be clearly seen that FAAs accumulated along with time, and these samples were clustered into three groups, representing the initial, mid and late stages of fermentation process. It was observed that the 20 °C and 25 °C batches reached the late stage faster (21 d for 20 °C, and 14 d for 25 °C) than that of lower temperature batches.

Regarding the fate of individual FAAs, most of them appeared to go through a sharp increase including branched-chain amino acids (valine, isoleucine and leucine). Branched-chain amino acids are regarded as the important precursors of aroma compounds, as they can be

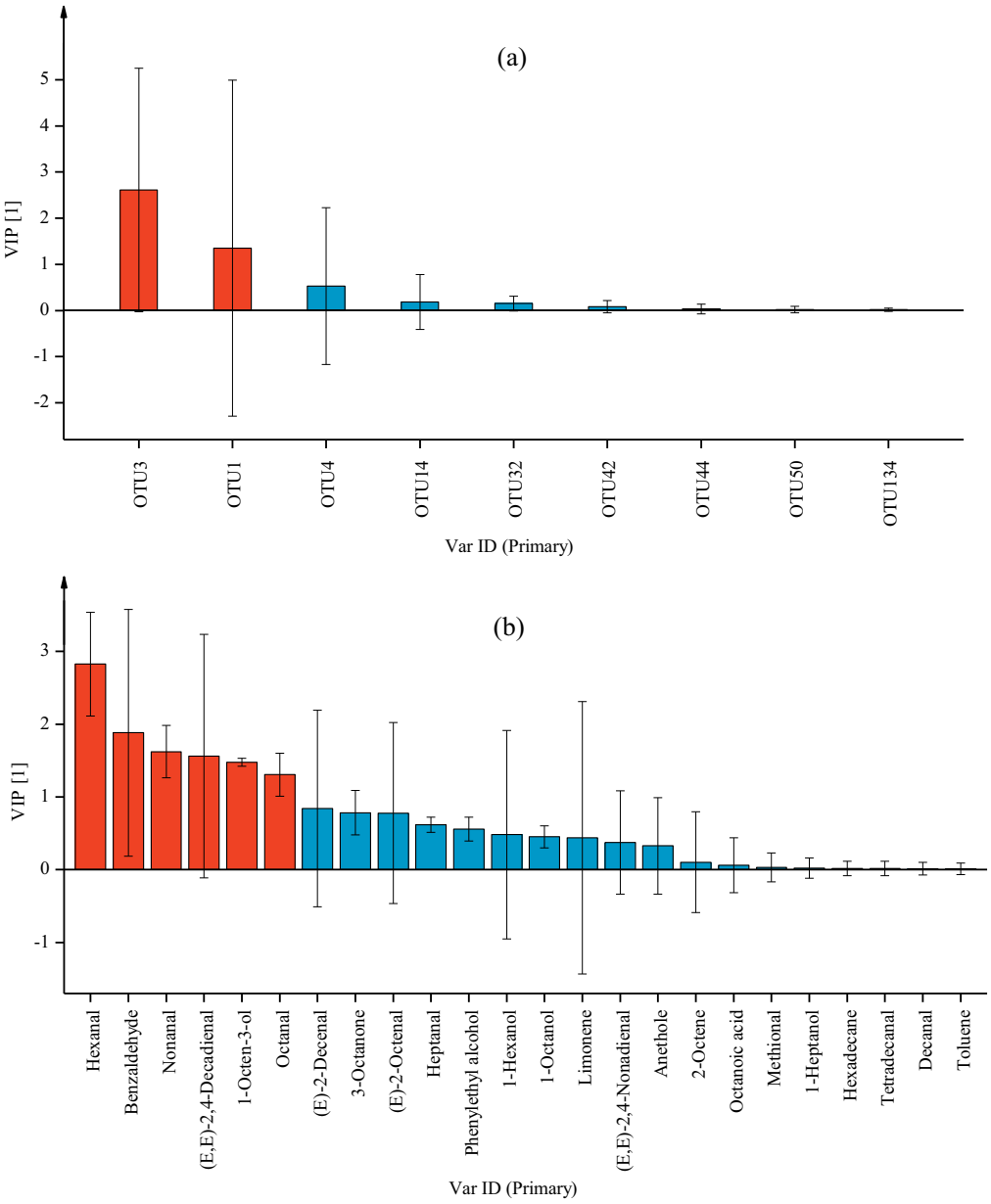

Fig. 5. Correlation analyses between microbiota and VOCs by O2PLS modeling during fermentation period. (a) VIP plot of *Lactobacillus* (defined as X matrix) vs. VOCs (OAV > 1, defined as Y matrix). (b) VIP plot of VOCs (OAV > 1, defined as X matrix) vs. *Lactobacillus* (defined as Y matrix). The microorganisms and VOCs with larger VIP values (> 1) were labeled in red, which indicate the important X variables for explaining Y variables. OTU 3 and OTU 1 represented *Lactobacillus plantarum* and *uncultured_bacterium_g_Lactobacillus*, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

converted into α-ketone acid by transaminase, then further metabolized to typical flavor compounds such as methyl-branched aldehydes, alcohols and acids in fermented meats (Gutsche et al., 2012; Zhao et al., 2016). The final concentrations of valine, isoleucine and leucine in sour meat showed maximums of 20.07-, 15.81- and 22.30-fold increments compared with unfermented sample (Table S3), which potential could greatly enrich the flavor in the final products.

3.4. Volatile organic compounds analysis

VOCs are formed essentially through the metabolism of proteins and lipids, as well as enzymatic oxidation and esterification (Montanari et al., 2016). Fig. 4 illustrates the VOCs in sour meats at 0 d and 28 d, respectively, and the specific contents can be found in Table S5. A total of 74 volatile compounds were identified in all the samples, which were grouped into chemical families: 12 alkanes, 5 alkenes, 12 alcohols, 2 ketones, 20 aldehydes, 2 acids, 12 esters and 9 others. 34, 39 and 51 VOCs were found in sour meats (28 d) fermented at 15 °C, 20 °C and 25 °C, which were far more than that of unfermented group (10 VOCs) and 10 °C group at 28 d (10 VOCs). As the fermentation temperature increased, the VOC profile became more diverse, suggesting the formation of more extensive/complex fermented flavors.

In addition, alkanes were found to be the largest group of VOCs (81.31%) in the sample at 0 d, while aldehydes were the most abundant VOCs in four fermented batches at 28 d, at 88.67%, 55.08%, 44.34% and 67.00% at 10 °C, 15 °C, 20 °C and 25 °C, respectively. Aldehydes are very important contributors to food aromas due to their low threshold values (Ansorena et al., 1998), their accumulation indicated that the fermentation imparted the sour meats with special flavors. Moreover, the clustering of heat map showed two categories of sour meats based on VOCs profiles. One category included samples at 0 d and samples on the 28th day fermented at 10 °C, the other category included samples at day 28th but fermented at higher temperature (15 °C, 20 °C and 25 °C). Clearly, sour meat was not fully fermented at 28 d at 10 °C, which showed a similar VOCs profiles like 0 d. Due to the high content and diverse VOCs in 20 °C and 25 °C groups, it could be concluded that these two groups underwent faster fermentation process than the “lower temperature” groups. This conclusion was also consistent with the results of analyses on microbial communities and amino acid changes.

OAVs were used to assess the influence of VOCs on sour meats

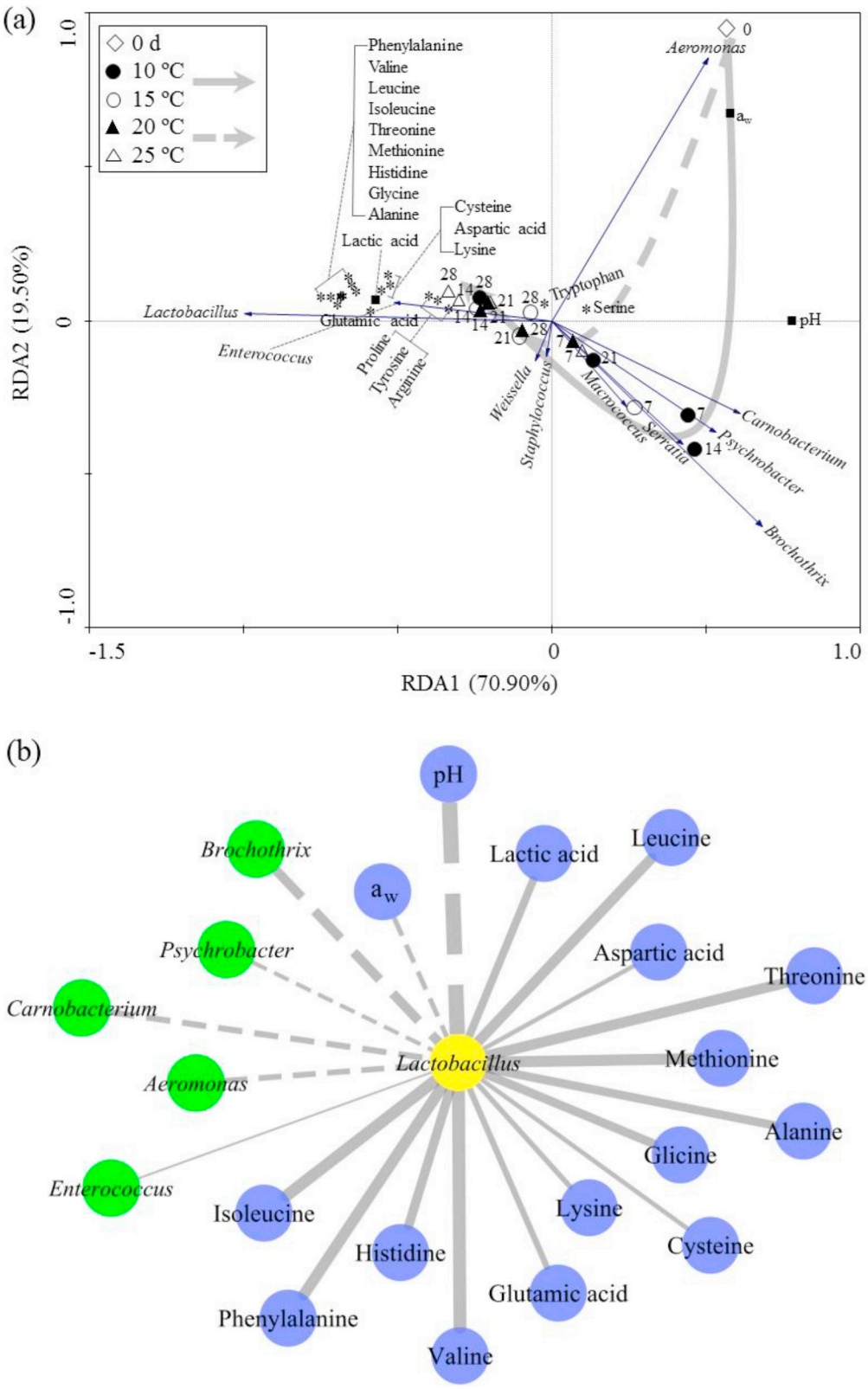

Fig. 6. (a) A RDA showing the correlations between relative microbial abundances and physicochemical characteristics at different temperature during sour meats fermentation. Numbers beside the symbols represent the fermentation time (days), asterisks represent free amino acids, squares represent pH, a_w and lactic acid. The directions and lengths of the straight arrows indicate the influence of the bacterial population on the sour meat samples. The thick curved arrows indicate the routes of sour meats fermentation at different temperature on the RDA triplot; (b) Pearson correlation analysis of *Lactobacillus* for sour meat fermentation ($p < 0.05$). The edge width is proportional to the correlation strength. The *Lactobacillus* was shown in yellow, other microorganisms were shown in green, and physicochemical parameters were shown in blue. The solid and dashed lines represented positive correlate and negative correlate, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Table S6). Except for two compounds with larger odor thresholds (42 μg/100 g for tridecane and 5 μg/100 g for tetradecane), 24 VOCs were found to have OAVs > 1, which were considered as the potent odorants that contributed substantially to the characteristic flavor of product (Huang et al., 2018).

It is reported that LAB can affect the flavor of fermented meat by releasing and degrading FAAs (Zhao et al., 2016). Moreover, *Lactobacillus* was the dominant genus in sour meat during the fermentation (Fig. 1). Therefore, we calculated the variable importance in the projection (VIP) values for 9 OTUs (*Lactobacillus*) vs. the 24 VOCs by O2PLS model, in order to evaluate the core *Lactobacillus* for producing VOCs (Fig. 5a) and the core VOCs conferred by *Lactobacillus* (Fig. 5b).

The R^2 and Q^2 of the models were 0.926 and 0.887 for Fig. 5a, 0.919 and 0.993 for Fig. 5b, suggesting the models fitted well with our data. The bacteria with larger VIP values (> 1) were OTU 3 (*Lactobacillus plantarum*) and OTU 1 (*uncultured_bacterium_g_Lactobacillus*), suggesting *L. plantarum* was a key microorganism in the ecosystem and played an important role in formation of the flavor components of sour meat. Meanwhile, hexanal, benzaldehyde, nonanal, (E,E)-2,4-decadienal, 1-octen-3-ol and octanal were considered as the key VOCs that were closely related to *Lactobacillus* in sour meat, which were also important components in other fermented meats (Kargozari et al., 2014; Latorre-Moratalla et al., 2011). Hexanal is described as an indicator of normal oxidation process in fermented meat (Fonseca et al., 2013), and nonanal, 1-octen-3-ol and octanal are also derived from lipid oxidation (Cruxen et al., 2018; Nowicka et al., 2017). These compounds derivated from lipid oxidation were deemed to have the characteristic odor of fresh grass, mushroom and citrus (Olivares et al., 2011). Additionally, benzaldehyde could originate from phenylalanine through bacterial conversion and oxidization. Benzaldehyde was considered as another typical flavor compound in fermented meats such as fermented sausage, pork jerky and fish sauce, and provided the smell of cherry-marzipan and burnt sugar (Hajaratul Najwa et al., 2012; Zhao et al., 2016) for those products. In conclusion, these core VOCs might be responsible for conferring typical and desirable flavor to the sour meat.

3.5. RDA of microbiota, pH, lactic acid, water activity and free amino acids

Fig. 6(a) showed the correlations between microorganisms (relative abundance of top 10 at genus level) and physicochemical characteristics during sour meat fermentation. Obviously, fermentation process of lower temperatures (10 °C and 15 °C) and higher temperatures (20 °C and 25 °C) presented two different routes, respectively. Genus *Aeromonas* was predominant in the samples at 0 d, other genus such as *Carnobacterium*, *Psychrobacter*, *Staphylococcus* and *Weissella* appeared subsequently, but most of them were replaced by *Lactobacillus* at the late stage of fermentation. At higher temperatures, the other microorganism were more rapidly replaced by *Lactobacillus*, which meant that these fermentation process were faster than that of lower temperatures, in accordance with the results of Fig. 1.

As displayed in RDA, most FAAs were produced at the later stages of fermentation, and they were positively correlated with *Lactobacillus* (Fig. 6(b)), indicating that *Lactobacillus* played an important role in releasing FAAs and contributing to the flavor formation of sour meat. *Lactobacillus* was also positively correlated with the content of lactic acid ($r = 0.65$, $p < 0.05$), while conversely, it was negatively correlated with pH ($r = -0.82$, $p < 0.05$), a_w ($r = -0.57$, $p < 0.05$) and other bacteria. It was known that higher content of lactic acid, lower value of pH and a_w could extend the shelf life of sour meats. Therefore, higher fermentation temperatures would be a better choice for sour meat production as more rapid growth of *Lactobacillus* could be achieved.

4. Conclusion

As fermentation time and temperature increased, a pH drop and lactic acid and FAA concentrations increase were observed ($p < 0.05$). Microorganism succession showed that *Lactobacillus* gradually replaced other genera during the fermentation, and higher the temperature, faster the process. Both the number and amounts of VOCs were higher when fermentation temperature was increased. Hexanal, benzaldehyde, nonanal, (E,E)-2,4-decadienal, 1-octen-3-ol and octanal were considered as the key VOCs produced by *Lactobacillus* in sour meat based on their high OAV and VIP analysis. RDA and Pearson correlation showed positive correlations between *Lactobacillus* and desired product characteristics, such as higher content of lactic acid, FAAs, VOCs, and lower value of pH, a_w, suggesting that favorable conditions for *Lactobacillus* growth may also ensure better quality and longer shelf life for the sour meat products. In conclusion, it is shown that higher fermentation temperatures (20 °C and 25 °C) should be used for sour meat production.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108286.

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