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Effect of combined application of *Lactiplantibacillus* strains and flavourzyme on improving the quality of fermented sour meat

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Abstract

This study explored the synergistic effects of lactic acid bacteria (LAB, *Lactiplantibacillus plantarum* LPJ1 and *Lactiplantibacillus pentosus* LPJ2) and flavourzyme on fermented sour meat quality. Five groups were compared: a natural fermentation control (CK), LAB only (L), and LAB with varying flavourzyme levels (F50, F75, F100). All treatments enhanced microbial safety, suppressing coagulase-positive staphylococci and Enterobacteriaceae to undetectable levels within 14–28 d and 3–7 d, respectively. Compared to CK, flavourzyme-treated groups accelerated pH and a_w declines and improved tenderness, while L and F50 reduced thiobarbituric acid reactive substances (TBARS) in later stages. Redundancy analysis showed positive correlations between LAB and product quality. At the stabilized fermentation stage (21 d), F50 group achieved optimal quality, including a strong electronic-nose response and an optimized flavor profile. Moreover, eight key volatile compounds were identified. Thus, combining LAB with flavourzyme, especially at the F50 ratio, effectively improves sour meat quality and shortens fermentation.

1. Introduction

Sour meat, a traditional fermented food renowned for its distinctive flavor and high nutritional value, has been widely consumed in Southwest China for over 2,000 years. Conventional techniques for producing sour meat generally involve mixing pork belly with ingredients such as rice flour, salt, and/or other spices, followed by sealing the mixture for fermentation. However, natural fermentation may lead to issues such as prolonged fermentation time and inconsistent product quality (Rossi et al., 2023). In recent years, much research has been conducted to enhance the quality and production efficiency of fermented meat products, including the application of starter cultures and enzymes.

Lactic acid bacteria (LAB) are known to inhibit spoilage organisms and pathogenic bacteria, as well as to exhibit antioxidant activity (Vougiouklaki et al., 2023), making them ideal starter cultures for enhancing the quality of fermented meat products. A study indicates that the addition of LAB to fermented sausages improves sensory attributes, enhances microbial safety, and extends shelf life (Shao et al., 2024). To better achieve the desired characteristics of fermented meats, different LAB strains are usually used as mixed starter cultures. For example, Zhang et al. (2020) demonstrated that in sour meat, inoculation with a mixed starter culture (*Lactobacillus curvatus* LAB26 and *Pediococcus pentosaceus* SWU73571) led to significant increases in the *L** value, *a** value, amino nitrogen content, and free amino acid content, while also improving product safety. Han et al. (2024) found that co-inoculation with *Lactobacillus plantarum* IMAUJBP3 and *Limosilactobacillus reuteri* IMAUJBR3 was superior to individual inoculation of either strain, yielding fermented mutton sausage with an enhanced flavor quality and a reduced content of biogenic amines, indicating the advantages of mixed-culture fermentation.

During the fermentation of meat products, proteolysis induced by endogenous enzymes and microbial enzymes constitutes another crucial reaction, serving as a key contributor to flavor formation (Sun et al., 2019). Given the limited enzyme content in the system, the appropriate supplementation of exogenous

enzymes represents a potential strategy to improve the flavor profile of meat products. Flavourzyme, a commercial protease derived from *Aspergillus oryzae*, possesses both exopeptidase and endopeptidase activities. It degrades proteins into peptides and amino acids, thereby improving the flavor of meat products. Similarly, the introduction of flavourzyme can facilitate proteolysis in fermented fish products, thus expediting the fermentation process while simultaneously enhancing their nutritional value and sensory quality (Xiao et al., 2022). Currently, many studies have confirmed the benefits of LAB or protease in fermented meat products, but their combined effects on sour meat have rarely been reported.

In this study, sour meat was added with different concentrations of LAB and flavourzyme, and microbial colony counts, water activity (a_w), thiobarbituric acid reactive substances (TBARS) values, color, texture, electronic nose (E-nose) profiles, and volatile organic compounds (VOCs) were monitored. The aim was to determine the optimal combination of LAB and flavourzyme in sour meat, thereby providing a scientific basis for improving the quality and production efficiency of sour meat and similar fermented meat products.

2. Materials and methods

2.1 Starter culture preparation

Lactiplantibacillus plantarum LPJ1 and *Lactiplantibacillus pentosus* LPJ2 were isolated and screened from commercially available sour meat. The previous research has demonstrated that two strains exhibit excellent acid-production, acid-tolerance, and antibacterial properties. They have been identified through 16S rDNA sequencing and stored in our laboratory. *Lpb. plantarum* LPJ1 and *Lpb. pentosus* LPJ2 were cultivated in MRS broth at 37 ℃ for 24 h and subcultured at a 2% inoculum. The bacterial cells were then collected via centrifugation (10,000×*g*, 10 min, 4 ℃), washed with sterile 0.9% NaCl solution, thoroughly resuspended, and adjusted to a concentration of 8–9 log CFU/mL.

2.2 Sour meat preparation

The preparation method for sour meats followed the previous study (Lv et al., 2023) with minor adjustments. Fresh pork belly, salt, and rice flour were sourced from a local supermarket (Zhengzhou, China), while flavourzyme (20 U/mg) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). The pork belly was cut into 5 cm × 3 cm × 1 cm pieces, and the rice flour was stir-fried until it became golden brown. The pork slices were then mixed with 10% rice flour, 4% salt, and additives (LAB, flavorzyme), compacted into jars and sealed for fermentation at 25 ℃. Five batches were manufactured: CK group (control without LAB or flavourzyme), L group (with mixed LAB strains, containing 5×10^6 CFU/g *Lpb. plantarum* LPJ1 and 5×10^6 CFU/g *Lpb. pentosus* LPJ2), F50 group (with the above mixed strains + 50 U/g flavourzyme), F75 group (with the above mixed strains + 75 U/g flavourzyme), F100 group (with the above mixed strains + 100 U/g flavourzyme). Under sterile conditions,

samples were collected from the center part of three parallel fermented jars on days 0, 3, 7, 14, 21, and 28 for subsequent analysis.

2.3 Enumeration of microorganisms

Five grams of sour meat were aseptically sampled and homogenized in 45 mL of 0.9% sodium chloride with a stomacher for 2 min. Decimal dilution series were prepared, and 200 μL aliquots were inoculated onto various agar plates as follows: LAB were cultured on de Man, Rogosa and Sharpe agar (HopeBio, Qingdao, China) at 37 ℃ for 48 h. Total aerobic bacteria were cultured on plate count agar (Aoboxing, Beijing, China) at 37 ℃ for 48 h. Staphylococci was cultured on Baird-Parker agar (HopeBio, Qingdao, China) at 37 ℃ for 48 h, and the positive strains were confirmed by the coagulase test. Enterobacteriaceae were cultured on violet red bile glucose agar (HopeBio, Qingdao, China) at 37 ℃ for 24 h. The colony counts were expressed as lg CFU per gram of sample.

2.4 Determination of pH, a_w and TBARS value

Homogenization of a 2 g sour meat sample with 20 mL of distilled water was performed, followed by pH measurement of the supernatant with a pH meter (FE28-Standard, Mettler Toledo, China). The a_w was measured at 25 ℃ using a water activity meter (HD-4, Wuxi Huake Instrument Co., Ltd., China). The TBARS value was determined following the method described by Cheng et al. (2020) to assess lipid oxidation in sour meat, with data expressed as mg MDA/kg of meat.

2.5 Texture and color analysis

The texture profile analysis (TPA) was conducted following Jiao's method (Jiao et al., 2025) with slight modifications. Sour meat samples were analyzed using a texture analyzer (TA.XT-plus, Stable Micro System, UK) fitted with a P/50R probe. The parameters of the texture analyzer were set to the following: compression ratio 50%, measurement interval 5 s, trigger force 5 g, pre-test speed 2.0 mm/s, test speed 1.0 mm/s, post-test speed 1.0 mm/s. The color of sour meat was measured regarding *L** (lightness), *a** (redness), and *b** (yellowness) using a handheld spectrophotometer (YS3010, Shenzhen ThreeNH Technology Co., Ltd., China). Measurements were performed under D65 standard illumination with a 10° standard observer after calibration using black and white reference tiles.

2.6 Electronic nose analysis

For E-nose analysis, the procedure described by Dong et al. (2024) was used, with slight adjustment. Sour meat samples were analyzed using a PEN3 E-nose system (Airsense Analytics Inc., Germany), which is fitted with 10 metal oxide sensors that capture different organic vapors. Briefly, a 2 g sample was sealed in a 20 mL headspace vial and heated at 60 ℃ for 30 min, then equilibrated at room temperature for 10 min. The following analytical parameters were used: chamber flow rate and injection volume at 300 mL/min, test time of 100 s, and clean time of 100 s. Sensor signals were stabilized to baseline levels before each test.

2.7 Analysis of volatile organic compounds

VOC analysis was conducted based on the protocol described by Nie et al. (2024) with slight modifications. Briefly, after steaming and homogenization, a 2 g sample was placed into a 20-mL headspace extraction vial, followed by the addition of 20 μL of a 50 mg/L cyclohexanone internal standard (Aladdin, China). Then, samples were incubated at 60 ℃ for 30 min, and their VOCs were extracted using solid-phase microextraction (SPME) for 40 min. GC-MS analysis was performed using an Agilent 7890B-5977B system, fitted with an HP-5MS column (30 m × 0.25 mm, 0.25 μm), following a temperature program: 35 ℃ (5 min) → 3 ℃/min to 50 ℃ (3 min) → 5 ℃/min to 150 ℃ → 20 ℃/min to 250 ℃ (5 min). The identification of VOCs was performed by comparing mass spectra against the NIST 14 library, with retention indices calculated based on C7–C30 n-alkanes (Sigma, American). Furthermore, the odor activity value (OAV) was calculated as the ratio of VOC content to its corresponding odor threshold.

2.8 Statistical analysis

Each experimental procedure was repeated at least three times. Significant differences were analyzed by Duncan's multiple comparisons following a one-way ANOVA using SPSS 26.0 (International Business Machines Corp., USA). Line charts, stacked column plots, and radar charts were generated using Origin 9.8 (OriginLab Corp., USA). Bubble plots were created with a program tool (TBtools, version 0.6652). Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed with SIMCA 14.1 (Umetrics, Sweden). Redundancy analysis (RDA) was conducted with Canoco5 (Biometris-Plant Research International, Wageningen, The Netherlands).

3. Results and discussion

3.1 Enumeration of microorganisms

Figure 1 presents the changes in microbial populations throughout the sour meat fermentation process. As fermentation progressed, the LAB counts in all sample groups increased rapidly from an initial range of 6.11–7.06 lg CFU/g to 6.49–9.47 lg CFU/g by day 3, followed by a gradual decline and eventual stabilization (Fig. 1a). The decline in LAB during the subsequent fermentation stage might be attributed to the gradual depletion of nutrients in the system (Chen et al., 2015). Furthermore, as facultative anaerobes, LAB continuously consumed oxygen in the sealed jars, which might also lead to a reduction in their growth rate. Similarly, total aerobic bacteria counts in all treatment groups initially increased, then declined and gradually stabilized, with their levels remaining overall higher than those observed in the control group (Fig. 1b).

As the fermentation time extended, the counts of coagulase-positive staphylococci and Enterobacteriaceae showed an overall decreasing trend, and the counts in the treatment groups were consistently lower than those in the CK group (Fig. 1c, d). In all treatment groups, coagulase-positive staphylococci fell below the detection limit (< 10 CFU/g) on day 14 (F100), day 21 (F50, F75), and day 28 (L). The rate of decrease in Enterobacteriaceae counts was significantly faster than that in the CK group. Particularly, in the groups where LAB and flavourzyme were added together (F50, F75, F100),

Enterobacteriaceae fell below the detection limit (< 10 CFU/g) by the 3rd day of fermentation. The reduction in coagulase-positive staphylococci and Enterobacteriaceae was likely due to competition from *Lactiplantibacillus* and other beneficial bacteria during sour meat fermentation, which produced lactic acid and antimicrobial compounds that exert an inhibitory effect against harmful microorganisms (Zang et al., 2025). Moreover, the proteolysis might promote LAB growth and metabolism by providing peptides and amino acids, resulting in a synergistic antibacterial effect (Wang et al., 2019). Similarly, previous studies have reported that adding flavourzyme to fermented fish products accelerates the dominance of *Lactiplantibacillus* and inhibits harmful bacteria (Yang et al., 2020).

3.2 Analysis of pH, a_w and TBARS value

The initial pH values of all samples fell within a range of 5.47 to 5.77 (Table 1), and decreased significantly ($p < 0.05$) within 7 days of fermentation. This pH reduction was primarily driven by the bacterial production of organic acids (e.g., lactic acid) from carbohydrates. Notably, before day 7, the pH dropped significantly more rapidly in the treatment groups than in the CK group, consistent with the finding of Xu et al. (2022) that adding LAB and proteases accelerated early pH decline in fermented grass carp. The final pH of all groups reached approximately 4.0 by the late fermentation stage. A previous study reported that the growth of harmful bacteria could be effectively inhibited when the pH of fermented meat was below 4.6 (Lv et al., 2022), which aligns with the findings presented in Fig. 1 (c, d).

Table 1
pH values, a_w, and TBARS values of sour meats during the fermentation.

	Group	Fermentation time (d)					
		0	3	7	14	21	28
pH	CK	5.47 ± 0.09^{Ac}	4.97 ± 0.04^{Ba}	4.70 ± 0.04^{Ca}	4.02 ± 0.01^{Db}	3.98 ± 0.01^{Db}	3.99 ± 0.04^{Db}
	L	5.61 ± 0.13^{Abc}	4.48 ± 0.08^{Bb}	4.25 ± 0.01^{Cb}	4.07 ± 0.01^{Da}	4.01 ± 0.01^{DEa}	3.95 ± 0.01^{Ea}
	F50	5.77 ± 0.01^{Aa}	4.04 ± 0.04^{Bd}	3.90 ± 0.03^{Cc}	3.93 ± 0.01^{Cc}	3.85 ± 0.02^{Ce}	3.78 ± 0.09^{Dc}
	F75	5.73 ± 0.07^{Aab}	4.18 ± 0.12^{Bc}	3.91 ± 0.02^{Cc}	3.86 ± 0.01^{Ce}	3.89 ± 0.01^{Cc}	3.71 ± 0.01^{Dc}
	F100	5.49 ± 0.05^{Ac}	4.06 ± 0.03^{Bcd}	3.92 ± 0.01^{Cc}	3.90 ± 0.01^{Cd}	3.86 ± 0.01^{De}	3.74 ± 0.01^{Ec}
a_w	CK	0.955 ± 0.001^{Aa}	0.943 ± 0.009^{Ba}	0.932 ± 0.001^{Cb}	0.912 ± 0.010^{Da}	0.913 ± 0.001^{Ca}	0.914 ± 0.006^{Da}
	L	0.954 ± 0.001^{Aa}	0.954 ± 0.007^{Aa}	0.947 ± 0.004^{Ba}	0.918 ± 0.001^{Da}	0.904 ± 0.001^{Cb}	0.905 ± 0.001^{Ea}
	F50	0.944 ± 0.006^{Ba}	0.952 ± 0.004^{Aa}	0.946 ± 0.004^{ABa}	0.888 ± 0.002^{Eb}	0.894 ± 0.001^{Cc}	0.895 ± 0.006^{Da}
	F75	0.953 ± 0.001^{Aa}	0.938 ± 0.009^{ABa}	0.912 ± 0.003^{CDc}	0.893 ± 0.005^{Db}	0.853 ± 0.001^{BCd}	0.854 ± 0.004^{Eb}
	F100	0.950 ± 0.007^{Aa}	0.937 ± 0.016^{Ba}	0.943 ± 0.002^{ABa}	0.890 ± 0.006^{Db}	0.884 ± 0.001^{Cd}	0.885 ± 0.001^{Dab}
TBARS (mg MDA/kg meat)	CK	0.038 ± 0.002^{CDb}	0.040 ± 0.002^{Ccd}	0.048 ± 0.001^{Bb}	0.036 ± 0.002^{Db}	0.072 ± 0.002^{Ac}	0.076 ± 0.002^{Aa}
	L	0.050 ± 0.003^{Ba}	0.054 ± 0.004^{Ab}	0.044 ± 0.002^{Cc}	0.018 ± 0.002^{Fc}	0.036 ± 0.002^{De}	0.030 ± 0.002^{Ed}
	F50	0.052 ± 0.001^{Ca}	0.061 ± 0.001^{Ba}	0.041 ± 0.002^{Dd}	0.065 ± 0.001^{Aa}	0.067 ± 0.002^{Ad}	0.016 ± 0.002^{Ee}

Values are presented as mean ± standard deviation (SD).

$^{a-e}$ Values in a column with different lowercase letters are significantly different ($p < 0.05$).

$^{A-F}$ Values in a row with different uppercase letters are significantly different ($p < 0.05$).

	F75	0.033 ± 0.001^{Dc}	0.038 ± 0.002^{Cd}	0.040 ± 0.002^{Cd}	0.037 ± 0.001^{Cb}	0.076 ± 0.001^{Ab}	0.058 ± 0.002^{Bc}
	F100	0.031 ± 0.001^{Fc}	0.044 ± 0.002^{Dc}	0.057 ± 0.001^{Ca}	0.038 ± 0.002^{Eb}	0.100 ± 0.001^{Aa}	0.070 ± 0.002^{Bb}

Values are presented as mean ± standard deviation (SD).

$^{a–e}$ Values in a column with different lowercase letters are significantly different ($p < 0.05$).

$^{A–F}$ Values in a row with different uppercase letters are significantly different ($p < 0.05$).

The a_w of all groups decreased from 0.944–0.955 at 0 d to 0.854–0.914 at 28 d. From day 14 onward, the a_w in each treatment group was significantly lower than that in the CK group ($p < 0.05$). The reduction in a_w helps maintain microbial stability and product safety, which might be related to the decrease in pH value. A lower pH may promote denaturation and gelation of muscle proteins, which in turn weakens water-holding capacity of the meat products (Shang et al., 2024).

The TBARS value is a widely used index for assessing the extent of secondary lipid oxidation in meat products, and it is generally accepted that this value should not exceed 5 mg MDA/kg (Xu et al., 2022). With ongoing fermentation, the TBARS value in the CK group showed an overall upward trend, increasing from 0.038 ± 0.002 mg MDA /kg to 0.076 ± 0.002 mg MDA/kg. However, TBARS values in all treatment groups initially increased and then decreased. Specifically, the L and F50 groups reaching 0.016 ± 0.002–0.067 ± 0.002 mg MDA/kg in the late fermentation stage (21d–28 d), significantly lower compared with the CK group (0.072 ± 0.002–0.076 ± 0.002 mg MDA/kg). The decrease in TBARS values observed in the treatment groups is likely attributed to the establishment of a LAB-dominated antioxidant system, as well as to the potential degradation of aldehydes (such as malondialdehyde) or their further reactions with other compounds during the later stages of fermentation (Vougiouklaki et al., 2023). The F75 and F100 groups, however, exhibited higher TBARS values at day 21 (0.076 ± 0.001 and 0.100 ± 0.001 mg MDA/kg, respectively) and remained significantly higher than L and F50 groups at day 28, suggesting that higher flavourzyme addition was associated with increased TBARS values during fermentation. The mechanism underlying this phenomenon needs to be explored in future studies.

3.3 Textural analysis and Color measurements

The texture parameters of sour meats are presented in Table S1. During fermentation, the hardness and chewiness increased across all groups, while the springiness decreased slightly, consistent with previous reports on sour meat (Lan et al., 2025). The increase in hardness primarily resulted from low pH-induced protein denaturation and aggregation, coupled with salt penetration and moisture loss, leading to a more compact structure (Wang et al., 2020). Compared to the CK group, the treatment groups, especially those supplemented with flavourzyme, exhibited lower hardness and chewiness along with slightly higher springiness. This textural modification was likely attributed to the hydrolysis of proteins by flavourzyme.

Over the fermentation period, all groups showed increases in *L** and *a** values but a significant decrease in *b** values ($p < 0.05$), with no significant differences between groups (Table S2). The observed increase in *L** values could be associated with the protein's hydrolysis, which enhances the light-scattering and surface reflectivity of the meat (Wang et al., 2022). The rise in the *a** values likely resulted from microorganisms such as staphylococci and LAB in the system that facilitate the production of nitrosylmyoglobin, thus enhancing the red color of the meat products. Conversely, the decline in *b** values was likely associated with the reduction of oxymyoglobin resulting from microbial oxygen consumption during fermentation (Chen et al., 2021).

Overall, the combined addition of LAB and flavourzyme improved the tenderness of sour meat while exerting no significant effect on the color, maintaining its bright and red appearance.

3.4 E-nose analysis

Figure 2 presents the E-nose response profiles to sour meat. During the early fermentation stage (0–3 d), the sensors W1S, W1W, W2S and W2W exhibited increased response values, indicating the production of methane, sulfur, terpenes, alcohol and aromatic compounds in the sour meats. This result aligns with the report on beef jerky (Wen et al., 2022). As fermentation proceeded, the overall E-nose responses exhibited a trend of first increasing and then decreasing across all sour meat groups. This trend likely resulted from the variations in volatile compounds, as has been observed in fermented oat lamb sausage, where the relative content of flavor compounds showed a comparable pattern (Wang et al., 2020). In the present study, the E-nose responses peaked at day 21, followed by a marked decrease at day 28, indicating that the maximum release of volatile compounds occurred at day 21. Notably, at this stage, the F50 group showed significantly higher response values in sensors W5S, W1S, W1W, W2S and W2W than other groups. This suggests that the F50 group likely contained higher levels of volatile compounds such as alcohols and aldehydes.

3.5 Redundancy analysis

The RDA triplot revealed that lactic acid bacteria were positively associated with *L** and *a**, but negatively associated with harmful bacteria (coagulase-positive staphylococci and Enterobacteriaceae), a_w, pH and *b** (Fig. 3), suggesting their beneficial role in improving sour meat quality. Meanwhile, the fermentation process diverged into two distinct trajectories: one for the CK group and another for the four treatment groups. The treatment group samples during mid-fermentation (14 d) showed close clustering with those from the CK group during late fermentation (21–28 d), indicating an accelerated fermentation process in the treatment groups. Furthermore, in the groups added with flavourzyme, sample positions converged at 21 d and 28 d, implying that the fermentation system had reached a relatively stable state by day 21.

3.6 Analysis of volatile organic compounds

Based on the RDA results (Fig. 3) indicating that the fermentation system had stabilized by day 21 and the E-nose data showing peak volatile release at day 21 (Fig. 2), VOCs were analyzed at this time point to characterize the flavor profiles of the stabilized sour meat products. A total of 33 VOCs were detected, including 5 alkanes, 14 aldehydes, 5 alcohols, 5 esters, 1 ketone, 2 acids, and 1 other compound (Table S3). Figure 4 shows that aldehydes accounted for the highest proportion (46.77%–78.69%) of VOCs in the samples from each group, followed by alcohols (9.27%–15.40%) and esters (0.33%–24.75%). These three types of compounds together constituted the main VOCs in all samples, a finding that is consistent with previous study on sour meat (Yang et al., 2020). Aldehydes, which feature high volatility and low odor thresholds, primarily arise from lipid oxidation and the subsequent reactions of their primary products (Shao et al., 2024). Of these, hexanal is generally recognized to be indicative of lipid oxidation. At low concentrations, it imparts a pleasant grassy aroma, while at higher concentrations it exhibits undesirable rancid or oxidized oil odors (Xiao et al., 2020). The hexanal content in the F50, F75, and F100 groups (180.15 ± 6.36 μg/100 g–366.32 ± 6.02 μg/100 g) was significantly reduced compared with the CK group (1228.19 ± 11.87 μg/100 g) and L group (741.67 ± 74.95 μg/100 g) (Table S3). This suggests that the use of LAB and flavourzyme might contribute to reducing the risk of rancid off-flavor development. In fermented meat products, alcohols originate from multiple metabolic sources, particularly lipid oxidation and amino acid metabolism, and serve as key precursors for diverse aroma compounds (Zhao et al., 2022). Esters, which contribute fruity and floral aromas, primarily arise from the esterification involving alcohols and acids, and their presence can help mask certain undesirable odors (Li et al., 2025). As shown in Fig. 5, only one ester was detected in both the CK and L groups, whereas the F50, F75, and F100 groups contained 3–5 esters. Notably, the F50 group exhibited the highest proportion (24.75%) and content (391.61 ± 4.38 μg/100 g) of esters (Fig. 4, Table S3), indicating that the combined addition of LAB and flavourzyme promoted ester formation. Moreover, cluster analysis based on the flavor profiles clearly separated the samples into two distinct categories: one containing the CK and the L groups, and the other comprising the flavourzyme-supplemented groups (F50, F75, and F100) (Fig. 5). Overall, the combined addition of LAB and flavourzyme increased the diversity of VOCs and optimized their relative composition in sour meat.

Additionally, OAVs were calculated for individual VOCs based on their content and odor threshold. Compounds with an OAV ≥ 1 were regarded as significant contributors to the flavor profile of sour meat. A total of 21 VOCs with OAVs greater than 1 were identified (Table S4), and variable importance in projection (VIP) scores were analyzed using an OPLS-DA model (Fig. 6). The OPLS-DA model yielded R^2 and Q^2 of 0.976 and 0.763, suggesting good explanatory and predictive ability. Eight compounds (VIP > 1) were identified as important VOCs in sour meats, including hexanal, ethyl decanoate, (Z)-2-heptenal, 1-octen-3-ol, 1-heptanol, ethyl octanoate, nonanal, and octanal. These compounds are key components in various fermented meat products. Among them, hexanal, (Z)-2-heptenal, 1-heptanol, nonanal, octanal, and 1-octen-3-ol, all originate from lipid oxidation and impart typical odors of grass, fatty, citrus, and mushroom (Liu et al., 2023). Ethyl decanoate and ethyl octanoate, which are medium-chain fatty acid ethyl esters derived from a condensation reaction between acyl-CoA and ethanol, contribute floral and

apple-like aromas, respectively (Hu et al., 2020). In summary, these core VOCs may be responsible for the distinctive flavor of sour meat.

4. Conclusion

This study investigated the combined effects of LAB (*Lpb. plantarum* LPJ1 and *Lpb. pentosus* LPJ2) and flavourzyme on the quality characteristics of fermented sour meat. During fermentation, the pH, a_w, and *b** values decreased, while the *L** and *a** values increased in all sour meat groups; the E-nose responses, however, first increased and then decreased. All the treatment groups enhanced microbial safety by rapidly suppressing coagulase-positive staphylococci and Enterobacteriaceae to undetectable levels. Compared to the control, the F50, F75 and F100 groups accelerated the decline in pH and a_w and improved tenderness, while the L and F50 groups exhibited significantly lower TBARS values in the later fermentation stage. RDA showed a positive correlation between LAB and product quality, and also indicated an accelerated fermentation process in all treated samples. At the stabilized fermentation stage (21 d), the F50 group achieved optimal quality, characterized by the strongest E-nose response and a favorable flavor profile. Moreover, hexanal, ethyl decanoate, (Z)-2-heptenal, 1-octen-3-ol, 1-heptanol, ethyl octanoate, nonanal, and octanal were considered key VOCs that contribute to the distinctive flavor of sour meat. These findings establish the LAB-flavourzyme synergy, particularly at the F50 ratio, as an effective strategy for quality improvement of the fermented meat products.

Declarations

Ethics statement

Ethics approval was not required as this study did not involve human or animal participants.

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Author Contribution

Jing Lv: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review and editing, Funding acquisition. Yu Wang: Data curation, Formal analysis, Writing – original draft, Visualization. Jingyun Li: Data curation, Formal analysis. Min Liang: Data curation, Formal analysis. Teng Cheng: Methodology, Investigation. Ke Li: Methodology, Investigation. Wuchao Ma: Methodology, Investigation. Chaofan Tang: Investigation, Data curation. Haojia Guo: Investigation, Data curation. Yanhong Bai: Supervision, Validation, Resources.

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Data Availability

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

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Figures

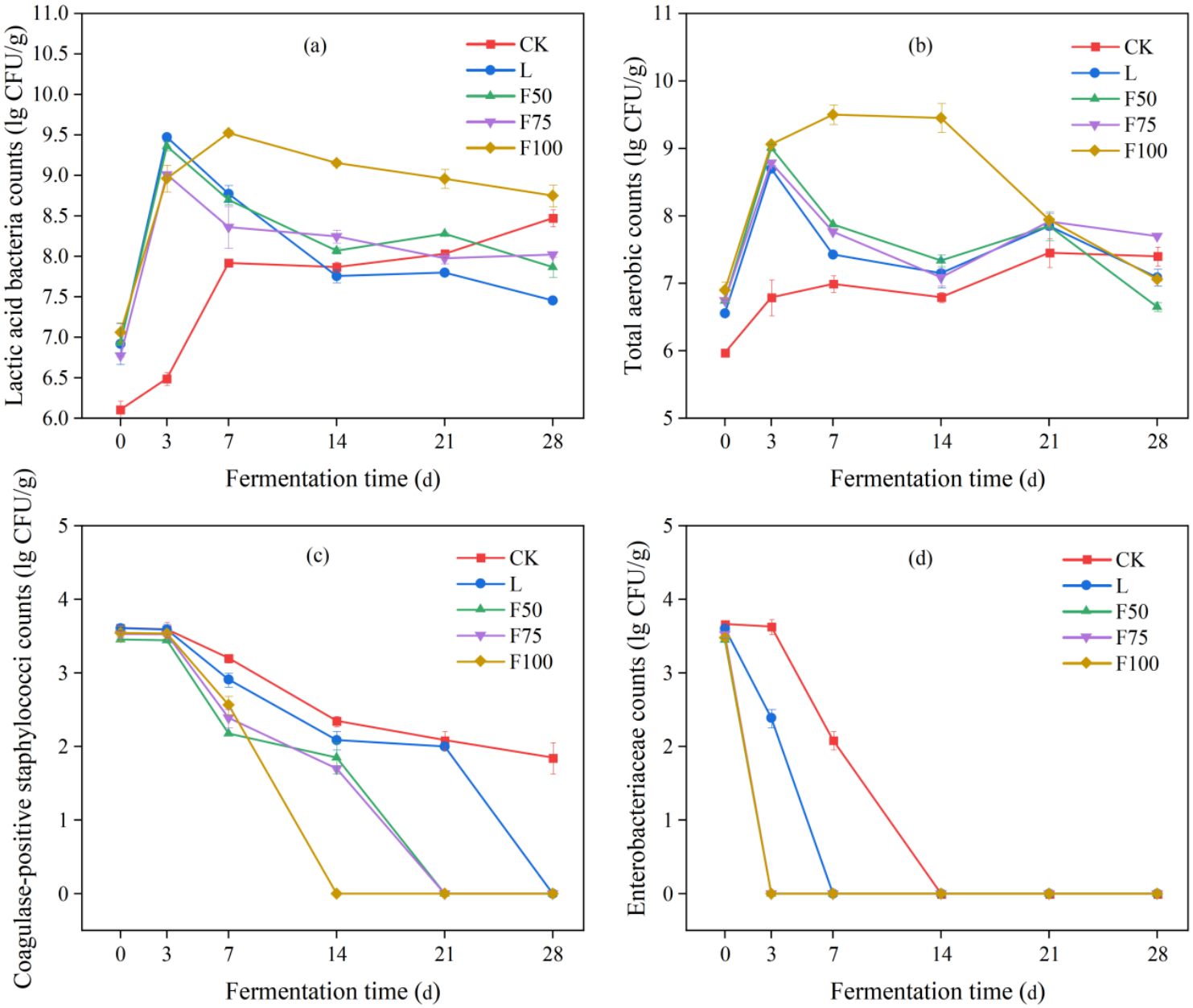


Figure 1

Changes in microbial counts during the fermentation of sour meat.

(a)

Array number	Sensors	Responsive substance
1	W1C	Aromatic compounds
2	W5S	Nitrogen oxides
3	W3C	Ammonia and aromatic compounds
4	W6S	Hydrogen
5	W5C	Hydrocarbons, aromatic compounds
6	W1S	Methane with broad range
7	W1W	Sulfur compounds, pyrazine, and terpenes
8	W2S	Alcohol with broad range
9	W2W	Aromatic components and sulfur compounds
10	W3S	Methane and other alkanes

(b)

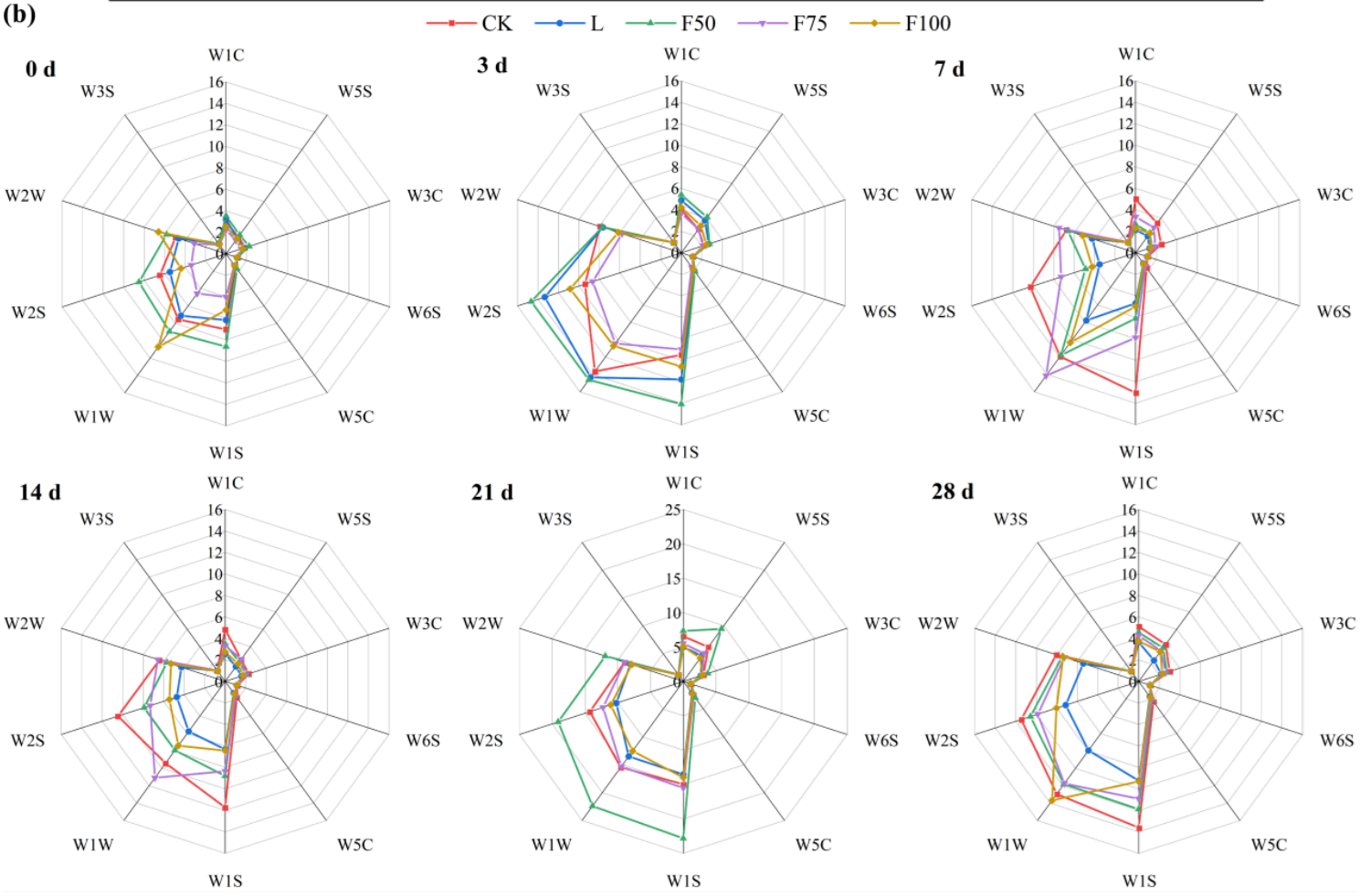


Figure 2

E-nose sensor information (a) and response profiles presented as radar charts during sour meat fermentation (b).

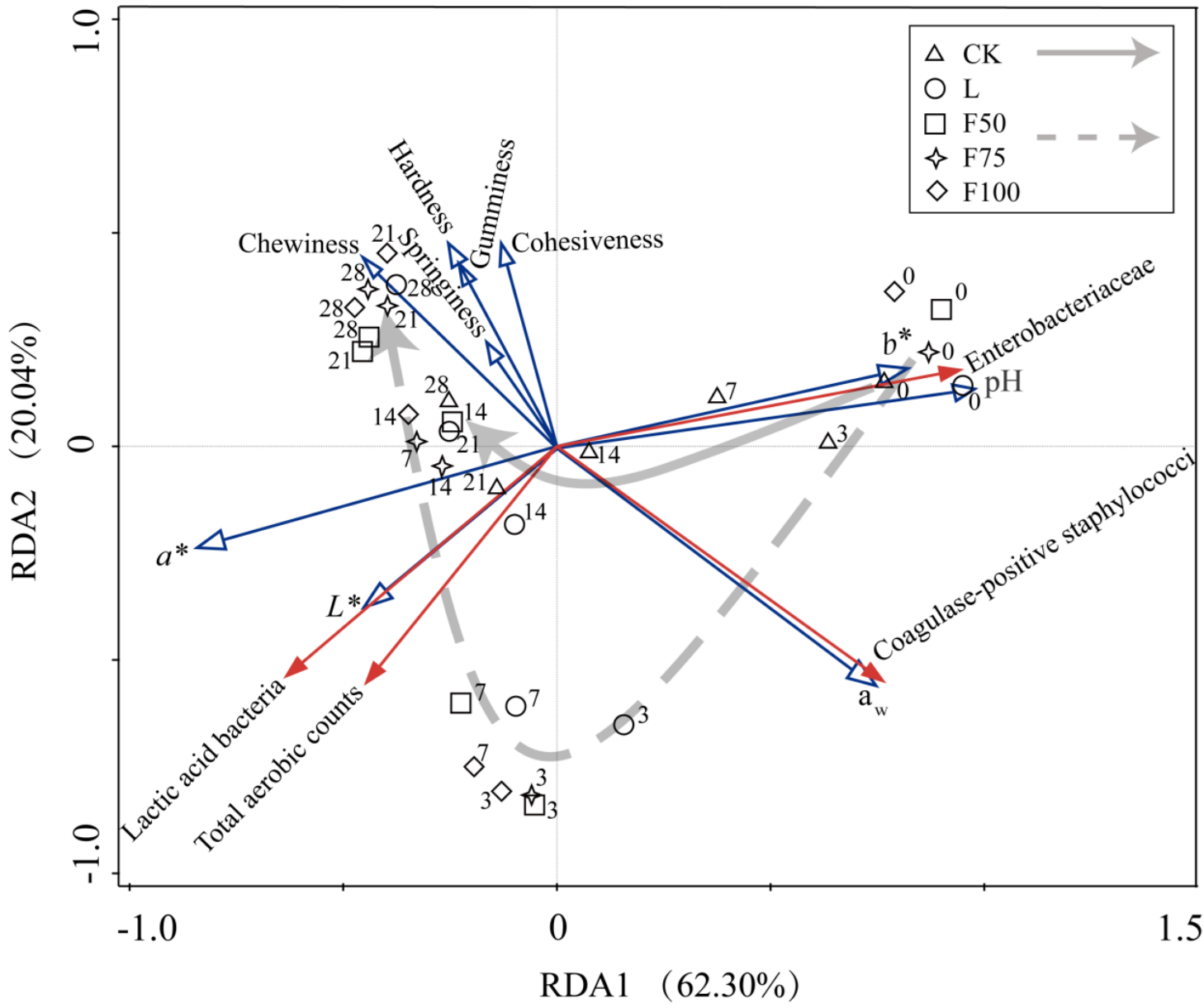


Figure 3

The RDA triplot illustrates the correlations among microbial counts, physicochemical properties, and sample clusters. Numbers beside the symbols represent the fermentation time (days). The directions and lengths of the straight arrows indicate the influence of the corresponding variables (microbial and physicochemical) on sample ordination. The curved arrows indicate the fermentation trajectories of different sample groups.

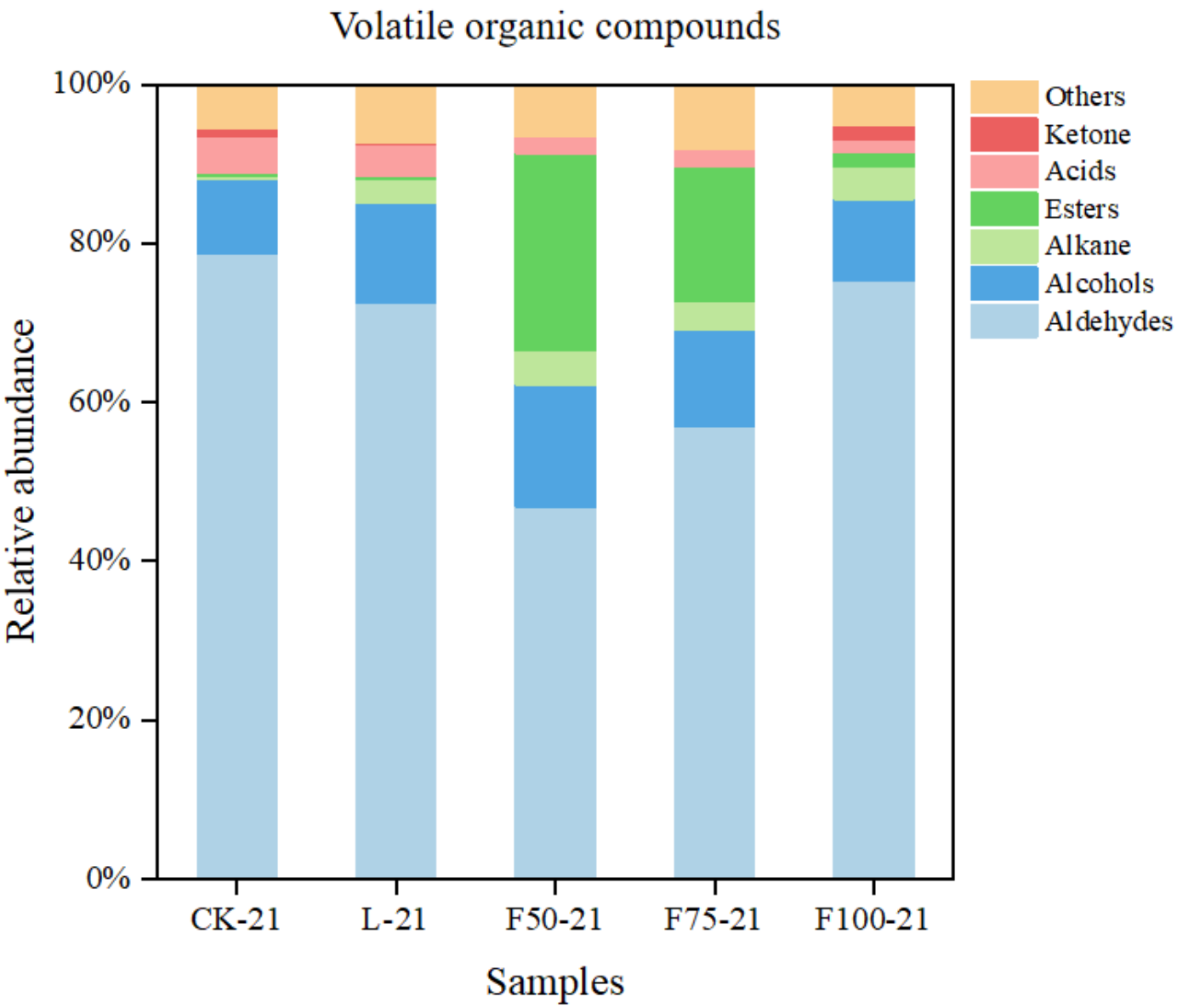


Figure 4

Relative abundance of VOCs in sour meats from different groups at 21 d.

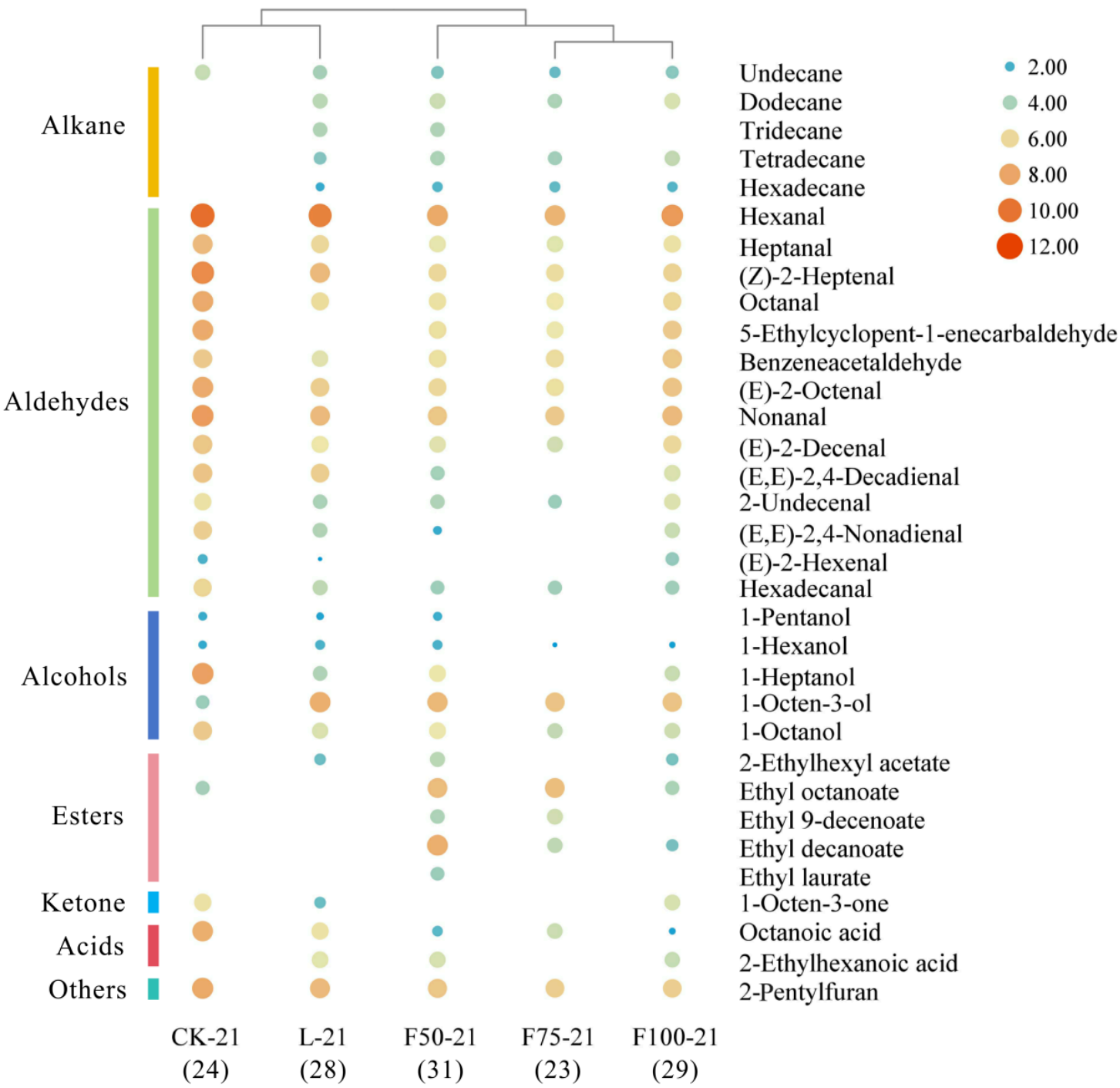


Figure 5

VOC composition in sour meats from different groups at 21 d. The clustering analysis was performed using PearsonDist distance and Complete linkage. Circle color and size correspond to the normalized mean levels, with blue/small and red/large indicating low and high values, respectively. Arabic numerals in brackets denote the number of VOCs identified in each sample.

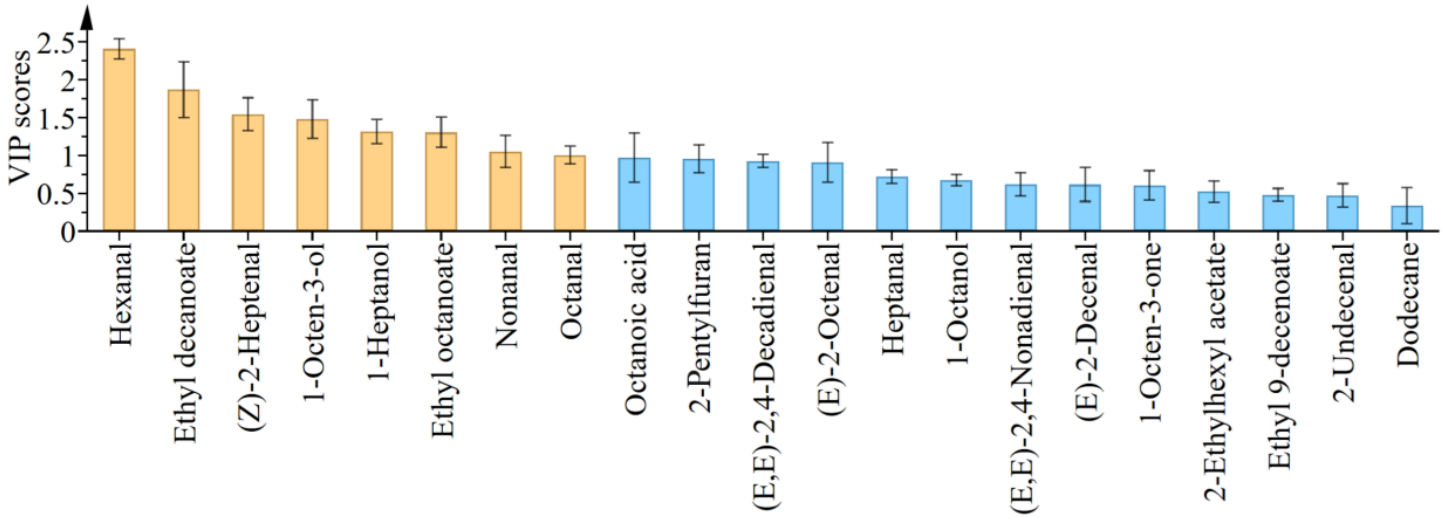


Figure 6

VIP scores of 21 VOCs (OAV>1) from an OPLS-DA model.

Supplementary Files

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