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Effect of a combination of probiotics on the flavor profiling and biogenic amines of composite fermented mutton sausages

Yana Liu, Mayinuer Mijiti, Zequan Xu, Batuer Abulikemu *

College of Food Science and Pharmacy, Xinjiang Agricultural University, Urumqi, 830052, China

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ABSTRACT

Microbial inoculants significantly influence the quality and safety of fermented sausages, making the selection of appropriate starter cultures a prominent research focus in the fermented food industry. This study investigated the impact of a combination of probiotics (*P. pentoosaceus* MJ11 and *L. pentosus* GM09) on the quality of mutton and chicken composite sausages. High performance liquid chromatography and gas chromatography-ion mobility spectrometry methods were employed to assess the degradation of biogenic amines by the starter cultures and their effect on sausage flavor. The results indicated that sausages inoculated with mixed starters exhibited a lower pH, inhibiting the growth of spoilage organisms and thus preventing the accumulation of biogenic amines during fermentation and storage. Moreover, the levels of thiobarbituric acid's reactivity was significantly lower in the mixed starters group compared to commercial and naturally fermented sausages. Additionally, the inoculation of compound starters promoted the enhancement of isoleucine and carbohydrate metabolism, thereby improving the flavor of fermented sausages. This study concluded that *P. pentoosaceus* MJ11 and *L. pentosus* GM09 are recommended as natural and desirable starter cultures to enhance both the flavor and safety of fermented sausages. This research established the feasibility and effectiveness of applying probiotics in fermented sausages.

1. Introduction

Fermenting sausages is considered one of the earliest meat preservation techniques (Ray et al., 2014). To preserve the raw meat long-term, it undergoes fermentation and drying subsequent to being stuffed into casings filled with a blend of minced meat, fat, salt, spices, and other food additives, encased in either natural or artificial casing (Hwang et al., 2023). Sausages are categorized based on water activity, with dry fermented sausages having an a_w value of less than 0.90, and semi-dry fermented sausages falling within the range of $0.90<a_w<0.95$ (Akansel et al., 2023). Dry fermented sausages have gained popularity as a result of the special taste, unrefrigerated consumption and wide shelf time (Kumar et al., 2017). Recently, there has been a diversification of raw meat used in fermented sausages beyond traditional pork, mutton, and poultry. Varieties such as rabbit or mixed meat sausages have emerged to meet the dietary preferences of consumers (Kim et al., 2014; Malekian et al., 2016). Concerns over the high fat and cholesterol content in pork have prompted interest in alternative meats like mutton and chicken (Kim et al., 2014). Mutton protein content is comparable to that of pork and beef, along with its essential amino acids and minerals (Malekian et al., 2016). Moreover, mutton fat contains comparatively lower levels of saturated fat and cholesterol, making it beneficial for the diets of elderly individuals and children (Malekian et al., 2016). Similarly, chicken contains less fat and cholesterol, along with higher production rates and lower prices, thus offset the relatively higher cost of mutton products (Kalibekkyzy et al., 2023). Therefore, incorporating mutton and chicken as mixed raw materials in fermented sausages not only broadens consumer choices but also aligns with trends towards healthier dietary options.

Traditional fermented sausages are prized for their unique flavors, which are largely influenced by microbial activity during processing. However, variations in microbial composition and environmental conditions can lead to inconsistent quality and safety concerns, resulting in substandard products in the market (Gong et al., 2024). Consequently, employing artificially inoculated starters can assist producers in effectively managing the fermentation process, thereby ensuring consistent safety and quality standards for their products (Rocchetti et al., 2023). Currently, the utilization of lactic acid bacteria (LAB), yeasts, and molds

* Corresponding author. No.311 Nongda East Road, Urumqi, Xinjiang Province, 830052, China.
E-mail address: 119890016@edu.xjau.cn (B. Abulikemu).

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as commercial starters is prevalent in dry sausages because of their importance in facilitating spontaneous fermentation (Stegmayer et al., 2023). Among these, the accumulation of acids produced by LAB during fermentation and ripening contributes to a decrease in pH, effectively inhibiting the growth of foodborne pathogens, thereby improving products safety (Xie et al., 2015). Additionally, specific LAB strains play an essential role in aroma and texture development, as well as color enhancement through acid production (El-Hadary et al., 2023). For example, lipolytic and proteolytic activities of *L. plantarum* and *L. sakei* improved sausage flavour profiles (Tian et al., 2023). Furthermore, sausages inoculated with *L. plantarum* and *L. sakei* displayed excellent capacity for nitrite reduction, effectively lowering residual nitrite levels (Chen et al., 2016). Beyond flavor and safety, some LAB strains exhibit probiotic properties, offering health benefits when consumed (Ray et al., 2014). Strains like *Lactobacillus* and *Pediococcus* have demonstrated potential as probiotics, offering antimicrobial, anti-inflammatory, anti-cancer, antioxidant, and lipid-lowering benefits (Liu et al., 2024, 2024). Among the emerging probiotic candidates, *P. pentosaceus* stood out as a promising option in the numerous established probiotics (Jiang et al., 2021). For example, *P. pentosaceus* AR243, separated from Chinese fermented foods, demonstrated a robust antioxidant properties and the ability to reduce cholesterol levels (Lin et al., 2018). *P. pentosaceus* GY23, derived from grass carp sausages, exhibited remarkable capacity for proteolysis and generated multi-amino acids for physiological nourishment or biological functions (Nie et al., 2014). The fermentation of seafood using *P. pentosaceus* FB145 and FB181 has reduced the toxicity of heavy metals (Cd) (Le & Yang, 2019). Therefore, probiotic strains were used for developing fermented meat products helping to meet the rising demand for functional foods in recent years.

Ensuring the safety of sausages is as critical as enhancing their nutritional value. Fermented sausages, rich in nutrients and diverse microbiota, are conducive to microbial decarboxylation, leading to the accumulation of biogenic amines (BA) (Wang et al., 2022). BA refer to a kind of alkaline compounds with a low molecular weight that contain nitrogen. BA, including histamine, tyramine and cadaverine, etc., frequently existed in fermented -0sausages (Jaguey-Hernandez et al., 2021). While BA play a role in normal body metabolism in small quantities, elevated levels can cause acute poisoning and even death (Torović et al., 2020). Regulatory guidelines prescribe limits for BA in food products, such as 1000 mg/kg for total BA, 100 mg/kg for histamine, and 100–800 mg/kg for tyramine (Wu et al., 2024, 2024). Since excessive BA cannot be metabolized by human body, reducing the residual BA in foods and controlling the intake are effective measures to BA prevent poisoning. Wang et al. (2024, 2024) discovered that extracts from spices could significantly inhibit the concentrations of BA, thereby decreasing their accumulation in reduced-salt dry sausages. Furthermore, Wu et al. (2024) observed a noteworthy decline in the levels of total and each of BA in fermented goat meat sausage by incorporating additives such as NaCl, $NaNO_2$, and glucose during processing. Given that BA are synthesized by microorganisms acting on free amino acids, the selection of starter cultures is paramount. Although the BA inhibitory effect of sausages inoculated with *P. pentosaceus* has been extensively studied, variability within the same species can affect their ability to degrade BA (Kantachote et al., 2016).

Based on the previous studies, *P. pentoosaceus* MJ11 and *L. pentosus* GM09, isolated from traditional Chinese dried lamb ham, were chosen as primary fermenting agents in sausage production. Physico-chemical characteristics of fermented sausages were focused on the study, particularly on aspects related to sausage safety. Therefore, the content of TBARS, nitrite and BA were evaluated during fermenting and maturing. Concurrently, GC-IMS analysis was employed to detect differences in volatile flavors of sausages in the final stage of maturation. This study aimed to develop new potential probiotic starters to produced high quality and safer fermented meat products.

2. Materials and methods

2.1. Starter cultures

The starter culture *P. pentoosaceus* MJ11 and *L. pentosus* GM09 were isolated from the air-dried lamb ham. The strains were kept in the College of Food Science and Pharmacy, Xinjiang Agricultural University. Strain was activated by anaerobic incubation in MRS medium at 30 °C for 24 h, then centrifuged thrice at 5000×*g* for 15 min, and then suspended in sterilised saline waiting to be inoculated.

2.2. Preparation of sausages

There were three groups of fermented sausages. (1) Sausages without any inoculation for natural fermentation labeled CK group. (2) Sausages inoculated with a combination of *P. pentoosaceus* MJ11 and *L. pentosus* GM09 (1:1, 10^8 CFU/g, inoculated 2.5%) for fermentation labeled GM group. (3) Sausages inoculated with commercial starter *P. pentoosaceus* CICC22227 (10^8 CFU/g, inoculated 2.5%) labeled SY group.

The following recipe was used to prepare the fermented sausages: Lamb hind leg: chicken breast: mutton fat = 2:2:1, 2.5% NaCl, 1.5% sugar, 0.015% nitrite. And the meat were mixed with the powder of black pepper, cumin and ginger (0.3%, 0.5% and 0.2%, respectively). Fermented sausage processing was carried out according to Fig. S1. Fermented samples were collected at 0, 2, 5, 9, 37–65 d for the analysis of microbial and BA. The samples on the 9th day of the maturing were used for texture, color, sensorial and volatile compound analysis.

2.3. Microbial quality analysis

The methods of microbial analysis were referred Wu, Gu, et al. (2024), with some modifications. Five g of each sample were taken from each fermented sausage under sterile conditions, mixed with 90% NaCl solution (45 mL) and homogenised at 5000×*g* for 5 min. Then, the sample was diluted by preparing serial 10-fold dilutions, where 9 mL saline diluted to 1 mL sample. Total microbial counts were conducted by culturing on Plate Count Agar, 37 °C for 48 h. The counts of LAB were conducted by culturing on Man Rogosa Sharpe Agar, 30 °C for 48 h.

2.4. Physico-chemical properties of fermented sausages

2.4.1. Moisture, pH and a_w analysis

Moisture content is a measure of the method of GB5009.3–2016.

pH was measured according to GB5009.237–2016 using a digital pH meter (pHS-3C, Shanghai Dapu Co., Shanghai, China).

The value of a_w was analyzed using water activity monitor (AKD-S1, Yangzhou Eckeride Instrument Co., Yangzhou, Jiangsu, China) after the samples were broken and spread it in a test box.

2.4.2. Thiobarbituric acid reactive (TBARS) and nitrite analysis

TBARS was measured according to Liu, Zhang, et al. (2023) with certain adjustments. Five g broken sample was mixed with 50 mL of trichloroacetic acid solution (75 g/L, including 0.1% EDTA). After vigorous shaking for 20 times, the mixture was filtered twice. The resulting supernatant (2.5 mL) was then combined with the same volume of thiobarbituric acid solution (0.01M TBA), then bathing in water at 90 °C for 30 min and subsequent cooling down to 25 °C. Subsequently, the mixture was subjected to centrifugation at a speed of 12,000×*g* for 7 min, and spectrophotometry (721, Shanghai Jinghua Technology Instrument Co., Shanghai, China) was used to detect supernatant's absorbance at 532 nm. By comparison with a standard curve generated from malondialdehyde, the TBARS content (mg/kg) was calculated.

The nitrite content of fermented sausages was detected using reagent kits (BC1495, Solarbio Co., Beijing, China).

2.4.3. Color and texture

The color of sausages was determined using method published by Hu et al. (2023). The fragmented sausages were compacted into a cylindrical shape measuring 2 cm in diameter and 1 cm in height. Subsequently, A colour meter (CM-600D, Konica, Co., Japan) was used to measure L^* (lightness), a^* (redness) and b^* (yellowness). The same sample was detected three times, and the mean values were computed.

Texture analysis was performed using the method reported in Liu, Zhang, et al. (2023). A texture analyser (TA-XT2i, Stable Micro Systems, Godalming, UK) coupled to a P/50 probe was used to perform texture profile analysis (TPA) on sausages. The sausages were then cut into 1 cm cubes. The compression ratio was 30% and the speed of test was 2 mm/s.

2.4.4. Biogenic amines analysis

The determination of biogenic amines was following the procedures described by Dias et al. (2020), with some changes. About 0.5 g–1.0 g sample was added in 5 mL 5% perchloric acid aqueous solution, then extracted with ultrasound for 30 min. Taking 1 mL of extraction solution added to 200 μL 2mol NaOH and 0.1 mL benzoyl chloride, then warmed at 40 °C for 30 min. Finally, stopped the process by adding 2 mL methanol. The solution was filtered in a 0.45-mm microfilter. Then, the filtrate was used for further machine analysis.

High performance liquid chromatography (HPLC, U3000, Thermo Fisher Scientific Co., Massachusetts, USA) was used to measure BA in sausages. The column was a C18 column. The HPLC reference conditions were as follows: UV detection was performed at a wavelength of 254 nm, column temperature was 35 °C, and the mobile phase consisted of 90% acetonitrile (A) and 10% acetonitrile (B), with 0.8 mL/min flow rate. Table S1 showed the process of gradient elution.

2.5. Volatile compounds analysis

Volatile compounds were analyzed via headspace solid-phase microextraction (HS-SPME) combined with GC-IMS (Flavourspec®, G. A.S, Germany). The 2 g sausage sample on the 9th day was added to headspace sample container, subsequently subjected to heating at 80 °C for 15 min to enhance the enrichment of volatile compounds. The reference conditions of GC-IMS could refer to Ma et al. (2023). The type of chromatographic column was FS-SE-54-CB-0.5 (15m ID:0.53 mm). The temperature in IMS ionization chamber and column were 45 °C and 60 °C, respectively. N_2 was used as the carrier gas. The drift gas flow was retained at 150 mL/min.The initial flow rate was 2 mL/min and maintained for 2 min. Then, the flow rates were 10 mL/min for 2 min, 20 mL/min for 2 min, 50 mL/min for 4 min, 80 mL/min for 2 min and 100 mL/min for 8 min. Finally, the NIST and IMS databases were used for the comparison and identification of substances.

2.6. Sensorial analysis

Fermented sausages were subjected to sensory analysis, evaluating their color, odor, chewiness, taste, and overall acceptability based on an evaluation criteria provided in Table S2 (Seleshe & Kang, 2021). Organoleptic evaluation participants comprising 10 experienced members (five men and five women) were involved in the assessment process. Prior to evaluation, all participants underwent 14 days training session focusing on the sausage sensory properties. Sausages were cut into 1 cm pieces and distributed among the assessors in a random manner. Cold water was used for rinsing their mouths between different samples.

2.7. Statistical analysis

SPSS 23.0 was used to analyse differences between treatments using one-way ANOVA with Tukey's test. Graphs were generated using GraphPad and SIMCA 14.1 software. Differences between means were considered statistically significant at 5% significance level (El-Hadary et al., 2022).

3. Results and discussion

3.1. Microbiological analysis

Fig. 1A shows the microbial growth during three stages of sausage processing (fermentation, drying and ripening, respectively). Overall bacterial counts exhibited a rapid increase in fermentation and then moderate increase in drying, followed by a gradual reduction in ripening. Especially, the bacterial counts in natural fermentation group (CK group) increased rapidly in early fermenting and consistently greater than GM and SY groups until the end of ripening. Ma et al. (2023) similarly observed that naturally fermented sausages exhibited the highest bacterial diversity, primarily originating from raw meat and the processing environment. Total bacterial counts in all groups declined alongside fermentation, influenced by environmental conditions and microbial competition (Ma et al., 2023). It also indicated that both inoculated commercial starter group and GM group showed excellent bacteriostatic effect during the fermentation process. For the counts of LAB, Fig. 1B shows a notable upward trend in the counts of LAB in each group after 2 days of fermentation, indicating favorable fermentation conditions conducive to the growth and proliferation of LAB. During the drying and ripening phases, it tended to remain stable and exhibited a slight decrease. Trends of LAB counts agreed with the studies of Wu, Gu, et al. (2024) on the process of air-dried goose. A reduction in moisture availability, pH and some other essential nutritional factors (carbohydrates, etc) could be responsible for the reduction of LAB growth observed during the end of maturing (Essid & Hassouna, 2013). Wu, Gu, et al. (2024) demonstrated that adding high levels of glucose (25 g/kg) significantly increased LAB counts compared to controls (10 g/kg) by day 14, highlighting the potential to enhance LAB quantities in industrial fermentation. After 9 days of maturing, LAB counts in CK, SY and GM group reached 7.26, 8.06 and 8.28 log CFU/g, respectively. It proposed that LAB were the dominant flora in GM group (inoculated *P. pentoosaceus* MJ11 and *L. pentosus* GM09) and it could have significant effect on the sausages characteristic.

3.2. Physico-chemical properties analysis

3.2.1. Moisture content, a_w, and pH analysis

During sausage processing, the water content and a_w in different groups decreased continuously, especially on the periods of drying and ripening (Table 1). The significant decrease in water content of sausage is related to the constant evaporation of water during the long fermenting process (Lorenzo & Franco, 2012). At the last stage of maturing, inoculated sausages had a significantly lower moisture content in comparison with the naturally fermented sausages ($p < 0.05$). It indicated that the pH of the inoculated groups (SY and GM) was closer to myoprotein isoelectric point, causing protein aggregation and low water-holding capacity. The changes of a_w were consistent with the moisture content, and a clear correlation had been demonstrated in moisture and a_w (Hu et al., 2019; Yin et al., 2021). The pH levels of three groups showed a tendency to decline and then rose during sausage processing, reaching their lowest point at the end of fermentation (2 d). At the beginning of fermentation, the sausage was properly hydrated and nutritious which was beneficial for the increases of LAB and the generation of lactic acid, causing a rapid decrease in pH. The pH of the inoculation groups were lower than that of the CK group, because LAB were the dominant strain ($p < 0.05$). A slight increase of pH value after fermentation can be attributed to alkaline ammonia produced by protein hydrolysis (Liu et al., 2024, 2024). Generally, there were no remarkable variations observed in moisture, a_w and pH values between commercial starter group (SY) and mixed starters group (GM), which agreed with findings on the trends of total bacterial and LAB counts. LAB as a dominant strain in SY and GM groups could effectively improve the quality of sausage.

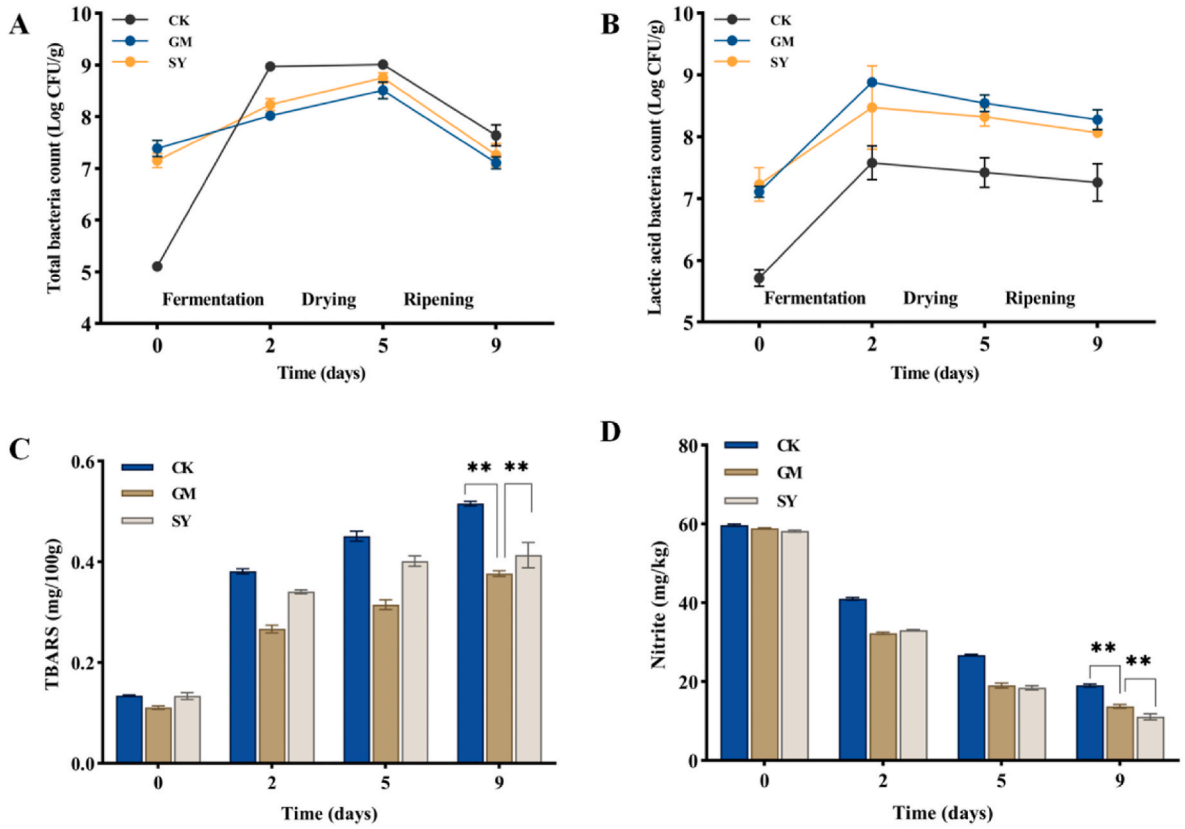

Fig. 1. Regular microbiological populations, TBARS and nitrite of fermented sausages in different groups. (A) Total bacteria count, (B) Lactic acid bacteria count, (C) The values of TBARS, (D) The values of nitrite.

Table 1
Moisture, pH and a_w content of fermented sausages during processing.

	Days	CK	SY	GM
Moisture content (%)	0	59.15 ± 0.17^{Aa}	60.02 ± 0.41^{Aa}	59.25 ± 1.12^{Aa}
	2	56.07 ± 0.09^{Ba}	54.28 ± 0.68^{Ba}	54.35 ± 1.40^{Ba}
	5	41.89 ± 0.08^{Ca}	36.15 ± 0.39^{Cb}	38.02 ± 0.55^{Cc}
	9	36.28 ± 0.22^{Da}	31.04 ± 0.66^{Db}	31.93 ± 1.02^{Db}
a_w	0	0.94 ± 0.01^{Aa}	0.94 ± 0.01^{Aa}	0.94 ± 0.002^{Aa}
	2	0.94 ± 0.004^{Aa}	0.93 ± 0.01^{Aa}	0.93 ± 0.01^{Aa}
	5	0.88 ± 0.005^{Ba}	0.85 ± 0.01^{Bb}	0.86 ± 0.003^{Bb}
	9	0.85 ± 0.002^{Ca}	0.84 ± 0.005^{Bab}	0.83 ± 0.01^{Cb}
pH	0	6.08 ± 0.05^{Aa}	6.11 ± 0.19^{Aa}	6.07 ± 0.13^{Aa}
	2	5.17 ± 0.03^{Ba}	4.81 ± 0.05^{Bb}	4.86 ± 0.17^{Bb}
	5	5.33 ± 0.07^{Ca}	4.93 ± 0.35^{Ba}	4.89 ± 0.12^{Ba}
	9	5.48 ± 0.05^{Da}	4.99 ± 0.12^{Bb}	5.11 ± 0.08^{Bb}

Values (a–c) in the same row with different letters are significantly different ($p < 0.05$). Values (A–D) in the same column with different letters are significantly different ($p < 0.05$).

Table 2
The alterations in TPA and color of fermented sausages at the end of ripening.

	CK	SY	GM
Hardness (g)	5899.66 ± 164.81^{a}	8128.16 ± 121.56^{b}	9364.27 ± 282.76^{c}
Chewiness (g)	578.65 ± 85.19^{a}	693.49 ± 65.80^{a}	883.04 ± 61.41^{b}
Springiness	0.56 ± 0.06^{a}	0.69 ± 0.09^{a}	0.66 ± 0.08^{a}
Cohesiveness	0.18 ± 0.09^{a}	0.28 ± 0.07^{a}	0.32 ± 0.13^{a}
Lightness (L^*)	32.81 ± 1.17^{a}	38.92 ± 0.63^{b}	41.19 ± 1.03^{c}
Redness (a^*)	13.17 ± 0.44^{a}	17.12 ± 1.17^{b}	18.09 ± 1.19^{b}
Yellowness (b^*)	8.16 ± 0.42^{a}	9.41 ± 1.26^{a}	9.05 ± 0.29^{a}

Values (a–c) in the same row with different letters are significantly different ($p < 0.05$).

3.2.2. TPA and color analysis

Table 2 shows the alterations in TPA (hardness, springiness, cohesiveness, chewiness) and color of fermented sausages at the end of ripening. Sausage samples inoculated with the commercial starter group (SY) and mixed starters group (GM) demonstrated significantly higher hardness values than control group on the 9th processing day ($p < 0.05$). Most studies reported that the hardness of fermented sausages was mainly influenced by the loss of moisture and the present work seemed to corroborate this viewpoint (Delgado-Pando et al., 2018; Lorenzo et al., 2014). At the end of maturity, pH reached the proteins isoelectric point, reducing the capacity of meat products to hold water, thereby increasing hardness and chewiness in fermented sausages. However, the degree of hardness and chewiness in GM group was remarkably greater than other groups. The differences may be connected with starter culture. This observation aligned with findings by Dias et al. (2020), suggesting that the introduction of a mixed starter culture enhances the textural stability of dry-cured pork belly. No differences were found among treatments in terms of springiness and cohesiveness ($p > 0.05$). Color serves as a paramount quality parameter influencing consumer acceptance (Seleshe & Kang, 2021). Table 2 shows an obvious influence of inoculated groups on the value of L^* and a^*, whereas b^* values showed no remarkable difference among groups ($p > 0.05$). Inoculated

groups exhibited higher values of redness (a^*), indicating that using fermenting agents positively affected the colouring. As indicated by our previous results, the relatively low pH and moisture content can facilitate nitroso myoglobin formation and color pigmentation, this was also concluded by Hu, Li, et al. (2022). Moreover, the low redness value is one of the sensory indicators reflecting the oxidation of meat (Seleshe & Kang, 2021). In this study, CK group showed the lowest values of redness, which was consistent with the trends of TBARS indicating that the naturally fermented sausage was oxidized.

3.2.3. TBARS and nitrite analysis

The value of TBARS serves as a crucial indicator for assessing the formation of peroxidation products, such as malondialdehyde, thereby reflecting the extent of oxidation of the lipids (Stadnik et al., 2022). Lipid peroxidation in meat products may lead to fat off-flavor, the change of color and nutrition, even harm the health (Rubén et al., 2019). The ability of LAB on scavenging free radicals and antioxidation had been verified by other studies (Stadnik et al., 2022). From Fig. 1C, TBARS values in all groups rose over time, implying that lipids undergo continuous oxidation during processing. The microbial inoculation groups (SY and GM) displayed remarkably lower TBARS values in comparsion with CK group ($p < 0.05$). Notably, GM group showed smaller TBARS values than SY group ($p < 0.05$), suggesting that starter in GM group had strong antioxidant capacity. Mei et al. (2022) observed that the participation of probiotics decreased lipid peroxidation significantly. Yu et al. (2020) suggested that sausage inoculated with *L. plantarum* and *S. simulans* exhibited good antioxidant properties because of the generation of antioxidant peptides. Typically, the acceptable range for TBARS levels in fermented sausages was considered to be between 0.6 and 2.8 mg MDA/kg (Seleshe & Kang, 2021). These results indicated that the TBARS values across all groups after maturing (from 0.37 to 0.51 mg MDA/kg) remained within this specified range.

Nitrite, as a food additive added in fermented sausages, helps to enhance meat color and flavor and has antibacterial and antioxidant effects. However, long-term ingestion of nitrites is harmful to human health, even cause cancer (Ferysiuk & Wójciak., 2020). Therefore, residual nitrite amounts in the meat product is a continuous food safety concern. During the sausages processing, the nitrite content exhibited a consistent decline in all samples, with significantly diminished levels observed in GM and SY groups than in CK group ($p < 0.05$) (Fig. 1D). Zhang et al. (2020) found that sausages inoculated with *L. curvatus* and *P. pentosaceus* exhibiting good nitrite reduction ability, because nitrite reductases generated by LAB degraded nitrite to nitrogen. Furthermore, Nitrite under acidic conditions decomposes to nitric oxide, which can bind to myoglobin and form bright red in meat products (Akansel et al., 2023). Due to the lowest pH level in SY group, the consumption of nitrite was higher than that of other groups. At the end of maturing, the concentrations of nitrite in all groups were 19.01, 13.71 and 11.07 mg/kg, all of them were below 30 mg/kg (restrictions on the residual nitrite content in China fermented meat products).

3.3. Biogenic amines analysis

BA are primarily produced by bacterial decarboxylation of free amino acids in traditional fermented foods. BA are notably prevalent in sausages because of the high-protein, diverse microorganisms, and low pH. Histamine, tyramine, putrescine and cadaverine are generally found in fermented foods (Ashaolu et al., 2023). From Fig. 2, seven BA were all detected in three groups, and cadaverine accumulation was the highest followed by putrescine, phenylethylamine, tryptamine, tyramine, spermidine and histamine.

Maximum daily dose of 600 mg tyramine and 50 mg histamine per meal is considered safe for healthy people (EFSA, 2011). Histamine and tyramine constants observed in all sausages were below the specified values. Histamine is a product of the enzyme histidine decarboxylase and is common in fish products, cheese, wine, and fermented products (Ye et al., 2021). From Fig. 2A, the content of histamine in natural fermented samples significantly increased at two days of fermentation, and reached the maximum 2.82 mg/kg on storage (65 d). It indicated that natural fermented sausages may have spoilt during storage. The

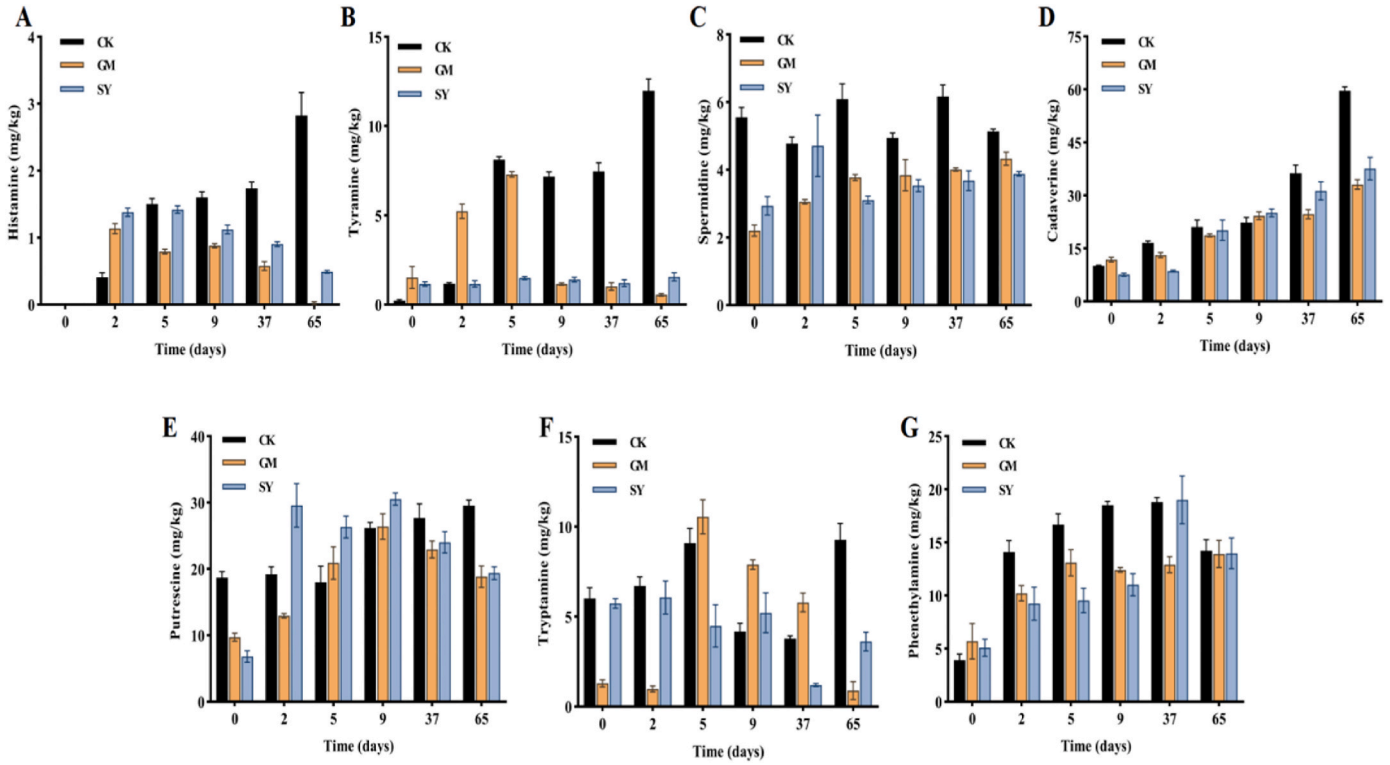

Fig. 2. The contents of biogenic amines in fermented sausages during processing and storage.

concentrations of histamine in GM and SY groups reached the maximum 1.13 and 1.42 mg/kg by day 2 and 5 respectively, and then dropped rapidly to 0.02 and 0.49 mg/kg on storage period (65 d). It was noted that the content of histamine in the GM group were consistently below the other two groups through drying, ripening and storage periods ($p < 0.05$). This suggested that *P. pentoosaceus* MJ11 and *L. pentosus* GM09 have a significant histamine reduction capacity in fermented sausages. The result agreed with Ren et al. (2022), who observed an obvious reduction in histamine levels in inoculated sausages compared to spontaneously fermented sausages. Tyramine is mainly formed by tyrosine decarboxylation. The initial tyramine concentrations in the CK group and GM group were 0.22 and 1.51 mg/kg, respectively, which subsequently increased to 8.12 and 7.29 mg/kg over 5-day fermentation period (Fig. 2B). And then the concentration in GM group dropped sharply to 0.55 mg/kg, while that of CK group increased to 11.97 mg/kg. However, the tyramine concentrations in the SY group fluctuated from 1.15 to 1.55 mg/kg. This result was similar to histamine, implying that *P. pentoosaceus* MJ11 and *L. pentosus* GM09 exhibited obvious advantages with the increase of storage period. Numerous studies have found that enzymolysis of *E. faecium* and *E. faecalis* was the major pathway to synthesize tyramine (Li et al., 2023). Consequently, a further explanation might be that mixed-strain can achieve the purpose of reducing the accumulation of tyramine via preventing the growth of germs. Zhu et al. (2020) also demonstrated a significant reduction in tyramine levels in inoculated *L. plantarum* than in uninoculated group.

Spermidine is endogenous biological amines and the concentrations rarely exceed 10 mg/kg in food products (Hu et al., 2023). The concentrations of spermidine in CK sample showed fluctuating trends during processing, and that in GM group continuously increased (Fig. 2C). Interestingly, the concentration of spermidine in the SY samples had a trend of gradual increase and then decreased. After 65 days of storage, the concentration of spermidine in the SY sausages (3.88 mg/kg) was lower than that in GM and CK sausages (4.33 and 5.13 mg/kg) significantly, which indicated that commercial starter was more effective in reduction of spermidine accumulation.

Fermented products often contain the diamines putrescine and cadaverine. Putrescine is notably abundant in fermented sausages compared to cadaverine (Fig. 2D). As time progressed and bacterial populations diversified, there was a noticeable increase in cadaverine content across all groups, reaching a peak of 3–6 times higher at day 65. The formation of diamines is likely linked to *Enterobacteria* and *Pseudomonas* presented on the outside of the sausages (Lorenzo et al., 2010). Comparatively, the cadaverine content in inoculated sausages remained markably lower than spontaneously fermented sausages at both 37 and 65 days of storage. This disparity suggested that LAB, as the predominant micro-organism, effectively suppressed the activity of the bacterial strains that produce cadaverine. A relatively high content of putrescine and cadaverine could increase the risk factor of histamine (Rabie et al., 2014). Results showed that the content of putrescine in CK group rose slowly in 5 d, and then grew rapidly (Fig. 2E). The putrescine content in GM and SY groups increased as processing time, and decreased with increase in storage time. At 65 d, no significant difference of putrescine were observed between SY and GM groups ($p > 0.05$). Van Ba et al. (2016) observed a positive relationship between the putrescine and pH. The sausages inoculated with *P. pentosaceus* and *S.carnosus* showed the minimum pH with the minimum putrescine concentration. It is belived that high acidity could inhibit putrescine bacteria growth (e.g., amino genic endogenous bacteria) in fermented sausages. Similarly, Lu et al. (2010) found the content of putrescine reduced by 30% in dry sausages inoculated with *P. pentosaceus*.

Enterococcus species are known producers of aromatic amines such as tryptamine and phenylethylamine (Benli et al., 2024). Except for SY groups, tryptamine levels decreased in all samples by day 9 of production and fluctuated during storage (Fig. 2F). By the end of the storage period (65 d), the GM group exhibited a notable decrease in tryptamine levels than other groups, likely due to bacterial degradation, as a nitrogen source. The decarboxylation of tryptamine, dependent on pyridoxal-phosphate, can be noncompetitively inhibited by tyrosine and phenylalanine (Suvajdžić et al., 2020). Regulations regarding tryptamine content typically fall within the extent of 5–27 mg/kg (Liu et al., 2023, 2023, 2023). Both inoculated groups maintained levels below 6 mg/kg throughout storage, comfortably within the specified limit. Non-specific tyrosine decarboxylase activity has been associated with the presence of phenylethylamine, and was simultaneous with accumulation of tyramine. Therefore, when tyramine is present, phenylethylamine is always at a higher level (Fan et al., 2015).

Phenylethylamine levels (Fig. 2G) initially increased at the end of processing and early storage, followed by a subsequent decrease in all groups during late storage period (65 d). However, phenylethylamine levels at the last stage of storage were similar between treatments, ranging from 13.91 to 14.21 mg/kg. The finding revealed that the commercial starter could decrease the rate of phenylethylamine formation during processing, while the inhibiting ability in all starters decreased over storage time.

Generally, different starter cultures can significantly influence the growth and activity of spontaneous microflora, thereby affecting the accumulation of BA throughout processing and storage. As anticipated, better outcomes (lower BA) were observed in the starter of *P. pentoosaceus* MJ11 and *L. pentosus* GM09 in comparison with commercial starter and natural fermentation. The two starter cultures exhibited quite different BA-elimination abilities. *P. pentoosaceus* MJ11 and *L. pentosus* GM09 had a significant inhibition on tryptamine, tyramine, cadaverine, putrescine, and histamine, while commercial starter performed better in reducing spermidine contents. This suggested that the observed reduction in BA in inoculated sausages could be attributed to the enzymatic degradation of BA by these strains. Furthermore, competitive inhibition of BA-producing bacteria may also play a role in reducing BA formation (Wang et al., 2024, 2024). Consequently, the mechanism of BA production or degradation by *P. pentoosaceus* MJ11 and *L. pentosus* GM09 in fermented sausages needs further investigation in the future.

3.4. Flavor compounds analysis

Volatile flavor compounds were identified using GC-IMS across three groups. In all, 49 compounds were identified, containing 12 aldehydes, 11 alcohols, 9 ketones, 6 acids, 4 hydrocarbon, 3 esters and 4 other compounds (Table S3 and Fig. 3C). Many of these compounds were generated through processes such as the degradation of carbohydrate and amino acid, as well as esterification and lipid oxidation (Hu et al., 2022, 2022b). To visually explore the diversity of sausage flavor, PCA was analyzed in Fig. 3A. Two principal components (PC1 and PC2) occupied 77.02% and 12.73% of the variances, respectively. A clear differentiation emerged between CK group and inoculated groups. The only group on the right of the x-axis was CK, with GM and SY on the negative side. The profiles of volatile flavors in the GM and SY groups partly overlapped, suggesting similarities in their aromatic characteristics. Fig. 3B shows the changes in seven kinds of flavor compounds of sausages. Aldehydes were the most common volatile compounds. Compared with natural fermented sausage, the contents of aldehydes, acids and alcohols remarkably increased in commercial starter group and mixed starter group, while the content of ketones and esters decreased. These results suggested that inoculated starters effectively enhance the flavor profiles of sausage products.

Aldehydes with low odor thresholds significantly influenced the flavor profile of fermented meat, primarily due to their production through lipid auto-oxidation. A variety of unsaturated aldehydes and saturated aldehydes were observed in this study. Among them, straight-chain aldehydes such as nonanal, hexanal, and heptanal originated from linoleic acid breakdown, while branched-chain aldehydes like 2-methylbutanal and 3-methylbutanal were derived from amino acid metabolism (Xing et al., 2023). Results showed that 2-methylbutanal, pentanal,

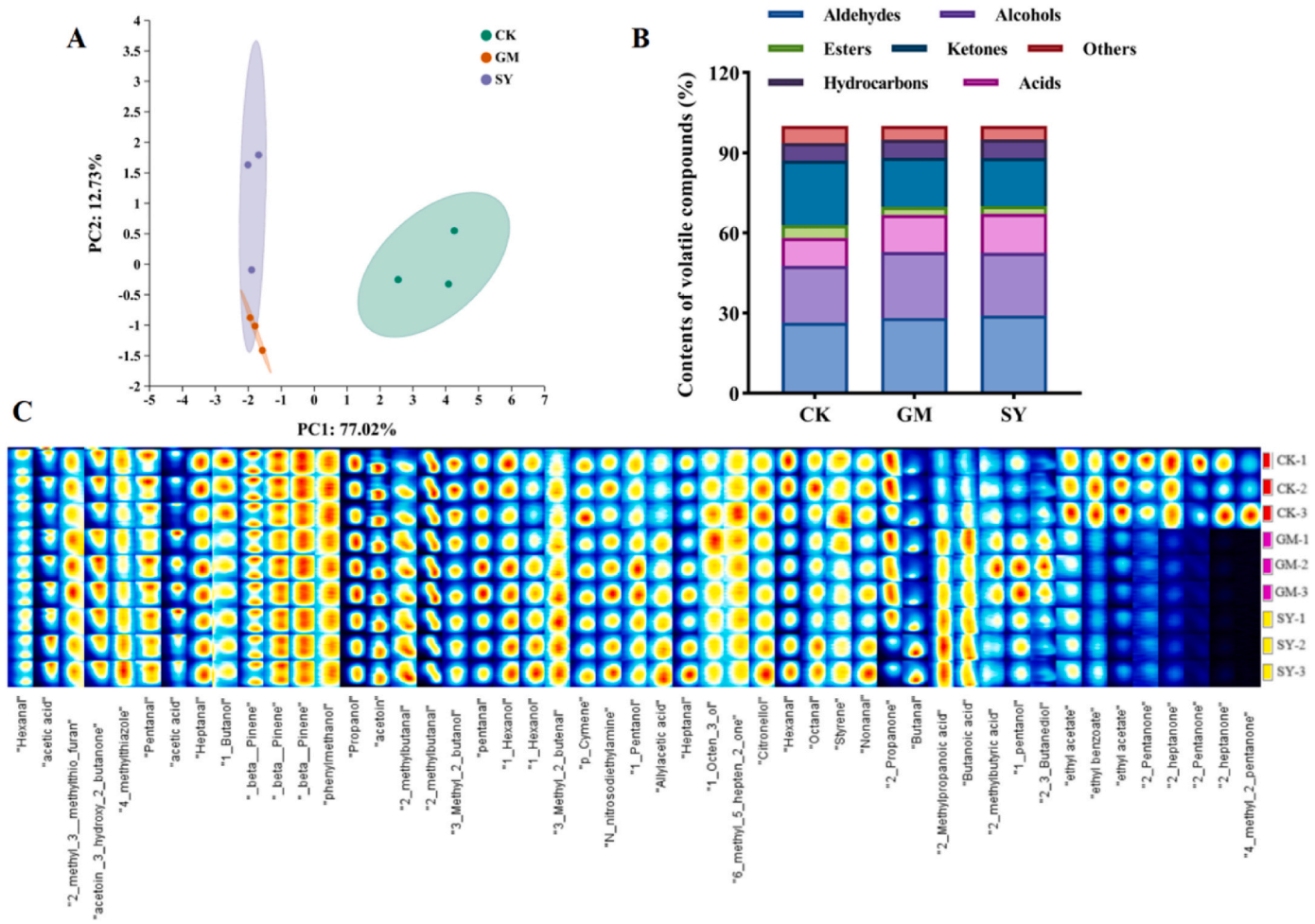

Fig. 3. (A) Principal Component Analysis (PCA) plots of volatile compounds for fermented sausages. (B) The relative level of total ester, acid, alcohol, ketone, aldehydes, hydrocarbon and other compounds in fermented sausages. (C) Gallery plot of spontaneously fermented sausages.

butanal and hexanal were the most common aldehydes found in sausages. Among them, the level of 2-methylbutanal was highest in all sausage groups, indicating that remarkable free amino acid degradation happened in fermented sausages. It was found to help develop fruity flavours in fermented sausages (Li et al., 2022, 2022). Hexanal, pentanal, and butanal, commonly used as indicators of lipid oxidation, imparted grassy, citrusy, and fatty notes to the samples (Murtaza et al., 2014). In the three sausage samples, no significant difference in the aldehyde content was observed, suggesting that no excessive fat oxidation was observed in sausages.

Alcohols, characterized by mild odors, constituted the second major category of flavor in samples. Apart from the oxidative decomposition of fat and amino acid, the metabolism of methyl ketones and carbohydrate also contributed to alcohol production in fermented meat products. Unsaturated alcohols (fruity and sweet) have great contributions on the odor of sausages because of their low odor thresholds, while saturated alcohols have little contributions and can be further oxidized to aldehydes or acids (Du et al., 2018). The concentration of propanol was notably the highest in the GM group, likely attributable to a higher LAB count. Tang et al. (2022) found that propanol had positive correlation with LAB in Baijiu fermentation. LAB could metabolize carbohydrates to produce ethanol or propanol by heterofermentation during fermentation (Gao et al., 2024). Fermentation with inoculation significantly improved the concentrations of most unsaturated alcohols in sausages, such as 2-3-butanediol, 1-pentanol, 1-octen-3-ol and 1-hexanol. Notably, 2, 3-butanediol, derived from carbohydrate metabolism, exhibited significantly higher levels in the GM group compared to other groups (Hu et al., 2022, 2022b). Additionally, 1-octen-3-ol was found in three types of sausages, which was from the oxidation of linoleic acid with strong mushroom aroma (Li et al., 2022, 2022). Furthermore, citronellol derives primarily from ginger added to the sausages.

6 acids were found in fermented sausages and they were all short-chain acids (C < 6) with a related low odor threshold. Of these, acetic acid (including monomers and dimers) were the greatest acids, primarily produced by LAB metabolism and contributed to the sour and cheesy aromas (Shao et al., 2024). The level of acetic acid in inoculated samples were remarkably higher compared to that of the nature fermentation sample ($p < 0.05$), as evidenced by the changes in the LAB values above. It was noted that 2-methylbutyric acid, which originated from isoleucine catabolism by LAB, was also greater in GM group than in CK and SY groups. It indicated that inoculated *P. pentoosaceus* MJ11 and *L. pentosus* GM09 in sausages contributed to the formation of sour and cheesy odors, aligning with the outcomes of Zhao et al. (2022).

Similarly, ketones are involved in the degradation of amino acid and microbial metabolism, representing a third category flavor compounds in sausages. The contents of 2-pentanone and 2-heptanone, from the fatty acids oxidation, exhibited an obvious reduction in the inoculated groups compared to naturally fermented group. Comparatively, the level of methyl ketones and 3-hydroxy-2-butanone obviously increased in GM and SY groups. 4-Methylthiazole was regarded as a precursor of fatty aromatic compound in meat products (Alim et al., 2019), which was most abundant in SY group. 3-Hydroxy-2-butanone, commonly found in sausages and primarily originating from carbohydrate metabolism by LAB, remained significantly higher in the GM and SY groups compared

to the CK group. This compound contributes to the rich sausage aromas characterized by creaminess and sweetness (Zhao et al., 2022). Additionally, 3-hydroxy-2-butanone can further be oxidized to 2,3-butanediol, which was also observed in the highest level in GM group.

Three esters were identified in fermented sausages, with ethyl acetate being the predominant ester that notably decreased in the inoculated groups. Esters typically arise from esterification between alcohols and organic acids, imparting floral and fruity notes. (Hu et al., 2020). Results showed that ethyl acetate levels were highest in the uninoculated group, likely due to minimal accumulation of acetic acid, suggesting that acetic acid was primarily utilized in ethyl acetate formation. Besides above volatile compounds, some hydrocarbons and other compounds were identified by GC-MS. Some of them were terpenes such as β-pinene, which largely came from the seasoning of black pepper (Liu et al., 2023, 2023, 2023). Heterocyclic compounds, such as 2-methyl-3--methylthio-furan was the richest in GM group, which was produced by Maillard reaction and was described as a typical meat flavor in sausages (Adams et al., 2011).

To better understand the flavor distinctions among CK, GM and SY sausages and identify characteristic flavor compounds, OPLS-DA analysis was employed (Fig. S2A). Fig. S2B demonstrates that the OPLS-DA models were rigorously validated using 200 random permutations, confirming their reliability and validity. Following OPLS-DA analysis, 31 volatile metabolites with VIP ≥1 were identified as characteristic volatile compounds for following analysis. These compounds were depicted in Fig. 4A and B, where heat maps and biplots from correlation analysis illustrated how different starters influence these compounds. As shown in Fig. 4A, there were two categories of the three groups of sausages, with CK one cluster and GM and SY the other. In Fig. 4B, samples in CK group were aggregated in quadrant IV, presenting high levels of 2-heptanone, 2-pentanone, ethyl acetate and 4-methyl-2-pentanone. Quadrant II primarily contained GM samples, exhibiting characteristic odors such as propanol, 2-3-butanediol and 2-methylbutyric acid, featuring characteristic flavor substances like nonanal, 1-butanol, butanal, and 4-methylthiazole, are mainly distributed in quadrant III. Alfaia et al. (2018) suggested that generating more volatile compounds was one of the choice criterion of commercial culture starters in fermented foods. Consequently, the SY group displayed higher levels of aldehydes with lower detection thresholds compared to other groups. However, the characteristic volatile compounds identified in the GM group primarily stem from the carbohydrates and amino acids metabolism by LAB, contributing to distinct aromas in fermented sausages. This highlighted the varied contributions of different starter cultures to flavor profiles (Gong et al., 2023), underscoring the potential for achieving desired flavors through optimal strain selection.

3.5. Sensory analysis

Sensory evaluation is essential for assessing the overall acceptability of a product. The sensory attribute scores of sausages were depicted in Fig. 4C. Generally, the sensory characteristics of inoculated groups differed significantly from those of naturally fermented sausages, particularly in terms of odor, color, and taste. Minor differences were observed between SY and GM samples in sensory qualities. The GM group had the highest odor score, possibly attributed to its lowest TBARS value. Liu, Zhang, et al. (2023) reported that TBARS value exhibited a strong positive correlation with the odor evaluation, the panelists could perceive the unpleasant taste. Color and chewiness scores matched the outcomes in Table 2. Overall, our findings indicate that the use of inoculated starter cultures positively influenced the odor, color, taste, and texture properties of fermented sausages.

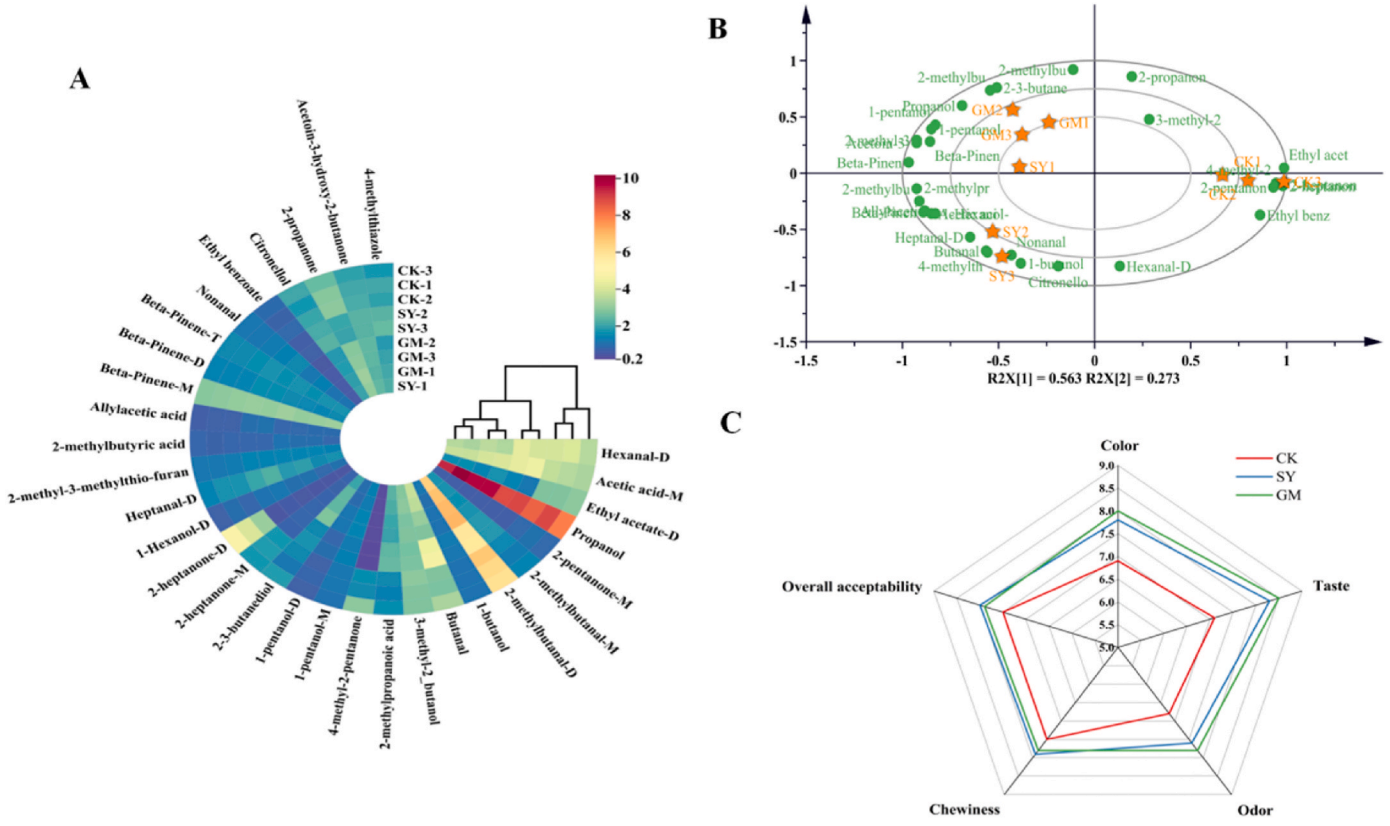

Fig. 4. (A) Heat map of clustering of flavor substances in different groups of fermented sausages. (B) Biplot of correlation analysis and different variance of volatile compounds in fermented sausage samples. Green circles represent characteristic volatile substances, and yellow color represents fermented sausage groups, (C) Sensory evaluation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusion

The present study demonstrated that sausages inoculated with a combination of probiotics (*P. pentoosaceus* MJ11 and *L. pentosus* GM09) exhibited slight differences in sensory quality compared to those inoculated with commercial starters. However, they exhibited a significant advantage in safety evaluation. The mixed strains (*P. pentoosaceus* MJ11 and *L. pentosus* GM09), dominant during sausage fermentation, created a more acidic environment that inhibited spoilage microorganisms. Remarkably, during storage, these sausages showed a significant reduction in histamine, tyramine, cadaverine, and tryptamine, which are highly toxic to humans. Moreover, inoculation with these mixed starters reduced levels of malonaldehyde and nitrite in sausages, thereby reducing fat peroxidation and enhancing product safety. Sausages fermented with *P. pentoosaceus* MJ11 and *L. pentosus* GM09 exhibited metabolic pathways associated with isoleucine and carbohydrate catabolism, contributing to the formation of sour and cheesy flavors. These findings suggested that these mixed starters are ideal for enhancing product quality and reducing BA in fermented sausages. Further elucidating the pathways through which microbes degrade biogenic amines using integrated omics techniques is necessary. Revealing the metabolic regulatory mechanisms of strains during fermentation could optimize sausage processing, enhance safety and quality, and improve the market competitiveness of fermented sausages.

CRediT authorship contribution statement

Yana Liu: Writing – original draft. **Mayinuer Mijiti:** Methodology, Investigation. **Zequan Xu:** Methodology. **Batuer Abulikemu:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104835.

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