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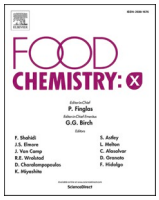

Effects of sorbitol-mediated curing on the physicochemical properties and bacterial community composition of loin ham during fermentation and ripening stages

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ABSTRACT

In this study, the impacts of loin ham with sorbitol-mediated curing on its physicochemical properties and bacterial community composition during fermentation and ripening were investigated. The salt content, pH, and water activity (a_w) were lower in the sorbitol group than in the control group throughout the fermentation and ripening stages ($P < 0.05$). In addition, the L* values were higher in the sorbitol group ($P < 0.05$). Additionally, microbial diversity diminished in all groups as the fermentation and ripening process proceeded, with *Lactobacillus* turning into the dominant genus in the control group and *Staphylococcus* and *Lactobacillus* becoming dominant in the sorbitol group. Pearson's correlation analysis confirmed that the physicochemical properties have been significantly correlated with the bacterial community. In conclusion, sorbitol-mediated curing not only facilitates salt reduction while prolonging the storage period of loin ham, but also improves the distribution of bacterial community in loin ham and enhances its quality.

1. Introduction

Traditional dry-cured fermented meat is generally prepared by rubbing high amounts amount of salt, spices, and sugar onto the surface of the meat product to cure it, and then naturally fermentation for a long time with the action of endogenous enzymes and beneficial microorganisms to form a meat product with unique flavor, color, and texture, as well as a long shelf life (Vidal, Bernardinelli, Paglarini, Sabadini, & Pollonio, 2019). The addition of large amounts of salt and moderate concentrations of starter cultures is essential not only to the texture and flavor of dry-cured meat but also to inhibit the growth of spoilage microorganisms, allowing to extend its shelf life and maintain its taste (Zhou, Pan, Cao, & Zhou, 2021; Zhou et al., 2022). However, this high salt content may induce oxidative stress in the body resulting in several chronic diseases (Mariutti & Bragagnolo, 2017), whilst excessive sodium intake directly increases the risk of cardiovascular disease (He, Tan, Ma, & MacGregor, 2020), which is extremely detrimental to human health.

Mediated curing (MC) is a new method for salt reduction in dry-cured meat products. MC refers to the systematic construction of an exogenous food additive as a medium to achieve a low sodium curing strategy for meat products by influencing the osmotic diffusion pathway of salt and the water migration in the matrix without reducing the amount of salt (Gong et al., 2022). The behavior of being able to change the osmotic diffusion of salt by mechanical means such as ultra-high pressure, ultrasound, tumbling, and electrical stimulation is termed physical mediation, while the behavior of changing the osmotic rate of salt and water migration by adding some chemical substance in the curing process is referred to as exogenous substance-mediated behavior, which is a chemical mediation. These differ from the traditional curing methods, as the salt does not diffuse freely.

Polyhydroxy alcohols can be applied as a curing medium in chemical MC due to their structure with multiple hydroxyl groups. These can bind to proteins in meat products and increase the polarity of certain groups in muscle proteins, converting some of the free water in myogenic fibers into bound water. This contributes to a change in the water distribution of the product and reduces water activity (a_w) (Liu et al., 2022). Furthermore, research suggests that polyhydroxy alcohols have an antibacterial effect, effectively inhibiting the growth of harmful

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microorganisms (Syafiq, Sapuan, Zuhri, Othman, & Ilyas, 2022). Sorbitol is a polyhydroxy alcohol, containing 6 hydroxyl groups, that can be hydrogen bonded with the hydroxyl groups in water, increasing water holding capacity (WHC), improving texture, decreasing a_w, and prolonging the storage life of meat products (Martins, Sentanin, & De Souza, 2019). Additionally, it can influence the microbial and enzymatic activity in the product, affecting protein and fat degradation, flavor formation, while also having antibacterial activity (Liu et al., 2022; Chai, Chen, He, Jiao, Cai, Dong, Liu, & Ren, 2022). There are few reports on the application of polyhydroxy alcohols for salt reduction in dry-cured fermented meat products. In this article, sorbitol, one of the polyhydroxy alcohols, was used as a medium to mediate curing.

Moderate salt reduction seems to affect microbial growth and lead to changes in physicochemical properties of fermented meat products. For instance, Gan, Zhao, Li, Tu, and Wang (2021) reported that during low-salt Chinese bacon processing the pH gradually decreased, *Lactobacillus* became the dominant genus, and the higher the KCl ratio, more rapid was the process. A study by Chen et al. (2019b) used KCl and selected amino acids to replace 30 % NaCl (w/w) in Harbin dry-cured sausages and analyzed the quality and microbial diversity of sausages. Results showed that the replacement salt did not negatively influence the physical properties of sausages, and the microbial diversity decreased during the fermentation of low-salt sausages. *Staphylococcus* and *Lactobacillus* became the dominant genera and the highest relative abundance of *Staphylococcus* at the end of fermentation. To the best of our knowledge, there is a lack of reports on the effects of salt reduction with sorbitol on the bacterial community of dry-cured fermented meat products. Hence, the aim of this research was to investigate the influence of sorbitol-mediated curing on the bacterial community of loin ham using a high-throughput sequencing technique. Moreover, the correlation between bacterial communities and physicochemical properties in loin ham was assessed to further explain how the quality of loin ham is affected by sorbitol in mediated curing.

2. Material and methods

2.1. Material

The pork loin was purchased from Huimin Fresh Supermarket in Huaxi District, Guiyang. The main components of the pigs' feed were corn, sorghum, and soybeans. After feeding a diet of about 250 kg and rearing for>365 days, the pigs are slaughtered (Tainong Xingwang Food Co., ltd.). Food-grade NaCl, spices, sugar, and glucose were purchased from Wal-Mart supermarket in Huaxi District, Guiyang. Food-grade sorbitol was purchased from Shandong Tianli Pharmaceutical Co., and the enteric coating was purchased from Yu Mu Group. The other chemicals and reagents were purchased from Aladdin (Shanghai, China).

2.2. Starter culture preparation

Lactobacillus plantarum SJ-4 (strain conservation number: CICC No. 11119 s) and *Staphylococcus simulans* QB7 (strain conservation number: CICC No. 11117 s). In a previous study conducted by our group, *L. plantarum* SJ-4 isolated from Chinese traditional fermented meats (i.e. Guizhou Jinping sour meat); a CNS strain, *S. simulans* QB7, was isolated in one of the Chinese dry-cured fermented sausages (i.e. Qianwufu). These two strains showed high proteolytic activity in degrading pork meat proteins. They were preserved by China Industrial Microbial Strain Conservation Management Center.

Man Rogosa Sharpe (MRS) broth medium was used to activate SJ-4, and Mannitol Salt (MSA) broth medium was used to activate QB7. After four generations of activation, the strains were washed thrice with 0.85 % (w/v) saline solution and resuspended until their concentration was adjusted to 10^9 CFU/mL for subsequent preparation of fermented loin ham.

2.3. Process of fermented loin ham

The preparation of fermented loin ham was performed as described by Chen et al. (2021) and Boumaiza, Najjari, Jaballah, Boudabous, and Ouzari (2021) with some modifications. The pork loin was cut equally into small square pieces of about 100 g (n = 24). The formulation of the curing ingredients was based on the mass of raw meat (w/w) with 3 % NaCl, 3 % food grade sorbitol (no addition in the control group), 0.3 % five spice powder, 0.3 % white pepper powder, 0.3 % pepper powder, 0.5 % white sugar and 0.5 % glucose, which were uniformly rubbed on the surface of the loin meat and left to cure at 4 °C for 24 h, to allow the spices mixture to be homogeneously distributed into the meat. The amounts of added salt and sorbitol, as well as the curing time, were determined through the physicochemical properties of loin ham during curing period, such as pH, salt content, cooking loss, centrifugal loss, and a_w. The activated SJ-4 and QB7 bacterial suspensions were inoculated into the loin at a ratio of 1:1 (10^7 CFU/g), and after vacuum tumbling for 30 min, the loin was stuffed into 45 mm diameter collagen casings and immediately suspended in the fermentation cabinet with constant temperature and humidity. The loin ham was fermented at 28 °C and 90 % relative humidity (RH) for the first 2 days, after which the fermentation cabinet was set to 15 °C and 80 % RH for ripening of the loin ham, for 20 days to the end. The finished loin ham was obtained at 22 days of processing. Samples, each with three parallel groups were taken on days 0, 2 (end of fermentation), 10 (mid- ripening), and 20 (end of ripening).

2.4. Determination of physicochemical properties

Minced meat sample (1 g) was weighed and diluted 10 times with ultrapure water and homogenized for 1 min at 2800 r/min using XHF-D homogenizer (Ningbo Xinzhi Biotechnology Co., Zhejiang, China), and the pH value was measured using digital pH meter (PHS-3C, Shanghai Yueping Scientific Instruments Co., China), while the NaCl content was determined using digital salinity meter (ES-421, ATAGO, Tokyo, Japan) and expressed as g/100 g meat.

Regarding a_w, 5 g of minced meat was weighed, spread evenly in a small petri dish and measured using an a_w measuring instrument (Huake HD-4B, Wuxi, China). The a_w instrument was calibrated using saturated NaCl and saturated magnesium chloride solutions.

Loin ham sample (3–5 g) was cut into cubes (1 × 1 × 1 cm^3) and weighed as M1, then put into a steaming bag and sealed. Afterwards, the sample was put in an 80 ℃ water bath and heated for 20 min. The surface of the meat sample was blotted dry with absorbent paper and weighed as M2. The cooking loss was expressed as percentage and determined as seen in the equation below:

$$\text{Cooking loss} = \frac{M1 - M2}{M1} \times 100\ \%$$

The convenient computerized colorimeter (NH350 Agilent, Shenzhen Sanenshi Technology Co., China) was used for color measurement. Loin ham samples were cut into slices of uniform thickness and their brightness (L*), redness (a*), and yellowness (b*) were measured.

2.5. Bacterial counts by a culture-dependent method

After removing the loin ham from the casings in the ultra-clean bench, the spices of the surface were stripped off, and samples (10 g) were taken from the center of the loin ham and added to sterile homogenization bags, then 90 mL of sterile saline solution (0.85 % (w/v) NaCl) was added, sealed, and homogenized by tapping (12.0/s, 5 min) (YM-08X, Shanghai Yuming Instruments Co., China). The homogenized liquid was diluted and coated on the culture media. Lactic acid bacteria (LAB) and total aerobic bacteria (TACs) were counted on MRS and Plate Count Agar (PCA) respectively after 36 h incubation at 37 ℃, while *Staphylococcus* were counted on MSA plates after 48 h incubation at 37

℃.

2.6. Bacterial diversity by a culture-independent method

The genomic DNA of the samples was extracted using a DNA extraction kit according to the manufacturers' instructions (D6356-F-96-SH), followed by the quantification of DNA using agarose gel electrophoresis and NanoDrop2000. Genomic DNA was used as the template, and PCR was performed using specific primers with barcode and Takara's Tks Gflex DNA Polymerase according to the selection of sequencing regions to ensure amplification efficiency and accuracy. Bacterial diversity was identified by analyzing the V3-V4 hypervariable regions of the 16S rRNA gene, which were amplified using the primers 343F and 798R, forward primer: 5′-TACGGRAGGCAGCAG-3′ and reverse primer: 5′-AGGGTATCTAATCCT-3′ (Nossa, 2010).

A two-step cycle PCR method was used for amplification (Zhang et al., 2021). Finally, aliquots were mixed according to PCR product concentrations, and 16S rRNA in the purified mixed samples was analyzed by high-throughput sequencing using the Illumina Novaseq6000, PE250 platform (Oebiotechnology Co. Ltd., Shanghai, China).

2.7. Bioinformatic analysis

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using cutadapt software to detect and cut off the adapter. After trimming, paired-end reads were filtering low-quality sequences, denoised, merged, and detect and cut off the chimera reads using DADA2 with the default parameters of QIIME2 (2020.11). At last, the software output the representative reads and the ASV abundance table.

The alpha diversity of the samples was calculated using QIIME, including the richness index (ASVs, ACE and Chao1), the diversity index (Shannon and Simpson), and the Coverage index. Stacked histograms, Circos, and clustered heatmaps of dominant genera among samples were visualized by R software.

2.8. Statistical analysis

Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, with differences being considered significant for *P*-values below 0.05. All data were analyzed using SPSS 17 software (SPSS, Chicago, IL, United States) and GraphPad Prism 8 software (GraphPad Software Inc., California, USA), with results being expressed as mean ± mean standard error (S.E.M). Pearson's correlation coefficient analysis between physicochemical properties and microorganisms was performed using origin software 2021 (OriginLab Corporation, MA, USA).

3. Results and discussion

3.1. pH and salt content

As expected, throughout the fermentation and ripening stages, the pH decreased from 5.28 and 5.26 to 4.95 and 4.31 in the control and sorbitol groups, respectively (Fig. 1A). The pH of the loin ham decreased rapidly during fermentation in both groups. During the ripening period, the pH slowly increased in the control group and slightly decreased in the sorbitol group until the end of ripening. The addition of *Lactobacillus* leads to the production of acid in meat products, resulting in a rapid pH decline. This fast acidification successfully inhibits the growth of microorganisms which cause the spoilage of food, being vital to enhance the quality and security of fermented meat products (Hu et al., 2022). In this study, the differences in pH were not significant during the fermentation period between the control and sorbitol groups ($P > 0.05$). However, at the cease of ripening period, the sorbitol group had a significantly lower pH than the control group ($P < 0.05$), which can be a result of the long-term oxidation of sorbitol in meat products, converting it into sorbic acid, and thus, resulting in lower pH (Leitmannová, Malá, & Červený, 2009).

Regarding the salt content, as seen in Fig. 1B, it rose constantly throughout the fermentation and ripening of loin ham. The salt content reached 6.34 g/100 g and 5.32 g/100 g in the control and sorbitol groups at the end of ripening, respectively. The increase in salt content may have been a result of salts' infiltration and consequent decrease in the moisture content of the samples. The salt content of the sorbitol group was significantly lower than the control group in both fermentation and ripening stages ($P < 0.05$), implying that the presence of sorbitol affected the pathway of salt permeation diffusion and water

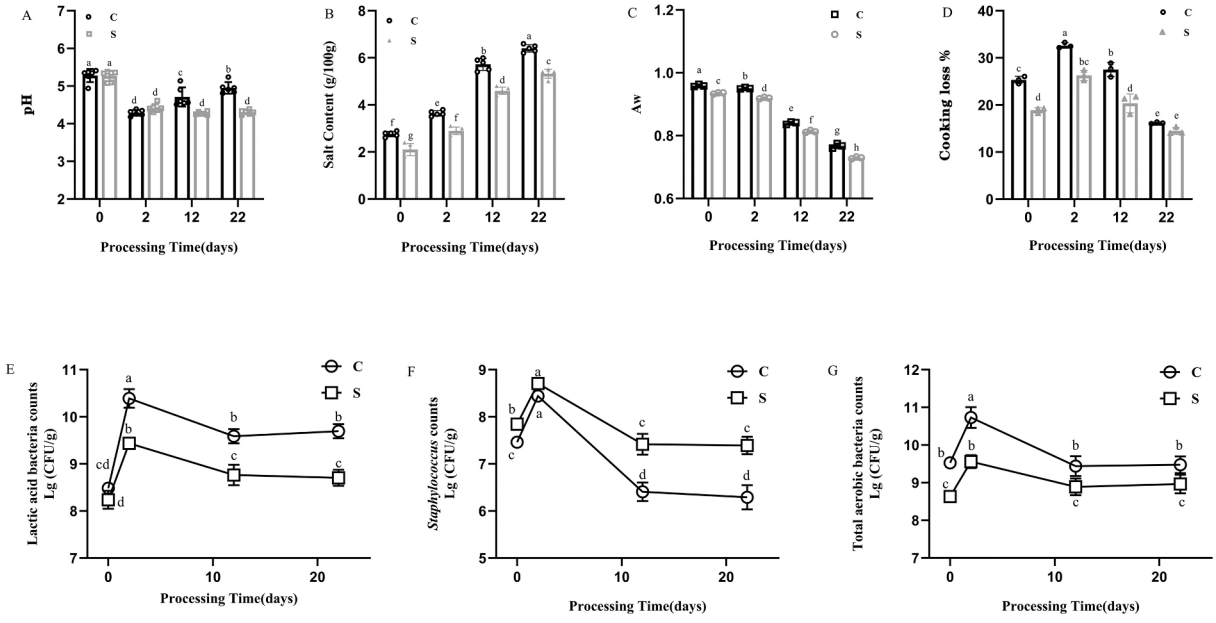

Fig. 1. Effects of sorbitol-mediated curing on pH (A), salt content (B), aw (C), WHC (D), lactic acid bacteria (E), *Staphylococcus* (F), and total aerobic bacteria (G) during fermentation and ripening of loin ham in different groups. C: control group, S: sorbitol group. Different lowercase letters (a-h) mean significant difference among different groups at different fermentation and ripening times ($P < 0.05$).

migration in the meat products. This could have happened due to sorbitol remaining on the cell surface due to its excessive molecular mass and high viscosity, and thus, diffusing slower than salt. As a result, ensuing in a higher extracellular osmotic pressure ensued, which created a high viscosity barrier of sorbitol on the product surface ("barrier effect"), forming a solute film that hindered the diffusion of sodium chloride, hence reducing the salt content (Sharma, Banipal, & Banipal, 2020; Gong et al., 2022). Another possible justification is the fact that the oxhydryl of the sorbitol is bonded to the oxhydryl of water in the matrix in the form of hydrogen bonds, and this interaction accelerated the diffusion rate of sorbitol, while slowing down the free diffusion of water. The quantity of water molecules interacting with NaCl is reduced, and consequently the amount of Na^+ coming into the cell is also reduced, which leads to a decrease in the salt content of the whole matrix (Chen, Zhang, Hemar, Li, & Zhou, 2020).

3.2. A_w and WHC

The a_w showed a reducing trend during the fermentation and ripening of loin ham (Fig. 1C). At the end of ripening, a_w decreased from an initial 0.965 and 0.935 to 0.764 and 0.729 in the control and sorbitol groups, respectively ($P < 0.05$). The a_w was significantly decreased in the sorbitol group compared with the control group during the fermentation and ripening stages ($P < 0.05$). This may be a result of sorbitol being a humectant with multiple oxhydryl in its molecular structure which, as previously mentioned, can bind to proteins in meat products and then extend the polarity of certain groups in muscle proteins, converting some of the free water in myogenic fibers into bound water, which would reduce the free water content (Meena & Kishore, 2021). This consequently leads to a change in the moisture distribution of the product and reduces a_w. These results stand in line with Liu et al. (2022) findings, in which they discovered that sorbitol significantly reduced a_w in minced pork tenderloin. The reduction of a_w inhibits microbial activities and chemical reactions, reducing the spoilage of food and prolonging its shelf life.

As shown in Fig. 1D, the cooking loss of the sorbitol group was significantly lower than that of the control group at days of 0 and 2 fermentation, and day 10 of ripening ($P < 0.05$), because sorbitol is a humectant and has the effect of increasing WHC in meat products. For instance, Fahrizal (2018) found that the addition of sorbitol, sucrose, and sodium tripolyphosphate enhanced the WHC of freshwater fish surimi. However, there was not a significant distinction in cooking loss between the control and sorbitol groups at the end of ripening ($P > 0.05$), due to the normal evaporation of moisture during the ripening stage, which happens to lead to a low water content at the end of the ripening stage.

3.3. Color analysis

It has been reported that the color of meat products depends primarily on protein composition, namely myoglobin content, as well as protein denaturation, moisture content, pH and fat content (Mancini & Hunt, 2005). As shown in Table 1, L* gradually decreased, while a* and b* increased in all groups as fermentation and ripening progressed ($P < 0.05$), which is consistent with the findings of Chen et al. (2019b). The decrease in L* may be attributed to water loss in meat products as the brightness is susceptible to moisture content, and the lower the moisture content, the lower the L* (Chai et al., 2022). The L* was consistently higher in the sorbitol group than in the control group ($P < 0.05$) during the fermentation and ripening stages, due to sorbitol containing six oxhydryl, which combine with the oxhydryl of water in the meat product in the form of hydrogen bonds to enhance its WHC.

The a* of all groups were progressively enhanced during the ripening period, which may be related to the formation of nitrosomyoglobin via bacterial action. This occurs mostly due to the NO_2^{-3} present being reduced to NO^{-2}, and the decomposition of NO^{-2} to NO to combine with myoglobin results in the formation of nitrosomyoglobin, giving the meat products a vibrant red color (Huang et al., 2020). In addition, the a* in the control group was consistently higher than in the sorbitol group during the ripening period ($P < 0.05$), which may be related to the presence of LAB and the increase in pH. The number of LAB in the control group was significantly higher than in the sorbitol group ($P < 0.05$), and studies have shown that some LAB can promote the formation of zinc protoporphyrin IX (ZnPP), a substance that can be used to enhance a* of fermented meat products (Kauser-Ul-Alam, Hayakawa, Kumura, & Wakamatsu, 2021). Furthermore, the amount of ZnPP formed in the presence of acid with pH > 4.75 was significantly increased (Wakamatsu, Kawazoe, Ohya, Hayakawa, & Kumura, 2020), so low pH in the sorbitol group may result in a lower a* than in the control group.

The increase in b* may be due to the presence of a yellow pigment. This pigment is a result of products of lipid oxidation with amines in phospholipid head groups or amines in proteins (Liu, Wang, Zhang, Wang, & Kong, 2019). The b* of the control group was higher than that of the sorbitol group during the ripening period ($P < 0.05$), which was likely due to the ability of sorbitol to retard lipid oxidation, reduce lipid oxidation-related products.

3.4. Bacterial counts

The numbers of LAB, *Staphylococcus* and TACs increased exponentially ($P < 0.05$) in the control and sorbitol groups during the fermentation period, with their highest levels being registered at the end of this process. Additionally, the numbers of LAB and *Staphylococcus* increased rapidly due to their adaptableness to the sarcomatrix (Fig. 1E and F), but the amounts of LAB, *Staphylococcus* and TACs decreased significantly in the ripening stage in contrast to the fermentation stage ($P < 0.05$). Since the conditions of the fermentation period present the optimal temperature and humidity for microbial growth, the microorganisms rapidly consume the nitrogen and carbon sources in loin ham, achieving rapid growth. However, the temperature and humidity at the ripening stage are not ideal for microbial growth, and, as plenty of the nitrogen, carbon and water sources in loin ham have already been consumed, the total number of bacteria was significantly lower than in the fermentation

Table 1
Color changes in the different groups of loin ham during fermentation and ripening.

Color	Groups	Fermentation time (d)		Ripening time (d)	
		0	2	10	20
L*-value	C	48.27 ± 0.97^{b}	42.65 ± 0.75^{c}	35.48 ± 0.57^{e}	33.68 ± 0.92^{e}
	S	50.94 ± 0.83^{a}	48.55 ± 1.26^{ab}	41.13 ± 1.43^{c}	38.62 ± 0.88^{d}
a*-value	C	9.41 ± 0.30^{d}	13.10 ± 0.40^{bc}	16.37 ± 0.77^{a}	17.08 ± 0.59^{a}
	S	9.34 ± 0.20^{d}	11.89 ± 0.26^{c}	13.38 ± 0.78^{b}	13.94 ± 0.58^{b}
b*-value	C	1.93 ± 0.26^{ef}	2.99 ± 0.38^{cd}	4.55 ± 0.22^{ab}	5.14 ± 0.36^{a}
	S	1.45 ± 0.14^{f}	2.50 ± 0.29^{de}	3.33 ± 0.29^{c}	4.13 ± 0.21^{b}

Note: C: control group, S: sorbitol group. Expressed as mean ± mean standard error (S.E.M). Different lowercase letters (a-f) mean significant difference among different groups at different fermentation and ripening times ($P < 0.05$).

stage. This is in accordance with studies by Chen et al. (2021b), who reported that sausages inoculated with *Lactobacillus* and *Staphylococcus xylosus* as fermenters, led to an exponential growth of *Lactobacillus* and *Staphylococcus* during the fermentation period, whilst the numbers of LAB and *Staphylococcus* decreased slightly during the ripening period.

From the end of fermentation to the end of ripening, the number of LAB in the control group was significantly higher than in the sorbitol group ($P < 0.05$), while the number of *Staphylococcus* was considerably lower than in the sorbitol group ($P < 0.05$). This may be due to sorbitol-mediated curing inhibiting the growth of LAB and promoting the growth of *Staphylococcus*. Moreover, TACs still showed higher numbers in loin ham, which indicated that other microorganisms also grew well in the meat products inoculated with fermenters. TACs were significantly higher in the control group than in the sorbitol group during the fermentation and ripening period ($P < 0.05$) (Fig. 1G), indicating that sorbitol-mediated curing can inhibit the growth of TACs.

3.5. Alpha diversity of the bacterial community during the fermentation and ripening of loin ham

In total, 1,777,040 high-quality and valid sequences were collected across all 24 samples of the fermented loin ham, among which 891,256 and 885,784 valid reads were obtained in the control and sorbitol groups, respectively. The alpha diversity index, ASVs richness, and sample coverage of bacteria are shown in Table 2. As can be seen from the table, all samples had a good coverage (>99.9 %), indicating that the sequencing depth had largely covered all species in the loin ham samples. The ASVs, ACE and Chao1 indexes are generally used to indicate the richness of bacterial communities, while the Shannon and Simpson indexes usually reflect the species diversity of bacterial communities. These values showed a downward trend during the fermentation and ripening period, indicating that the abundance and diversity of microorganisms gradually decreased as fermentation and ripening proceeded.

Table 2
Alpha diversity indexes in samples of loin ham.

Sample	ASVs	Species abundance index		Species diversity index		Coverage
		ACE	Chao1	Simpson	Shannon	
C0	260.33 ± 64.71^{a}	261.04 ± 65.65^{a}	260.95 ± 64.16^{a}	0.98 ± 0.01^{a}	6.72 ± 0.62^{a}	99.994 % ± 0.00003^{ab}
C2	120.00 ± 19.87^{bc}	120.60 ± 19.99^{bc}	119.99 ± 19.89^{bc}	0.56 ± 0.04^{bc}	1.96 ± 0.91^{cd}	99.999 % ± 0.00001^{a}
C10	153.00 ± 69.78^{bc}	154.15 ± 70.37^{bc}	153.02 ± 69.89^{bc}	0.42 ± 0.03^{bc}	1.45 ± 0.09^{cd}	99.998 % ± 0.00001^{a}
C20	83.33 ± 9.03^{c}	84.03 ± 12.7^{c}	83.50 ± 8.97^{c}	0.37 ± 0.04^{c}	1.10 ± 0.20^{d}	99.996 % ± 0.00001^{a}
S0	186.00 ± 39.00^{ab}	187.71 ± 39.45^{ab}	186.09 ± 38.91^{ab}	0.86 ± 0.03^{a}	4.53 ± 0.41^{b}	99.996 % ± 0.00001^{b}
S2	124.33 ± 24.23^{bc}	125.16 ± 24.71^{bc}	124.62 ± 24.34^{bc}	0.62 ± 0.15^{b}	2.37 ± 0.74^{c}	99.997 % ± 0.00001^{a}
S10	102.67 ± 22.53^{bc}	103.26 ± 21.91^{bc}	102.65 ± 22.59^{bc}	0.59 ± 0.16^{b}	2.14 ± 0.79^{cd}	99.999 % ± 0.00000^{a}
S20	89.00 ± 18.38^{bc}	88.89 ± 17.13^{bc}	88.94 ± 18.36^{bc}	0.62 ± 0.04^{b}	1.94 ± 0.11^{cd}	99.999 % ± 0.00001^{a}

Note: expressed as mean ± mean standard error (S.E.M). Different lowercase letters (a-d) in the same column indicate significant differences ($P < 0.05$). C0: control group fermented for 0 day; *C2*: control group fermented for 2 days; C10: control group ripened for 10 days; C20: control group ripened for 20 days; S0: sorbitol group fermented for 0 day; S2: sorbitol group fermented for 2 days; S10: sorbitol group ripened for 10 days; S20: sorbitol group ripened for 20 days.

Furthermore, Simpson index was significantly higher in the sorbitol group than in the control group by the end of the ripening process, implying that the microbial diversity was higher in the sorbitol group when compared with the control group ($P < 0.05$). This result indicates that sorbitol-mediated curing can increase the microbial community diversity. Moreover, there were no significant differences among the groups in the observed ASVs, ACE and Chao1 indexes at the end of ripening ($P > 0.05$). As the samples were fermented by inoculation with fermenters, it is plausible that a subset of bacteria became dominant in the loin ham samples (Zhang, Zhang, Zhou, Wang, & Li, 2021).

3.6. Bacterial community composition during the fermentation and ripening of loin ham

The relative abundance and association heatmap of bacterial communities at the phylum level along with their species phylogenetic trees and ASV abundance maps are presented in Fig. 2. As seen in these results, regardless of the stage of the process, most of the bacterial communities in the loin ham belonged to three phyla: Firmicutes, Bacteroidota and Proteobacteria (Fig. 2C). However, the percentages of phylum distribution changed during the fermentation and ripening period, as well as the bacterial community relative abundance in the loin ham. The bacterial community diversity was the most abundant in the samples fermented at day 0. Firmicutes, Bacteroidota and Proteobacteria were observed in the C0 group, accounting for 39.39 %, 36.62 %, and 19.22 % of the whole sequences, respectively; and in the S0 group, Firmicutes, Bacteroidota and Proteobacteria, accounting for 42.75 %, 16.20 % and 21.64 % of the whole sequences, respectively (Fig. 2A). With increasing fermentation and ripening times, the relative abundance of Firmicutes increased in all groups and was significantly higher than other phyla ($P < 0.05$). For instance, in the control and sorbitol groups it increased from 39.39 % and 42.75 % at the initial stages of fermentation to 98.94 % and 98.58 % at end of ripening (Fig. 2A). On the other hand, the relative abundance of Bacteroidota and Proteobacteria gradually decreased (Fig. 2B). This indicates that Firmicutes dominated the loin ham samples from fermentation to the end of ripening, which is consistent with the findings of Gan et al. (2021). One reason for the dominance of Firmicutes is that these can produce budding spores, which can resist dehydration and extreme environments. Another reason is that Firmicutes contains both the *Lactobacillus* and *Staphylococcus* genera, which is consistent with the results on the relative abundance of bacterial communities at the genus level.

The relative abundance, correlation heatmap, and Circos figure of bacterial communities at the genus level are shown in Fig. 3. It is clear that *Lactobacillus*, *Staphylococcus*, *Muribaculaceae*, *Ralstonia*, *Prevotellaceae_UCG-001*, *Bacteroides*, and *Lachnospiraceae_NK4A136_group* in the day 0 of fermentation were all groups present in high relative abundance, with *Lactobacillus* being the most abundant in the C0 and S0 groups with 15.72 % and 28.58 %, respectively. At the end of fermentation and ripening stages the relative abundance of the genera *Muribaculaceae*, *Ralstonia*, *Prevotellaceae_UCG-001*, *Bacteroides*, *Lachnospiraceae_NK4A136_group*, *Sphingomonas*, *Prevotella*, *Alloprevotella*, *Alistipes*, *Faecalibacterium*, *Acinetobacter*, *Clostridia_UCG-014* and *Escherichia-Shigella* decreased rapidly (Fig. 3A). These microorganisms are associated with spoilage, including *Acinetobacter*, *Clostridia_UCG-014* and *Escherichia-Shigella* and were all inhibited in loin hams inoculated with fermenters. These bacteria are considered spoilage factors in meat as they lead to the manufacturing of undesirable metabolites and off-flavor compounds (Zhu, Wang, Zhang, Li, Zhang, Ji, Zhao, Zhang, & Chen, 2022). Controlling spoilage flora is an effective way to improve the quality of meat products. In this study, the amount of *Lactobacillus* is higher than that of *Staphylococcus*, and the large growth of *Lactobacillus* can inhibit the proliferation of other pathogenic microorganisms, which has a positive effect on the safety of low-salt ham. As fermentation and ripening proceed, the numbers of other microorganisms decreased while *Lactobacillus* and *Staphylococcus* gradually increased and became the

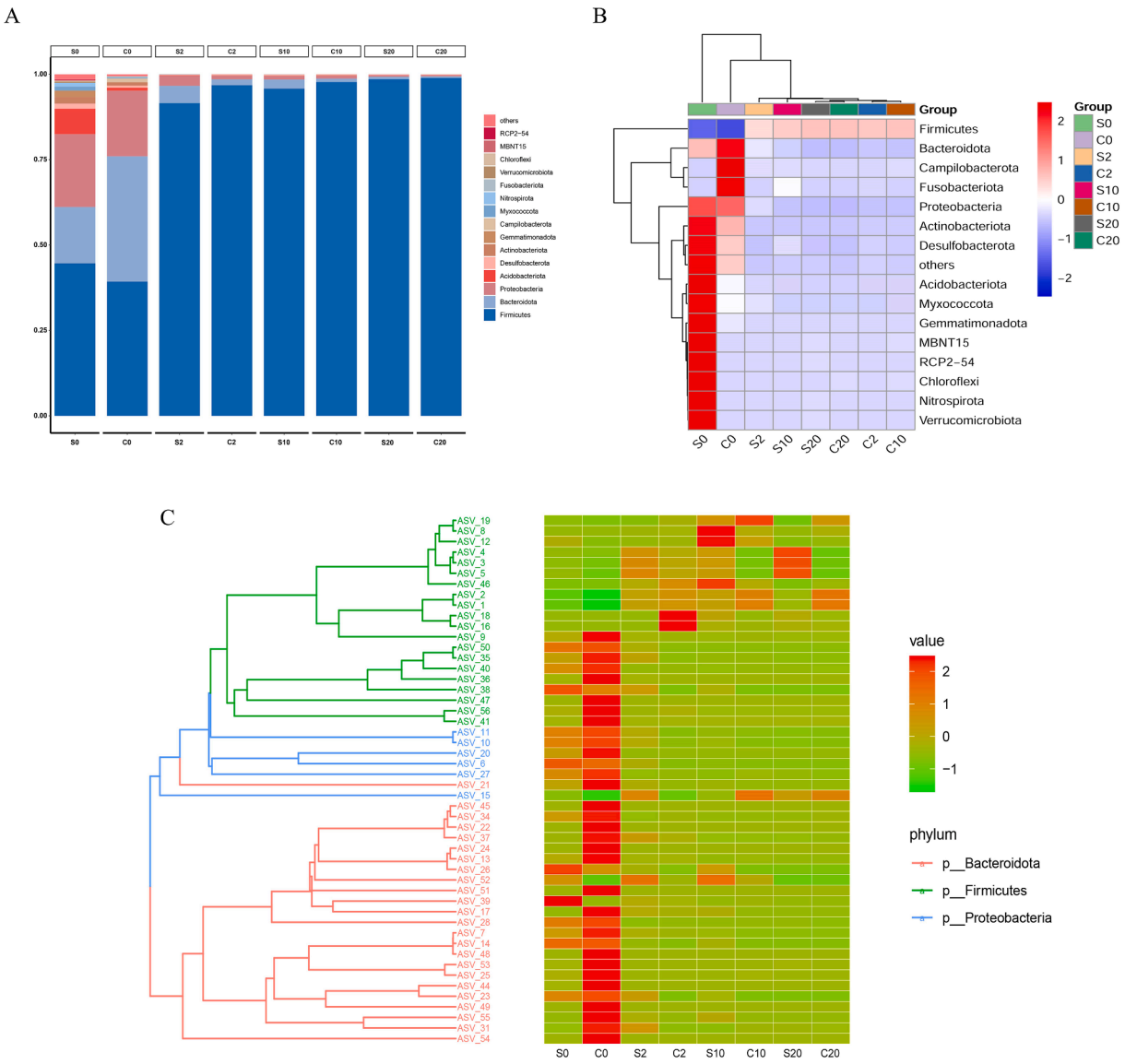

Fig. 2. Bacterial taxonomic compositions at phylum level (TOP15) during the manufacturing process of loin ham (A). phylum level Heatmap (B). The red color indicates a higher relative abundance of species, and the blue color indicates a lower relative abundance of species. Top50 species evolutionary tree and ASV abundance map (C). S0: sorbitol group fermented for 0 day; S2: sorbitol group fermented for 2 days; S10: sorbitol group ripened for 10 days; S20: sorbitol group ripened for 20 days; C0: control group fermented for 0 day; *C*2: control group fermented for 2 days; C10: control group ripened for 10 days; C20: control group ripened for 20 days.

most dominant bacteria in all groups. This could have been due to the inoculation of fermenters in all groups, which helped increase the antimicrobial metabolites produced by *Lactobacillus* and *Staphylococcus*, as well as their increased competition with other bacteria for nutrients, causing them to inhibit the growth of other bacteria.

During the fermentation and ripening stages, *Lactobacillus* was extensively higher in the control group than in the sorbitol group ($P < 0.05$), while *Staphylococcus* was significantly higher in the sorbitol group than in the control group ($P < 0.05$). It can also be seen from the heatmap that the relative abundance of *Lactobacillus* was higher than that of *Staphylococcus* in the control group, while in the reverse happened in the sorbitol group (Fig. 3B). Circos figure showed that sorbitol-mediated curing altered the relative abundance of bacteria genera levels in the loin hams (Fig. 3C). Especially at the end of ripening, the percentage of *Lactobacillus* reached 95.14 % and 51.90 % in the control and sorbitol groups, respectively, while the percentage of *Staphylococcus* reached 3.57 % and 46.37 % in the control and sorbitol groups, respectively. This indicates that sorbitol-mediated curing can inhibit *Lactobacillus*, while potentially promoting the growth of *Staphylococcus*, which may be because sorbitol has an antibacterial effect and can inhibit the growth of some microorganisms (Beyler Çiğil, Şen, Birtane, & Kahraman, 2022), while *Staphylococcus* has antibacterial activity and can resist the bacteriostatic effect of sorbitol (Kanjan & Sakpetch, 2020). Thus, *Lactobacillus* and *Staphylococcus* were evenly distributed in the sorbitol group.

In dry-cured fermented meat products, the microflora contributes greatly to their fermentation, especially all through the ripening stage (Zhang et al., 2021). In addition to *Lactobacillus*, which is the ideal microorganism, *Staphylococcus* also belongs to the main genus in meat fermentation and is necessary throughout the fermentation and ripening of meat products (Wang et al., 2022). Under the action of proteolytic enzymes and lipases, these bacteria can also expand the flavor of the product and prevent off-flavor and sourness owing to their antioxidant activity (Tu, Wu, Lock, & Chen, 2010). It has been demonstrated that *Staphylococcus* is the first dominant genus in the fermentation process of low-sodium ham, and due to its sturdy resistance, it gradually becomes

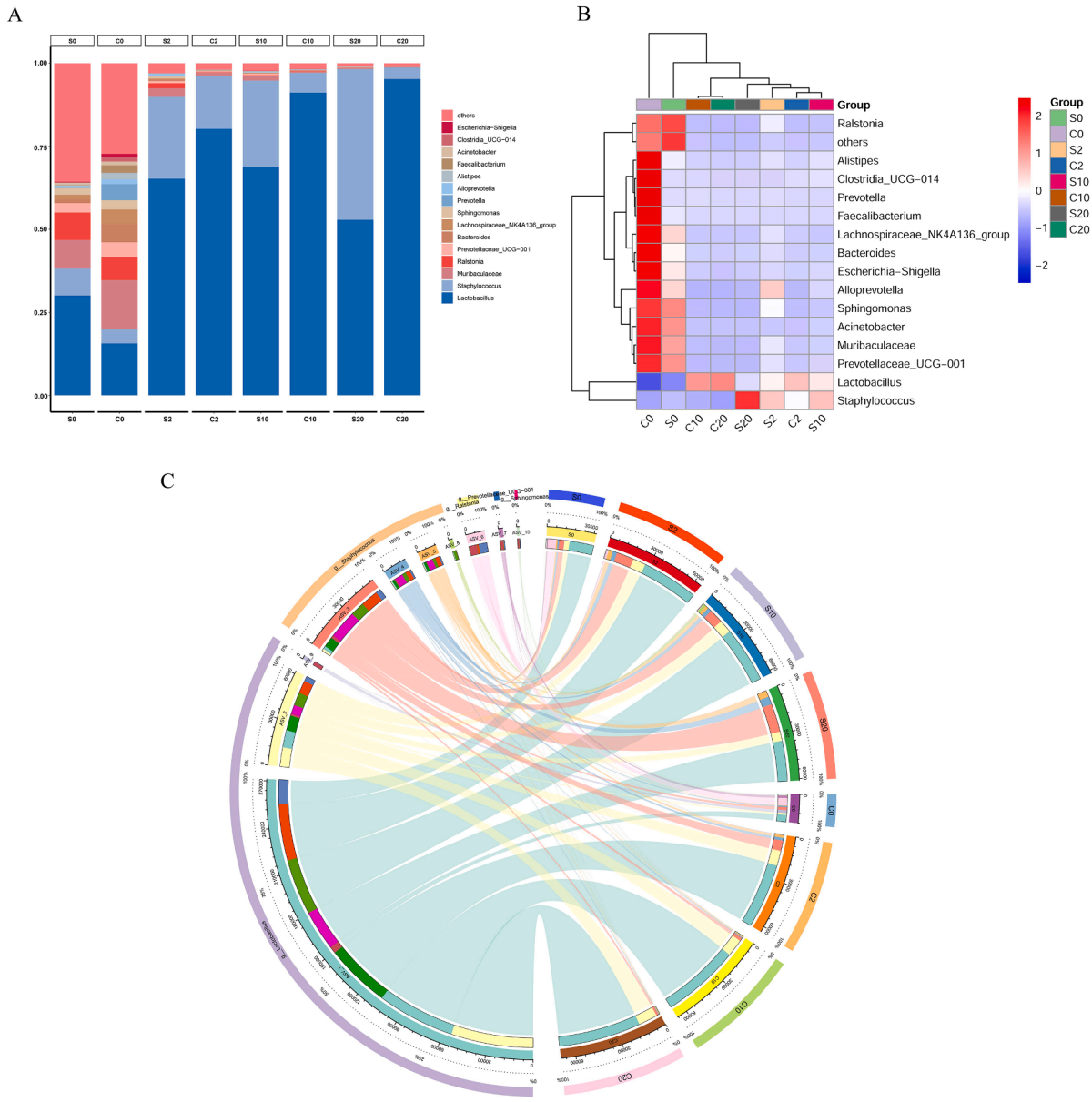

Fig. 3. Bacterial taxonomic compositions at genus level (TOP15) during the manufacturing process of loin ham (A). genus level Heatmap (B). The red color indicates a higher relative abundance of species, and the blue color indicates a lower relative abundance of species. the Circos figure of loin ham (C). S0: sorbitol group fermented for 0 day; S2: sorbitol group fermented for 2 days; S10: sorbitol group ripened for 10 days; S20: sorbitol group ripened for 20 days; C0: control group fermented for 0 day; *C2*: control group fermented for 2 days; C10: control group ripened for 10 days; C20: control group ripened for 20 days.

the dominant genus during the fermentation process. This allows for it to compete with other spoilage or pathogenic microorganisms, inhibiting their growth and enhancing the ham's safety for consumption. The loin ham by sorbitol-mediated curing was inoculated with *L. plantarum* SJ-4 and *S. simulans* QB7, which not only enhanced the competitive ability of two dominant bacteria (*Lactobacillus* and *Staphylococcus*), but also multiplied the relative abundance of beneficial bacteria in loin ham, while inhibiting the growth of harmful bacteria (pathogens and spoilage bacteria), which helped to improve the quality of loin ham.

3.7. Comparison of microbial communities between different groups

LEfSe analysis (LDA log score threshold $\geq$ 4) of loin hams was assessed at the beginning of fermentation and the end of ripening (Fig. 4A). It was possible to clarify the similarities and differences in community composition between groups at any taxonomic level. Bacteroidota was the dominant bacterial phylum in group C0 and Firmicutes was the dominant bacterial phylum in group C20, while Proteobacteria was the dominant bacterial phylum in group S0 (Fig. 4B). Additionally, *Lactobacillus* was the key bacterial genus, dominating the C20 group (Fig. 4B). LAB is a major flora in the ripening stage of dry-cured meat products (Hu et al., 2022). Traditional naturally fermented hams are susceptible to contamination by undesirable microorganisms, while inoculation with fermenters enhances the competitiveness of predominant bacteria, while also inhibiting the growth of unwanted bacteria. Furthermore, taking into account the adaptation of LAB to the sarcomatrix, the number of LAB increases rapidly and dominates the microflora (Xiao, Liu, Chen, Xie, & Li, 2020). *Lactobacillus* and *Staphylococcus* are regarded as the main bacteria involved in the lipolysis and proteolysis processes in meat products, which contributes to the formation of flavor in meat products (Hu, Wang, Kong, Wang, & Chen, 2021). However, the overgrowth of *Lactobacillus* in the C20 group also

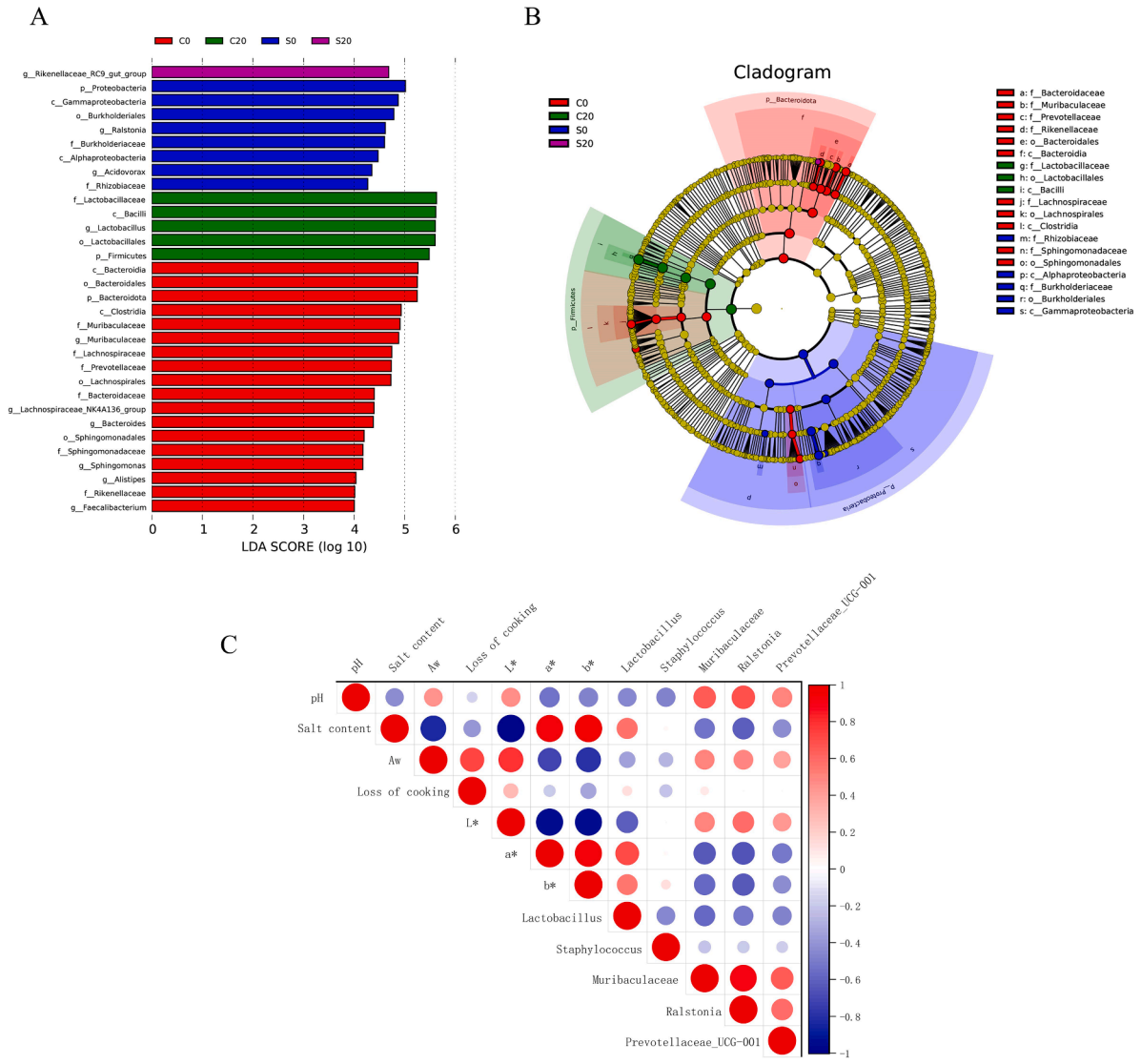

Fig. 4. LEfSe analysis of the key phyla and genera of the bacterial community of loin ham. The histogram shows the LDA scores (A) calculated for features with different abundances between groups, with higher scores and longer bands having greater influence and importance. The clade plot (B) shows that the yellow points are the unimportant bacteria in any group; the other colored points are the important bacteria in the groups marked with the same color; the shaded colors cover the highest taxonomic units when the difference is significant corresponding to the highest abundance of the group. Pearson's correlation matrix calculated from physicochemical properties and relative abundance of major microorganisms (C). The positive correlations are marked in red, and the negative correlations are in blue. The size of each circle and color intensity are proportionate to the correlation coefficient. The bar on the right indicates the correlation coefficient and the corresponding color.

inhibited *Staphylococcus*, which is a genus that contributes extensively to flavor. Sorbitol-mediated curing had a significant impact on the bacterial community of the loin ham, which resulted in an even distribution of the dominant microorganisms (*Lactobacillus* and *Staphylococcus*) present. Therefore, sorbitol-mediated curing improved the quality of loin ham.

3.8. Correlation between microorganisms and physicochemical changes

It has been reported that microbial growth is highly correlated with physicochemical changes in fermented meats (Zhang et al., 2021). In this study, we evaluated the relationships between microorganisms (Top 5 relative abundances at the genus level), between physicochemical properties, as well as between microorganisms and physicochemical properties in loin ham using Pearson's correlation analysis (Fig. 4C). *Lactobacillus*, *Staphylococcus*, *Muribaculaceae*, *Ralstonia* and *Prevotellaceae_UCG-001* were dominant in the early fermentation of loin ham. However, in the ripening stage, the genera which were dominant at the beginning of fermentation were replaced by *Lactobacillus* in the control group, while in the sorbitol group, these were replaced by *Lactobacillus* and *Staphylococcus*. Therefore, *Lactobacillus* and *Staphylococcus* showed significant negative correlation with other microbial genera due to this increase ($P < 0.05$). This result corroborates the conclusion that sorbitol-mediated curing may promote the growth of *Staphylococcus* or inhibit the overgrowth of *Lactobacillus*, leading to *Lactobacillus* and *Staphylococcus* becoming the dominant bacteria. The pH was negatively correlated with *Lactobacillus* ($P < 0.05$), suggesting that dominant *Lactobacillus* can lead to lower pH in loin ham, with *Lactobacillus* acidifying meat directly by producing organic acids (Hu et al., 2022). *Lactobacillus* can also demonstrate lipase activity, leading to the release of free fatty acids, and thus acidifying the meat and inhibiting the growth of other unwanted genera and spoilage bacteria

(Gao, Jiang, Xu, & Xia, 2018). The a* was positively correlated with *Lactobacillus*, indicating that *Lactobacillus* may promote the formation of zinc protoporphyrin IX (ZnPP), which can be used to improve the a* of fermented meat products. Additionally, salt content showed a positive correlation with *Lactobacillus* and a negative correlation with other genera, while a_w showed a negative correlation with *Lactobacillus* and *Staphylococcus* and a positive correlation with other genera, indicating that the increase in salt content and the decrease in a_w could significantly inhibit the growth of undesirable microorganisms whilst favoring dominant bacteria in loin ham.

4. Conclusion

This study revealed that the effects of sorbitol-mediated curing on physicochemical properties and bacterial community composition of loin ham. The physicochemical results of the loin hams demonstrated that sorbitol-mediated curing did not negatively influence the physicochemical properties of the loin ham. Sorbitol-mediated curing of loin ham led to a significant decrease in salt content and a_w ($P < 0.05$), which facilitated the salt reduction of loin ham and prolonged its shelf life. Moreover, *Lactobacillus* gradually dominated in the control group, while both *Lactobacillus* and *Staphylococcus* were evenly distributed in the sorbitol group throughout the fermentation and ripening stages. This indicates that sorbitol-mediated curing may promote the growth of *Staphylococcus* or inhibit the overgrowth of *Lactobacillus*, resulting in an even distribution of dominant microorganisms in the loin ham and, therefore, improving the quality of loin ham. Our study provides a preliminary perspective on the potential development of a salt-reduced fermented meat product in the food industry, with promising results.

CRediT authorship contribution statement

Yeling Zhou: Conceptualization, Visualization, Formal analysis, Writing – original draft. **Ying Zhou:** Conceptualization, Formal analysis, Writing – review & editing. **Jing Wan:** Conceptualization, Formal analysis, Writing – review & editing. **Qiujin Zhu:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Linggao Liu:** Conceptualization, Writing – review & editing. **Sha Gu:** Data interpretation. **HongYing Li:** Writing – review & editing.

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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