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Research Article

Evaluation of Changes in Sensory Quality and Bacterial Community Composition of Cold-Eating Rabbit Meat Stored under Different Temperatures

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In Sichuan cuisine, cold-eating rabbit meat is highly regarded for its very strong taste and historical legacy. This study is aimed at evaluating the quality and bacterial diversity of cold-eating rabbit meat during storage. Under different storage time and temperatures, cold-eating rabbit meat underwent a decrease in pH, whereas the contents of thiobarbituric acid reactive substances, total volatile basic nitrogen, and total viable count increased. Moreover, the loads of lactic acid bacteria and *Staphylococcus aureus* increased. Furthermore, 20 different bacterial genera were identified throughout the stages of raw meat processing and storage. Among these, *Tardiphaga* was the most abundant species during processing and storage. *Lactobacillus* was found to dominate the bacterial community associated with spoilage alterations in cold-eating rabbit meat. Thus, cold-eating rabbit meat can be safely stored at 4°C for up to 42 days. These findings offer valuable insights into the microbial processes underlying spoilage of cold-eating rabbit meat and serve as a guide for the development of strategies to prevent spoilage during processing and storage of cold-eating rabbit meat.

1. Introduction

Rabbit meat is rich in protein containing high levels of essential amino acids and is low in fat with a favorable proportion of saturated, monounsaturated, and polyunsaturated fatty acids [1]. Over the last two decades, rabbit meat products have rapidly developed and become increasingly popular worldwide, particularly in China [2]. In 2019, approximately 675 million rabbits were marketed in Asia and 164 million in Europe, with numbers increasing in 2020. The European Union has a production scale of 180 million rabbits [3], and the rabbit meat industry showed a stable growth in 2021, with a stronger trend for development and growth [4].

Cold-eating rabbit meat is a traditional meat product made from fresh rabbit meat supplemented with spices such as chili pepper and ginger [5]. The steps involved in cold-eating rabbit meat processing include dicing, pickling, frying, refrying, stir-fry, cooling, vacuum packing, and high-temperature steaming (Figure 1). However, cold-eating rabbit meat is considered an oil-soaked product with high fat and water content, being thus prone to contamination by undesirable microorganisms, which may lead to spoilage [6].

During processing, transportation, and storage, meat products may become contaminated with different types of microorganisms, but spoilage only occurs when the growth

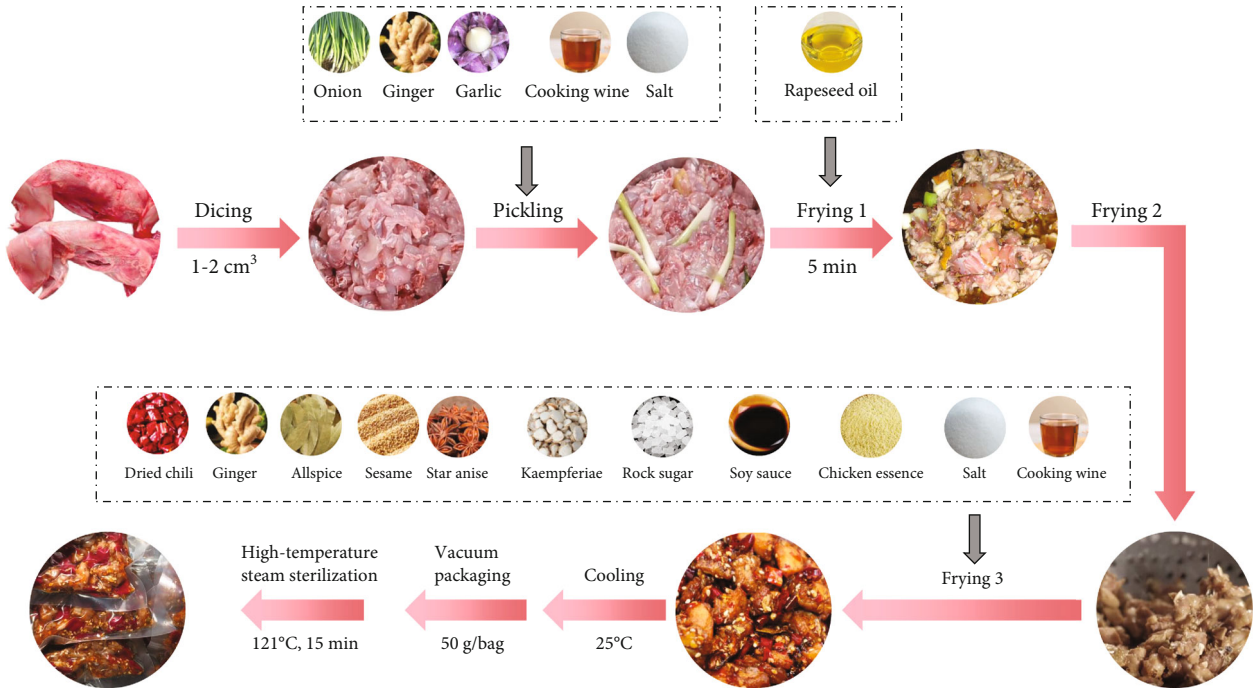


Figure 1: Cold-eating rabbit production process.

of specific spoilage microorganisms (SSOs) is favored [7]. The characteristic and dominant SSOs in various meat products have been previously described [8]. This microbiota undergoes a dynamic process of succession, and the number and ratio of characteristic SSOs may be low at the beginning of storage. With prolonged storage time, SSOs gradually dominate the microbiota due to their strong adaptability to specific storage environmental conditions and fast metabolic activity [9]. Furthermore, various types of meat products, along with the specific methods used for processing and storage, can impact the prevalence of certain species of spoilage bacteria [10].

In a previous study, *Staphylococcus warneri* and *Bacillus subtilis* were described among specific spoilage bacteria leading to spoilage of cold-eating rabbit meat, and *Staphylococcus warneri* showed stronger spoilage ability [11]. At present, only few studies have reported the changes in the bacterial community structure of high-temperature meat products during storage, mostly by employing conventional culture-dependent methods combined with 16S rDNA sequencing. However, studies on the dynamic changes among spoilage bacteria in these types of meat products are still lacking.

In the present study, changes in the microbial community structure of cold-eating rabbit meat were analyzed using high-throughput sequencing technologies. In addition, changes in sensory and quality attributes of cold-eating rabbit meat stored at different temperatures were evaluated. The findings discussed herein provide a theoretical basis for controlling microbial species and load in cold-eating rabbit meat products at the source, which is expected to extend their shelf life and contribute to diversifying the associated product range.

2. Materials and Methods

2.1. Materials. Nutrient agar, LB broth, plate counting agar, MRS agar, and Baird-Parker agar bases were supplied by Beijing Aoboxing Biotech Co., Ltd. (Beijing, China). NaCl, Tris, EDTA-2Na, hexadecyltrimethylammonium bromide (CTAB), 50x TAE, snail enzyme, and lysozyme were supplied by Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). PCR premix, high-purity low-electroosmotic agarose, and DL5000 DNA marker were supplied by Beijing Tsingke Biotech Co., Ltd. (Beijing, China).

2.2. Sample Collection and Treatment. Cold-eating rabbit meat from the same batch was randomly collected in a food processing facility in Zigong City, China, placed in bags, and stored at 4, 25, and 37°C (150 g/bag; 21 bags prepared for each temperature; 63 bags in total). Determination of quality attributes and sensory analysis of cold-eating rabbit meat was performed on day 6, day 3, and day 2 of storage in three parallel experiments. The sampling points for the determination of bacterial diversity were as follows: J1, raw rabbit meat; J2, raw rabbit meat after preboiling and curing; C1~C5, cold-eating rabbit meat samples stored at 25°C for 6, 9, 12, 15, and 18 days, respectively; and C6, cold-eating rabbit meat samples at the end of spoilage.

2.3. Sensory Analysis. The sensory properties of cold-eating rabbit meat were assessed according to the relevant literature [12–15]. The sensory evaluation panel consisted of eight panelists who judged cold-eating rabbit meat stored at different temperatures based on color and luster, organization form, taste and flavor, and mouthfeel using a 10-point scale, in which 10 indicates excellent, 8 good, 6 medium, 4 poor,

and 2 bad. The weighting factor for color and luster, organization form, taste and flavor, and mouthfeel was 25%, 20%, 25%, and 30%, respectively, and the final results were calculated as the average of reported scores. The detailed criteria adopted for sensory analysis are shown in Table 1. The sensory scores were calculated as follows:

$$\text{Sensory value} = C \times 25\% + O \times 20\% + T \times 25\% + M \times 30\%, \quad (1)$$

where C indicates color and luster, O indicates organization form, T refers to taste and flavor, and M refers to mouthfeel.

2.4. Determination of Microbial Load. The following standard methods were used to perform microbial enumeration: for total viable counts (TVC), [16]; for lactic acid bacteria (LAB), [17]; and *Staphylococcus aureus* (*S. aureus*), [18]. Briefly, 25 g of the sample was obtained under aseptic conditions and placed in a flask, and 225 mL of 0.16 mol/L sterile NaCl solution was added, followed by shaking for 5 min for homogenization. All microbial enumerations were performed using the pour plate method, and the results were expressed as log CFU/g. Plates for TVC were incubated at 37°C for 48 h, for LAB at 37°C for 72 h, and for *S. aureus* at 37°C for 48 h.

2.5. Determination of Chemical Characteristics. Cold-eating rabbit meat was subjected to pH measurements following the method proposed by Charmpi et al. [19]. Thiobarbituric acid reactive substances (TBARS) were determined following the method proposed by Qiu et al. [20]. Total volatile basic nitrogen (TVB-N) content was determined according to Liu et al. [21]. All analyses were performed in triplicate.

2.6. High-Throughput Sequencing of Spoilage Microbial Community. Total bacterial DNA was extracted using the CTAB method as described previously [22]. DNA concentration and purity were determined using horizontal gel electrophoresis in 1% agarose gels. The extracted genomic DNA was used as a template to amplify the hypervariable V3~V4 region of the 16S rDNA. After amplification, samples were submitted to horizontal gel electrophoresis in 2% (*w*/*v*) agarose gels, and purified samples were quantified using the Tiangen™ DNA detection kit (Tiangen Biotech Co. Ltd., Beijing, China). The products were recovered and sent to Shanghai Meiji Biomedical Technology Co., Ltd., for high-throughput sequencing. All bioinformatic analyses were performed on the I-Sanger cloud platform (http://www.i-sanger.com).

2.7. Statistical Analysis. Data were analyzed by one-way analysis of variance using SPSS Statistics 27.0.1 software (SPSS Inc., Chicago, IL, USA), and significance was analyzed by Duncan's multiple comparisons method, with $P < 0.05$ representing a statistically significant difference. Plotting was performed using Origin 2021 (OriginLab Co. Ltd., Northampton, MA, USA.).

3. Results and Discussion

3.1. Changes in Microbial Load. Figure 2 depicts microbial loads in cold-eating rabbit meat under different storage temperatures.

The initial microbial load in cold-eating rabbit meat was 1.57 log CFU/g (Figure 2(a)), primarily due to the high temperature applied in cold-eating rabbit meat processing, which inactivated most microorganisms, and only bacterial spores or damaged bacterial cells survived [23]. TVC of cold-eating rabbit meat increased significantly ($P < 0.05$) with storage time at the three storage temperatures tested, and the higher the storage temperature, the faster the increase in TVC. Specifically, TVC of cold-eating rabbit meat was 5.14, 5.27, and 5.24 log CFU/g under storage at 4°C for 36 days, 25°C for 15 days, and 37°C for 8 days, respectively. The relevant standards indicate that meat products with TVC higher than 5.00 log CFU/g are not edible [24].

The initial load of LAB was 0.7 log CFU/g, which increased during storage (Figure 2(b)). LAB load at 4°C observed the following trend: 0-6 days of slow growth ($P > 0.05$), 6-18 days of faster growth ($P < 0.05$), 18-24 days of slow growth ($P > 0.05$), 24-36 days of faster growth ($P < 0.05$), and LAB load increased to 1.85 log CFU/g on day 36. Conversely, at 25°C, LAB grew rapidly ($P < 0.05$) on days 0-6, then slowly ($P > 0.05$) on days 6-9, and then rapidly ($P < 0.05$) on days 9-18, to reach a final LAB load of 2.48 log CFU/g on day 18. Moreover, at 37°C, LAB grew rapidly ($P < 0.05$) from days 0-10 and then stabilized ($P > 0.05$) after day 10, with a final LAB load of 3.16 log CFU/g on day 12. Thus, these findings indicate that storage at lower temperatures had an inhibitory effect on LAB growth [25].

Finally, *S. aureus* was not detected in cold-eating rabbit meat at the beginning of storage (Figure 2(c)). *S. aureus* counts showed a stable upward trend ($P < 0.05$) from days 0-36 to finally reach 2.36 log CFU/g when stored at 4°C. At 25°C, *S. aureus* grew rapidly during days 0-3 ($P < 0.05$), followed by a small decrease during days 3-6 from 0.87 log CFU/g to 0.80 log CFU/g ($P < 0.05$) and then showed an upward trend during days 6-18 ($P < 0.05$), reaching 2.76 log CFU/g on day 18. At 37°C, *S. aureus* grew rapidly on days 0-8 ($P < 0.05$) and peaked at 3.48 log CFU/g on day 8, followed by a decreasing trend on days 8-12, reaching 3.33 log CFU/g on day 12. At the end of storage at 37°C, *S. aureus* load continued to decline, which might be due to the proliferation of LAB and the significant decrease in the pH of the meat matrix, which inhibited staphylococcal growth [26].

3.2. Changes in Chemical Characteristics. Figure 3 depicts the chemical properties of cold-eating rabbit meat submitted to different storage temperatures, including pH, TBARS, and TVB-N.

With the increase in storage time, the pH value fluctuated slightly at first and then showed a downward trend (Figure 3(a)). The pH value of cold-eating rabbit meat stored at 4°C tended to decrease and increase repeatedly ($P < 0.05$), which can be illustrated by the following trend: decrease on

Table 1: Sensory quality rating scales.

	Color and luster (25%)	Organization form (20%)	Taste and flavor (25%)	Mouthfeel (30%)
Excellent (10 points)	Golden yellow, lustrous	The meat is firm, free of focal and mildew spot	Strong spicy or fresh fragrance, no other peculiar smell	Chewy, refreshing, and not lingering, with a long aftertaste
Good (8 points)	Color is good, more lustrous	The meat is a little firm, free of focal and mildew spot	Strong spicy or fresh fragrance, no other peculiar smell	More chewy, more aftertaste
Medium (6 points)	Yellow, normal luster	The meat is a little loose, free of focal and mildew spot	A little strong spicy or fresh fragrance, no other peculiar smell	No chewy, no aftertaste
Poor (4 points)	Yellow, lusterless	The meat is loose, without coke spot, with small mildew spot	No fragrance, slight spoilage	
Bad (2 points)	White, lusterless	The meat is loose, without coke spot, with large mildew spot	A serious smell of corruption	

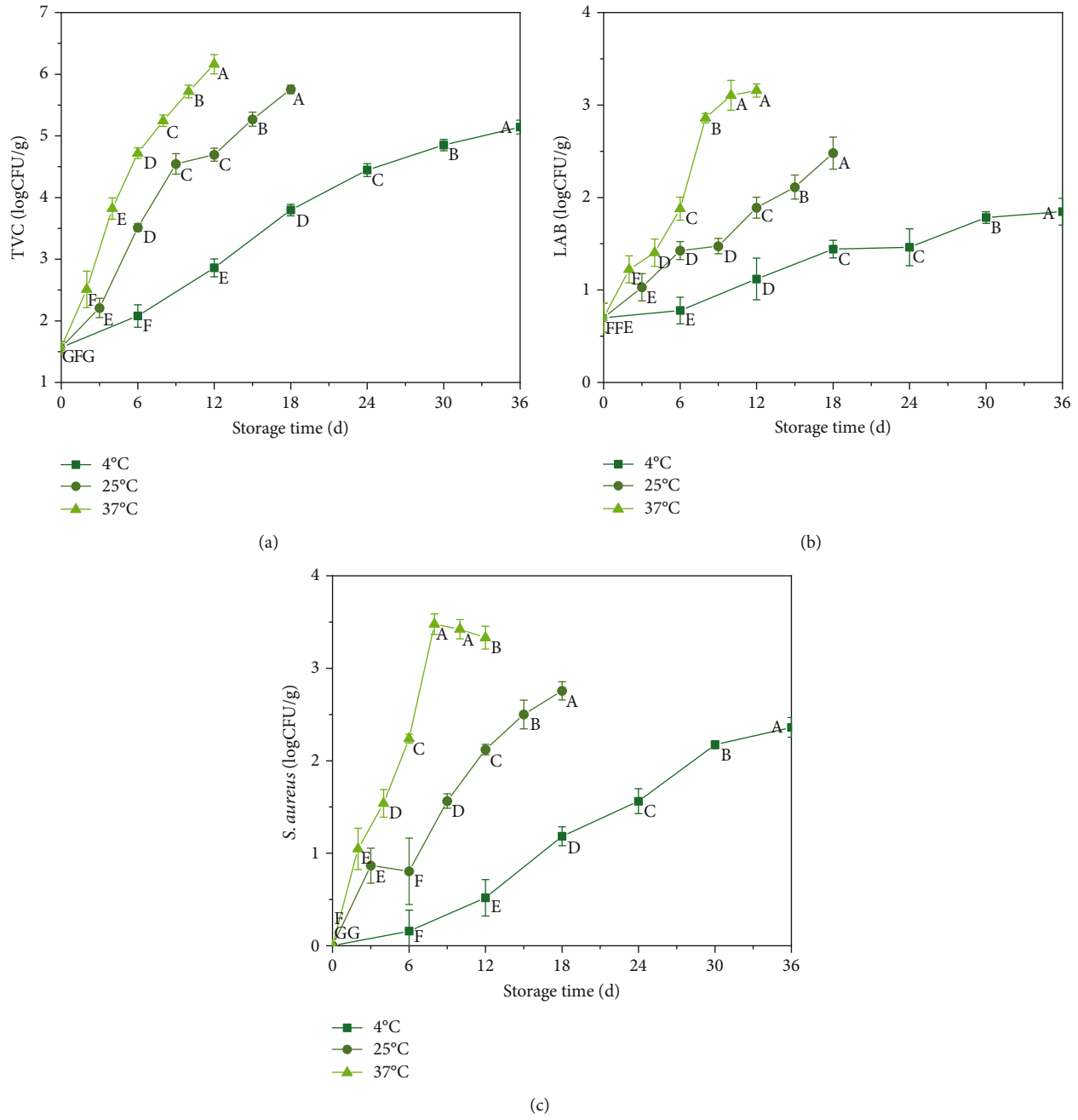


Figure 2: (a) TVC, (b) LAB, and (c) *S. aureus* of cold-eating rabbit meat during storage at 4, 25, and 37°C.

days 0-6, increase on days 6-12, decrease on days 12-24, increase on days 24-30, and decrease on days 30-36. Conversely, the pH value of cold-eating rabbit meat stored at 25°C or 37°C showed an initial downward trend followed by an increase and then a final decrease ($P < 0.05$). Specifically, changes in pH value under storage at 25°C were as follows: decrease on days 0-3, increase on days 3-12, and decrease on days 12-18. The specific changes under storage at 37°C were as follows: decrease on days 0-2, increase on days 2-4, and decrease on days 4-12. pH values of cold-eating rabbit meat stored at 4°C and 25°C fluctuated slightly, but pH decreased significantly when rabbit meat was stored at 37°C. During early storage, pH fluctuated between 6.4 and 6.6, probably due to the degradation of carbohydrates into organic acids, thus leading to pH reduction. On the contrary, protein degradation leads to pH increase, which reflects as fluctuation in pH values [27]. In mid- and late storage, the rapid growth of acid-producing bacteria further accelerated nutrient decomposition, thus resulting in a continuous decrease in pH value. Compared with storage at 4 and 25°C, pH of cold-eating rabbit meat decreased more rapidly during storage at 37°C, thus indicating that storage under higher temperatures can accelerate the degradation of glycogen and ATP [28].

Malondialdehyde (MDA) content reflects the degree of lipid oxidation of meat products. Figure 3(b) shows the

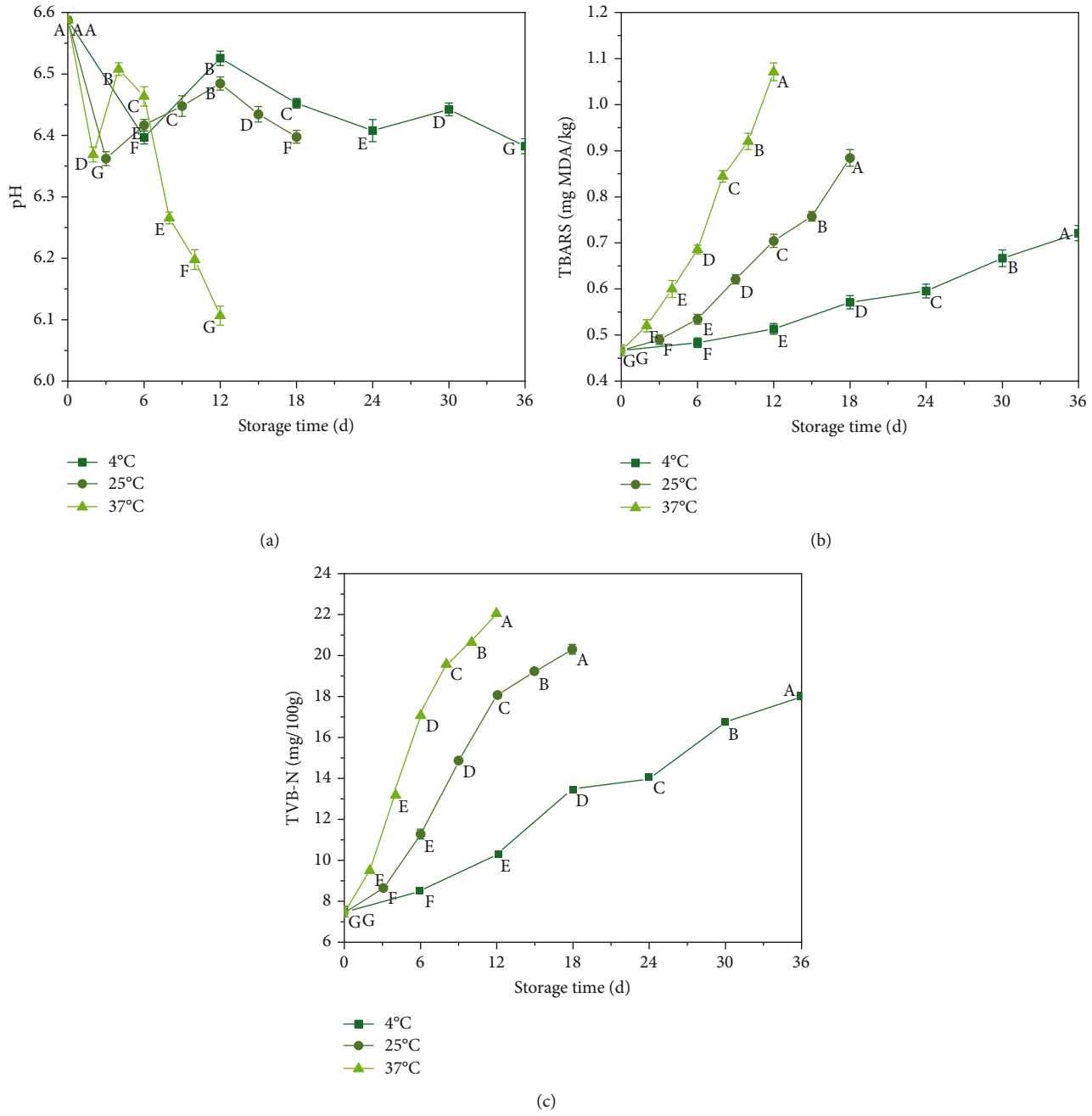


Figure 3: (a) pH, (b) TBARS, and (c) TVB-N of cold-eating rabbit meat during storage at 4, 25, and 37°C.

changes in TBARS values of cold-eating rabbit meat at different storage temperatures. The TBARS values of cold-eating rabbit meat stored at all tested temperatures increased continuously with the extension of storage time ($P < 0.05$), and the higher the storage temperature, the faster the TBARS values increased. The TBARS values of cold-eating rabbit meat stored at 4, 25, and 37°C increased by 0.72 mg MDA/kg, 0.88 mg MDA/kg, and 1.07 mg MDA/kg, respectively, compared with the initial TBARS value of 0.46 mg MDA/kg. Thus, lower storage temperatures can effectively inhibit lipid oxidation of cold-eating rabbit meat.

Figure 3(c) shows the changes of TVB-N content in cold-eating rabbit meat stored at different temperatures. The TVB-N values of the cold-eating rabbit meat stored at all the tested temperatures increased continuously ($P < 0.05$) with the extension of the storage time, and the higher the storage temperature, the faster the increase of the TBARS value. It has been reported that TVB-N content in meat products ≤ 15 mg/100 g corresponds to first-grade freshness, 15-25 mg/100 g to second-grade freshness, and ≥25 mg/100 g to spoilage [29]. When stored at 37°C for 6 d, TVB-N content in cold-eating rabbit meat increased to 15 mg/100 g, whereas during storage at 37°C for 12d, TVB-N content was 22 mg/100 g. During storage at 25°C, TVB-N content increased to 15 mg/100 g on day 8 and to 20 mg/100 g on day 18. In contrast, TVB-N content increased slowly during storage at 4°C, reaching 18 mg/100 g

Table 2: Changes in sensory scores of cold-eating rabbit meat at different storage temperatures.

Storage temperature (°C)	Storage time (d)	Color (5%)	Tissue morphology (20%)	Taste and flavor (25%)	Mouthfeel (30%)	Overall score
4	0	7.89 ± 0.30^{a}	8.98 ± 0.37^{a}	9.01 ± 0.37^{a}	9.09 ± 0.29^{a}	8.80 ± 0.25^{a}
	7	7.51 ± 0.30^{a}	8.68 ± 0.23^{a}	8.78 ± 0.20^{ab}	8.80 ± 0.33^{a}	8.43 ± 0.27^{a}
	14	6.99 ± 0.34^{b}	8.21 ± 0.22^{b}	8.36 ± 0.18^{b}	8.30 ± 0.24^{b}	7.88 ± 0.35^{b}
	21	6.35 ± 0.19^{c}	7.68 ± 0.13^{c}	7.83 ± 0.21^{c}	7.66 ± 0.21^{c}	7.29 ± 0.30^{c}
	28	5.91 ± 0.20^{d}	7.10 ± 0.24^{d}	7.23 ± 0.35^{d}	7.09 ± 0.25^{d}	6.78 ± 0.25^{d}
	35	5.43 ± 0.15^{e}	6.54 ± 0.18^{e}	6.75 ± 0.31^{e}	6.48 ± 0.21^{e}	6.30 ± 0.31^{e}
	42	4.81 ± 0.27^{f}	6.00 ± 0.27^{f}	6.55 ± 0.25^{e}	6.04 ± 0.30^{f}	5.76 ± 0.27^{f}
25	0	7.89 ± 0.30^{a}	8.98 ± 0.37^{a}	9.01 ± 0.37^{a}	9.09 ± 0.29^{a}	8.80 ± 0.25^{a}
	3	7.63 ± 0.32^{a}	8.68 ± 0.23^{ab}	8.86 ± 0.21^{a}	8.89 ± 0.20^{a}	8.49 ± 0.22^{ab}
	6	7.35 ± 0.21^{b}	8.25 ± 0.23^{b}	8.50 ± 0.29^{ab}	8.56 ± 0.23^{ab}	8.14 ± 0.24^{b}
	9	6.67 ± 0.24^{c}	7.65 ± 0.30^{c}	8.10 ± 0.32^{bc}	8.29 ± 0.34^{bc}	7.51 ± 0.24^{c}
	12	6.11 ± 0.34^{d}	6.98 ± 0.32^{d}	7.64 ± 0.30^{c}	7.70 ± 0.33^{c}	6.96 ± 0.37^{d}
	15	5.41 ± 0.18^{e}	6.09 ± 0.36^{e}	7.04 ± 0.38^{d}	6.95 ± 0.39^{d}	6.05 ± 0.42^{e}
	18	4.51 ± 0.12^{f}	5.70 ± 0.19^{e}	6.39 ± 0.64^{e}	6.28 ± 0.64^{e}	5.41 ± 0.49^{f}
37	0	7.89 ± 0.30^{a}	8.98 ± 0.37^{a}	9.01 ± 0.37^{a}	9.09 ± 0.29^{a}	8.80 ± 0.25^{a}
	2	7.38 ± 0.17^{b}	8.59 ± 0.29^{a}	8.63 ± 0.27^{ab}	8.64 ± 0.31^{ab}	8.50 ± 0.33^{a}
	4	6.81 ± 0.22^{c}	8.06 ± 0.27^{b}	8.31 ± 0.16^{b}	8.06 ± 0.22^{bc}	7.93 ± 0.41^{b}
	6	6.13 ± 0.32^{d}	7.11 ± 0.25^{c}	7.76 ± 0.39^{c}	7.58 ± 0.55^{c}	6.91 ± 0.52^{c}
	8	5.54 ± 0.25^{e}	5.20 ± 0.28^{d}	6.78 ± 0.32^{d}	6.56 ± 0.64^{d}	6.06 ± 0.36^{d}
	10	4.66 ± 0.23^{f}	4.56 ± 0.25^{e}	6.08 ± 0.21^{e}	5.63 ± 0.59^{e}	5.24 ± 0.38^{e}
	12	4.19 ± 0.20^{g}	3.81 ± 0.31^{f}	5.19 ± 0.40^{f}	4.65 ± 0.45^{f}	4.36 ± 0.23^{f}

Different lowercase letters indicate significant differences between the same columns ($P < 0.05$).

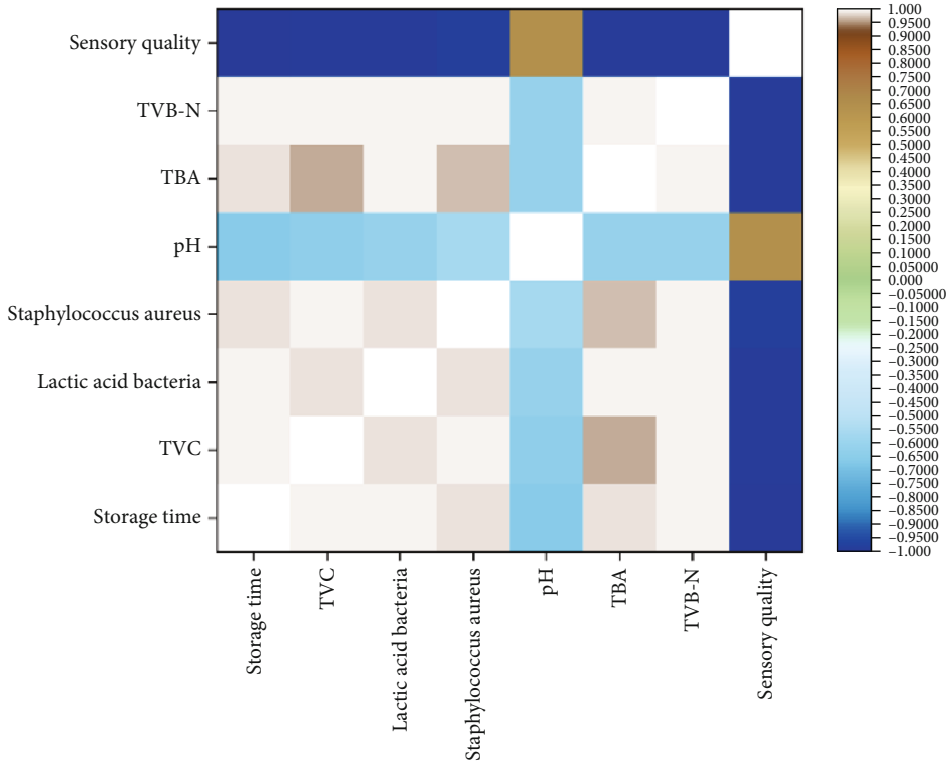
on day 36. Taken together, the findings showed that temperature had a significant effect on TVB-N content during storage of cold-eating rabbit meat.

3.3. Changes in Sensory Quality. The sensory quality of cold-eating rabbit meat stored at 4, 25, and 37°C was assessed on the basis of meat color and luster, organization form, taste and flavor, and mouthfeel (Table 2). The sensory score was a comprehensive evaluation of the degree of spoilage and deterioration of cold-eating rabbit meat.

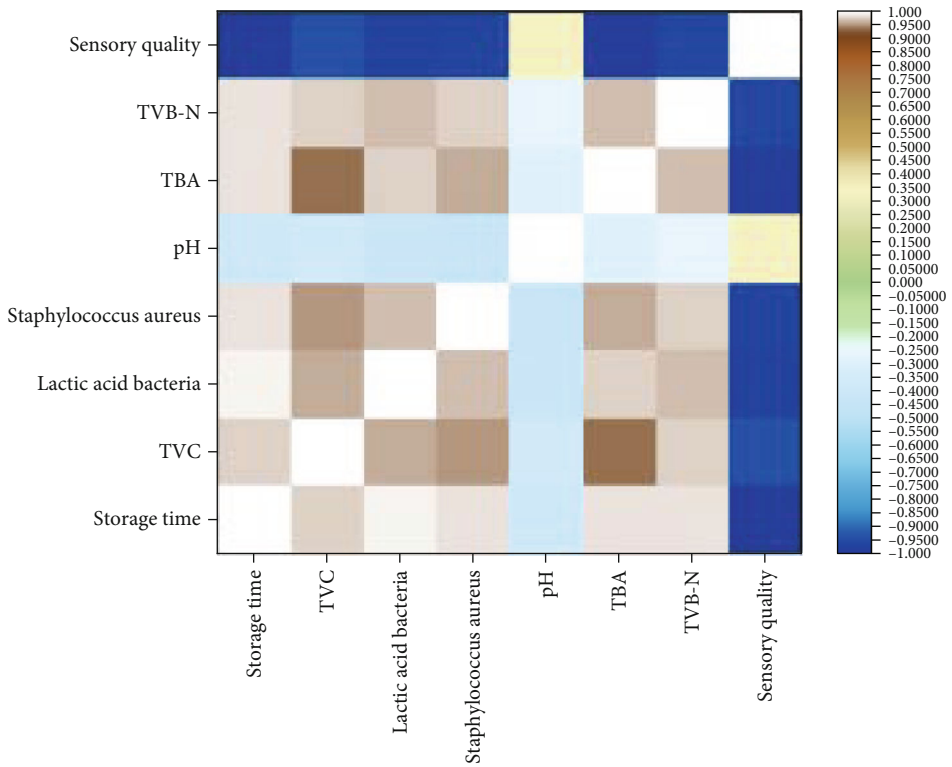
Sensory scores of cold-eating rabbit meat stored at different temperatures did not significantly differ ($P > 0.05$) on days 0-7 and showed a steady decrease ($P < 0.05$) on days 7-42. Color scores were consistently lower than scores related to tissue morphology, flavor, taste, and texture. The rate of decline in sensory quality was slower in the early stages of storage and faster in mid- and late stages of storage. After 8 days of storage at 37°C, a total sensory score of 6.06 was observed, color changed from golden to yellow, and loss of luster and connectivity was identified, while taste and general odor were retained and reached the lowest level of consumer acceptance (total score < 6). The overall sensory score was very close to or above 6 under storage at 37°C on day 8, at 25°C on day 15, and at 4°C on day 42, at which point eating cold rabbit was considered inedible. A continuous decline in sensory quality was observed in cold-eating rabbit meat at different storage temperatures, which may be caused by lipid oxidation and microbial proliferation, leading to deterioration [30].

Correlations were found between sensory scores and related quality indexes of cold-eating rabbit meat at different storage temperatures (Figure 4). According to Figure 4(a), organoleptic scores of cold-eating rabbit meat showed the strongest correlation with storage time under 4°C. This indicates that the sensory quality of cold-eating rabbit meat decreases slowly at lower temperatures due to the inhibition of oxidation and microbial reproduction. However, the sensory quality of cold-eating rabbit meat showed a continuous decline with prolonged storage time [31]. Sensory evaluation showed the strongest correlation with TBARS when cold-eating rabbit meat was stored at 25°C and 37°C (Figures 4(b) and 4(c)). Moreover, these two temperature conditions exerted a significant effect on food quality, probably due to the fact that cold-eating rabbit products are rich in lipids, which are oxidized under high-temperature conditions, thus generating low-molecular-weight aldehydes, ketones, acids, and other compounds. With prolonged storage time, MDA content increased, which triggered a strong unpleasant odor and sensation of spicy, bitter, and astringent taste, leading to a significant decrease in the sensory score of cold-eating rabbit meat [32].

3.4. High-Throughput Sequencing Analysis. Eight samples of raw materials as well as rinsed and pickled samples stored at

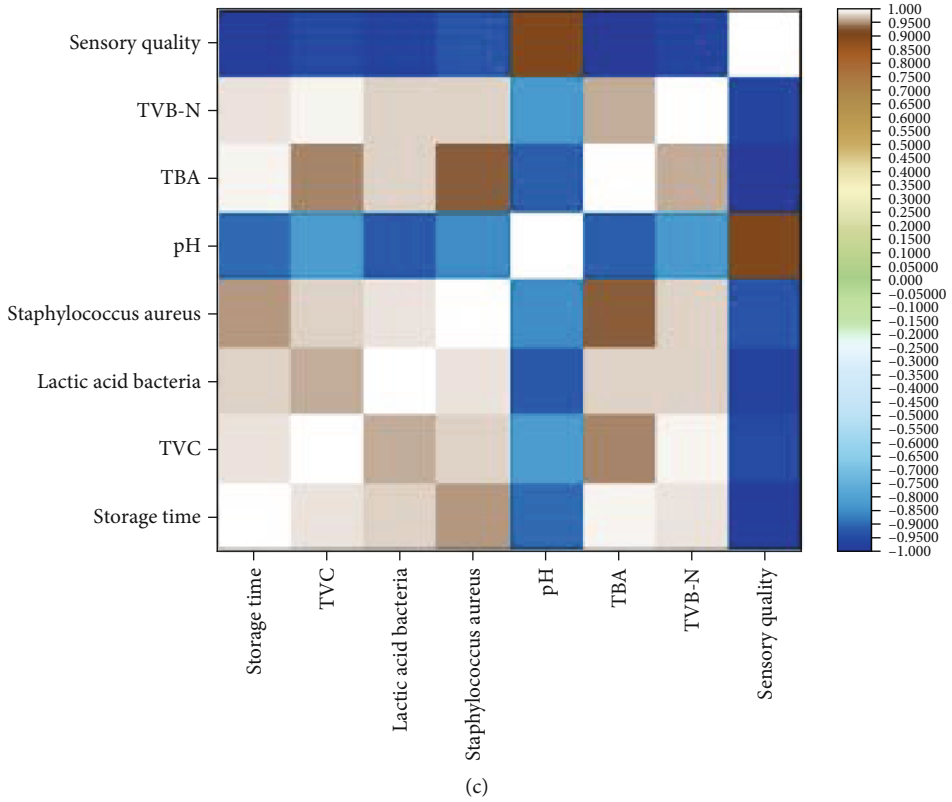


(a)



(b)

Figure 4: Continued.



(c)

Figure 4: Heat map of correlation cluster analysis of indicators for cold-eating rabbit meat stored at (a) 4, (b) 25, and (c) 37°C.

Table 3: Alpha diversity index based on ASVS.

Samples	ACE index	Chao index	Shannon index	Simpson index	Coverage index
J1	122	127	3.31	0.06	0.9995
J2	104	103	3.24	0.06	0.9998
C1	179	178	2.04	0.36	0.9994
C2	300	298	2.72	0.25	0.9989
C3	244	244	3.27	0.17	1.0000
C4	164	163	1.91	0.44	0.9997
C5	149	147	2.31	0.23	0.9995
C6	11	11	0.62	0.72	1.0000

J1 and J2 represent raw materials, rinsing and curing; C1~C6 are stored at 25°C for 6, 9, 12, 15, and 18 d and spoilage end point, respectively.

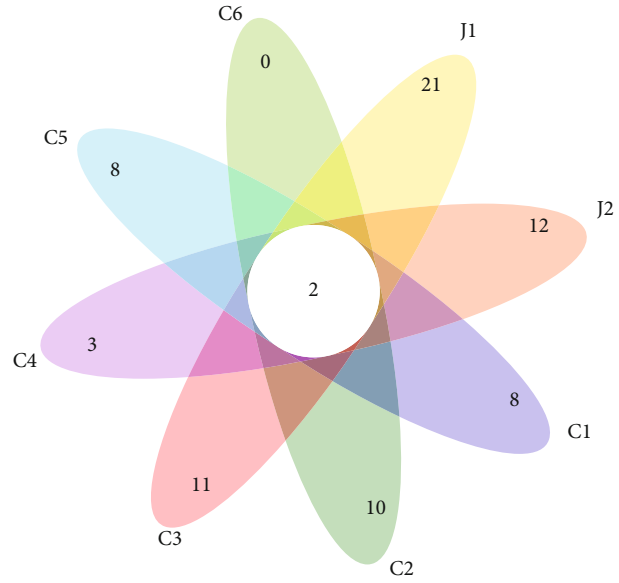


Figure 5: Venn plots of 9 horizontal bacteria.

25°C (on days 6, 9, 12, 15, and 18) and at the spoilage endpoint were submitted to sequencing. In total, 115,616 sequences were obtained by V3-V4 sequencing. After DADA2 denoising using QIIME2 software, 224,154 clean sequences were obtained. The resulting sequences were analyzed at 100% similarity level, and 1,311 amplicon sequence variants (ASVs) were obtained. Interestingly, the number of ASVs initially increased and then decreased gradually with prolonged storage time at 25°C.

The alpha diversity index was calculated to assess microbial community diversity in samples, whereas Chao and ACE indices were calculated to assess microbial community richness; the greater the reported value, the higher the abundance in the microbial community. In addition, Shannon and Simpson indices were calculated to assess microbial community diversity, with a higher Shannon index (or a lower Simpson index) indicating higher microbial community diversity. The coverage index was used to reflect community coverage, and a higher value indicated a higher probability of sequence detection in the sample [33]; a coverage index above 0.99 indicates that sequencing results are representative of the actual situation of bacterial community

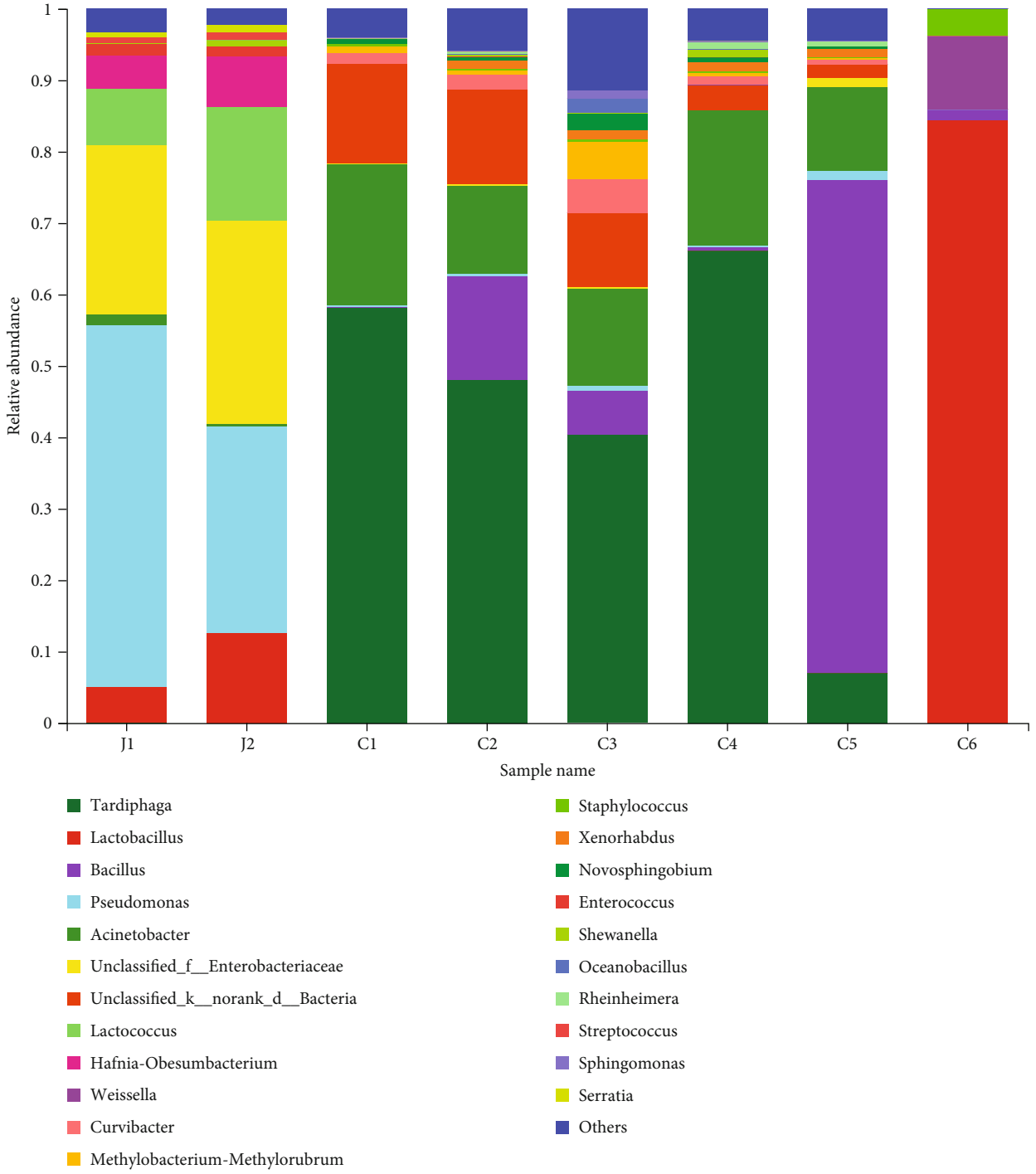


Figure 6: Bar diagram of horizontal bacteria of genus.

in the sample (Table 3). According to the joint analysis of Chao and ACE indices, sample J1 exhibited higher microbial abundance compared to sample J2. During storage, microbial abundance in cold-eating rabbit meat samples decreased in the following order: C2, C3, C1, C4, C5, and C6. Additionally, Shannon and Simpson indices indicated that sample J1 exhibited higher microbial community diversity in comparison to sample J2. Throughout storage at 25°C, microbial community diversity in chilled rabbit meat was most significant in sample C3 followed by C2, C5, C1, C4, and C6.

Furthermore, a Venn diagram was constructed based on ASVs (Figure 5). At the species level, 75 ASVs were annotated with 21, 12, 8, 10, 11, 3, and 8 ASVs per sample, respectively. Two ASVs were annotated at the species level in sample C6 which are commonly shared among all samples. Thus, washing and marinating treatments significantly reduced both richness and diversity in the microbiota of raw materials. During storage, microbial community richness initially increased and then decreased, while diversity showed a fluctuating trend. In six samples taken on days 6,

9, 12, 15, and 18 during storage, as well as in spoilage endpoint, certain ASVs were commonly shared while others were unique. These samples exhibited rapid changes in bacterial community composition with a fluctuation pattern. Under storage at 25°C, the number of bacterial species in samples increased during the storage period of C1-C3, and TVC, LAB and *S. aureus* counts, pH, TBARS, and TVB-N also increased, while the sensory score decreased. This may be due to the fact that, at this stage, bacteria in cold-eating rabbit meat are metabolically active and multiplying, and an environment rich in nutrients is conducive to the growth and reproduction of bacteria, which then gradually dominate [34]. Interestingly, during the storage period of C3-C6, the number of bacterial species in samples showed an upward trend followed by a downward trend, which may be related to antagonistic, mutualistic, and other interactions among different microorganisms. Nevertheless, TVC, LAB and *S. aureus* counts, pH, TBARS, and TVB-N showed an upward trend, which suggests that bacteria continued to absorb and decompose nutrients in cold-eating rabbit meat. However, sensory scores and pH decreased during the C3-C6 storage period, indicating that cold-eating rabbit meat gradually showed signs of spoilage with prolonged storage time, which resulted in lower sensory scores. In particular, the sudden decrease in pH may be attributed to an increase in the number of LAB, leading to an increase in the production of lactic acid [35].

During processing and storage, the relative abundance of bacterial species in cold-eating rabbit meat in decreasing order was as follows: *Tardiphaga*, *Lactobacillus*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, unclassified *Enterobacter*, unclassified *Bacteria*, *Lactococcus*, *Hafnia-Obesumbacterium*, *Weissella*, *Gracilis*, *Staphylococcus*, *Methylobacterium*, *Budapestensis*, *Novosphingobium*, *Enterococcus*, *Shewanella*, *Oceanobacillus*, *Sphingomonas*, and *Serratia* (Figure 6).

The bacterial community of samples which were not processed under high temperatures was similar, consisting mainly of *Pseudomonas*, unknown *Enterobacter*, *Lactococcus*, *Lactobacillus*, and *Hafnia-Obesumbacterium*; the relative proportion of these bacterial species was 50.60%, 28.91%, 23.81%, 28.47%, and 7.78% in sample J1 and 15.91%, 5.08%, 12.59%, 4.75%, and 7.06% in sample J2, respectively. After roasting, frying, and thermal treatment at high temperature, *Pseudomonas*, unclassified *Enterobacteriaceae*, and *Hafnia-Obesumbacterium* were virtually inactivated in cold-eating rabbit meat, and the relative abundance of *Lactococcus* and *Lactobacillus* was greatly reduced. Importantly, the structure of the microbiota of cold-eating rabbit meat changed significantly, and *Tardiphaga*, *Acinetobacter*, *Bacillus*, and unclassified *Bacteria* were the dominant bacterial genera after high temperature frying, deep frying, and subsequent inactivation processes. In particular, *Tardiphaga* and *Bacillus* dominated during early storage; the relative proportion of *Tardiphaga* in samples C1, C2, C3, and C4 was 58.21%, 48.10%, 40.40%, and 66.12%, respectively; in C5, the relative abundance of Bacillus increased significantly, becoming the most abundant genus (68.69%), while *Tardiphaga* accounted for only 7.00%. In contrast, the relative abundance of *Acinetobacter* did not change considerably throughout storage, and the abundance of unclassified *Bacteria* decreased gradually with prolonged storage time. Moreover, the bacterial community of cold-eating rabbit meat at the spoilage endpoint was mainly composed of *Lactobacillus*, *Weisseria*, *Staphylococcus*, and *Bacillus*, accounting for 84.39%, 10.27%, 3.81%, and 1.48%, respectively, whose proportions when combined corresponded to 99.95% of the microbiota.

Interestingly, *Lactobacillus* was found only in raw meat and in spoiled final products, with no occurrence during storage, thus indicating that *Lactobacillus* in spoiled products may originate from raw meat. *Tardiphaga*, *Bacillus*, *Acinetobacter*, and other bacteria can produce extracellular enzymes, such as high-activity proteases and lipases, that can effectively degrade fat, protein, polysaccharides, and other molecules in cold-eating rabbit meat into smaller molecules, thus laying the foundation for the rapid growth of *Lactobacillus*. After *Lactobacillus* became abundant in the microbiota, the pH value in the meat matrix decreased with the increase in acid production, which in turn inhibited the metabolic activity of *Tardiphaga*, *Bacillus*, and *Acinetobacter*.

In addition, *Staphylococcus* and *Weisseria* were found throughout the storage period (<1%) but did not occur in raw meat or during rinsing and curing, which may be due to the fact that the relative abundance of other bacteria was significantly high, and *Staphylococcus* was classified into others, or it contaminated cold-eating rabbit meat products in later stages of processing. Finally, *Pseudomonas aeruginosa* and *Acinetobacter* are frequently isolated from various types of chilled meat, and the unique bacteria found in chilled meat may be due to contamination during processing and storage [36].

4. Conclusion

Herein, it was described for the first time the bacterial diversity and composition of cold-eating rabbit meat using a high-throughput sequencing approach. Herein, it was shown that the sensory quality of cold-eating rabbit meat decreased significantly as storage time and temperature increased. Compared with storage at 4°C and 25°C, pH showed the highest rate of decrease and TBARS value increased at the fastest rate in cold-eating rabbit meat stored at 37°C. Additionally, microbial community diversity in cold-eating rabbit meat stored at 25°C was the highest in sample C2 (9 days of storage) and the lowest in sample C6 (spoilage end point). Moreover, during early storage, the most abundant bacteria were *Tardiphaga*, *Acinetobacter*, *Bacillus*, and unclassified *Bacteria*. Finally, towards the end of spoilage, *Lactobacillus*, *Weisellosis*, *Staphylococcus*, and *Bacillus* dominated the microbiota in cold-eating rabbit meat. This study explored changes in the structure of bacterial microbiota during storage of high-temperature-processed meat products, thus providing a theoretical basis for developing strategies for extending the shelf life of these food products.

Data Availability

The research data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

This supplement is a certificate of English language embellishment. *(Supplementary Materials)*

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