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Research Article

Study on the Growth, Decline, and Permeability of Dominant Microorganisms in Chilled Pork under Different Temperature Conditions

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ABSTRACT

Chilled meat has a high water activity and is rich in nutrients, so it is prone to microbial contamination during industrial production, and the microorganisms contaminated are widespread and complex. In order to address its biosafety issues and potential food safety risks, the experiment was conducted to investigate the variation of dominant spoilage bacteria in chilled meat under storage conditions of 2 ± 2°C, 10 ± 2°C and 20 ± 2°C, respectively. At the same time, the variation of spoilage bacteria in four layers of chilled meat under three temperature conditions was detected. The results showed that when chilled pork was stored at 2 ± 2°C and 10 ± 2°C, *Pseudomonas* sp., Thermosporaceae, and *Enterobacteriaceae* were the dominant spoilage bacteria in chilled pork, while when stored at 20 ± 2°C, Thermosporaceae, *Enterobacteriaceae*, *Pseudomonas* sp., and *Lactobacillus* sp. became the dominant spoilage bacteria in chilled pork. When chilled pork was stored at 2 ± 2°C and 10 ± 2°C, the order of proliferation of different types of spoilage microorganisms in the inner layer was as follows: Fusarium thermophila>*Enterobacteriaceae*>*Lacto bacillus*>*Pseudomonas*>Yeast/Mold>*Staphylococcus/Micrococcus*; When stored at 20 ± 2°C, the order of the number of proliferation of different types of spoilage microorganisms is as follows: Thermomycetes>*Enterobacteriaceae*>*Lactob acillus*>Yeast/Yeast>*Pseudomonas*>*Staphylococcus/Micrococcus*. Finally, through the change rules of dominant spoilage bacteria under three temperatures and the penetration of spoilage bacteria in chilled meat, it provides a theoretical basis for enterprises to improve precision sterilization and key control point technology.

Keywords: Chilled meat; Spoilage microorganisms; Temperature; Permeation situation

INTRODUCTION

Chilled meat, also known as cold meat or acid-removing meat, is a nutritious and tasty food that is an important part of meat consumption in China [1]. However, during industrial production, chilled meat is susceptible to microbial contamination due to its high water activity and nutrient-rich composition. This can lead to shortened shelf life and potential food safety risks [2]. In order to address these issues, it is important to analyze the changes in dominant spoilage bacteria in chilled meat under different storage conditions [3]. The growth characteristics of spoilage microorganisms differ depending on environmental conditions, packaging, and storage conditions. Moreover, the ability of different types of spoilage microorganisms to adapt to the surface and inner layers of chilled meat also varies [4].

The purpose of this study is to determine the dominant spoilage bacteria in chilled pork under different storage temperatures (2 ± 2°C, 10 ± 2°C, and 20 ± 2°C) and to investigate the variation of spoilage bacteria in four layers of chilled pork [5]. By understanding the growth patterns and dominance of different spoilage microorganisms in chilled meat, it is possible to identify key control points for targeted preservation of chilled meat and extend its shelf life. Therefore, the aim of this experiment is to measure the number of different types of spoilage microorganisms in chilled meat during storage, analyze the differences between microorganisms in the surface and inner layers of chilled meat [6], and determine the proliferation rate and growth pattern of

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different spoilage microorganisms in chilled meat to identify the more dominant spoilage microorganisms [7].This paper will provide a theoretical basis for improving precision sterilization and key control point technology in enterprises dealing with chilled meat.

MATERIALS AND METHODS

Materials, reagents and instruments

The experimental samples were provided by Shuanghui Investment and Development Co. from the slaughterhouse in Luohe City, Henan Province.

The following media were used are plate counting agar medium, nutrient agar medium, *Pseudomonas* agar medium, VRBGA agar medium, MSA agar medium, STAA agar medium, MRS agar medium, and Bengal red agar medium. These were obtained from Beijing Road and Bridge Technology Co Ltd. Petri dishes were purchased from BRAND (Germany). Sterile homogenization bags and sterile gun tips were purchased from Beijing Road and Bridge Technology Co. Ltd. BUG agar medium, GN/GP-IF inoculum, and GenIII microtiter plates were obtained from Biolog (USA).

The instruments and equipment used are listed in Table 1.

Sample preparation and storage

The longest back muscle, weighing approximately 200 g, was obtained from the slaughterhouse of Luohe City, Henan Province, Shuanghui Investment and Development Co. The muscle was processed using the normal procedure on the splitting line and then tray packed. The packed samples were stored at three different temperature conditions of 2 ± 2°C, 10 ± 2°C and 20 ± b 2°C. The numbers of total bacteria, *Enterobacteriaceae*, *Pseudomonas*, Thermospora, *Lactobacillus*, *Staphylococcus*, *Micrococcus* and Mycobacterium/yeast in the cooled pork were determined every 2 days, 1 day, and 6 hours to determine the pattern of decay of spoilage microorganisms in the cooled pork.

The cooled pork was then divided into four layers under aseptic conditions, with each layer having a thickness of approximately 1 cm. The total number of bacteria, *Enterobacteriaceae*, *Pseudomonas* spp., *Pseudomonas*, Thermospora, *Lactobacillus*, *Staphylococcus/Micrococcus*, and Mycobacterium/yeast in each layer was determined to evaluate the penetration of spoilage microorganisms in the cooled pork.

Microbiological analysis

The total bacterial count of the samples was determined according to the national standard GB4789.2-2016 "Microbiological test for food hygiene-Determination of total bacterial count".

Data analysis

Each experiment was conducted three times, and the data were analyzed using SPSS16.0 software. The results were expressed as Mean (M) ± Standard Deviation (SD). The mean values were compared using Duncan's multiple range test ($P<0.05$).

RESULTS AND DISCUSSION

Analysis of the initial bacterial composition of spoilage microorganisms in cooled pork

The total number of bacteria and the number of colonies of six types of dominant spoilage bacteria were detected in the cooled pork samples using selective media based on the national standard GB4789. The results are presented in Table 2.

Table 1: Instrumentation used in the experiments.

No.	Instrument name	Company
1	Ultra clean bench (Hf safe-1200)	Hong Kong Likang Development Limited (China)
2	Slap homogenizer (BagMixer 100/400/3500)	Interscience Corporation (France)
3	Oscillator (MS 3 basic)	IKA Corporation (Germany)
4	Incubator (LRH-250A)	Guangdong Medical Equipment Factory (China)
5	Autoclave (MLS-3780)	SANYO Corporation (Japan)
6	Biochemical incubator (LRH-250A)	Guangdong Medical Equipment Factory (China)
7	Automated Microbiological Analysis System (Biolog)	Biolog Corporation (USA)
8	Dedicated turbidity meter (Biolog)	Biolog Corporation (USA)
9	Single-channel pipettes (Eppendorf)	Eppendorf Corporation (Germany)
10	Eight-channel electric pipettes	Biolog Corporation (USA)
11	Electronic balances	Sartorius Corporation (Germany)

Table 2: Initial microflora constitution of chilled pork .

Bacterial phase	Quantity CFU/g	Log value lg(A)	Proportion%
Total number of bacteria	3.33×10^4~4.53×10^4	4.57 ± 0.07	100%
Enterobacteriaceae	4.07×10^3~4.67×10^3	3.62 ± 0.02	8.47~12.96%
Lactobacillus spp.	5.23×10^3~1.43×10^4	3.91 ± 0.19	15.07~29.80%
Pseudomonas spp.	1.19×10^3~1.66×10^4	3.12 ± 0.05	3.44~3.91%
Thermokill Soxhlet	2.17×10^4~2.64×10^4	4.30 ± 0.04	55.01~62.38%
Staphylococcus/Micrococcus	2.93×10^2~1.01×10^3	2.66 ± 0.33	0.61~2.90%
Yeast/Mold	1.13×103~1.27×10^3	3.07 ± 0.03	2.64~3.26%

Note: Ratio=the number of each bacterium /the sum of the number of the 6 species

The microbial quantity of cooled pork produced by the normal process of this enterprise was well controlled, and the initial total colony range was generally between 3.33-4.53 × 10^4 CFU/mL. Among the different categories of microorganisms in cooled pork, *S. thermophilus* was detected in the highest amount, reaching 2.17 × 10^4 to 2.64 × 10^4 CFU/g, accounting for more than 55% of the composition of the bacterial phase. *Staphylococcus/Micrococcus* had the least amount, ranging from 2.93 × 102 to 1.01 × 10^3 CFU/g, accounting for less than 3.00% of the composition of the bacterial phase. Based on Table 2, the initial microbial profile of cooled pork was as follows: the highest amount of *S. thermophilus* was detected, followed by Enterobacter and *Lactobacillus*, less *Pseudomonas* spp. and *Staphylococcus/Micrococcus*, and the lowest amount of *Staphylococcus/Micrococcus* was detected.

Pattern of decay of spoilage microorganism growth and comparative analysis in cooled split pork under 2 ± 2°C Condition

As shown in Figure 1, the total number of bacteria and various spoilage microorganisms in cooled pork stored at 2 ± 2°C increased to different degrees with the extension of storage time. In the first 3 days of storage, there was almost no growth, and then it entered a period of rapid growth. Among them, *S. thermophilus* had the highest initial number, and it also maintained the highest number during storage. The initial number of *Enterobacteriaceae* was significantly smaller than that of *S. thermophilus*, but it grew rapidly during storage and was second only to the number of *S. thermophilus*. The number of colonies of *Pseudomonas* spp. was low at the beginning of storage, but it proliferated faster during storage and became one of the main dominant bacteria under this temperature condition. Although the initial number of *Lactobacillus* was higher, it gradually lost its numerical advantage during storage. Mold/Yeast grew more slowly during storage and gradually stabilized after 7 days. *Staphylococcus/Micrococcus* had the lowest number and growth rate during storage, as shown in Figure 1. Based on the analysis of Figure 1, the main dominant spoilage bacteria of cooled pork at 2 ± 2°C were *S. thermophilus*, *Enterobacteriaceae*, and *Pseudomonas* spp (Table 3).

The results from Table 3 and Figure 1 suggest that during the storage of cooled pork at 2 ± 2°C, the proportion of Enterobacteriaceae and *Pseudomonas* spp. increased significantly, indicating that these two microorganisms have a high growth rate at this temperature condition and are important factors contributing to pork spoilage. On the other hand, the proportion of *Lactobacillus*, *Staphylococcus/Micrococcus*, and Mycobacterium/yeast decreased during storage, indicating that these microorganisms were not dominant in the spoilage of cooled pork at this temperature condition. The results also suggest that *S. thermophilus*, which had the highest initial number, remained the dominant microorganism throughout the storage period. Overall, the results indicate that controlling the growth of *Enterobacteriaceae* and *Pseudomonas* spp. during the storage of cooled pork at 2 ± 2°C is crucial for extending the shelf life of the product.

The study analyzed the microbial changes in different layers of chilled pork during storage at 2 ± 2°C. The results showed that the first layer of cooled pork had the fastest growth rate and the largest number of microorganisms, with the total number of colonies exceeding 108 CFU/mL on the 9th day of storage. The second layer had a slower growth rate, with the total number of colonies of Thermokill Soxhlet exceeding 10^6 CFU/mL and the number of colonies of *Enterobacteriaceae* increasing rapidly and approaching 10^6 CFU/mL. The third layer had the slowest growth rate, with the total number of colonies of Thermokill Soxhlet close to 10^4 CFU/mL and the number of colonies of *Enterobacteriaceae* still higher than that of *Pseudomonas*. The fourth layer had relatively lower numbers of microorganisms, with the total number of Thermokill Soxhlet colonies close to 10^3 CFU/mL and the number of *Enterobacteriaceae* colonies being the second highest

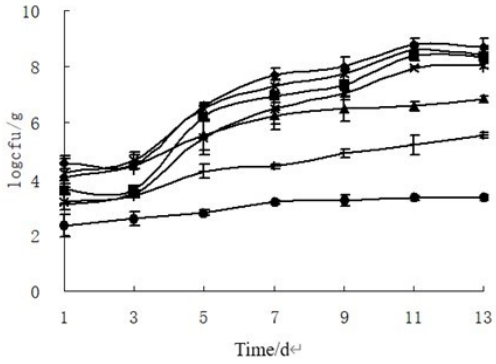

Figure 1: The growth of spoilage microorganism in chilled pork stored at 2 ± 2°C. **Note:** :Total number of bacteria; :Enterobacteriaceae; :Lactobacillus spp.; :*Pseudomonas spp.*; :Thermkill Soxhlet; : Staphylococcus/Micrococcus; : Yeast/Mold

Table 3: The change of microflora constitution of chilled pork storage at 2 ± 2°C.

Bacterial phase	Time/d						
	1	3	5	7	9	11	13
Enterobacteriaceae	8.47%	4.75%	25.24%	33.98%	32.67%	35.43%	33.06%
Lactobacillus spp.	29.80%	33.48%	9.78%	5.23%	4.17%	0.54%	0.94%
Pseudomonas spp.	3.47%	3.11%	9.41%	10.62%	10.34%	12.14%	15.25%
Thermokill Soxhlet	55.01%	55.68%	55.22%	50.10%	52.75%	51.86%	50.69%
Staphylococcus/ Micrococcus	0.61%	0.45%	0.01%	0.01%	<0.01%	<0.01%	<0.01%
Yeast/Mold	2.64%	2.53%	0.33%	0.07%	0.08%	0.03%	0.05%

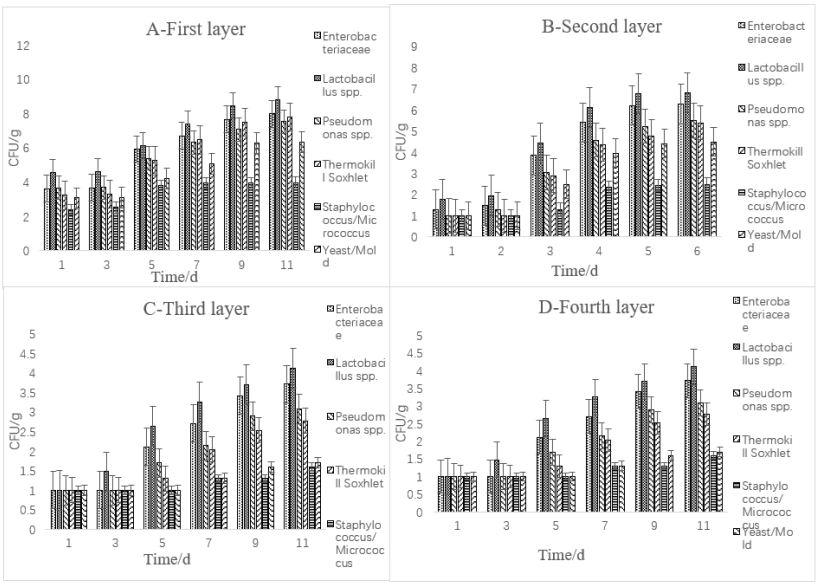

Figure 2: The spoilage microorganism quantity in 4-layer chilled meat at 2 ± 2°C.

after Thermokill Soxhlet (Figure 2).

Overall, the results suggest that the growth rate and number of microorganisms in chilled pork are influenced by the storage conditions and location in the meat. Therefore, proper storage conditions and handling practices are essential to ensure the safety and quality of chilled pork. It appears that under the storage conditions of 2 ± 2°C, the growth rate and number of colonies of Thermokill Soxhlet and Enterobacter were relatively high in all four layers of the chilled pork. In contrast, *Pseudomonas* and lactic acid bacteria showed weaker permeability under these conditions, possibly due to changes in oxygen availability. It is worth noting that the permeability of microorganisms can vary depending on the storage conditions, as well as the specific strain of microorganism in question. Therefore, it is important to monitor the microbial changes and adjust the storage conditions accordingly to ensure the safety and quality of the chilled pork.

Growth and decline law and comparative analysis of spoilage microorganisms in cooled and segmented pork at 10 ± 2°C

When cooled pork is stored at 10 ± 2°C, the total number of bacteria and the number of spoilage microorganisms increase to varying degrees with the extension of storage time, as shown in Figure 3. The indicators remain stable for the first 2 days of storage before entering a period of rapid growth. The growth tends to stabilize after 5 days. Among the spoilage microorganisms, Thermokill Soxhlet had the highest initial number and maintained the highest number throughout storage. The initial number of *Enterobacteriaceae* was significantly smaller than that of Thermokill Soxhlet, but it grew rapidly in storage and became the second highest number after Thermokill Soxhlet. *Pseudomonas* had a high proliferation rate in the middle and late stages of storage, exceeding the number of lactic acid bacteria on the 3rd day. Although the initial number of lactic acid bacteria was high, it lost its numerical advantage at the 4th day. Mold/yeast growth was slower in storage, and *Staphylococcus/Micrococcus* had the lowest number and growth rate in storage, both significantly lower than those of Thermokill Soxhlet, *Enterobacteriaceae* and *Pseudomonas*, as shown in Figure 3. Thus, under these storage conditions, Thermokill Soxhlet, *Enterobacteriaceae* and *Pseudomonas* are the dominant spoilage bacteria for cooled pork (Figures 3 and 4, Table 4).

When cooled pork was stored at 10 ± 2°C, the proportion of *Enterobacteriaceae* and *Pseudomonas* in the bacterial community increased significantly, with *Enterobacteriaceae* increasing from 12.12% to more than 24.48% and *Pseudomonas* increasing from 3.91% to 13.44%. In contrast, the proportion of Thermokill Soxhlet remained stable, accounting for about 60% of the community. However, the proportions of lactic acid bacteria, *Staphylococcus/Micrococcus* and mold/yeast decreased over time, with lactic acid bacteria decreasing from 21.73% to less than 2.00%, *Staphylococcus/Micrococcus* decreasing from 1.17% to less than 0.01%, and mold/yeast decreasing from 3.06% to less than 0.05%. This decline was mostly replaced by *Enterobacteriaceae* and *Pseudomonas*. Combining Figure 3 and Table 4, it can be concluded that Thermokill Soxhlet, *Enterobacteriaceae*, and *Pseudomonas* are the dominant spoilage microorganisms in cooled pork when stored at 10 ± 2°C.

The number of spoilage microorganisms present in different layers of cooled pork stored at 10 ± 2°C is depicted in Figure 4. The microbial changes tend to stabilize after 5 days of storage as compared to 2 ± 2°C. The results revealed that, on the 5th day of storage, the total number of Thermokill Soxhlet colonies in the first layer of cooled pork was close to 10^9 CFU/mL, while the total number of colonies of Lactic Acid bacteria, *Enterobacteriaceae* and *Pseudomonas* exceeded 10^7 CFU/mL, and the total number of colonies of *Staphylococcus/Micrococcus* exceeded 10^4 CFU/mL. In the second layer, the total number of Thermokill Soxhlet and *Enterobacteriaceae* colonies was close to 10^6 CFU/mL, while the number of Lactic Acid bacteria and *Pseudomonas* was close to 105 CFU/mL, and the growth of *Staphylococcus/Micrococcus* and mold/yeast was the slowest, with the total number of colonies being about 102 CFU/mL. In the third layer, the total number of Thermokill Soxhlet colonies was close to 10^4 CFU/mL, followed by the number of *Enterobacteriaceae* colonies, which was significantly higher than that of *Pseudomonas*, and the total number of *Staphylococcus/Micrococcus* and mold/yeast colonies was close to 10^1 CFU/mL. In

the fourth layer, the total number of Thermokill Soxhlet colonies was close to 10^3 CFU/mL, and the number of *Enterobacteriaceae* colonies was second only to Thermokill Soxhlet. The number of *Lactobacillus* species was higher than the number of *Pseudomonas*, and the total number of mold/yeast colonies was more than 10^1 CFU/mL, while the total number of *Staphylococcus/Micrococcus* colonies was less than 10^1 CFU/mL.

It can be inferred from Figure 4 that when cooled pork is stored at 10 ± 2°C, the ability of some dominant spoilage bacteria to penetrate the 4 layers of cooled pork is as follows: Thermokill So xhlet>*Enterobacteriaceae*>Lactic Acid bacteria>*Pseudomonas*>Yeast/ Yeast>*Staphylococcus/Micrococcus*.

Growth and decline law and comparative analysis of spoilage microorganisms in cooled and segmented pork at 20 ± 2°C

Figure 5 shows that as the storage time increases, the total number of bacteria and various spoilage microorganisms in cooled pork stored at 20 ± 2°C also increase, with minimal growth in the first 12 hours and a period of rapid growth afterward. The growth rate tends to slow down after 42 hours. Thermocidae has the highest initial number and maintains a high number throughout the storage period. *Enterobacteriaceae* grows rapidly and has the second-highest number after Thermocidae. Lactic acid bacteria have a higher initial number but grow slightly slower than *Enterobacteriaceae*. The initial number of *Pseudomonas* is significantly smaller than that of the other bacteria but has a high growth rate in storage, ranking second only to lactic acid bacteria. Mold/yeast growth is slower, while *Staphylococcus/Micrococcus* has the lowest number and growth rate compared to the other bacteria, including Thermocida, *Enterobacteriaceae*, Lactic Acid Bacteria, and *Pseudomonas* species. Therefore, it can be concluded that Thermocida, *Enterobacteriaceae*, *Pseudomonas*, and Lactic Acid Bacteria are the dominant spoilage bacteria in cooled pork stored at 20 ± 2°C (Figure 5).

Actually, the information you provided is about chilled pork stored at 20 ± 2°C, not cooled pork. It is important to note that there is a difference between "chilled" and "cooled" pork. Chilled pork refers to meat that has been cooled to a temperature just above freezing, typically between 0 and 2°C, while cooled pork refers to meat that has been cooled to a higher temperature, usually between 4 and 10°C.

The study you mentioned showed that *Enterobacteriaceae* and *Pseudomonas* increased in proportion during storage of chilled pork at 20 ± 2°C, while the proportion of Thermokill Soxhlet lactic acid bacteria, *Staphylococcus/Micrococcus*, and mold/yeast decreased. This suggests that *Enterobacteriaceae* and *Pseudomonas* are dominant spoilage bacteria in chilled pork under these conditions, with lactic acid bacteria also playing a role in spoilage. It is important to note that the dominant spoilage bacteria may differ under different storage conditions, such as temperature and packaging, and that proper storage and handling of meat is necessary to prevent spoilage and foodborne illness (Table 5).

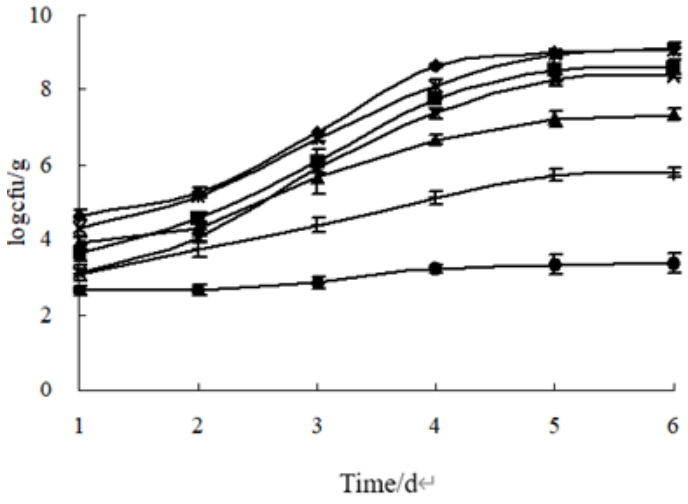

Figure 3: The growth of spoilage microorganism in chilled pork stored at 10 ± 2°C. **Note:** :Total number of bacteria; :Enterobacteriaceae; :Lactobacillus spp.; :*Pseudomonas spp.*; :Thermkill Soxhlet; : Staphylococcus/Micrococcus; : Yeast/Mold

Table 4: The change of microflora constitution of chilled pork storage at 10 ± 2°C.

Bacterial phase	Time/d					
	1	2	3	4	5	6
Enterobacteriaceae	12.12%	17.66%	18.64%	26.72%	25.83%	24.48%
Lactobacillus spp.	21.73%	9.91%	8.73%	2.25%	1.24%	1.31%
Pseudomonas spp.	3.91%	5.89%	11.37%	11.32%	12.91%	13.44%
Thermokill Soxhlet	58.00%	63.52%	60.92%	59.64%	59.98%	60.74%
Staphylococcus/ Micrococcus	1.17%	0.22%	0.01%	<0.01%	<0.01%	<0.01%
Yeast/Mold	3.06%	2.80%	0.32%	0.06%	0.04%	0.04%

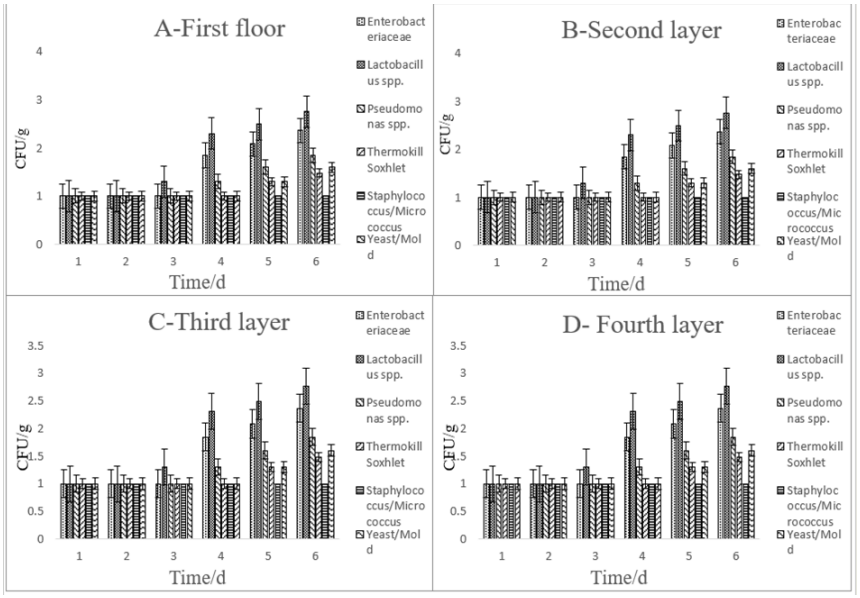

Figure 4: The spoilage microorganism quantity in 4-layer chilled meat at 10 ± 2°C.

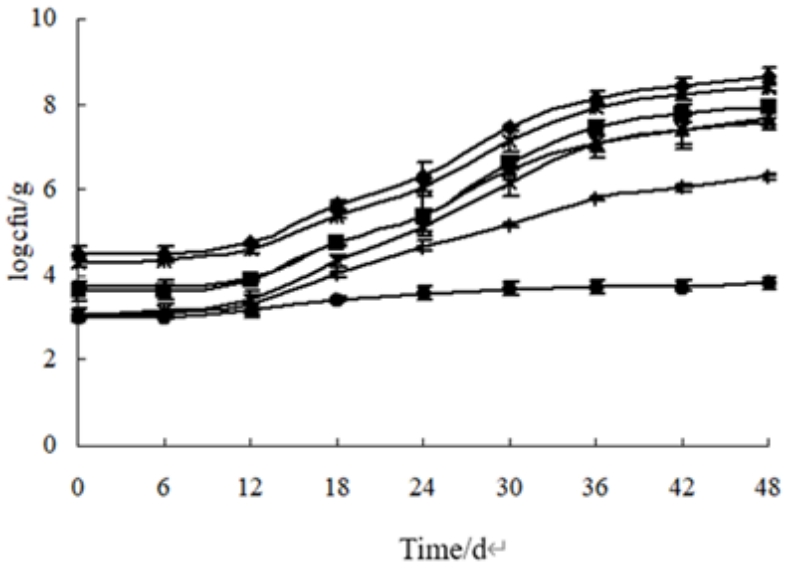

Figure 5: The growth of spoilage microorganism in chilled pork stored at 20 ± 2°C. **Note:** :Total number of bacteria; :Enterobacteriaceae; :Lactobacillus spp.; :*Pseudomonas spp.*; :Thermkill Soxhlet; : Staphylococcus/Micrococcus; : Yeast/Mold

Table 5: The change of microflora constitution of chilled pork storage at 20 ± 2°C.

Bacterial phase	Time /h								
	0	6	12	18	24	30	36	42	48
Enterobacteriaceae	12.96%	12.61%	12.91%	14.20%	18.79%	18.76%	19.68%	20.03%	20.51%
Lactobacillus spp.	15.07%	15.29%	13.80%	13.95%	12.69%	12.78%	11.11%	11.14%	11.11%
Pseudomonas spp.	3.44%	4.20%	4.58%	5.48%	6.59%	7.30%	8.41%	9.53%	9.63%
Thermospora	62.38%	61.69%	62.70%	63.11%	59.35%	60.51%	60.37%	58.92%	58.27%
Staphylococcus/ Micrococcus	2.90%	2.91%	2.42%	0.65%	0.20%	0.02%	<0.01%	<0.01%	<0.01%
Mycobacterium/yeast	3.26%	3.31%	3.58%	2.62%	2.37%	0.63%	0.43%	0.36%	0.47%

The microbial changes in the 4-layer part of pork during storage at 20 ± 2°C. From the results, it appears that the microbial growth tends to stabilize at the 30th hour of storage. The first layer of cooled pork had the highest number of microorganisms, with Thermokill Soxhlet showing the most rapid growth rate and highest number of colonies. The second and third layers also showed high numbers of colonies of *Enterobacteriaceae* and *Lactobacillus* species. In contrast, the fourth layer had relatively lower numbers of colonies of all microorganisms except for Thermokill Soxhlet.

The results suggest that Thermokill Soxhlet is the most important spoilage bacteria in all four layers of meat, while *Enterobacteriaceae* and lactic acid bacteria are also significant spoilage microorganisms. *Pseudomonas*, *Staphylococcus/Micrococcus*, and mold/yeast had lower total colony numbers and weaker proliferation capacity at 20 ± 2°C for 36 hours. Overall, the study provides important insights into the microbial changes in pork during storage at ambient temperatures, which can help in the development of better preservation strategies to prevent spoilage and improve food safety (Figure 6).

Chilled meat is a nutrient-rich food that is susceptible to microbial contamination starting from the slaughtering process. Segmentation further exacerbates the secondary microbial contamination of chilled pork [8]. During subsequent storage and sales, variations in temperature, environmental hygiene, and cross-contamination by personnel can accelerate the proliferation of microorganisms. Microbial contamination and proliferation are the main causes of spoilage in chilled meat. When the bacterial count on the surface of chilled meat exceeds 10^7 CFU/cm^2, it begins to deteriorate, and these spoilage characteristics are closely related to the biological and decay characteristics of the spoilage microorganism's themselves [9]. Different types of spoilage microorganisms have varying growth and reproduction characteristics under different environmental conditions. They are influenced by factors such as nutrition, temperature, pH, competition, and coexistence, which produce unique microbial effects [10].

A study conducted by Jing, et al. revealed that the major spoilage bacteria in chilled meat were *Pseudomonas* (25%~26%), *Enterobacteriaceae* (19%~25%), Lactic acid bacteria (20%~21%), *Micrococcus* and *Staphylococcus* (12%~15%), and Cyclofilium thermodylidae (12%~13%) [3]. This finding differed from the results of the first detection ratio of Soxobacterium colonies in our study. Novakovic, et al. suggests that *Pseudomonas* is the dominant bacteria in cooling pork, while other scholars indicate that *Zoobacterium*, Moraxella, *Enterobacteriaceae*, and Soxobacter thermocidal can also be the dominant bacteria under different conditions [11,12]. The differences may be attributed to variations in production areas, meat processing enterprises, pork slaughtering environments, carcass segmentation equipment technology, and management measures of industry-related enterprises, resulting in initial contamination of different microorganisms and strain variations.

Experimental findings indicate that when stored at 2 ± 2°C and 10 ± 2°C, the dominant spoilage bacteria in cooling pork are thermocidactylus, *Enterobacteriaceae* and *Pseudomonas*, with the highest proportion of *Pseudomonas* and *Enterobacteriaceae* [13]. While the initial number of lactic acid bacteria is higher, it is inhibited to some extent during storage. When stored at 20 ± 2°C, lactic acid bacteria grow more and become one of the dominant spoilage bacteria in addition to the above three. A study on the penetration of pork microorganisms at different levels of cooling and segmentation found that when stored at 2 ± 2°C and 10 ± 2°C, the proliferation of different species of spoilage microorganisms in the inner layer was as follows: Thermokill Soxhlet>*Enterobacteriaceae*>Lactic acid bacteria>*Pseudomonas*>Yeast/Yeast>*Staphylococcus/Micrococcus*. When stored at 20 ± 2°C, the proliferation of different species of spoilage microorganisms was as follows: Thermokill Soxhlet>*Enterobacteriaceae*>Lactic acid bacteria>Yeast/Yeast>*Pseudomonas*>*Staphylococcus/Micrococcus*, mainly due to changes in oxygen levels affecting *Enterobacteriaceae*, lactic acid bacteria, and *Pseudomonas* [14].

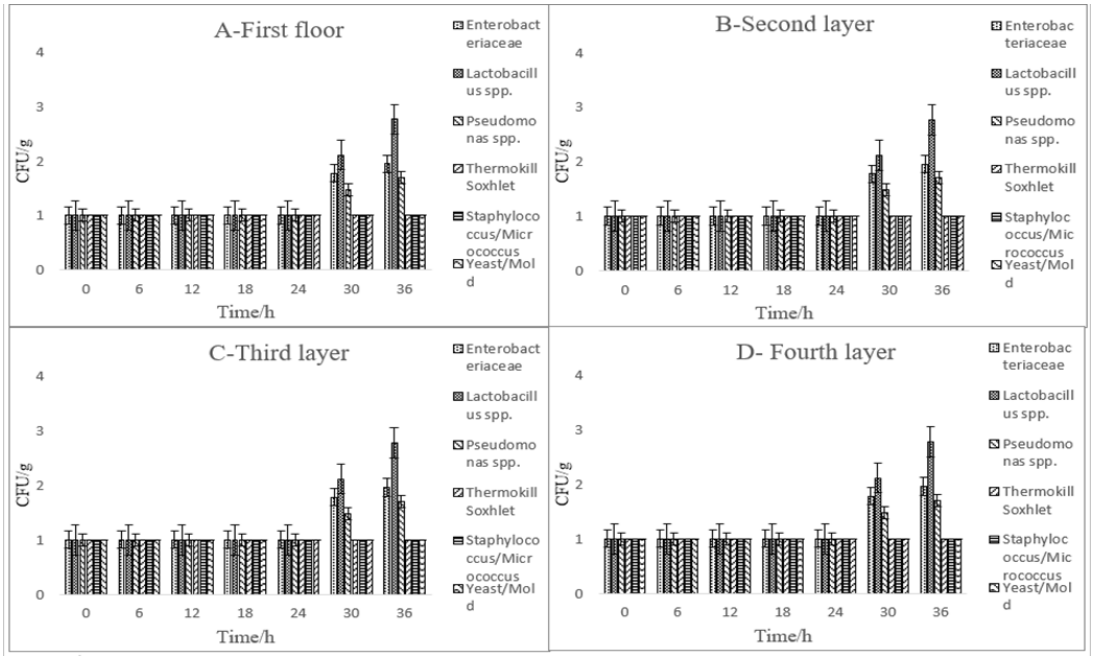

Figure 6: The spoilage microorganism quantity in 4-layer chilled meat at 20 ± 2°C.

Carcass contamination is the primary cause of cooled pork pollution, occurring when animals are first slaughtered and microorganisms are mainly distributed on the carcass surface. Subsequent segmentation breaks the carcass structural system, leading to secondary contamination of cooled pork. During the initial stages of segmentation, microorganisms are concentrated on the surface and have not yet penetrated the food's interior. However, further refinement of some cooled pork is necessary for production and consumer demand, leading to structural degradation and contamination of the inner layer [15]. Nevertheless, the overall number of microorganisms in the segmented cooled pork's interior is minimal or even sterile. Different types of spoilage microorganisms have distinct growth characteristics, adaptation abilities to cooling pork's surface and interior, and penetration rates from the surface to the inner layer. Facultative anaerobic microorganisms, such as Thermokill Soxhlet, *Enterobacteriaceae*, and lactic acid bacteria, have high proliferation rates in cooling pork. *Pseudomonas* is aerobic and proliferates at a relatively slow rate, while *Pseudomonas* aeruginosa is aerobic or facultative anaerobic with certain penetration abilities. Yeast/mold has slow reproduction rates but survive in hypoxic environments and have some penetration ability [16]. The proliferation rate of spoilage microorganisms is also influenced by factors such as oxygen partial pressure, nutrient utilization capacity, antagonism, and the composition of spoilage microorganisms in cooled pork [17].

CONCLUSION

This experimental study demonstrates that facultative anaerobic microorganisms have significant penetration ability, with growth occurring in each layer of cooling meat, and some microorganisms are in the late storage stages to grow in the inner layer of cooling meat. In general, the proliferation of Thermokill Soxhlet and *Enterobacteriaceae* is the highest, followed by lactic acid bacteria. Although one of the dominant spoilage microorganisms on the cooling meat surface, the number of yeast/mold proliferation is significantly lower than the above microorganisms. Enterprises should target sterilization to control the proliferation of cooled meat microorganisms and improve food safety.

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CONFLICT OF INTEREST

No conflict of interest declared.

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