



Evaluation of the microbiological shelf life and sensory quality of rabbit meat produced in a slaughterhouse in Algeria

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Abstract

Rabbit meat, known for its nutritional qualities, is often considered a more dietetic and healthier alternative to other types of meat. This study aims to evaluate the microbiological shelf-life and sensory quality of rabbit meat from a rabbit slaughterhouse in Algeria. A shelf-life test was conducted on twenty-four carcasses, divided into four lots, to assess contamination by hygiene indicator microorganisms, such as *Escherichia coli*, *Enterobacteriaceae*, and potential pathogens including *Salmonella spp.* and coagulase-positive staphylococci. The analyses were performed at three different time points : Day 1 (24 hours post-slaughter), Day 3, and Day 7, at storage temperatures of 4°C and 8°C. The results showed that the meat met the microbiological criteria for *Salmonella spp.*, *E. coli*, and coagulase-positive staphylococci. However, while *Enterobacteriaceae* levels were satisfactory at Days 1 and 3, they were non-compliant at Day 7, limiting the microbiological shelf-life to less than seven days. In contrast, the sensory quality of the meat remained acceptable throughout the storage period. The enumeration of *Enterobacteriaceae* proved to be a useful indicator for assessing enteric contamination, a critical factor affecting the hygienic quality of the meat. Low initial contamination contributes to an extended microbiological shelf-life, highlighting the importance of maintaining good hygiene practices throughout the production chain.

Keywords : Microbiological shelf-life, sensory quality, rabbit meat, slaughterhouse.

1. Introduction

Meat is considered the most nutritious and energy-rich food product, providing the human body with essential amino acids and micronutrients necessary for growth and development (Wood and Enser, 2017).

Rabbit meat is often perceived as healthier than other meats due to its low saturated fat and cholesterol content (Adeyemi et al., 2022). Its low caloric value, high protein content (17-26%) (Kumar et al., 2023), and rich supply of minerals such as potassium, calcium, and phosphorus (Nistor et al., 2013) make it a food with excellent nutritional properties. These characteristics can also contribute to the prevention of cardiovascular diseases, atherosclerosis and hypertension (Hermida et al., 2006; Bernstein et al., 2010).

Algeria stands out among the top ten rabbit producing countries in the world. In 2021, it ranks fifth, behind China, the Republic of Korea, Egypt and France, with a production of 8,474 tons (FAOSTAT, 2023).

The commercialization of rabbit meat is crucial in the livestock sector. It is based on the increasing importance of food quality, organoleptic and sensory properties, while addressing food safety and human health concerns (Hoffman et al., 2004).

Slaughter and meat preparation conditions in abattoirs, if properly managed, play a key role in ensuring product safety. Hygiene control during slaughter is an essential element in the management of all biological hazards (Clinquart et al., 2022). Improper handling during slaughter and carcass preparation can result in significant surface contamination. This not only affects the bacteriological quality of the meat, but also alters its organoleptic properties, which may be detrimental to the health of the consumer (Dennaï et al., 2001).

This study was conducted in response to a request from the owner of a rabbit abattoir in Algeria, who sought to determine the microbiological shelf life of the rabbit meat produced in his facility. The objective was to optimize the commercialization of his product.

2. Materials and Methods

2.1 Slaughtering of the Animals

Our study began with a visit to the rabbit slaughterhouse in question, located in Tizi-Ouzou, approximately 100 kilometers east of Algiers. The aim of this visit was to observe the different slaughtering steps while identifying any potential anomalies in order to propose corrective measures if necessary.

At this facility, an average of fifty rabbits are slaughtered daily. The rabbits are slaughtered manually by exsanguination, in accordance with the appropriate procedures for Muslim ritual slaughter. This step is followed by a five-minute draining process. The skinning of the carcasses is done manually using the dressing method, which involves two operators, as described by Kpodékon et al. (2000). The rabbits are then suspended by their hind legs at the hock joint. The carcass is opened along the abdominal wall to remove the viscera, while the head, liver, kidneys, and heart remain attached. The carcasses are then placed in the drying room and stored in a cold chamber at a temperature between 0°C and +2°C for 24 hours.

2.2 Packaging

The slaughterhouse produces whole carcasses intended for commercial sale. These carcasses are wrapped in a flexible plastic film, which seals them tightly. Once packaged, the carcasses are placed in secondary packaging consisting of plastic crates, ensuring both protection and product identification during transport. These containers comply with the CEE/UN standard (Anonymous, 2013).

2.3 Sample Collection

A total of twenty-four rabbit carcasses, divided into four groups of six, were included in this study. Each group was transported to the National Veterinary School of Algiers using a refrigerated truck, maintained at a temperature between 0°C and 4°C. 2.4 Sensory Evaluation

The sensory evaluation of rabbit meat was carried out at different times : upon receipt of the carcasses, 24 hours after slaughter (Day 1), and during storage in the laboratory at Days 3 and 7. Two storage temperatures were tested : 4°C and 8°C. A thorough inspection of the carcasses was conducted upon receipt. This inspection included checking for the presence of clots or bloodstains, bruises, bone residues, and any visible foreign matter (dirt, hair, etc.).

The sensory quality of the rabbit meat was determined based on a quality ranking system, a method primarily applied to assess the freshness quality of rabbit meat. This approach involves evaluating key characteristics such as color, odor, and texture, with a demerit scoring system ranging from 0 to 3 (Rodríguez-Calleja et al., 2004), as shown in Table 1.

Table 1. Sensory quality system developed for rabbit meat (Rodríguez-Calleja et al., 2004).

| Parameter | Characteristic | Demerit points |
|----------------------|----------------------------------|----------------|
| Color | Characteristic rabbit meat color | 0 |
| | Opaque | 1 |
| | Yellowish | 2 |
| Odor (fresh) | Normal | 0 |
| | Neutral | 1 |
| | Trace of “off” odor | 2 |
| Odor (boiled) | Normal | 0 |
| | Neutral | 1 |
| | Abnormal, urine | 2 |
| Flesh texture | Firm | 0 |

| | | |
|--|----------------------------------|-----------|
| | Slightly soft | 1 |
| | soft | 2 |
| Wetness | Normal | 0 |
| | Slightly dry or slightly wet | 1 |
| | Moderately dry or moderately wet | 2 |
| | Excessive dry or excessive wet | 3 |
| Contamination (hair, feces, blood patches) | None | 0 |
| | Slight | 1 |
| | Moderate | 2 |
| Fat appearance | White | 0 |
| | Opaque | 1 |
| | Yellowish | 2 |
| Rancidity (odor) | Absent | 0 |
| | Slight | 1 |
| | Moderate | 2 |
| Maximum demerit points for fresh rabbit carcasses | | 17 |

2.5 pH Measurement

pH was measured 24 hours after slaughter (Day 1), and then on Days 3 and 7, at two tested storage temperatures (4°C and 8°C), using a digital pH meter (testo 205, Testo SE & Co. KGaA, Germany). The method involved immersing the pH meter electrode into the relevant muscle of the carcass to a depth of 2 cm, specifically at the level of the biceps femoris. The device was carefully cleaned before each measurement.

2.6 Microbiological Analyses

To evaluate the microbiological shelf life of the rabbit meat produced at the abattoir in question, we conducted a shelf-life test on the four batches received, following the NF V 01-003 (2018) standard. The steps followed for each batch are illustrated in Figure 1.

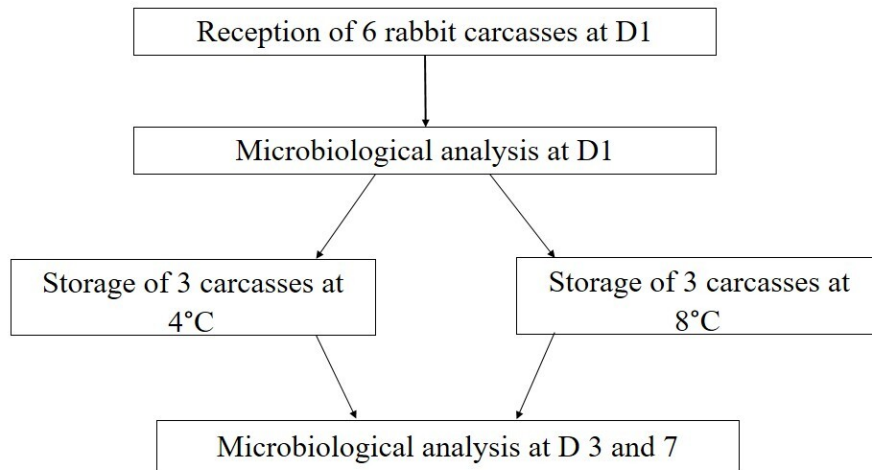


Figure 1. Stages of the shelf-life test.

The microbiological analyses conducted focused on the detection of hygiene indicator organisms, such as *Enterobacteriaceae* and *Escherichia coli*, as well as potential pathogens, including *Salmonella spp.* and coagulase-positive staphylococci, that may be present in the samples. This approach is in accordance with the recommendations established in the Official Journal of the Democratic People's Republic of Algeria (Anonymous, 2017).

Rabbit meat samples and serial dilutions were prepared in accordance with ISO 6887-2 (2003) standards. For each microorganism tested, 25 g of rabbit meat were aseptically collected from five distinct sites on each carcass : the thigh (*Biceps femoris*), abdominal wall, loin (*Longissimus lumborum*), chest, and shoulder, with 5 g taken from each site. These samples were then transferred into sterile stomacher bags, to which 225 mL of buffered peptone water (BPW) (Pronadisa, Madrid, Spain) was added. The samples were homogenized using a peristaltic stomacher at 250 cycles for two minutes. The samples were left at room temperature for 5 minutes, resulting in a 10^{-1} primary suspension. From this suspension, serial decimal dilutions were made.

The enumeration of *Enterobacteriaceae* at 30°C was carried out on Violet Red Bile Glucose Agar (VRBG) (Pronadisa, Madrid, Spain), incubated for 24 hours according to ISO 21528-2 (2004). For the isolation of *Escherichia coli*, chromogenic Tryptone Bile X-glucuronide Agar (TBX) (Pronadisa, Madrid, Spain) was used, incubated at 37°C for 24 hours in accordance with ISO 16649-2 (2001). *Salmonella spp.* detection was carried out according to the ISO 6579-1 (2002) guidelines, by isolation on Xylose-Lysine-Desoxycholate Agar (XLD) (Pronadisa, Madrid, Spain) and Hektoen Enteric Agar (Liofilchem Srl, Italy), both incubated at 37°C for 24 hours. Suspected colonies were then subjected to biochemical testing using Triple Sugar Iron Agar (TSI) (Difco, BD). Coagulase-positive staphylococci were isolated on Baird-Parker Agar (Pronadisa, Madrid, Spain), containing egg yolk and potassium tellurite. Coagulase presence was confirmed using rabbit plasma (Bio-Rad, France), according to ISO 6888-1 (1999).

2.7 Statistical Analysis

Statistical analysis was performed using SPSS software (version 26). The normality of the data distribution was assessed using the Shapiro-Wilk test, while the homogeneity of variances between groups was determined using Levene's test. Due to the sample size and deviations from normal distribution assumptions, non-parametric tests were preferred for the subsequent analysis. Comparisons of bacterial load and pH for each study day (Day 1, Day 3, and Day 7) were conducted using the Mann-Whitney U test. Repeated measures ANOVA was used to evaluate the bacterial growth dynamics in rabbit meat over Days 1, 3, and 7 at 4°C and 8°C. pH variations in rabbit meat during the experimental days (Day 1, Day 3, and Day 7) at 4°C and 8°C were analyzed using the Friedman test. All analyses were conducted at a significance level of $p < 0.05$.

3. Results

3.1 Slaughtering of the Animals

During our visit to the rabbit slaughterhouse, no anomalies were observed during the slaughter procedure, and the different stages of the process are illustrated in Figure 2.



Figure 2. Steps of rabbit slaughtering at the rabbit slaughterhouse.

3.2 Sensory Evaluation

For each characteristic, demerit points were assigned, and the scores for all characteristics were summed to obtain an overall sensory score, which should not exceed 17 points (Rodríguez-Calleja et al., 2004). The lower the score, the better the quality.

The results of the sensory evaluation of the rabbit carcasses on Days 1, 3, and 7, at different storage temperatures (4°C and 8°C), revealed that the average scores obtained were below the maximum threshold of 17 demerit points, as shown in Figure 4.

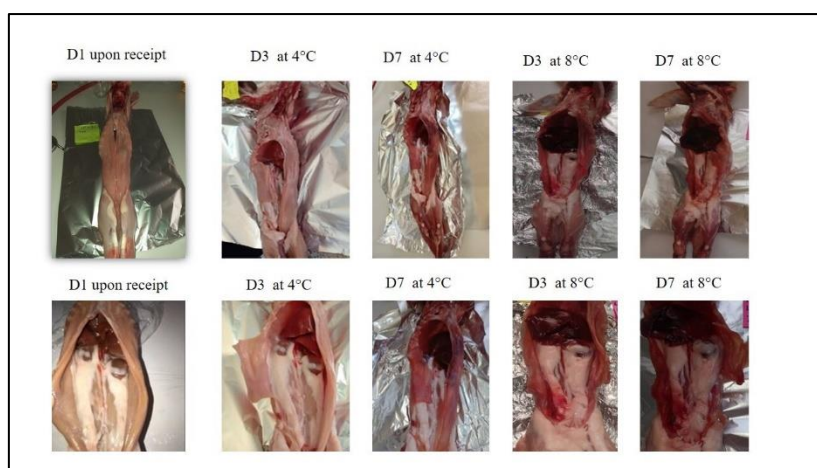


Figure 3. Sensory evaluation of rabbit carcasses.

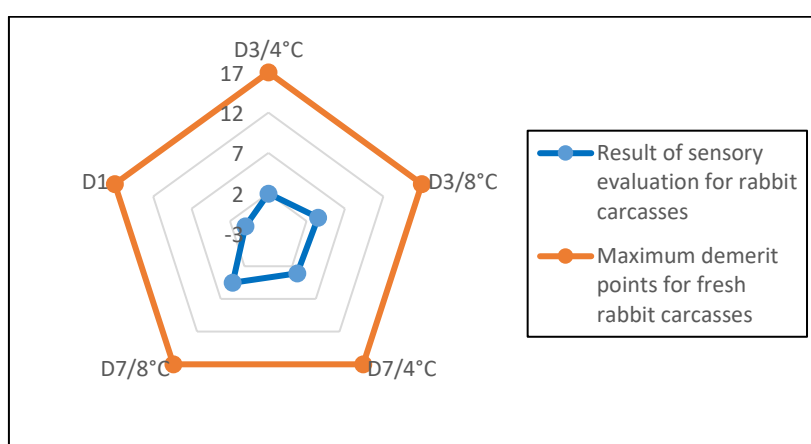


Figure 4. Results of the sensory evaluation of rabbit carcasses.

3.3 pH Measurement

The results of the pH measurements are presented in Table 2.

Table 2. pH evolution of rabbit meat on Days 1, 3, and 7 at 4°C and 8°C (M±SD)

| Temperature | Days | | |
|-------------|-------------------------|-------------------------|-------------------------|
| | D1 | D3 | D7 |
| 4 °C | 6,02±0,26 ^{aA} | 5,8±0,12 ^{bA} | 6,11±0,19 ^{aA} |
| 8 °C | 6,02±0,26 ^{aA} | 6,14±0,31 ^{aB} | 6,07±0,19 ^{aA} |

Changes of superscript small letters in the same row indicates a significant difference between the days (P<0.05).

Changes of superscript small letters in the same column indicates a significant difference between the temperature (P<0.05).

3.4 Microbiological Analyses

The results of the shelf-life test are presented in Table 3.

Table 3. Evolution of bacterial growth in rabbit meat on Days 1, 3, and 7 at 4°C and 8°C, expressed as Log₁₀ CFU/g (M±SD)

| Bacteria | Temperature | Days | | |
|----------------------------|-------------|-------------------------|-------------------------|--------------------------|
| | | D1 | D3 | J7 |
| <i>Enterobacteriaceae</i> | 4 °C | 3,64±1,27 ^{aA} | 3,78±1,48 ^{aA} | 4,64±1,11 ^{aA} |
| | 8 °C | 3,64±1,27 ^{aA} | 3,88±1,32 ^{aA} | 4,81±0,99 ^{bcA} |
| <i>E. coli</i> | 4 °C | 2,77±0,53 ^{aA} | 2,92±1,14 ^{aA} | 3,34±0,76 ^{aA} |
| | 8 °C | 2,77±0,53 ^{aA} | 3,15±0,8 ^{aA} | 3,85±1,43 ^{aA} |
| <i>Staphylocoques spp.</i> | 4 °C | 4,72±1,05 ^{aA} | 5,16±1,05 ^{ba} | 4,28±0,75 ^{acA} |
| | 8 °C | 4,72±1,05 ^{aA} | 5,33±0,98 ^{aA} | 4,75±0,9 ^{aA} |

Changes of superscript small letters in the same raw indicates a significant difference between the days ($P<0.05$).

Changes of superscript small letters in the same column indicates a significant difference between the temperature ($P<0.05$).

4. Discussion

The quality of meat is a comprehensive concept that encompasses not only safety, in terms of eliminating and reducing physical, chemical, and microbiological hazards, but also sensory and technological properties (Alarcon-Rojo et al., 2019).

The shelf life of meat is defined as the period during which it remains fit for consumption before it deteriorates, primarily due to bacterial activity (Borch et al., 1996). Meat is considered spoiled and should be removed from the commercial market if it is rejected by the consumer due to unacceptable color or odor, or if the microorganism concentration becomes too high and problematic.

Numerous tools are available to estimate the microbiological evolution of perishable refrigerated foods, with the microbiological shelf life aiming to ensure safety and wholesomeness (Anonymous, 2005). Temperature and storage duration are crucial parameters influencing microbial growth. Microbiological aging tests are conducted on food while maintaining their properties and storage conditions. In our study, the shelf life was set to seven days, with two temperatures to be evaluated : 4°C and 8°C.

The temperature of 4°C was selected under the assumption that the rabbit meat would be stored within a fully controlled cold chain. It is important to note that the internal temperature of rabbit meat must be consistently maintained between -1.5°C and +4°C following refrigeration post-slaughter (Anonymous, 2013).

The second temperature, 8°C, was chosen to simulate a storage environment where the cold chain is partially controlled. In this scenario, the shelf life of the product is influenced by various factors, such as transportation, storage, display conditions, as well as the act of purchase, including home transport and consumer storage (Anonymous, 2018).

Sensory evaluation is a scientific method designed to evoke, measure, analyze, and interpret responses to products as perceived by the senses : sight, smell, touch, taste, and hearing (Stone and Sidel, 1993).

The evaluation of "freshness" and potential consumer acceptance relies on several characteristics, such as appearance, color, odor, and texture. Sensory attributes are crucial for consumers and influence purchasing decisions (Rodríguez-Calleja et al., 2004; Prache et al., 2022). In our study, the scores assigned to each parameter ranged from 0 to 2 over the course of the storage period. The average scores obtained were well below the maximum threshold of 17 demerit points, indicating good sensory quality of the carcasses analyzed.

pH is also a key indicator of the freshness and shelf life of meat (Chwastowska-Siwiecka et al., 2013), as it reflects the degree of protein degradation (Bhat and Pathak, 2012). Several factors influence the final pH of rabbit meat, including pre-mortem stress, transportation procedures, fasting prior to slaughter, and slaughter conditions (Larivière-Lajoie et al., 2021). pH variations are also linked to microbial growth processes, which lead to the release of carbon dioxide (Liu et al., 2022).

In live rabbits, muscle pH is close to neutrality, but it decreases rapidly after slaughter, dropping from 6.4 to 5.8 within 30 minutes to 24 hours when the carcasses are cooled to 4°C. Thus, 20 hours post-slaughter, the pH is considered to be final (Zhoulin et al., 2024).

In our study, the average final muscle pH at the *Biceps femoris* (BF), measured 24 hours post-slaughter, was 6.02 ± 0.26 . According to Larivière-Lajoie (2021), BF pH values 24 hours post-mortem range between 5.80 and 6.83. Additionally, research conducted by Zhoulin et al. (2024) revealed that pH values of rabbit meat stored at 4°C for 8 days ranged from 6.12 to 6.61. In our study, the pH variation at the same temperature over a 7-day period ranged from 5.8 ± 0.12 to 6.11 ± 0.19 . Moreover, Cullere and Dalle Zotte (2018) noted that the normal pH range for rabbit meat at the BF is between 5.8 and 6.3. The values observed in our study at days 1, 3, and 7, ranging from 5.8 ± 0.12 to 6.14 ± 0.31 , fall within this normal range, indicating good product quality.

Microbial contamination is a limiting factor for the safety and shelf life of meat. The slaughtering process can lead to widespread contamination of muscle tissues by a wide range of microorganisms (Mahmoud et al., 2022).

The microbiological quality of rabbit meat raises concerns, as, like many other raw animal products, it can be contaminated by a broad spectrum of microorganisms, including agents responsible for foodborne illnesses such as *Staphylococcus aureus* and *Salmonella spp.* (Morshdy et al., 2023).

The results of the enumeration revealed that none of the rabbit meat samples contained *Salmonella spp.* Similar results were reported by Rodríguez-Calleja et al. (2006) in Spain, Bobbitt (2002) in Australia, and Ben Jaafar et al. (2002) in Tunisia. However, in Italy, Cerrone et al. (2004) isolated the bacterium in 0.81% of rabbit carcasses. Furthermore, Mahmoud et al. (2022) and Morshdy et al. (2023) reported *Salmonella* isolation rates of 16% and 20% to 35%, respectively, in Egypt.

The results of our study regarding the enumeration of *Staphylococcus spp.* ($4.72 \pm 1.05 \log_{10}$ CFU/g) at Day 1 were higher than those observed by Morshdy et al. (2021), who recorded $4.13 \pm 0.18 \log_{10}$ CFU/g after 12 hours. During the storage period at 4°C, the counts at Days 3 and 7 were $5.16 \pm 1.05 \log_{10}$ CFU/g and $4.28 \pm 0.75 \log_{10}$ CFU/g, respectively, compared to $4.85 \pm 0.21 \log_{10}$ CFU/g at Day 3 and $4.93 \pm 0.19 \log_{10}$ CFU/g at Day 6 reported by Morshdy et al. (2021). On the other hand, Guedes et al. (2024) reported a lower mean count than ours, at $2.59 \pm 0.70 \log_{10}$ CFU/g.

However, no isolate was identified as coagulase-positive staphylococci after confirmation, indicating that the meat was satisfactory in this regard. This contrasts with studies conducted in Spain, which revealed an average count of $1.37 \pm 0.79 \log_{10}$ CFU/g of *S. aureus* in 52.9% of rabbit meat samples (Rodríguez-Calleja et al., 2006). Similarly, Morshdy et al. (2023) reported isolation rates of this pathogen of 20% and 24% in rabbit breast and thigh samples, respectively, in Egypt.

The isolation of *E. coli* from food samples is of critical importance for public health (Sapna-Kumari et al., 2001). Indeed, there has been a regular emergence of new pathogenic strains (pathovars), which have acquired virulence factors through genetic exchange (Kaper et al., 2004). The enumeration results at Day 1 were $2.77 \pm 0.53 \log_{10}$ CFU/g. A progressive increase in bacterial load was observed over time, at Days 3 and 7, for both storage temperatures. At Day 7, the counts reached $3.34 \pm 0.76 \log_{10}$ CFU/g at 4 °C, and $3.85 \pm 1.43 \log_{10}$ CFU/g at 8 °C. It is important to note that, for both storage conditions, the average counts remained below the maximum threshold ($M = 5.10^4$ CFU/g, or $4.7 \log_{10}$ CFU/g) (Anonyme, 2017), making the product satisfactory. These results are in agreement with those reported by Bello et al. (2018), who also observed satisfactory levels. Overall, *E. coli* was detected at low levels in rabbit meat, as indicated by the work of Rodríguez-Calleja et al. (2004). However, the study conducted by Mahmoud et al. (2022) reported the presence of *E. coli* in rabbit meat samples at levels considered unsatisfactory.

The detection of *Enterobacteriaceae* in meat is a key indicator of enteric contamination, revealing inadequate hygiene conditions (Görner and Valík, 2004). The initial enumeration of *Enterobacteriaceae* at Day 1 was $3.64 \pm 1.27 \log_{10}$ CFU/g, which is higher than the results reported by Rodríguez-Calleja et al. (2005), who found $2.76 \pm 0.51 \log_{10}$ CFU/g, as well as those by Morshdy et al. (2021), who observed $3.25 \pm 0.22 \log_{10}$ CFU/g. At Days 3 and 7, the counts at 4 °C were $3.78 \pm 1.48 \log_{10}$ CFU/g and $4.64 \pm 1.11 \log_{10}$ CFU/g, respectively, remaining lower than the values of $4.63 \pm 0.24 \log_{10}$ CFU/g and $4.96 \pm 0.22 \log_{10}$ CFU/g reported by Morshdy et al. (2021) after three and six days of storage.

During the storage period, the counts of *Enterobacteriaceae* in our study showed a tendency to increase, reaching $4.64 \pm 1.11 \log_{10}$ CFU/g at 4 °C and $4.81 \pm 0.99 \log_{10}$ CFU/g at 8 °C. These results exceed the marginal limit of $M = 10^4$ CFU/g, or $4 \log_{10}$ CFU/g (Anonymous, 2017), rendering the product unsatisfactory at Day 7 for both storage temperatures. Therefore, the microbiological shelf life of the rabbit carcasses studied is less than seven days, which

aligns with the findings of Rodríguez-Calleja et al. (2005), who estimated the average shelf life of rabbit meat to be 6, 8 days.

5. Conclusion

This study highlights several factors influencing meat quality, both microbiologically and sensorially. The results show that good hygiene and manufacturing practices are followed in the slaughterhouse visited. The sensory quality of the meat remains satisfactory throughout the storage period, and the pH values stay within the normal range for rabbit meat. The microbiological shelf life of the rabbit meat is less than seven days, due to an average *Enterobacteriaceae* count deemed unsatisfactory on the seventh day for both storage temperatures. Thus, low initial contamination contributes to extending the microbiological shelf life of the product.

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