Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.30522

Shelf-life extension of chilled beef by sodium lactate enhanced with Natamycin against discoloration and spoilage

Tian TIAN[1#], Yang LIU[2#], Xinhui WANG[2*]

Abstract

The efficacy of sodium lactate combined with Natamycin against discoloration and spoilage of beef stored at 4 °C was evaluated. The beef was treated with 3 g/L sodium lactate combined with 3 g/L Natamycin, and 3 g/L sodium lactate treatment and 3 g/L Natamycin treatment were performed as control. The spoilage of beef samples was monitored by pH, total volatile basic nitrogen (TVB-N), discoloration, sensory analysis and microbial quality. Sodium lactate exhibited a remarkable efficacy against discoloration together with antibacterial efficacy against spoilage. Natamycin has no contribution to delaying discoloration but displayed a remarkable antibacterial efficacy. These results revealed that sodium lactate combined with Natamycin was more conducive to hygiene levels improvement. In the sample treated by sodium lactate combined with Natamycin, *Lactobacillus* spp. and *Weissella* spp. sharply displaced the *Ralstoni* spp. with high potential spoilage and became the predominant bacteria, and the proliferation of *Serratia* spp. as the representatives' genus of psychrotrophic Enterobacteriaceae were completely controlled. As a result, the acceptable shelf life of beef treated by sodium combined with Natamycin reached 7 days. While the acceptable shelf life of beef treated by sodium lactate and Natamycin was 6 and 4, respectively.

Keywords: chilled beef; discoloration; Natamycin; sodium lactate; spoilage.

Practical Application: In this study, comparative efficacy of sodium lactate, Natamycin and sodium lactate combined with Natamycin against discoloration and spoilage of chilled beef was systematically evaluated. The findings revealed that the acceptable shelf life of fresh beef treated by sodium lactate, Natamycin and sodium lactate combined with Natamycin reached 6 d, 4 d and 7 d, respectively. Sodium lactate displayed the best efficacy against discoloration and spoilage of chilled beef enhanced by Natamycin.

1 Introduction

Fresh beef as a perishable product is easy to discoloration and spoilage during storage at room temperature. Thus, it is essential to perform adequate preservation technologies against discoloration and spoilage to promote its quality and safety. Frozen storage as a common commercial preservation method has been applied for fresh beef. The tenderness, juiciness, flavor and appearance acceptable of frozen beef are likely to be reduced due to the freeze and thaw process (Coombs et al., 2017). As a result, frozen beef is less popular with consumers compared with chilled meat (Lagerstedt et al., 2008). Whereas, chilled beef has a limited shelf life, resulting in a limiting for distribution and long distance transport (Yang et al., 2016). Thus, it is critical to apply adequate preservation technologies to prolong the shelf life of chilled beef. Recently, chemical preservatives such as salts are extensively applied in meat production such as sausages and bacon, but it is not suitable for fresh meat preservation. Furthermore, salts may bring some potential risks to customers, especially for patients with hypertension. Therefore, it is essential to further explore preservatives which meet food safety and are suitable for fresh meat preservation.

Sodium lactate 'Generally Recognized as commonly Safe' (GRAS) preservative agent is widely used in meat products. It has been reported that sodium lactate could reduce microbial growth by allowing a greater membrane permeability due to the pH conditions, increasing proton and lactic acid accumulation inside the bacterial cells. Moreover, sodium lactate has been revealed to maintain an acceptable color appearance of fresh meat against discoloration during chilled storage (Papadopoulos et al., 1991). Natamycin with a broad spectrum of antimicrobial activity has been recognized as an effective preservative with a good antiseptic effect, and has been approved by the Food and Drug Administration (FDA) as a safe food additive used in meat (Magrinyà et al., 2015). Although successful applications of sodium salt or Natamycin in meat products shelf life extension are available, a few systematic studies have been concentrated on fresh beef quality enhancement and the synergism between sodium lactate and Natamycin on the freshness promotion of fresh meat during chilled storage (Beristain-Bauza et al., 2017; Tenderis et al., 2021).

Discoloration and spoilage are mainly responsible for the short shelf life of fresh beef under chilled storage. Therefore, in this study, the efficacy of sodium salt or Natamycin against

Received 02 Feb., 2022
Accepted 09 Apr., 2022
[1] *Chengdu Agricultural College, Chengdu, Sichuan, China*
[2] *School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan, China*
*Corresponding author: renhongyang@swpu.edu.cn
[#] *Equal contribution*

discoloration and spoilage was focused on, and the synergism between sodium lactate and Natamycin on shelf life extension was systematically studied. Spoilage was monitored by pH, total volatile basic nitrogen (TVB-N), discoloration, sensory analysis and microbial quality. Discoloration was detected by colorimetric evaluation combined with consumer-defined color acceptability. Microbial quality was evaluated by five principal microbiological indicators determined by culture dependent methods, namely total viable counts (TVC) and Lactic acid bacterial (LAB) counts, *Brochothrix* spp. counts, *Pseudomonas* spp. counts and *Enterobacteriaceae* counts, combined with bacterial community structure determined by high throughput sequencing technology.

2 Materials and methods

2.1 Samples collection and treatments

Longissimus lumborum muscle of fresh beef (20 kg) under at 0-4 °C was supplied by a local market of agricultural products located in Chengdu, Sichuan Province, China. After transportation to the laboratory, the fresh beef was immediately divided into cuts (10 cm × 10 cm × 1.5 cm) with about 100 g under chilled conditions. Subsequently, all cuts were equally divided into 3 groups for experiments, and labeled as Group SL, Group NA, and Group SLNA, respectively. The Group SL and Group NA were dipped into (3 g/L) sodium lactate and (3 g/L) Natamycin for 1 min, respectively. Correspondingly, the Group SLNA was dipped into (3 g/L) sodium lactate combined with (3 g/L) Natamycin for 1 min. After treatment, all samples were immediately packaged into polyethylene sterile bags (254 mm × 330 mm) and stored at 4 ± 1 °C for 7 days. Samples were removed in triplicate for physiochemical analyses and sensory evaluation on day 0, 1, 2, 3, 4, 5, 6 and 7, and for microbial examination on day 0, 3, 5 and 7.

2.2 Physicochemical analyses

pH measurement

At each time point, the pH value of beef samples was measured by a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with an automatic temperature compensation (NTC) electrode according to the method described by Wang et al. (2015a). Before measurement, the pH probe was calibrated using buffers at pH 4.00 and 7.00 at room temperature. Inserting the electrode directly into beef samples, triplicate readings were obtained from three different random locations and then averaged.

Total Volatile Basic Nitrogen (TVB-N) measurement

The TVB-N content was measured using a steam distillation method according to the Chinese National Food safety standard methods GB 5009.228-2016 described by Chen et al. (2019). The TVB-N content was calculated according to the formula described by Chen et al. (2020) and was expressed as mg/100 g of sample.

Color measurement

The surface color of samples was measured by using a colorimeter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China), which measuring head consisted of D65 lighting source, 8 mm aperture, and a 10° standard observer angle, according to the method described by Wang et al. (2015b) and reported as lightness (L*), redness (a*) and yellowness (b*) as CIELab coordinates. At each time point, each sample was measured in triplicate and then triplicate readings were averaged.

2.3 The NADH/NAD⁺ ratio determination

The NADH and NAD^+ concentration was determined using the coenzyme I NAD (H) content test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions of coenzyme I NAD (H) content test kit. The NADH/NAD^+ ratio was calculated by the specific value of NADH concentration and NAD^+ concentration.

2.4 Relative myoglobin content measurement

The spectrophotometer records reflectance values in the range of 360 to 740 nm at 10 nm intervals. The reflectance values (R) were calculated by integrations of the measurement at 473, 525, 572 and 700 nm, and the R was converted to reflex attenuance (A) according to the equation: A = log (1/R). Then the percentage of myoglobins, namely metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), was determined by the formulas as described by Wu et al. (2020) (Equations 1-3).

$$\%\text{MetMb} = \left(1.395 - \frac{A572 - A700}{A525 - A700}\right) \times 100 \quad (1)$$

$$\%\text{DeoxyMb} = \left[2.35 \times \left(1 - \frac{A473 - A700}{A525 - A700}\right)\right] \times 100 \quad (2)$$

$$\%OxyMb = 100 - \left(\%MetMb + \%DeoxyMb\right) \quad (3)$$

Where A473, A525, A572 and A700 is the reflex attenuance at 473, 525 and 700 nm, respectively.

2.5 Sensory evaluation

Sensory evaluation of beef freshness was undertaken according to the methods described by Chen et al. (2019). The sensory panel was established according to ISO 8586–1 standard (1993) by selected students with the average age of 22 years old in the School of Food science and Technology, Chengdu University, Chengdu, China. All samples were coded with three-digit numbers and presented to 11 trained sensory panelists (5 females and 6 males) who evaluated the beef samples for their organoleptic characteristics, namely color, odor, texture, appearance and viscosity, using a 5-point scale based on attribute degrees as shown in Table S1. A total score of 20.0 points was considered fresh and the scores decreased according to spoilage to 9.0, which was the lowest acceptable limit.

2.6 Microbial analyses

Bacterial counts

Microbial quality of beef samples was monitored by culture-dependent methods based on plate counts. Total viable counts (TVC), lactic acid bacteria (LAB) counts, *Pseudomonas* spp. counts, *Bronchothrix* spp. counts and *Enterobacteriaceae* counts were undertaken according to the methods reported by Yang et al. (2016). Briefly, 10 g of sample was removed aseptically to sterile bags containing 90 mL 0.1% sterile peptone water, and then was mixed in a blender for 2 min. After mixing, serial decimal dilutions were performed for microbial analysis. Thereafter, plate count agar (PCA, Sangon Biotech Co. Ltd, Shanghai, China), De Man Rogosa Sharpe Agar (MRS, Sangon Biotech Co. Ltd, Shanghai, China) Steptomycin Thallous Acetate Agar (STAA, Shandong Tuopu Biological Engineering Co., Ltd., Shandong, China), *Pseudomonas* selective Agar (Shandong Tuopu Biological Engineering Co., Ltd., Shandong, China) and Violet Red Bile Glucose Agar (VRBGA, Shandong Tuopu Biological Engineering Co., Ltd., Shandong, China) were undertaken to determine total viable counts (TVC), lactic acid bacteria (LAB) counts, *Pseudomonas* spp. counts, *Bronchothrix* spp. counts and Enterobacteriaceae counts, respectively. TVC and LAB counts were all incubated at 37 °C for 48 h, and *Pseudomonas* spp. counts and *Bronchothrix* spp. counts were all incubated at 25 °C for 48 h. Enterobacteriaceae counts were enumerated after incubation at 37 °C for 48 h. The results of bacterial counts were expressed as $\log_{10}$ CFU/g sample.

Bacterial community analyses by HST technology

The bacteria were collected from the beef samples according to previous method as described by Liu et al. (2018) and Wang et al. (2018a), and the total bacterial genomic DNA was extracted by using the E.Z.N.ATM Mag-bind Soil (OMEGA,USA) according to the manufacturer's instructions. After the determination of the DNA concentration and detection of the integrity of DNA, the V4 region of bacterial 16S rRNA gene was amplified with the primer pairs 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Thereafter, the systems and conditions of PCR amplification were undertaken according to a previous method as described by Wang et al. (2018b). After amplification, the PCR products were purified and quantified, and then were sequenced by the high throughput sequencing technology on the llumina MiSeq platform (Beijing Novogene Technology Co. Ltd, Beijing, China).

The raw sequencing data were merged and screened as previously described (Chen et al., 2019; Wang et al., 2019). After chimera and other interrogative sequences were removed, high quality sequences were clustered and regarded as operational clustering method (OTUs) based on the SILVA reference gene database (Quast et al., 2013; Edgar, 2010) at an identity threshold of 97%. Alpha diversity was performed to identify bacterial community richness based on Chao1 and Abundance-based coverage (ACE), diversity based on Shannon and Simpson indices, and sequencing depth based on Good's coverage. Beta diversity was conducted to get principal coordinates and to visualize the differences in microbial communities. All these indices were calculated using the QIIME software (Version 1.7.0).

2.7 Statistical analyses

Each test was performed in triplicate and these results were reported by means with standard deviation. Data were displayed as mean values accompanied with the standard deviation. Duncan's multiple range test (significance was defined at $p < 0.05$) was employed for the independence of error terms using the SPSS statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussions

3.1 Physicochemical examining results

pH values

The changes of pH values in 3 samples during storage at 4°C are shown in Figure 1a. Initial pH value in Group SL, Group NA and Group SLNA was 5.81, 5.57 and 5.79, respectively. During

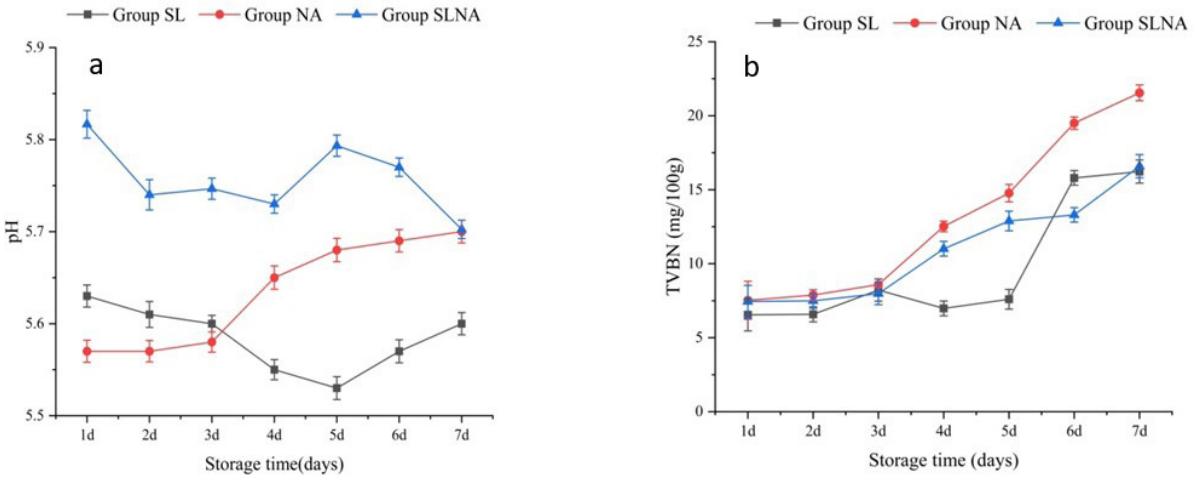

Figure 1. Changes in the pH values (a) and total volatile basic nitrogen (TVB-N) levels (b) of chilled beef during storage at 4 °C for 7 days.

storage, the pH values in Group NA displayed an increase and reached to 5.94 on the 7th day. In contrast, the pH values in Group SL maintained at a stable level with a range of 5.67 to 5.80, and the pH values in Group SLNA exhibited the best pH stability within a narrow range of 5.70 to 5.79.

As is well known, the pH value plays a critical role in raw beef quality during storage, and any deviations from the normal in terms of pH will affect color intensity and water-holding capacity of fresh meat (Han et al., 2021). Once the pH value reduced to 5.2-5.5, which is close to the isoelectric point of protein in muscle and the attraction between muscle protein and water is extremely small, resulting in a weak water holding capacity. Moreover, the low pH plays a negative effect on the color characteristics of beef due to the transition of the easily oxidized Fe^{2+}Mb into Fe^{3+}MetMb, resulting in a darker red color. Obviously, beef with sodium lactate treatment and sodium lactate combined with Natamycin treatment, suffered a delayed glycolysis process, and avoided a rapid acidification in the initial stage of storage. Subsequently, the pH will increase with the storage extension. Once the pH is over 6, autolysis will undergo and protein is further decomposed and produces a large number of amino acids, which is conducive to the bacterial growth, resulting in spoilage (Holman et al., 2016). In this study, sodium lactate combined with Natamycin treatment effectively inhibited the pH increase at middle and late stage of storage, suggesting that the synergism of sodium lactate and Natamycin would be more conducive to pH stabilization for beef during chilled storage.

TVB-N contents

The changes of TVB-N levels in 3 samples during storage at 4 °C are shown in Figure 1b. The initial TVB-N level was 7.15 mg/100 g, 7.53 mg/100 g and 7.45 mg/100 g in Group SL, Group NA and Group SLNA, respectively, which is indicative of good meat quality (Wang et al., 2021; Tian et al., 2017). TVB-N has been used as a direct quality indicator of meat freshness/deterioration of fresh meat in China (Zhang et al., 2009) which is positively correlated with the growth of spoilage bacteria (Chen et al., 2019). According to the National Food Safety Standard of China (GB 2707-2016), 15 mg/100 g of TVB-N content is set as the upper limit for fresh level and reaches the threshold of acceptability. In this study, referring to the threshold (≤ 15 mg/100 g), the Group SL, Group NA, Group SLNA reached the rejection level on the 6th, 7th and 7th day, respectively. The acceptable shelf life according to the threshold of TVB-N level was highly consistent with the acceptable shelf life according to sensory evaluation. Obviously, these findings revealed that both sodium lactate and Natamycin were conducive to reducing the TVB-N content, and sodium lactate exhibited a better efficacy. As a result, sodium lactate combined with Natamycin displayed a superior effect on inhibiting the TVB-N increase. Thus, the synergism of sodium lactate and Natamycin is best beneficial to the shelf life extension of chilled beef.

Color values

The changes in color parameters L^*, a, and b values of 3 samples during storage at 4 °C are shown in Table S2. Preservative treatments displayed no significant impact ($p > 0.05$) on the L^* and b values of chilled beef. Natamycin treatment has no significant impact ($p > 0.05$) on the redness (a^*) value, while sodium lactate and sodium lactate combined with Natamycin treatments had positive effects on the a^* value along with storage. The a^* value of Group SLNA was the highest followed by Group SL.

Intensity of color appearance of the 3 beef samples during storage at 4 °C is shown in Figure 2. The initial color appearance of all beef samples was bright cherry red on the first day, suggesting a desirable color acceptability. Subsequently, the color appearance of Group SL and Group SLNA turned to bright red on the second day, and stayed this color until the 6th and 7th day, respectively.

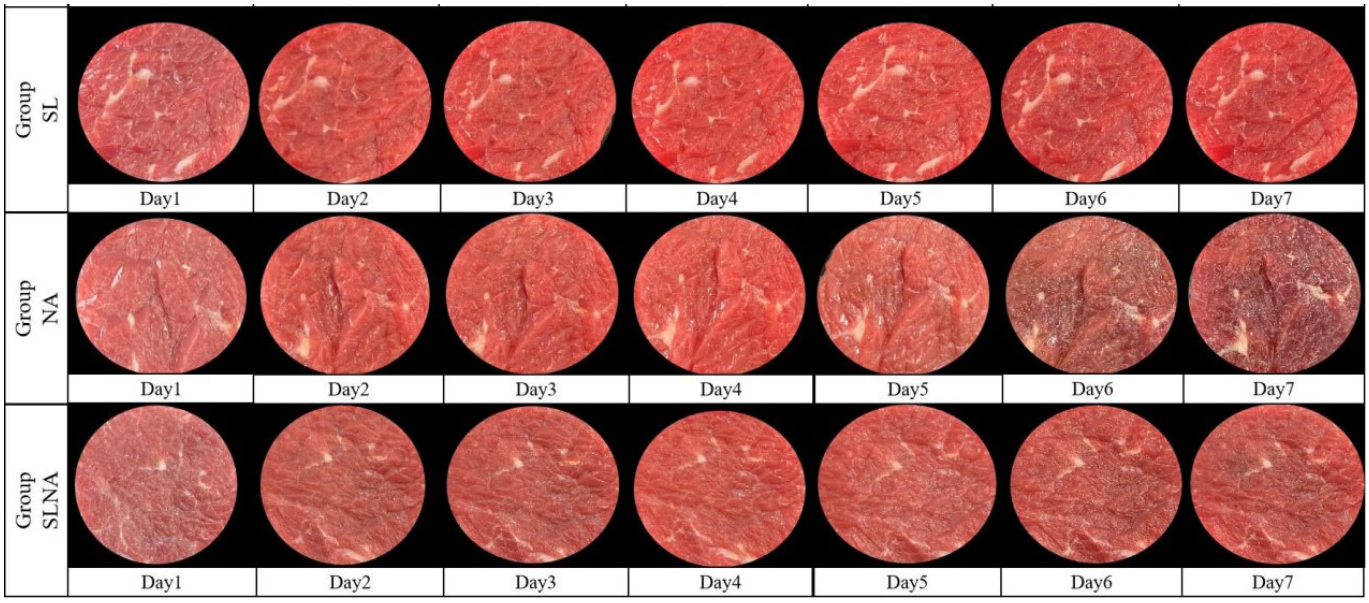

Figure 2. Comparative effects of sodium lactate, Natamycin and sodium lactate combined with Natamycin on color appearance of chilled beef samples during storage at 4 °C for 7 days.

Whereas, the color appearance of Group NA turned to dull red on the 3rd and the color appearance became dark red on the 4th day, and the color appearance was undesirable on the 5th day. These results were in well line with the results of instrumental color *a* values.

Meat color has been acknowledged as a critical indicator for consumers to evaluate meat quality and make a purchase decision, and a bright red color is considered a positive attribute for freshness and superior quality of beef (Holman et al., 2016). According to the relationship between the colorimetric evaluation and the consumer-defined beef color acceptability, when a* < 14.5 the beef color was considered unacceptable (Holman et al., 2017). In this study, the a* value of Group NA decreased to 13.17 and reached the rejection level on the 5th day. In contrast, a* value of Group SL and Group SLNA stayed above this threshold ($a^* > 14.5$) for the entire storage period, which a* values that were in the range of 18.49-31.28 and 18.89-32.31, respectively. These results indicate that sodium lactate has a positive effect on slowing down the rate of discoloration and color stability. Furthermore, its efficiency of color protection of chilled beef was enhanced by Natamycin.

3.2 Proportions of myoglobin redox forms

Metmyoglobin reducing ability is crucial for meat color stability. The state of myoglobin was monitored by its three redox forms, namely MetMb, OxyMb and DeoxyMb in raw beef during storage at 4 °C as shown in Figure 3. The initial proportion of OxyMb was 67%, 70% and 69% in Group SL, Group NA and Group SLNA, respectively, showing a high proportion in all samples (> 50%), which can be beneficial to beef color with bright redness. Subsequently, the proportion of OxyMb in Group NA dropped significantly ($p > 0.05$) from 70% to 42% on the 4th day, meanwhile the proportion of MetMb which can result in a dark redness increased sharply from 22% to 50%. In contrast, the proportion of OxyMb in Group SL maintained a high level with a range from 70% to 61%, and the proportion of MetMb maintained a low level, occupying for 13% to 35% along with the storage. Likewise, the similar trend of changes in proportion of OxyMb and MetMb appeared in Group SLNA. These results revealed that sodium lactate usage had a significant effect ($p < 0.05$) on the state of myoglobin and was conducive to maintaining a high proportion of OxyMb. These results were well in agreement with the results of redness (*a*), which can partially explain the reason why the color appearance was more acceptable in Group SL and Group SLNA.

3.3 The NADH/NAD⁺ ratio

The changes of NADH/NAD⁺ ratio in the 3 samples during the storage are shown in Table S3. The initial NADH/NAD⁺ was in a range from 0.52 to 0.58 in all samples, showing a good reduction oxidation system. As the storage extended, the Group NA displayed different NADH/NAD⁺ profiles compared with the Group SL and Group SLNA. The NADH/NAD⁺ ratio in Group NA continued to rise, and reached a level approximately five times on the 3rd day compared with the initial NADH/NAD⁺ ratio. In contrast, NADH/NAD⁺ ratio in Group SL displayed

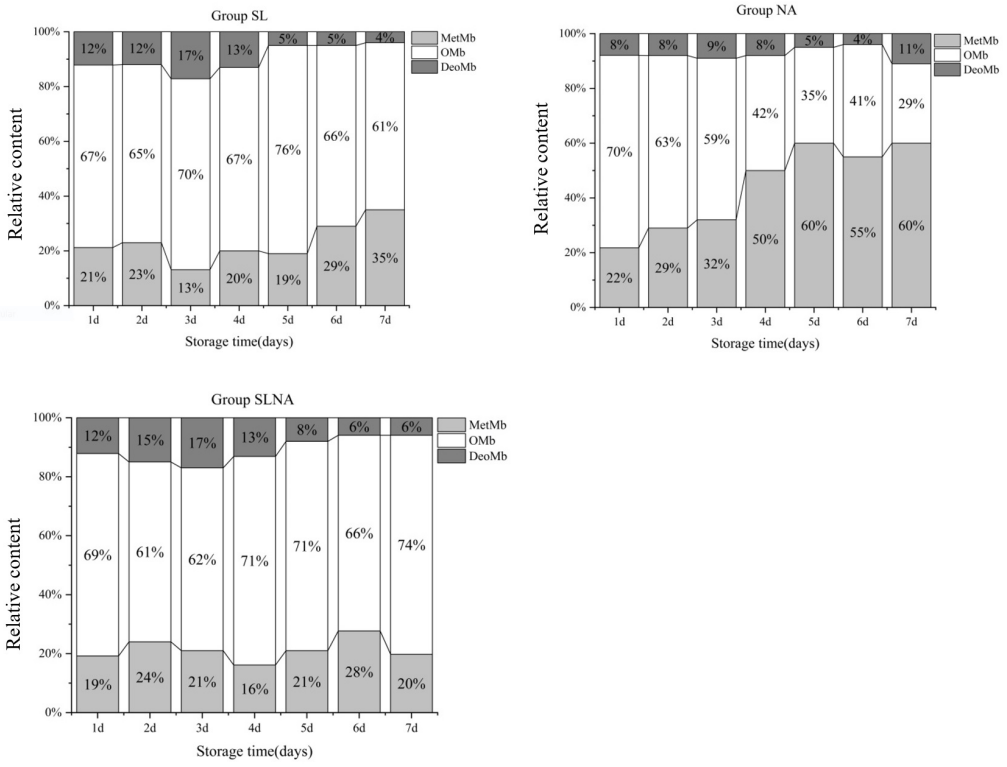

Figure 3. Relative content of three myoglobin forms, including Deoxymyoglobin (DeoxyMb), Oxymyoglobin (OxyMb) and Metmyoglobin (MetMb) of Group SL, Group NA and Group SLNA during storage at 4 °C for 7 days.

a small reduction with 0.38 on the second day. Subsequently, NADH/NAD^+ ratio in Group SL maintained at a stable level with a range of 0.25 to 0.37 until the 6th day. Likewise, a similar result of the changes of NADH/NAD^+ ratio was found in Group SLNA.

The NADH and NAD^+ have been acknowledged as the primary redox carriers involved in metabolism. A balance in the rates of oxidation and reduction of these nucleotides is a prerequisite for the continuation of both catabolism and anabolism (Gao et al., 2014). The high NADH/NAD^+ ratio indicates insufficient oxidation of NADH, resulting in a poor reduction effect. Fe^{+3}-MetMb is reduced to Fe^{+2}-Mb by NADH oxidation via mitochondrial respiration. The high NADH/NAD^+ ratio in Group NA indicates insufficient reducing power. As a result, Fe^{+3}-MetMb was insufficiently restored to Fe^{+2}-Mb, resulting in a high proportion of MetMb and discoloration. Indeed, as shown in Figure 3, the proportion of MetMb in Group NA was higher compared with the Group SL and Group SLNA. Clearly, the sodium lactate usage was conducive to stabilizing the cellular NADH/NAD^+ ratio, which is probably the main positive contribution against discoloration.

3.4 Microbial quality

The microbial quality of the 3 samples during storage at 4 °C was monitored by the culture-dependent method based on plate counts and culture-independent method based on bacterial community analysis through high-throughput sequencing technology.

Five principal microbiological indicators, namely total viable counts (TVC), LAB counts, *Brochothrix* spp. counts, *Pseudomonas* spp. counts and Enterobacteriaceae counts, were used to evaluate the microbial quality. The results of bacterial enumeration of all samples during storage are shown in Figure 4. The Enterobacteriaceae was not detected in all samples through the storage($\log_{10}$ CFU/g < 1).The initial bacterial TVC counts in all samples was approximately 4.0 $\log_{10}$ CFU/g, indicating good hygienic quality of the tested beef samples. As storage time extended, the bacterial TVC value in Group SLNA increased slowly and reached 6.3 $\log_{10}$ CFU/g on the 7th day, and was the lowest followed by Group NA.

The TVC counts as a direct quality indicator of fresh meat is positively correlated with the spoilage process, and the value of 7 $\log_{10}$ CFU/g has been defined as a threshold of microorganism counts for good quality fresh meat by the International Commission on Microbiological Specifications for Foods (ICMSF). In this study, the bacterial TVC counts in Group SL, Group NA and Group SLNA reached to above 7.0 $\log_{10}$ CFU/g on the 7th, 8th and 8th day, respectively. Likewise, the growths of *Brochothrix* spp. and *Pseudomonas* spp. were significantly inhibited($p < 0.05$) by sodium lactate and Natamycin as shown in Figures 4c-4d. Obviously, both sodium lactate and Natamycin exhibited a

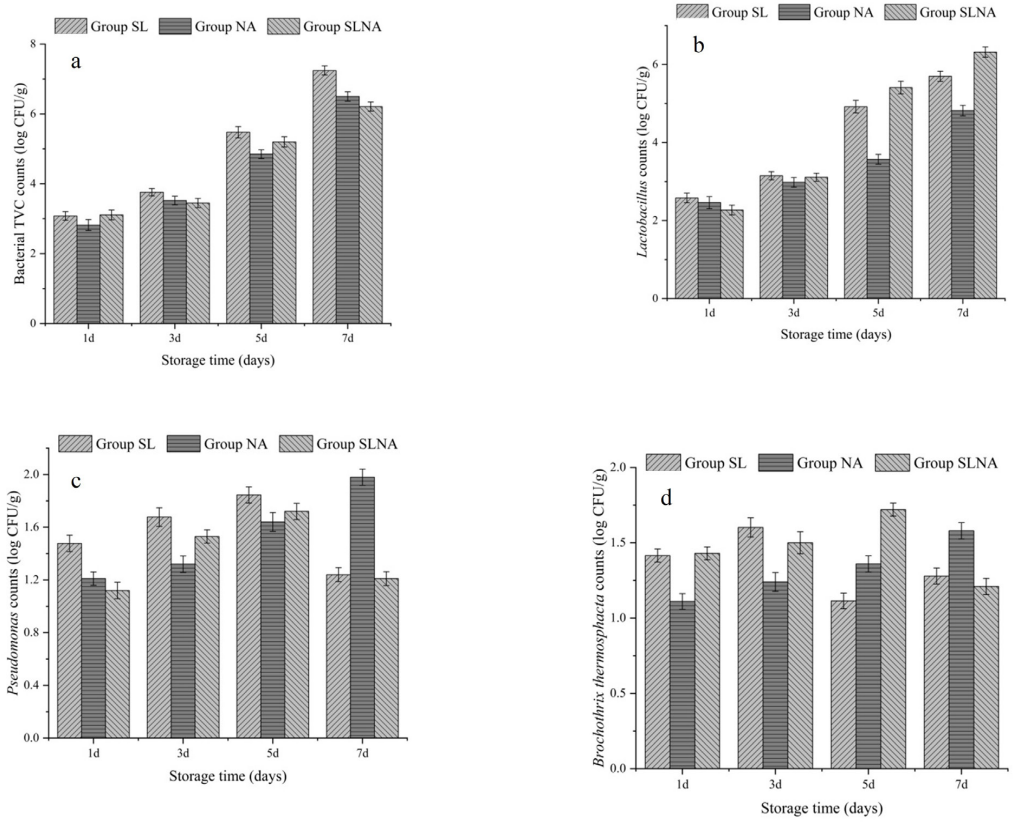

Figure 4. Comparative effects of sodium lactate, Natamycin and sodium lactate combined with Natamycin on the total viable counts (TVC) (a) and lactic acid bacterial (LAB)counts (b), *Brochothrix* spp. counts (c) and *Pseudomonas* spp. counts (d) of chilled beef samples during storage at 4 °C for 7 days.

good bacteriostatic efficacy, except for LAB, and Natamycin was more effective in delaying the bacterial growth compared with sodium lactate. As a result, the bacteriostatic efficacy was enhanced by the synergistic effect of Natamycin and sodium lactate. Therefore, sodium lactate combined with Natamycin will be an alternative preservative used for freshness promotion.

Microbial data associated with spoilage collected from conventional cultural methods are not available often enough to unravel the characteristics of microbial spoilage. Therefore, high throughput sequencing data were provided to analyze the bacterial community of chilled beef during storage at 4 °C as shown in Figure 5. Initially, *Ralstonia pickettii* was the most predominant bacteria in all beef samples with a 93.2-93.5% of relative abundance. Subsequently, the succession of the bacterial community was significantly influenced ($p < 0.05$) by the preservative treatments during storage at 4 °C for 7 days. As the storage extended, *Lactobacillus sakei* (33.9%-78.7%) and *Weissella ceti* (4.5%-18.1%) underwent a dramatic increase and dominated during the middle and late stage of the storage period (5-7 days) in Group SL, Group NA and Group SLNA, while *Serratia* spp. was nearly undetectable during the whole storage period.

Ralstonia pickettii as an opportunistic pathogen is widely distributed in the environment and can cause primary and secondary infections. *Serratia* spp. as the representatives' genus of psychrotrophic Enterobacteriaceae and *Pseudomonas* spp. with high spoilage potential are easy to cause deterioration of fresh beef under chilled conditions (Liu et al., 2020). *Lactobacillus sakei* and *Weissella ceti* granted GRAS (Generally Recognized as Safe) status has been applied in fermented foods as starter cultures (Zhang et al., 2021). Combined with the results of the culture-independent method, these findings indicate that both sodium lactate and Natamycin can display antibacterial efficacy, and Natamycin has a better antibacterial activity. Moreover, the bacteriostatic efficacy was enhanced by the synergistic effect of Natamycin and sodium lactate. Thus, these results suggested that sodium lactate combined with Natamycin treatment is one of the effective ways to obtain reliable shelf life of fresh beef by hygiene levels improvement during storage at 4 °C.

3.5 Sensory evaluation

The sensory evaluation of all samples stored at 4 °C for 7 days are shown in Figure 6. The sodium lactate treatment and sodium lactate combined with Natamycin treatment had positive effects ($p < 0.05$) on the sensory evaluation compared with the control and Natamycin treatment. All samples presented acceptable sensory characteristics during the first 3 days. On the 4th day, the appearances of Group NA were undesirable, while the appearances of Group SL and Group SLNA were still with a good acceptance until on the 7th and 8thday, respectively. In terms of the sensory evaluation, all samples had a score of 20 on the first day, suggesting that all samples were very fresh. As storage extended, the sensory evaluation score got the lowest in Group NA, and the highest in Group SLNA followed by Group SL. These results were well in line with the results of the TVB-N level and color. According to the sensory evaluation, both sodium lactate and sodium lactate combined with Natamycin prolonged the shelf life by 3-4 days. Furthermore, sodium lactate combined with Natamycin exhibited the best acceptability. These results indicate that sodium lactate could delay discoloration together with the antibacterial efficacy against spoilage enhanced by Natamycin.

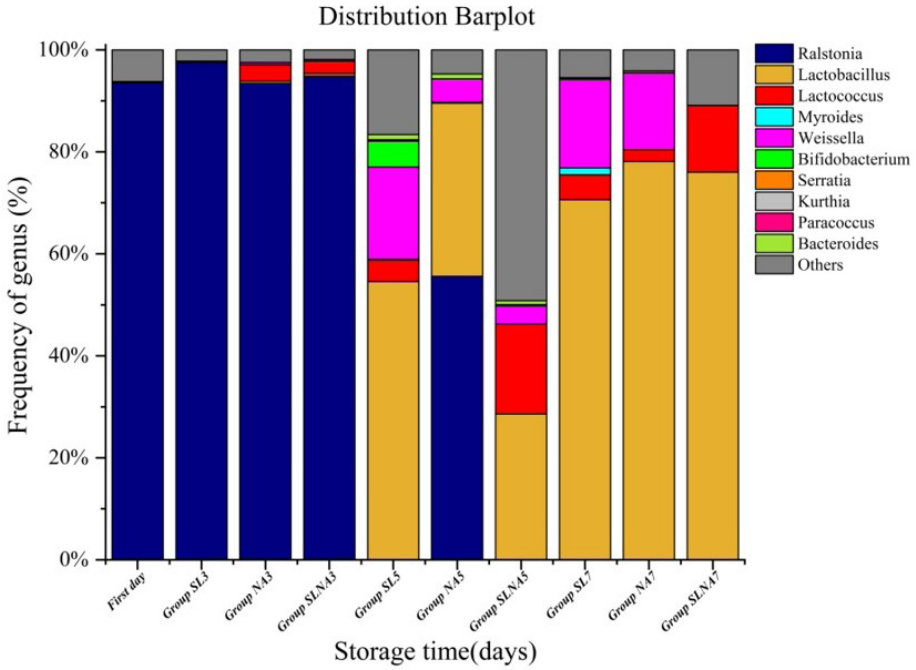

Figure 5. Relative abundance at the genus level based on the classification of partial 16S rRNA genes sequences of bacteria from Group SL, Group NA and Group SLNA during storage at 4 °C for 7 days.

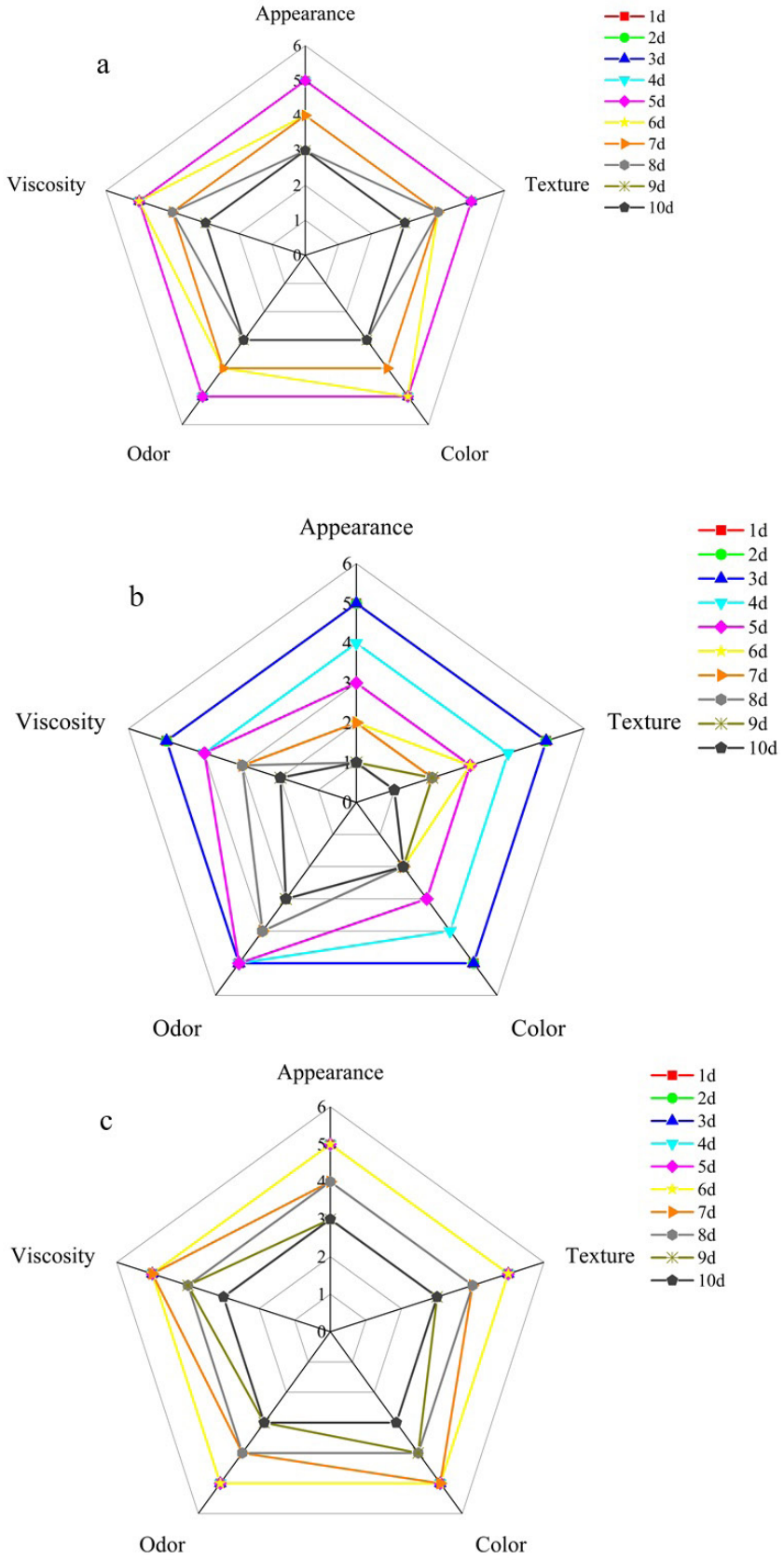

Figure 6. The sensory evaluation including color, odor, texture, appearance and viscosity of Group SL (a), Group NA (b) and Group SLNA (c) during storage at 4 °C for 7 days.

4 Conclusions

Based on the results of TVB-N, color and bacterial TVC counts, the acceptable shelf life of chilled beef stored at 4 °C treated by sodium lactate, Natamycin and sodium lactate combined with Natamycin reached 6 days, 4 days and 7 days, respectively. Sodium lactate exhibited a remarkable efficacy against discoloration together with antibacterial efficacy against spoilage. Natamycin has no contribution to delaying discoloration but displayed a remarkable antibacterial efficacy. As a result, sodium lactate is suggested to be suitable for use to promote the freshness of fresh meat during chilled storage, while Natamycin is unsuitable for use to preserve fresh meat. Sodium lactate displayed a best efficacy against discoloration and spoilage of chilled beef enhanced by Natamycin.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31772093), the Research Fund Project of Chengdu Agricultural College (20ZR110), and Application foundation project of Sichuan Science and Technology Department (2020YFS0504). The authors thank Novogene Biotech for technical assistance in carrying out the analyses.

References

Beristain-Bauza, S. C., Mani-López, E., Palou, E., & López-Malo, A. (2017). Antimicrobial activity of whey protein films supplemented with *lactobacillus sakei* cell-free supernatant on fresh beef. *Food Microbiology*, 62, 207-211. http://dx.doi.org/10.1016/j.fm.2016.10.024. PMid:27889150.

Chen, X., Zhang, Y. M., Yang, X. Y., Hopkins, D. L., Zhu, L. X., Dong, P. C., Liang, R. R., & Luo, X. (2019). Shelf-life and microbial community dynamics of super-chilled beef imported from Australia to China. *Food Research International*, 120, 784-792. http://dx.doi.org/10.1016/j.foodres.2018.11.039. PMid:31000298.

Chen, X., Zhu, L. X., Liang, R. R., Mao, Y. W., Hopkins, D. L., Li, K., Dong, P. C., Yang, X. Y., Niu, L. B., Zhang, Y. M., & Luo, X. (2020). Shelf-life and bacterial community dynamics of vacuum packaged beef during long-term super-chilled storage sourced from two Chinese abattoirs. *Food Research International*, 130, 108937. http://dx.doi.org/10.1016/j.foodres.2019.108937. PMid:32156384.

Coombs, C. E. O., Holman, B. W. B., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: a review. *Meat Science*, 125, 84-94. http://dx.doi.org/10.1016/j.meatsci.2016.11.025. PMid:27918929.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460-2461. http://dx.doi.org/10.1093/bioinformatics/btq461. PMid:20709691.

Gao, X. G., Wang, Z. Y., Tang, M. T., Ma, C. W., & Dai, R. T. (2014). Comparsion of the effects of succinate and nadh on postmortem metmyoglobin redcutase activity and beef colour stability. *Journal of Integrative Agriculture*, 13(8), 1817-1826. http://dx.doi.org/10.1016/S2095-3119(14)60754-1.

Han, J., Liu, Y., Zhu, L., Liang, R., Dong, P., Niu, L., Hopkins, D. L., Luo, X., & Zhang, Y. (2021). Effects of spraying lactic acid and peroxyacetic acid on the quality and microbial community dynamics of vacuum skin-packaged chilled beef during storage. *Food Research International*, 142, 110205. http://dx.doi.org/10.1016/j.foodres.2021.110205. PMid:33773680.

Holman, B. W. B., Mao, Y., Coombs, C. E. O., Van de Ven, R. J., & Hopkins, D. L. (2016). Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. *Meat Science*, 121, 104-106. http://dx.doi.org/10.1016/j.meatsci.2016.05.002. PMid:27294520.

Holman, B. W. B., Van de Ven, R. J., Mao, Y., Coombs, C. E., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science*, 127, 57-62. http://dx.doi.org/10.1016/j.meatsci.2017.01.005. PMid:28130985.

Lagerstedt, A., Enfält, L., Johansson, L., & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef M. longissimus dorsi. *Meat Science*, 80(2), 457-461. http://dx.doi.org/10.1016/j.meatsci.2008.01.009. PMid:22063353.

Liu, Y., Wang, X. H., Fang, Y., Huang, M. H., Chen, X. Y., Zhang, X. Y., & Zhao, H. (2018). The effects of photoperiod and nutrition on duckweed (Landoltia punctata) growth and starch accumulation. *Industrial Crops and Products*, 115, 243-249. http://dx.doi.org/10.1016/j.indcrop.2018.02.033.

Liu, Y., Zhang, F. X., Zhu, B. W., Ruan, X. R., Yi, X. M., Li, J., Gao, Y. Y., & Hui, G. H. (2020). Effect of sodium lactate coating enriched with nisin on beef strip loins (M. *Longissimus lumborum*) quality during cold storage and electronic nose rapid evaluation. *Journal of Food Measurement and Characterization*, 14(6), 2998-3009. http://dx.doi.org/10.1007/s11694-020-00548-4.

Magrinyà, N., Terjung, N., Loeffler, M., Gibis, M., Bou, R., & Weiss, J. (2015). Influence of fat addition on the antimicrobial activity of sodium lactate, lauric arginate and methylparaben in minced meat. *International Journal of Food Microbiology*, 215, 86-94. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.08.017. PMid:26344644.

Papadopoulos, L. S., Miller, R. K., Ringer, L. J., & Cross, H. R. (1991). Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. *Journal of Food Science*, 56(3), 621-626. http://dx.doi.org/10.1111/j.1365-2621.1991.tb05343.x.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590-D596. PMid:23193283.

Tenderis, B., Kılıç, B., Yalçın, H., & Şimşek, A. (2021). Controlling growth of Listeria monocytogenes and Pseudomonas fluorescens in thermally processed ground beef by sodium lactate, encapsulated or unencapsulated polyphosphates incorporation. *LWT*, 144, 111169. http://dx.doi.org/10.1016/j.lwt.2021.111169.

Tian, X., Wu, W., Yu, Q., Hou, M., Gao, F., Li, X., & Dai, R. (2017). Bacterial diversity analysis of pork *longissimus lumborum* following long term ohmic cooking and water bath cooking by amplicon sequencing of 16S rRNA gene. *Meat Science*, 123, 97-104. http://dx.doi.org/10.1016/j.meatsci.2016.09.007. PMid:27665069.

Wang, X. H., Ren, H. Y., Wang, W., Bai, T., & Li, J. X. (2015a). Evaluation of key factors influencing histamine formation and accumulation in fermented sausages. *Journal of Food Safety*, 35(3), 395-402. http://dx.doi.org/10.1111/jfs.12187.

Wang, X., Ren, H., & Zhan, Y. (2018a). Characterization of microbial community composition and pathogens risk assessment in typical Italian-style salami by high-throughput sequencing technology. *Food Science and Biotechnology*, 27(1), 241-249. http://dx.doi.org/10.1007/s10068-017-0200-5. PMid:30263746.

Wang, X., Ren, H., Wang, W., Zhang, Y., Bai, T., Li, J., & Zhu, W. (2015b). Effects of inoculation of commercial starter cultures on the quality and histamine accumulation in fermented sausages. *Journal of Food Science*, 80(2), M377-M383. http://dx.doi.org/10.1111/1750-3841.12765. PMid:25588615.

Wang, X., Wang, S., & Zhao, H. (2019). Unraveling microbial community diversity and succession of Chinese Sichuan sausages during spontaneous fermentation by high-throughput sequencing. *Journal of Food Science and Technology*, 56(7), 3254-3263. http://dx.doi.org/10.1007/s13197-019-03781-y. PMid:31274892.

Wang, X., Zhang, Y., Ren, H., & Zhan, Y. (2018b). Comparison of bacterial diversity profiles and microbial safety assessment of salami, Chinese dry-cured sausage and Chinese smoked-cured sausage by high-throughput sequencing. *LWT*, 90, 108-115. http://dx.doi.org/10.1016/j.lwt.2017.12.011.

Wang, X., Zhang, Y., Sun, J., Pan, P., Liu, Y., & Tian, T. (2021). Effects of starter culture inoculation on microbial community diversity and food safety of Chinese Cantonese sausages by high-throughput sequencing. *Journal of Food Science and Technology*, 58(3), 931-939. http://dx.doi.org/10.1007/s13197-020-04607-y. PMid:33678876.

Wu, S., Luo, X., Yang, X., Hopkins, D. L., Mao, Y., & Zhang, Y. (2020). Understanding the development of color and color stability of dark cutting beef based on mitochondrial proteomics. *Meat Science*, 163, 108046. http://dx.doi.org/10.1016/j.meatsci.2020.108046. PMid:31954335.

Yang, X., Zhang, Y., Zhu, L., Han, M., Gao, S., & Luo, X. (2016). Effect of packaging atmospheres on storage quality characteristics of heavily marbled beef *longissimus* steaks. *Meat Science*, 117, 50-56. http://dx.doi.org/10.1016/j.meatsci.2016.02.030. PMid:26946476.

Zhang, H., Kong, B., Xiong, Y. L., & Sun, X. (2009). Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4 °C. *Meat Science*, 81(4), 686-692. http://dx.doi.org/10.1016/j.meatsci.2008.11.011. PMid:20416570.

Zhang, J., Song, H. S., Zhang, C., Kim, Y. B., Roh, S. W., & Liu, D. (2021). Culture-independent analysis of the bacterial community in Chinese fermented vegetables and genomic analysis of lactic acid bacteria. *Archives of Microbiology*, 203(7), 4693-4703. http://dx.doi.org/10.1007/s00203-021-02375-7. PMid:34189594.

Supplementary Material

Supplementary material accompanies this paper.

Table S1 Sensory scheme for evaluating the beef sample quality.

Table S2 Effects of sodium lactate combined with Natamycin on the instrumental color values of chilled beef samples.

Table S3 Effect of sodium lactate, Natamycin and sodium lactate combined with Natamycin on the NADH/NAD+ ratio of beef samples during chilled storage at 4 °C for 7 days.

This material is available as part of the online article from https://www.scielo.br/j/cta