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Sodium lactate promotes freshness of chilled beef through colour stability and antibacterial activity

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Abstract

In the present work, fresh beef was treated with 3 g/L sodium lactate, while fresh beef without treatment served as negative control. Based on the comprehensive analyses of pH, total volatile basic nitrogen (TVB-N), colour, and microbial quality, the acceptable shelf life of chilled beef treated with sodium lactate was six days, while the control was acceptable for three days. Sodium lactate usage was conducive to hygiene levels improvement. *Lactobacillus* spp. and *Weissella* spp. sharply displaced the *Ralstoni* spp. with high potential spoilage, and became the predominant bacteria. Moreover, the proliferation of *Serratia* spp. was completely controlled by sodium lactate treatment. Sodium lactate usage exhibited a better pH stability and outstanding colour stability together with antibacterial efficacy. Furthermore, we suggested the mechanistic insights on colour stability and antibacterial efficacy of sodium lactate due to its roles in the regeneration of reduced nicotinamide adenine dinucleotide (NADH) together with pH regulation.

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Introduction

Fresh beef is a large part of human diet in many countries. However, due to its rich nutrition, fresh beef is an ideal substrate for the growth of spoilage microorganisms, thus resulting in a perishable product with short shelf life (Liu *et al*., 2020). Due to spoilage such as off-colour, off-odour, or off-texture, the annual losses of beef could reach to approximately 20% of the initial beef production (Chen *et al*., 2020). Consequently, the hygiene and safety of fresh beef become a major concern to consumers, processors, retailers, and meat industries. Therefore, it is essential to implement hygiene procedures and adequate preservation technologies to promote freshness of fresh beef to maintain its quality and safety.

The spoilage of raw beef is largely dependent on the initial microbial load, as well as packaging and storage conditions. Chilled and frozen storage are the common commercial preservation methods used for fresh beef. Frozen storage is a safe way to store meat and extend shelf life, but the tenderness, juiciness, and appearance of beef is likely to be reduced, and it is less popular with consumers when compared with chilled beef. However, chilled beef has limited shelf life, hence a limiting factor for distribution during long distance transport. As a result, the way to extend shelf life of chilled beef and retain better quality is of great importance for both customers and the meat industries.

Recently, meat quality enhancement by preservative usage during chilled storage has been receiving much attention. Sodium lactate ($C_3H_5NaO_3$, MW = 112.06) has been approved by Food and Drug Administration (FDA) as a safe food addictive (Liu *et al*., 2020). To date, although sodium lactate has been applied in sausage and manufactured meat to control food spoilage, it is rarely used in fresh meat for freshness improvement. Additionally, there are few reports on mechanistic insights of freshness promotion for meat by sodium lactate.

Therefore, in the present work, sodium lactate was used as preservative for fresh beef under chilled storage. The relationship between shelf life extension, colour preservation, and antimicrobial activity enhanced by the preservation treatment was systematically evaluated. Spoilage was monitored by pH, total volatile basic nitrogen (TVB-N), discoloration, sensory analysis, and microbial quality.

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Discoloration was detected by colorimetric evaluation combined with consumer-defined colour acceptability. Microbial quality was evaluated by five principal microbiological indicators determined by culture-dependent method, namely total viable counts (TVC) and lactic acid bacteria (LAB) counts, *Brochothrix* spp. counts, *Pseudomonas* spp. counts, and Enterobacteriaceae counts, combined with bacterial community dynamics determined by high-throughput sequencing technology. Furthermore, based on the results of the colour stability promotion and antibacterial efficacy, the mechanistic insights on colour stability and antibacterial efficacy enhanced by sodium lactate were discussed systematically, and the simplified schematic diagram of mechanistic insights was prepared.

Materials and methods

Beef sample collection and treatments

Fresh beef *longissimus lumborum* muscle was obtained from a local market of agricultural products located in Chengdu, Sichuan Province, China, within 24 h after slaughter. After purchase, the fresh beef was immediately kept in an insulation ice box, and transported to laboratory within 15 min. Then, the fresh beef was diced into samples (10 × 10 × 1.5 cm) of approximately 100 g each. Subsequently, all samples were equally divided into two groups. One group was dipped into (3 g/L) sodium lactate for 3 min, and labelled as Group SL, while another group was labelled as Group C which was the negative control. After treatment, all samples were immediately packed with polyethylene sterile bags, and stored at 4°C for 7 d. Samples were taken in triplicate for physiochemical analyses and sensory evaluation every day; and for microbial examination, every other day during chilled storage.

Physicochemical analyses

pH

The pH values of samples were measured using a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with automatic temperature compensation (NTC) electrode according to Wang *et al.* (2015a). Before measurement, the pH probe was calibrated in buffers at pH 4.00 and 7.00 at room temperature. Then, the pH probe was inserted directly into samples, and all measurements were performed in triplicate at each time point, and the average was calculated.

TVB-N

The TVB-N content of samples was measured using an automatic azotometer (KDN-1000, Shanghai Xin Rui Instrument and Meter Co. Ltd., Shanghai, China) following China Standard Protocols GB/T 5009.44-2003, which is for the analysis of hygienic standard of meat and meat products (Liu *et al*., 2020). The TVB-N level was expressed as mg/100 g sample.

Colour

The surface colour of samples was determined using a colorimeter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China) according to Wang *et al*. (2015b), and reported as lightness (L^*), redness (a^*), and yellowness (b^*) as CIELab coordinates. At each time point, the surface colour of samples was determined at three random locations, and then triplicate readings were averaged.

Relative myoglobin content

A spectrophotometer was used to record the reflectance values (R) in the range of 360 to 740 nm at 10 nm intervals, and R was calculated by integrations of the measurement at 473, 525, 572, and 700 nm, then the R was converted to reflex attenuance (A) following the equation: A = log (1/R). Next, the percentages of myoglobin, namely metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb), and oxymyoglobin (OxyMb) were determined using Eqs. 1, 2, and 3 as described by Wu *et al*. (2020):

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

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

$$\%\mathrm{OxyMb} = 100 - (\%\mathrm{MetMb} + \%\mathrm{DeoxyMb}) \quad \text{(Eq. 3)}$$

where, A473, A525, A572, and A700 = reflex attenuance at 473, 525, and 700 nm, respectively.

NADH/NAD$^+$ ratio

The reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide (NAD^+) concentrations were determined using the coenzyme INAD (H) content test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. The NADH/NAD^+ ratio was calculated

by the specific value of NADH concentration and NAD^+ concentration.

Sensory evaluation

At each time point, the freshness of samples was evaluated by sensory analyses according to Chen *et al*. (2020). Based on colour, odour, texture, appearance, and viscosity, 11 sensory panellists were trained using random order to evaluate the freshness, who then scored each on a scale from 1 - 5 points based on attribute degrees, in which a 5.0 - 4.0 indicated good quality, 4.0 - 3.0 indicated acceptable quality, 3.0 - 2.0 indicated unacceptable quality, and 2.0 - 0.0 indicated a complete spoiled state. A total score of 20.0 points was considered fresh, whereas total score lower than 10.0 was considered as unacceptable and spoiled.

Microbiological quality analyses

Microbial quality was monitored by culture-dependent method based on plate counts including TVC counts, LAB counts, *Pseudomonas* spp. counts, *Brochothrix* spp. counts, and Enterobacteriaceae counts, and culture-independent method through high-throughput sequencing technology.

Bacterial counts

The TVC counts, LAB counts, *Pseudomonas* spp. counts, *Brochothrix* spp. counts, and Enterobacteriaceae counts were performed according to Yang *et al*. (2016). Briefly, 10 g of sample was aseptically placed in stomacher bag containing 90 mL sterile 0.1% peptone water, and homogenised using a stomacher. Following homogenisation, a 10-fold dilution series was performed for microbiological analysis. TVC counts were determined on plate count agar (PCA; Sangon Biotech Co. Ltd., Shanghai, China), and incubated at 37°C for 48 h. LAB counts were determined on De Man, Rogosa, Sharpe agar (MRS; Sangon Biotech Co. Ltd., Shanghai, China), and incubated at 37°C for 48 h. *Pseudomonas* spp. counts were determined on *Pseudomonas* CFC selective agar (Sangon Biotech Co. Ltd., Shanghai, China), and incubated at 25°C for 48 h. *Brochothrix* spp. counts were determined on Streptomycin Thallous Acetate (STAA) agar (Land Bridge Co. Ltd., Beijing, China), and incubated at 25°C for 48 h. Enterobacteriaceae counts were determined on Violet Red Bile Glucose agar (VRBGA; Land Bridge Co. Ltd., Beijing, China), and incubated at 37°C for 24 h. Results of bacterial counts were expressed as $\log_{10}$ CFU/g sample.

Bacterial community analyses by high-throughput sequencing technology

Extraction of total genomic DNA of the bacteria collected directly from the beef samples were carried out according to Liu *et al*. (2018), Wang *et al*. (2018a), and Chen *et al*. (2020). Total genomic DNA of bacteria was extracted following the manufacturer protocol of the E.Z.N.ATM Mag-bind Soil (OMEGA, USA). The DNA concentration and quality were monitored using the micro-volume Bio-Spec Nano spectrophotometer (Shimadzu, Japan) and 0.8% agarose gel electrophoresis, respectively.

After the determination of DNA concentration and quality, the forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for PCR amplification of V4 region of the 16S rDNA. The system and conditions of PCR amplification were performed according to Wang *et al.* (2018b). Following amplification, the amplified products were purified and quantified. Then, the amplified products were pooled in equal proportions, and sequenced by the high-throughput sequencing technology on the llumina MiSeq platform at Beijing Novogene Technology Co. Ltd. (Beijing, China).

After high-throughput sequencing, the raw sequencing data were merged and screened as previously described (Chen *et al*., 2019; Wang *et al.*, 2019). Then the high-quality sequences were clustered and regarded as operational taxonomic units (OTUs) based on the SILVA reference gene database (Quast *et al.*, 2013) at an identity threshold of 97%, generating the community diversity (Shannon index) and bacterial community richness (Ace estimator), simultaneously, by using QIIME software. Furthermore, a community structural component diagram was generated using the R programming language.

Statistical analyses

Each test was performed in triplicate. Data were then analysed separately using Linear Mixed Models in Genstat described by Biffin *et al*. (2019). Results were then presented as mean ± standard error. Statistical significance ($p < 0.05$) was analysed by One-way analysis of variance using SPSS software (Version 15.0, SPSS Inc., Chicago, IL, USA).

Results

pH

Sodium lactate treatment had an effect ($p < 0.05$) on the pH values of chilled beef stored at 4°C as shown in Figure 1A. The initial pH values in Group C and SL were 5.43 and 5.63, respectively. During storage, the pH values in Group C fluctuated dramatically, varying over a range of 5.47 - 5.71. In contrast, the pH values in Group SL maintained at a stable level within a range of 5.53 - 5.63, which was normal during the storage. These results revealed that sodium lactate treatment effectively inhibited the increase in pH value in chilled beef, especially in the middle and late storage period.

pH plays a critical role in raw beef quality during storage which can affect colour appearance by influencing oxygen consumption, metmyoglobin reducing activity, water holding capacity, and bacterial growth (Mancini and Ramanathan, 2014). After slaughter, the pH value in the normal muscles of fresh beef is in the range of 5.7 - 5.8. Like pH, water holding capacity of muscle proteins becomes greater which can cause swelling of fibres and shrinkage of the space between muscle fibrils. Once the pH value is over 6, autolysis will occur, and protein is further decomposed, thus producing a large number of amino acids, which is conducive for bacterial growth, which in turn leads to spoilage (Liu *et al.*, 2020).

In the present work, the fluctuation of pH values in beef treated with sodium lactate was within a narrow range from 5.53 - 5.63. In contrast, the fluctuation of pH values in the control was relatively larger with a range from 5.47 - 5.71. In the first three days, the pH value in the control was lower, when compared with the beef treated with sodium lactate. As a result, the low pH value negatively affected the colour characteristics of beef due to the transition of the easily oxidised Mb fraction into MetMb, thus resulting in darker red colour. Moreover, in the first three days, the pH values in the control with a range of 5.47 - 5.50 was close to isoelectric point (5.2 - 5.5) of protein, thus resulting in a weak water holding capacity. Meanwhile, in the final four days, the pH value in the control was higher than that of the beef treated with sodium lactate. A high pH value would lead to a more closed structure muscle fibres swell, thus resulting in the formation of a barrier against oxygen diffusion, together with the inhibition of oxygen binding to Mb. As a result, the formation of red OMb will be inhibited, thus resulting in weaker colour intensity. Therefore, any deviations from the norm in terms of pH will affect colour intensity. pH value in normal range and stabilisation is conducive to form of an acceptable colour for beef. These results indicated that sodium lactate as preservative could stabilise the pH value to delay spoilage of chilled beef during storage at 4°C. These results were consistent with those results reported by Sallam and Samejima (2004) and Liu *et al*. (2020) who found that sodium lactate was conducive for pH stabilisation of meat during storage at 2 - 4°C.

TVB-N

The TVB-N levels in chilled beef during storage at 4°C are shown in Figure 1B. The initial TVB-N content was 7.18 and 6.55 mg/100 g in Groups C and SL, respectively, which was indicative of good meat quality (Tian *et al*., 2017; Wang *et al*., 2021). During storage, the TVB-N level in Group C increased sharply, and reached 19.71 mg/100 g on the third day, while the TVB-N level in Group SL increased slowly and reached 15.80 mg/100 g on the sixth day.

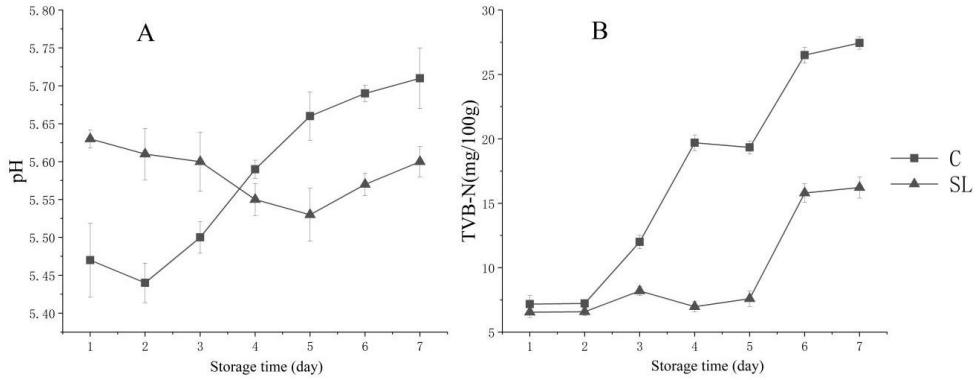

Figure 1. Changes in pH values **(A)** and total volatile basic nitrogen (TVB-N) levels **(B)** of control (Group C) and treatment (Group SL) during storage at 4°C for seven days.

The TVB-N is mainly composed of ammonia, biogenic amine, and trimethylamine, which originate from microorganisms and enzyme activity (Lu *et al*., 2015). Therefore, the TVB-N is used as a direct quality indicator of meat freshness or 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 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 the threshold of acceptability. In the present work, referring to the threshold (≤ 15 mg/100 g), Group C reached the rejection level on the third day, whereas Group SL reached the rejection level on the sixth day. The acceptable shelf life based on the threshold of TVB-N level was well consistent with the acceptable shelf life based on sensory evaluation.

These findings indicated that sodium lactate treatment could prolong the shelf life. Bacterial activity and endogenous enzymes are mainly responsible for the increase in TVB-N levels (Cai *et al*., 2011). Consequently, sodium lactate treatment could inhibit the growth of microorganisms and inactivate activity of endogenous enzymes in beef, thus resulting in shelf life extension (Lu *et al*., 2015). In the present work, indeed, the results of TVB-N were in agreement with the results of microbiological and other physicochemical analyses. Moreover, these results were in line with those studies reported by Schelegueda *et al*. (2016) and Liu *et al*. (2020) who reported that sodium lactate had superior effects on inhibiting TVB-N than that of the control.

Colour properties

Instrumental colour values

The changes in colour parameters *L**, *a**, and *b** values of beef samples during storage at 4°C are shown in Table 1. The initial *L** and *a** values of all beef samples was above 20 and 31, respectively, thus suggesting that all beef samples were fresh at the beginning of storage. Subsequently, both the *L** and *a** values of Group SL were significantly higher ($p < 0.05$) than that of the control during storage. Meat colour has been considered as an indicator of freshness and quality of meat, which influences the consumer preference to purchase a meat product. A bright red colour is considered a positive attribute for freshness and superior quality of beef (Holman *et al*., 2016). The degree of *a** of chilled beef is associated with the concentration of reduced MetMb or Mb or OxyMb (Krasulya *et al*., 2021). Therefore, the indicator *a** is of the greatest interest in the analysis. Based on the relationship between colorimetric evaluation and consumer-defined beef colour acceptability (Holman *et al*., 2017), when the *a** value is equal to or greater than 14.5 (with 95% acceptance), beef colour is desirable. In the present work, the *a** value of Group SL remained above this threshold (*a** > 14.5) for the entire storage period with a range of 35.6 - 29.7. Meanwhile, the *a** value of Group C decreased to 13.8, and reached the rejection level on the fifth day. These results revealed that sodium lactate treatment was conducive to stabilise colour parameters of raw beef, especially the *a** value. These results were in line with the previous experimental studies reported by Liu *et al*. (2020).

Table 1. Effect of sodium lactate treatment on the instrumental colour values control (Group C) and treatment (group SL) during storage at 4°C for seven days.

		Storage time (day)						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Group C	*L**	24.3 ± 0.5	24.4 ± 0.3	24.8 ± 1.2	25.4 ± 0.2	26.5 ± 0.3	24.6 ± 0.5	23.8 ± 0.5
	*a**	31.4 ± .0.9	30.5 ± 0.6	34.4 ± 1.6	21.2 ± 0.3	18.1 ± 0.8	13.8 ± 0.5	12.1 ± 0.9
	*b**	-3.6 ± 0.2	-2.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.4	2.1 ± 0.2	4.5 ± 0.2	6.1 ± 0.4
Group SL	*L**	20.8 ± 0.4	21.6 ± 0.4	22.8 ± 0.3	21.3 ± 0.3	23.5 ± 1.4	22.6 ± 0.2	24.1 ± 1.7
	*a**	33.1 ± 1.5	34.2 ± 0.4	32.4 ± 1.3	34.2 ± 1.4	35.6 ± 1.7	32.1 ± 0.3	29.7 ± 0.4
	*b**	-6.0 ± 0.3	-3.4 ± 0.3	-0.06 ± 0.5	1.5 ± 0.1	1.5 ± 0.1	1.9 ± 0.3	2.8 ± 0.1

Visual colour evaluation

The intensity of colour appearance of beef samples during storage at 4°C is shown in Figure 2. The initial colour appearance of all beef samples was bright cherry red on the first day, thus suggesting desirable colour acceptability. Subsequently, the colour appearance of Group SL turned to bright red on the second day, and stayed this colour until the sixth day. Meanwhile, the colour appearance of Group C turned to dull red on the third day, became dark red on the fourth day, and was undesirable on the fifth day. These results were in line with the results of instrumental colour *a** values, and revealed that sodium lactate treatment was conducive to delaying the discoloration when compared with the control.

Relative myoglobin content

Metmyoglobin reducing ability is crucial for meat colour 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. Sodium lactate usage had a significant effect ($p < 0.05$) on the state of myoglobin, and was conducive to maintaining high proportion of OxyMb. The initial %DeoxyMb, %OxyMb, and %MetMb in Groups C and SL were 7.51 and 61.33, 11.31 and 9.92, 53.68 and 17.23, respectively, showing that %OxyMb, which is beneficial to beef colour of bright redness, was the highest. Subsequently, the %OxyMb of Group C significantly decreased from 53.13 - 20.87 on the fourth day, while the %OxyMb of Group SL increased from 54.42 - 61.09. As storage extended, the %OxyMb of Group C was significant lower ($p < 0.05$) than that of the Group SL from the fifth to seventh day, while the changes in %MetMb, which is responsible for the dark redness, showed opposite trend. These results were in agreement with the results of redness ($a*$), which could partially explain the reason why the colour appearance of Group SL was more acceptable when compared with Group C.

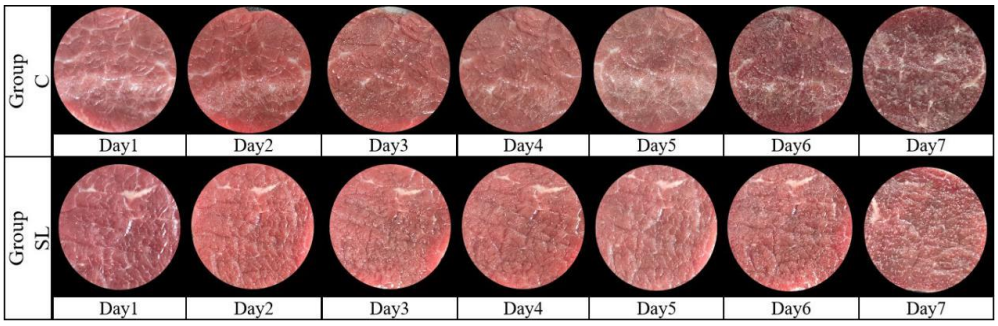

Figure 2. Effect of sodium lactate treatment on colour appearance of control (Group C) and treatment (Group SL) during storage at 4°C for seven days.

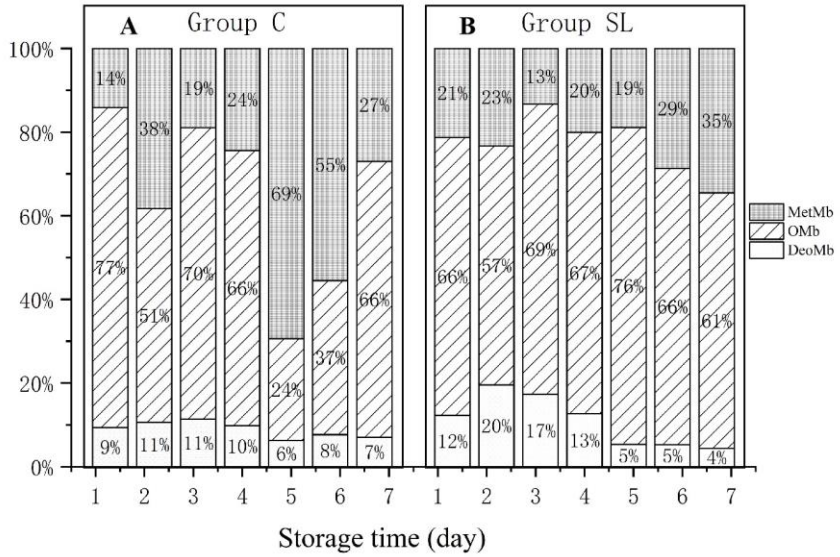

Figure 3. Relative content of three myoglobin forms namely deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb) of control (Group C) and treatment (Group SL) during storage at 4°C for seven days.

NADH/NAD$^+$ ratio

The initial NADH/NAD$^+$ ratio in Groups C and SL were 0.59 and 0.58, respectively. As the storage extended, Group C displayed much different NADH/NAD$^+$ profiles when compared with Group SL. The NADH/NAD$^+$ ratio in Group C continued to increase, and reached a level approximately five times on the third day when compared with the initial NADH/NAD$^+$ ratio. In contrast, NADH/NAD$^+$ ratio in Group SL displayed a small decrease at 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 sixth day.

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. The high NADH/NAD$^+$ ratio indicates insufficient oxidation of NADH, thus 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 C indicates insufficient reducing power. As a result, Fe^{+3}-MetMb is insufficiently restored to Fe^{+2}-Mb, thus resulting in a high proportion of MetMb and discoloration. Indeed, as shown in Figure 3, the proportion of MetMb in Group C was higher when compared with Group SL. Clearly, the sodium lactate usage was conducive to stabilising the cellular NADH/NAD$^+$ ratio, which was probably the main positive contribution against discoloration. Furthermore, we suggested the mechanistic insights on colour stability through MetMb-reducing activity to illustrate the contributions of NADH.

Microbial quality

The microbial quality of raw beef during storage at 4°C was monitored by culture-dependent method based on plate counts, and culture-independent method based on bacterial community analysis by high-throughput sequencing technology. Five principal microbiological indicators, namely TVC, LAB, *Brochothrix* spp., *Pseudomonas* spp., and Enterobacteriaceae counts are given in Figure 4. Enterobacteriaceae was not detected in all samples during storage. The initial TVC counts in Groups C and SL were 3.45 and 3.08 $\log_{10}$ CFU/g, respectively, thus indicating good hygienic quality, which was well in agreement with the production requirements of chilled beef. Subsequently, the TVC counts in Group C increased sharply, and reached to 4.95 $\log_{10}$ CFU/g on the third day, and 7.78 $\log_{10}$ CFU/g on the fifth day, while the TVC counts in Group SL increased slowly, and reached to 6.84 $\log_{10}$ CFU/g on the seventh day. The TVC counts as a direct quality indicator of fresh meat is positively correlated with spoilage process, and the value of 7 $\log_{10}$ CFU/g has been defined as threshold of microbial counts for good quality fresh meat by the International Commission on Microbiological Specifications for Foods (ICMSF). Therefore, based on the results of TVC counts, sodium lactate exerted a good bacteriostatic effect, and could prolong the shelf life by two days when compared with the control. Likewise, except for LAB counts, *Brochothrix* spp. and *Pseudomonas* spp. counts in Group SL were significant lower ($p < 0.05$) than that of Group C, thus suggesting good inhibition of microbial spoilage by sodium lactate.

Microbiological data associated with spoilage collected from conventional cultural methods are often not sufficient enough to indicate the extent of microbial spoilage. Therefore, high throughput sequencing was performed to analyse the bacterial community of beef during storage at 4°C as shown in Figure 5. At the genus level, initially, *Ralstoni* spp. were the most predominant bacteria in all beef samples with a relative abundance of 93.2 - 93.5%. Subsequently, the succession of the bacterial community was significantly influenced ($p < 0.05$) by sodium lactate treatment. In Group C, *Ralstoni* spp. were the predominant bacteria, accounting for 93.2% on the first day. Subsequently, their relative abundance decreased to 0.1%, and *Lactococcus* spp. suddenly reached a maximum of 81.4% on the third day and then gradually decreased to 43.15% on the seventh day. Meanwhile, *Myroides* spp. showed an increase from the third day, and became predominant with a relative abundance of 37.7% on the seventh day. Moreover, *Serratia* spp. were observed on the seventh day, accounting for 4.8% of the total abundance. Instead, in Group SL, *Ralstoni* spp. predominated at the initial stage of storage, and were displaced by *Lactobacillus* spp. (54.51 - 70.70%) and *Weissella* spp. (18.07 - 17.32%) from middle to later of storage, which was well in agreement with the results reported by Liu *et al*. (2020). Moreover, *Serratia* spp. were nearly undetectable in Group SL at all storage time points.

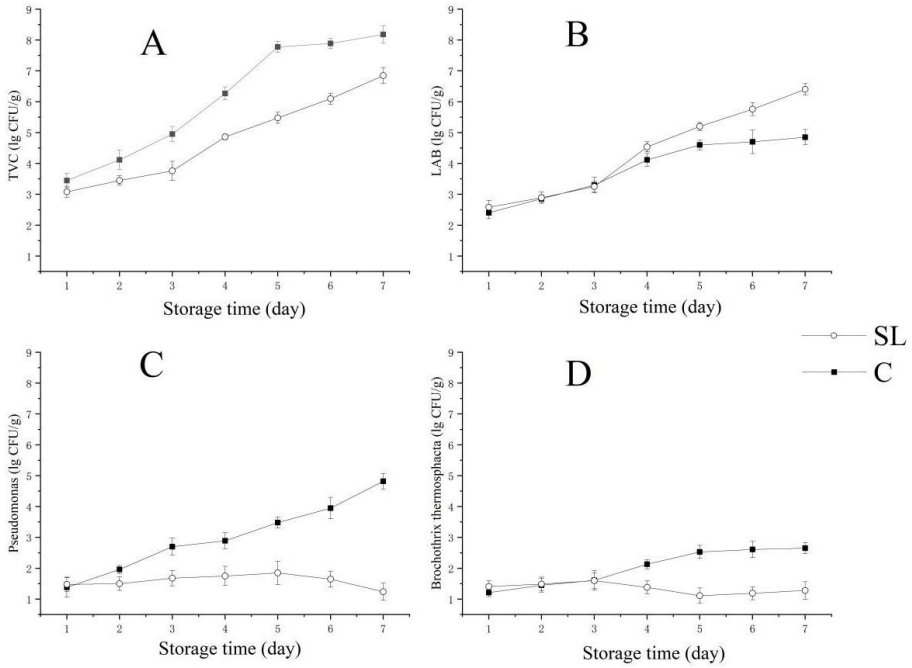

Figure 4. Effect of sodium lactate treatment on total viable counts (TVC) **(A)**, lactic acid bacterial (LAB) counts **(B)**, *Brochothrix* spp. counts **(C)**, and *Pseudomonas* spp. counts **(D)** in control (Group C) and treatment (group SL) during storage at 4°C for seven days.

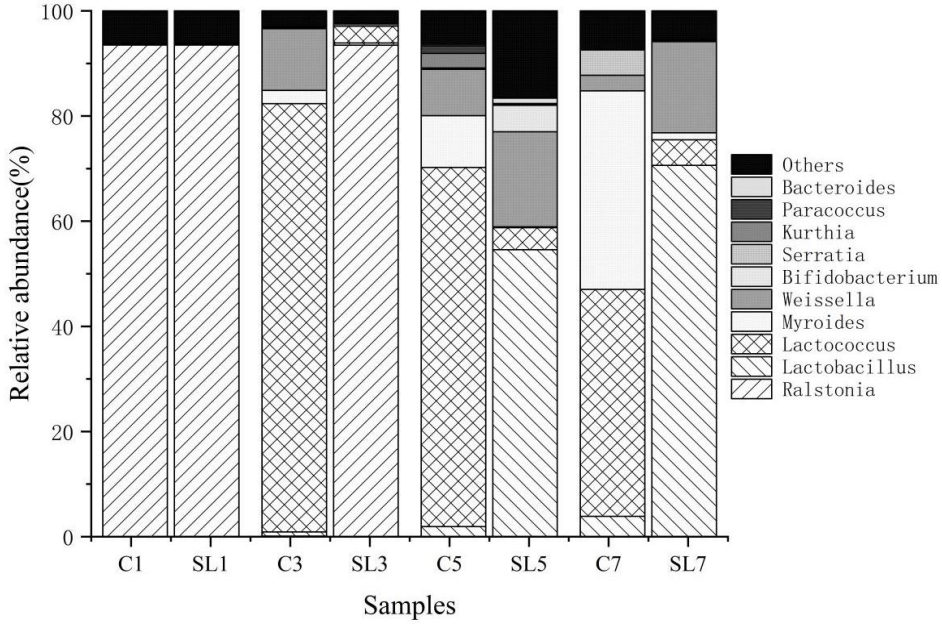

Figure 5. Relative abundance at the genus level based on the classification of partial 16S rRNA genes sequences of bacteria from control (Group C) and treatment (group SL) during storage at 4°C for seven days.

Both *Lactobacillus* spp. and *Weissella* spp. can inhibit the growth of spoilage bacteria such as *Serratia* spp. and *Pseudomonas* spp. due to their antibacterial and anti-oxidative activities (Zhang *et al.*, 2016). *Serratia* spp. as the representative genus of psychrotrophic Enterobacteriaceae, and *Pseudomonas* spp. with high potential spoilage are easy to cause deterioration of fresh meat under chilled conditions. These results revealed that sodium lactate was conducive that *Lactobacillus* spp. and *Weissella* spp. became the dominant bacteria, thus resulting in competitive inhibition of the growth of *Serratia* spp.

and *Ralstoni* spp., which are sub-branches of *Pseudomonas* spp. Indeed, *Serratia* spp. were hardly detected in Group SL during the whole storage period. Meanwhile, small amount of *Serratia* spp. (0.24%) was detected on the fifth day, and a high level (4.79%) was presented on the seventh day in the control. Furthermore, combined with the findings of *Brochothrix* spp. and *Pseudomonas* spp. counts, sodium lactate was conducive to controlling the rate of proliferation of spoilage bacteria, which might be attributed to *Lactobacillus* spp. which can produce bacteriocins. These results suggested that sodium lactate treatment could be one of the effective ways to prolong shelf life of fresh beef by hygiene level improvement during storage at 4°C. However, further study to verify this hypothesis is required, and their direct relationship and effects on the final shelf life need further exploration.

Sensory evaluation

Sodium lactate treatment had positive effect ($p < 0.05$) on all the consumer panel scores as shown in Figure 6, when compared with the control. In Group C, the results of the sensory evaluation including colour, odour, texture, appearance, and viscosity significantly decreased ($p < 0.05$) as storage time extended. Meanwhile, Group SL displayed better sensory attributes than that of control during storage. Sensory attributes have been employed to evaluate the shelf life of beef in some countries (Chen *et al*., 2020). In the present work, the beef treated with sodium lactate maintained high consumer acceptance and good acceptable appearance, and exhibited longer shelf life in term of sensory attributes when compared with the control, which was well consistent with the results in term of TVB-N values, colour values, and microbial quality.

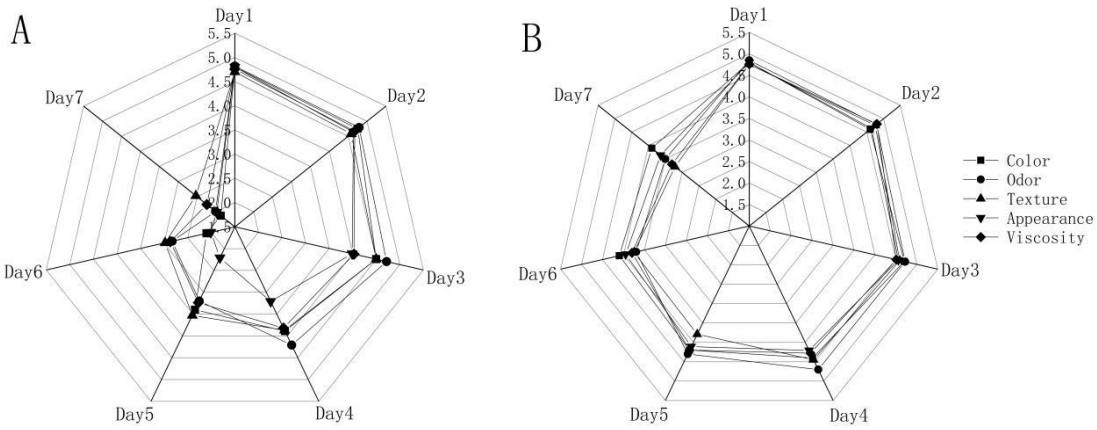

Figure 6. Sensory evaluation of colour, odour, texture, appearance, and viscosity of control (Group C) **(A)** and treatment (Group SL) **(B)** during storage at 4°C for seven days.

Discussion

Mechanistic insights on colour stability enhanced by sodium lactate

A key indicator of quality and safety of fresh meat is appearance which is primarily determined by colour. Visual appearance and colour of fresh meat could indicate deterioration during biochemical processes occurring in post-slaughter period and chilled storage. At biochemical level, colour of fresh meat is mainly determined by the concentration of pigment and Mb, which is a myofibrillar protein capable of actively binding oxygen (Olga *et al*., 2021), and the ratio of redox forms of Mb, namely MetMb, DeoxyMb, and OxyMb in muscle tissue. It has been acknowledged that the variation of the ratio of redox forms of Mb is the crucial factor in the progress of discoloration of fresh meat post-slaughter and during storage (Wu *et al*., 2020; Krasulya *et al*., 2021).

The saturation of Mb with oxygen leads to bright pink colour of fresh meat as OxyMb. At the same time, due to the oxidation of oxygen, Fe^{+2} in OMb will be oxidised to Fe^{+3}, which leads to the formation of dark brown Mb (MetMb), thus resulting in a loss of consumer acceptability. Subsequently, due to the action of MetMb reduction system and antioxidant systems of fresh meat such as vitamin B_{12}, ascorbic acid, and glutathione, Fe^{+3} is restored to Fe^{+2}, and MetMb is converted to Mb, thus resulting in more acceptable colour appearance. Over time, the substrates which can maintain MetMb reduction

system will be gradually depleted, thus resulting in MetMb-reducing activity, and becomes insufficient for the transformation of MetMb into Mb. Therefore, maintaining a durative MetMb-reducing activity by supplementing with substrates and energy sources, such as nicotinamide adenine dinucleuotide (NADH) as reducing equivalents, is considered as an effective and feasible way.

In the present work, based on the results of colour properties, the fresh beef treated with sodium lactate exhibited significant ($p < 0.05$) more acceptable colour appearance and stability during storage at 4°C when compared with the control, thus indicating an effective reduction of discoloration. We suggest that the colour stability efficacy by sodium lactate is based on the increasing amount of NADH through the previous step of gluconeogenesis pathway, that is, lactic acid ($CH_3CHOHCOOH$) is catalysed to pyruvic acid ($CH_3COCOOH$) by lactate dehydrogenase (LDH-B) in mitochondrion and cytoplasm of muscle tissue together with pH changes. In order to draw out the mechanistic suggestions of the colour stability, a simplified schematic diagram of maintaining a durative MetMb-reducing activity by supplementing with sodium lactate was prepared to illustrate the contributions of the changes in pH value and (NADH) level which is conducive for colour stability.

The state of Mb is closely determined by the MetMb-reducing system. Mb is as a carrier of oxygen to mitochondria in muscles of living animals. However, mitochondria continue to metabolise oxygen in the post-mortem state, thus resulting in a competitive relation with Mb through oxygen uptake. As a result, OMb levels are decreased, and the bright pink colour of meat becomes weaker. Furthermore, the low oxygen partial pressure resulting from an increase in mitochondrial activity maintains Mb in a deoxygenated state. Meanwhile, radicals generated by mitochondrial electron transport pathways will promote the oxidation of Fe^{+2} to Fe^{+3} along with NADH depletion, thus resulting in an acceleration of accumulation of MetMb. Under this state, the fresh meat begins the discoloration. Therefore, reduction of MetMb to Mb will contribute to maintaining colour stability. In fact, MetMb can be restored to Mb by the MetMb reductase system, which consists of cytochrome b5 and NADH as a donor. Cytochrome b5 reduces Fe^{+3}-MetMb to Fe^{+2}-Mb as follows: MetMb (Fe^{+3}) + NADH → cytochr b5 (reduced) + NAD^+. The NADH replenishment provided by the reaction that lactic acid is catalysed to pyruvic acid will promote the reduction Fe^{+3}-MetMb to Fe^{+2}-Mb, thus resulting in an acceleration of accumulation of Fe^{+2}-Mb. In the present work, the significant higher ($p < 0.05$) NADH level and the ratio of Fe^{+2}-Mb than that of the control was detected in chilled beef treated with sodium lactate. Therefore, a suggestion is reached that the addition of sodium lactate as the substrates of the reaction that lactic acid is catalysed to pyruvic acid promotes NADH regeneration and replenishment for MetMb reductase system, thus resulting in an increase of Fe^{+2}-Mb accumulation which is conducive to forming a characteristic bright pink colour.

Additionally, it has been reported that the colour intensity of fresh meat is also determined by pH value, except for the amount and saturation of Mb (Sallam and Samejima, 2004). In the present work, the fluctuation in pH values in beef treated with sodium lactate was within a narrow range from 5.53 - 5.63. In contrast, the fluctuation in pH values in the control was relatively larger with a range from 5.47 - 5.71. In the first three days, the pH value in the control was lower when compared with the beef treated with sodium lactate. As a result, the low pH led to negative effect on colour characteristics of beef due to the transition of the easily oxidised Mb fraction into MetMb, thus resulting in a darker red colour. Meanwhile, in the final four days, the pH values in the control were higher than that of the beef treated with sodium lactate. A high pH value leads to a more closed structure muscle fibres swell, thus resulting in forming a barrier for oxygen diffusion, together with inhibition of oxygen binding to Mb. As a result, the formation of red OMb will be inhibited, thus resulting in weaker colour intensity. Therefore, any deviations from the norm in terms of pH will affect colour intensity. The pH value in normal range and stabilisation is conducive to formation of an acceptable colour. Therefore, in the present work, we suggest that another role of colour contribution by sodium lactate is pH regulation. Specifically, $CH_3CHOHCOO^-$ from sodium lactate will form lactic acid ($CH_3CHOHCOOH$) which belongs to weak electric acid through combination with H^+, thus resulting in a decrease in the amount of H^+ in muscle tissue, and a slightly higher pH value. Subsequently, $CH_3CHOHCOOH$ is catalysed to $CH_3COCOOH$ by LDH-B. In $CH_3CHOHCOOH$ production, $CH_3CHOHCOOH$ is partially ionised into $CH_3CHOHCOO^-$, and releases a small amount H^+

into muscle tissue, thus resulting in a slight increase in pH value along with the H^+ supplement. Therefore, the pH is well regulated and maintains stability.

Based on the above studies, sodium lactate as the substrate of LDH-B is an important factor for colour stability of fresh beef due to its roles in the regeneration of NADH together with pH regulation.

Mechanistic insights on antibacterial activity enhanced by sodium lactate

In the present work, the beef treated with sodium lactate exhibited a better microbial quality when compared with the control, especially in the middle and later period of storage, which suggested that sodium lactate could have antibacterial activity. Based on the results of microbial quality, the growths of spoilage bacteria such as *Pseudomonas* spp., *Brochothrix* spp., and *Serratia* spp. were effectively inhibited, and *Lactobacillus sakei*. and *Weissella* spp. were promoted to predominant bacteria which have been granted GRAS (Generally Recognized as Safe) status. We suggest that antibacterial efficacy is mainly due to the fact that $CH_3CHOHCOO^-$ hinders the formation of electrochemical gradient in microbial cell, thus resulting in reduced growth of bacteria with weak acid resistance, which in turn promotes the interspecific competition by *Lactobacillus* spp. and *Weissella* spp. In order to illustrate the contribution of antibacterial efficacy by supplemented with sodium lactate, a simplified schematic diagram was prepared. Specifically, $CH_3CHOHCOO^-$ with short chains can easily penetrate the microbial cell membrane in its undissociated form, and then dissociates into free proton and acid anion in the cytoplasm of microbial cell, thus leading to acidification. As a result, a decrease in intracellular pH occurs, which significantly impacts cell metabolism, thus resulting in reduced microbial growth such as *Pseudomonas* spp., *Brochothrix* spp., and *Serratia* spp. whose suitable acid-base environment for growth is neutral, with a pH value of 7, or slightly alkaline, and has a poor acid resistance. However, under such conditions, *Lactobacillus sakei* and *Weissella* spp. can still grow well due to its good acid tolerance, and gradually become the dominant bacteria, showing interspecific competition, thus resulting in a further inhibition of the growth of spoilage bacteria such as *Pseudomonas* spp., *Brochothrix* spp., and *Serratia* spp. Additionally, sakacin P as a bacteriocin, is possible produced by *Lactobacillus sakei*, which has been recognised as a food bio-preservative by the World Health Organization, and exhibits synergetic antibacterial efficacy.

Conclusion

Based on the changes in pH values, sodium lactate usage exhibited better pH stability. The fluctuation of pH in beef treated with sodium lactate was within a narrow range of 5.53 - 5.63, while the fluctuation of pH in the control sample was relatively larger in a range of 5.47 - 5.71. As is well known, any deviations from the norm in terms of pH will affect colour intensity and water-holding capacity of fresh meat. Therefore, pH value in normal range and stabilisation are important in the formation of acceptable colour and appearance for fresh beef. We suggest that the pH stability could have been due to pH regulation by the biochemical reaction as follows: $CH_3CHOHCOO^- + H^+ \rightarrow CH_3CHOHCOOH \rightarrow CH_3COCOOH \rightarrow CH_3COCOO^- + H^+$.

Based on the changes in TVB-N levels, sodium lactate usage extended shelf life. The TVB-N level reached the rejection level (≥ 15 mg/100 g) on the sixth day in sample treated with sodium lactate, while the TVB-N level reached the rejection level on the third day in the control sample.

Based on the results of colour properties combined with the relative Mb content and $NADH/NAD^+$ ratio, sodium lactate usage displayed outstanding colour maintenance effect. The *a** value, as a critical indicator of degree of redness for fresh meat, remained above the rejection threshold (≤ 14.5) for the entire storage period within a range of 35.6 - 29.7 in sample treated with sodium lactate, while *a** value decreased to the rejection threshold on the fifth day in the control sample. The sample treated with sodium lactate showed desirable colour appearance for seven days, while the control sample showed undesirable colour appearance on the fourth day. Further, a significantly higher ($p < 0.05$) $Fe^{+2}Mb$ ratio was detected in the sample treated with sodium lactate. We suggest that the colour stability promotion could have been attributed to the regeneration of NADH together with pH regulation by sodium lactate.

The regeneration of NADH is the major supplementary source which will promote the reduction of Fe^{+3} MetMb to $Fe^{+2}Mb$ with NADH supplementation, thus resulting in a higher %OxyMb which will contribute to maintaining the colour

stability. Meanwhile, pH stability promotion will also be conducive to colour stability.

Sodium lactate usage exhibited antibacterial efficacy. Except from LAB, the TVC, *Brochothrix* spp., and *Pseudomonas* spp. counts were lower than that of the control sample. Based on the analysis of the bacterial community, *Lactobacillus* spp. (54.51 - 70.70%) and *Weissella* spp. (18.07 - 17.32%) were the dominant bacteria, and *Serratia* spp. were nearly undetectable in the sample treated with sodium lactate. We suggest that the antibacterial efficacy could have been attributed to $CH_3CHOHCOO^-$ which hindered the formation of electrochemical gradient in microbial cell, thus resulting in reduced growth of bacteria with weak acid resistance, which then promoted the interspecific competition by *Lactobacillus* spp. and *Weissella* spp.

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