



Effects of sodium lactate on glycolytic activity and color stability of fresh beef during chilled storage

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Abstract

Fresh beef was treated with 3 g/L sodium lactate (Group SL) and the fresh beef without treatment was sampled as negative control (Group C). Based on the comprehensive analyses of pH, color and glycolytic rate, sodium lactate usage exhibited a better pH and color stability, which is conducive to maintaining the meat quality. The Group SL had lower glycogen content and higher lactate content than that of Group C. Meanwhile, the Group SL had significantly higher ($p < 0.05$) lactate dehydrogenase and hexokinase activity than that of Group C during chilled storage. Moreover, NADH/NAD⁺ ratio in Group SL maintained at a stable level with a range from 0.54 to 0.68 during chilled storage. In contrast, Group C showed a high NADH/NAD⁺ ratio which indicated insufficient reducing activity. These results revealed that the sodium lactate treatment had a positive relationship with glycolytic rate and promoted the increase of glycolysis rate. These results may be partially explained the reason why sodium lactate usage can promote color stability via increase of glycolysis rate.

Keywords: chilled beef; color stability; glycolysis; sodium lactate.

Practical Application: In this study, the effect of sodium lactate on glycolytic activity and color stability of chilled beef was investigated. These results revealed that the sodium lactate usage was a good way to maintain the color stability of chilled beef. Our work indicated that the sodium lactate addition had a positive relationship with glycolytic rate and promoted the increase of glycolysis rate, which was conducive to maintaining color stability of chilled beef.

1 Introduction

Fresh beef is a large part of the human diet in many countries. However, due to spoilage characterized by color deteriorations or off-odors or texture decomposition, these annual losses of beef reach to approximately 20% of the initial beef production (Chen et al., 2020). Recently, the meat quality enhancement by preservatives usage during chilled storage has been received much attention (Magrinyà et al., 2015; Rumape et al., 2022). During the chilled storage, the energy metabolic activities are still ongoing. After slaughter, the chilled beef will metabolize via glycolysis for supplying energy due that oxygen is limiting (Yang et al., 2018). Glycolysis changes glycogen in muscle into lactate. Then the lactate is converted back to pyruvate with nicotinamide adenine dinucleotide (NADH) production (Giraud et al., 2018). Thus, glycolysis is a very important metabolic pathway in beef muscle cells after slaughter, which induces the accumulation of lactate in muscle tissue to avoid rapid drop of pH value (Yang et al., 2018). Moreover, glycolysis can produce NADH which is essential for the initiation of the reduction reaction of Fe⁺³-MetMb which can negatively affect the color appearance of chilled beef. Both the pH decline and NADH production critically affect ultimate beef quality (Wen et al., 2022). Thus, the changes in glycolytic activity can be very important factors in meat quality.

Sodium lactate (C₃H₅NaO₃, MW = 112.06) has been approved by Food and Drug Administration (FDA) as a safe food additive (Liu et al., 2020). To date, although sodium lactate has been applied in sausage and manufactured meat to control food spoilage, while sodium lactate is rarely used in fresh meat for freshness improvement. Additionally, there are few reports in detail of mechanistic insights on freshness promotion of meat by sodium lactate. Sodium lactate can combine with H⁺ to form lactate (CH₃CHOHCOOH) which is a member of the glycolytic pathway. Therefore, the addition of sodium lactate will affect glycolytic activity, resulting in influencing the meat quality.

Thus, in this study, sodium lactate was added into fresh beef before chilled storage to investigate the effect on color stability and pH, and to evaluate the influence of glycogen, lactate content, lactate dehydrogenase and hexokinase activity on glycolytic rate and meat quality of fresh beef during chilled storage.

2 Materials and methods

2.1 Sample processing

Fresh beef was supplied by a local market of agricultural products located in Chengdu, Sichuan Province, China, within 12 h after slaughter. Then the fresh beef was divided into samples

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(10 cm × 10 cm × 1.5 cm) with about weight of 100 g for experiments. Subsequently, all samples were equally divided into two groups. One group was dipped into (3 g/L) sodium lactate for 2 min marked as Group SL, and another group was marked as Group C without sodium lactate treatment as the negative control. After treatment, all samples were immediately packed with polyethylene sterile bags and stored at 4 °C for 7 days.

2.2 pH measurement

The pH of chilled beef was measured according to the method described by Wang et al. (2015b) using a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with automatic temperature compensation (NTC) electrode. The pH probe was calibrated in buffers at pH 4.00 and 7.00 at room temperature before measurement, and then the pH probe was inserted directly into beef to measure the pH value. All measurements were performed in triplicate at each time point and an average was calculated.

2.3 Color measurement

The color of chilled beef was evaluated as Lightness (L^*), redness (a^*) and yellowness (b^*) as CIELab coordinates, according to the method described by Wang et al. (2015a) using an auto color chromameter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China). At each time point, the color of each sample was determined at three random locations and then triplicate readings were averaged.

2.4 Glycolytic activity determination

The glycolytic activity in chilled beef was characterized by muscle glycogen and lactate content. Muscle glycogen content was measured with the Muscle Glycogen Analysis Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to its instruction. The lactate content was measured with the Lactate Analysis Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to its instruction.

2.5 Lactate dehydrogenase activity analysis

Lactate dehydrogenase activity of chilled beef was analysed according to the method described by England et al. (2014). Briefly, 5 g sample was homogenized in 100 mM K_2HPO_4 at a 1:3 ratio (wt/vol). Aliquots of the muscle homogenate were added to a reaction buffer containing 200 mM MES (pH 5.0), 60 mM KCl, 10 mM KH_2PO_4 , 5 mM $MgCl_2$, 2 mM ATP, 0.5 mM ADP, 0.1 mM AMP, 0.5 mM NAD^+ , 25 mM carnosine, 30 mM creatine and 10 mM sodium acetate. The treatment group contained 4 mM fructose 1,6-bisphosphate while the control treatment did not. Aliquots were taken at 0, 180, 360 and 1440 min for lactate quantification.

2.6 Hexokinase activity analysis

The hexokinase activity of chilled beef was analysed according to the method described by Dogra et al. (2018) using glucose as substrate. The final concentrations of the reaction mixture were 8.3 mM glycylglycine, 17 mM ATP, 0.0011% cresol red, 14 mM

magnesium chloride and 27 mM glucose. For hexokinase activity determination, the reaction mixture was to the cellular extracts of chilled beef and the decrease in A_{560} nm was measured for 5 minutes.

2.7 The NADH/ NAD^+ ratio

The NADH and NAD^+ concentration was determined according to method described by Tian et al. (2022) using the coenzymeINAD (H) content test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The NADH/ NAD^+ ratio was calculated by the specific value of NADH concentration and NAD^+ concentration.

2.8 Sensory evaluation

The freshness of all samples was assessed by human sensory analyses according to the method described by Wang et al. (2022b). Briefly, the freshness of beef samples was evaluated by their organoleptic characteristics, namely color, odor, texture, appearance and viscosity, using a 5-point scale based on attribute degrees by 11 experienced sensory panelists.

2.9 Statistical analysis

Three replicates were performed for all samples and these results were expressed as mean ± standard deviation (SD) unless otherwise mentioned. Student's *t* test was used to calculate the significance, accepting $p < 0.05$ as the level of significance using the SPSS 15.0 statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussion

3.1 The effect of sodium lactate on the pH value of chilled beef

The pH values in samples during chilled storage are shown in Figure 1. The initial pH value in Group C and Group SL

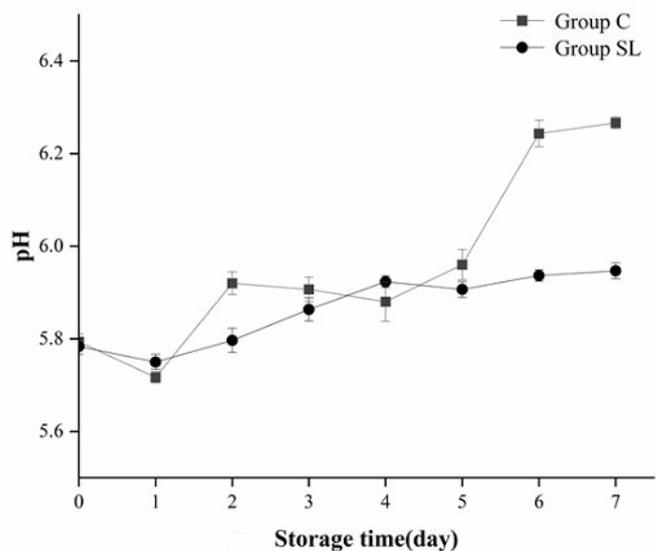


Figure 1. The effect of sodium lactate on the pH value of chilled beef during storage at 4 °C.

was 5.73 and 5.74, respectively. The pH value both in Group C and Group SL was decreased slightly on the first day of storage to 5.66 and 5.68, respectively, which may be attributed to the anaerobic metabolism of chilled beef within 24 h after slaughter with the lactate production. As storage time extended, the pH values in Group C increased significantly ($p < 0.05$) and reached up to 6.24 on the 7th day. In contrast, the pH value in Group SL maintained at a stable level with a range from 5.68 to 5.91 from 2 to 7 d, which was within the normal range during the whole storage. These results revealed that sodium lactate treatment effectively inhibited the increase of pH value in chilled beef, especially in the middle and late storage period.

The pH value plays a critical role in fresh beef quality, which could affect color stability, the water holding capacity and microbial growth (Ramanathan et al., 2013; Wang et al., 2022a). Once the pH is over 6, proteins in chilled beef are further decomposed and produce a large number of amino acids, which are conducive to the bacterial growth, resulting in spoilage and unacceptance (Liu et al., 2020). In this study, the fluctuation of pH values in chilled beef treated with sodium lactate was within a narrow range from 5.68 to 5.91. Whereas, the pH values in the control was unacceptance. The pH value

in normal range and stabilization is conducive to forming an acceptable appearance. These results indicate that sodium lactate can stabilize the pH value. The result was consistent with those results reported by Liu et al. (2020) and Wang et al. (2022b), who found that sodium lactate was conducive to pH stabilization for meat during chilled storage.

3.2 The effect of sodium lactate on the color stability of chilled beef

The changes in color parameters L , a^* and b^* values of chilled beef during storage at 4 °C are shown in Figure 2. The initial L , a^* and b^* values of the Group C and Group SL were nearly identical. The L , a^* and b^* values of Group C and Group SL significantly increased ($p < 0.05$) from 1 to 3 d. Suddenly, the L , a^* and b^* values of Group C and Group SL significantly decreased ($p < 0.05$) on the 4th day. However, the L , a^* and b^* values of Group SL were always significantly higher ($p < 0.05$) than that of Group C. Correspondingly, the intensity of color appearance of Group SL was also better compared with the Group C (Figure 3).

Meat color has been considered as an indicator of freshness and quality of meat, which influences the consumer preference to purchase fresh meat (Wang et al., 2021; Wang et al., 2018).

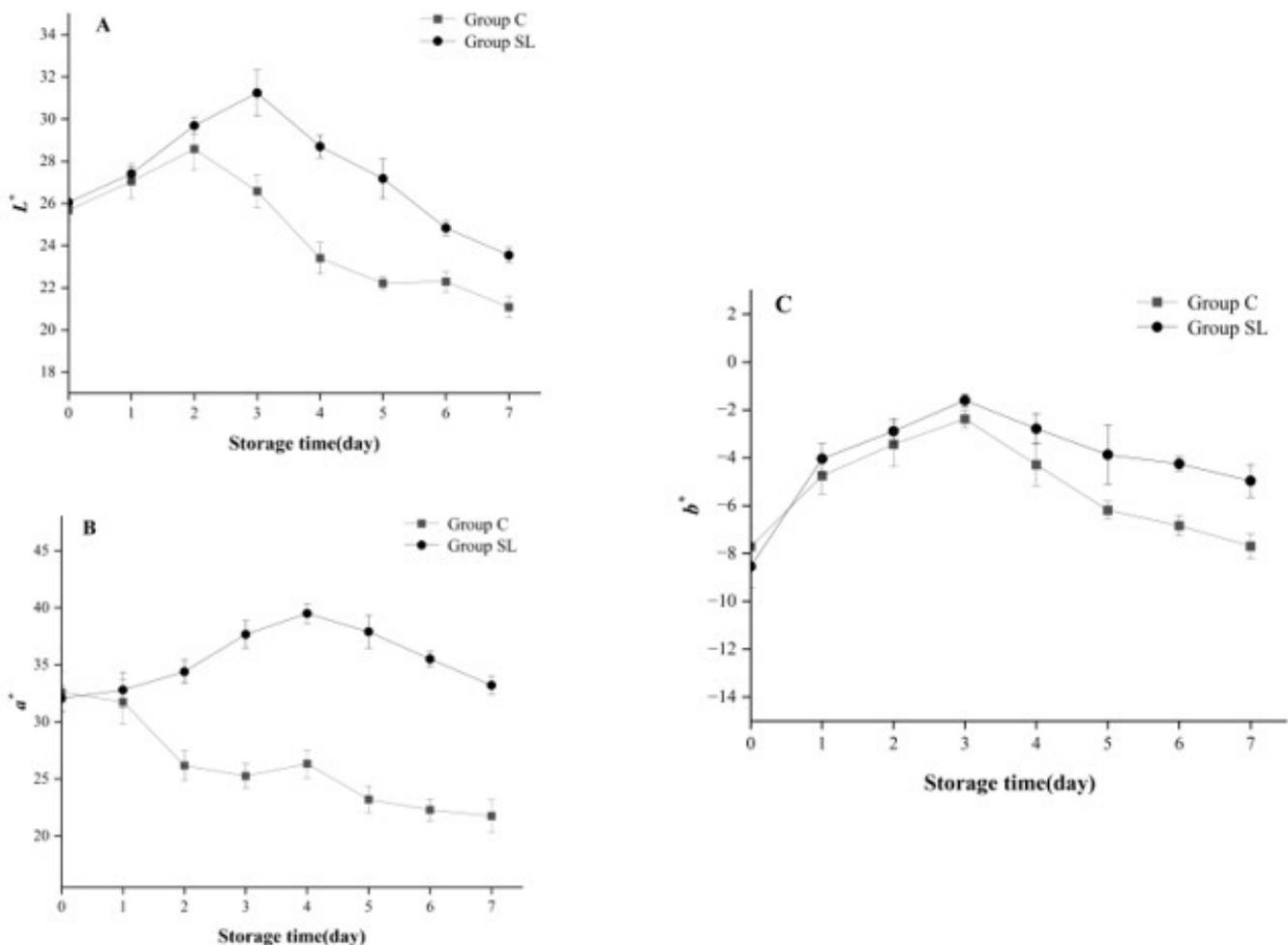


Figure 2. The effect of sodium lactate on the color parameters L (A), a^* (B) and b^* (C) values of chilled beef during storage at 4 °C.

Particularly, a bright red color is considered as a positive attribute for freshness and superior quality of beef (Holman et al., 2016; Wang et al., 2022a). It has been reported that a^* value is associated with consumer-defined fresh beef color acceptability (Holman et al., 2017). In this study, the a^* value of Group SL maintained stability for the entire storage period with a range from 38.23 to 32.12. In contrast, the a^* value of Group C decreased greatly and reduced to 20.82 on the 7th day. The results revealed that sodium lactate treatment was conducive to stabilizing color parameters of chilled beef, especially for a^* value. These results are in well line with the color appearance of samples.

3.3 The effect of sodium lactate on the glycolytic activity of chilled beef

Glycolysis is the main energy source of meat after slaughter, which plays a key role in the color stability of fresh beef. The glycolytic rate of chilled beef was evaluated by glycogen and lactate content. The changes in glycogen and lactate content

between Group C and Group SL during chilled storage are shown in Figure 4. There were significant differences ($p < 0.05$) between the two groups for glycogen and lactate content. The Group SL had significantly lower glycogen content ($p < 0.05$) than that of Group C during chilled storage. Correspondingly, the Group SL had significantly higher lactate content ($p < 0.05$) than that of Group C during chilled storage. It has been reported that glycogen content decreases as time goes by, and lactate content shows an opposite tendency due to the metabolism of glycogen via glycolysis after slaughter (Choe et al., 2008). Thus, the lower glycogen and higher lactate content in chilled beef indicated a much faster glycolytic rate in Group SL than that of Group C.

Moreover, the changes of lactate dehydrogenase activity and hexokinase activity, which are the key enzymes for glycolytic rate, in chilled beef during storage are shown in Figure 5. There were significant differences ($p < 0.05$) between the two groups for lactate dehydrogenase and hexokinase activity. The Group SL had significantly higher ($p < 0.05$) lactate dehydrogenase and



Figure 3. The changes in color appearance of group C (A) and group SL (B) during chilled storage at 4 °C for 7 days.

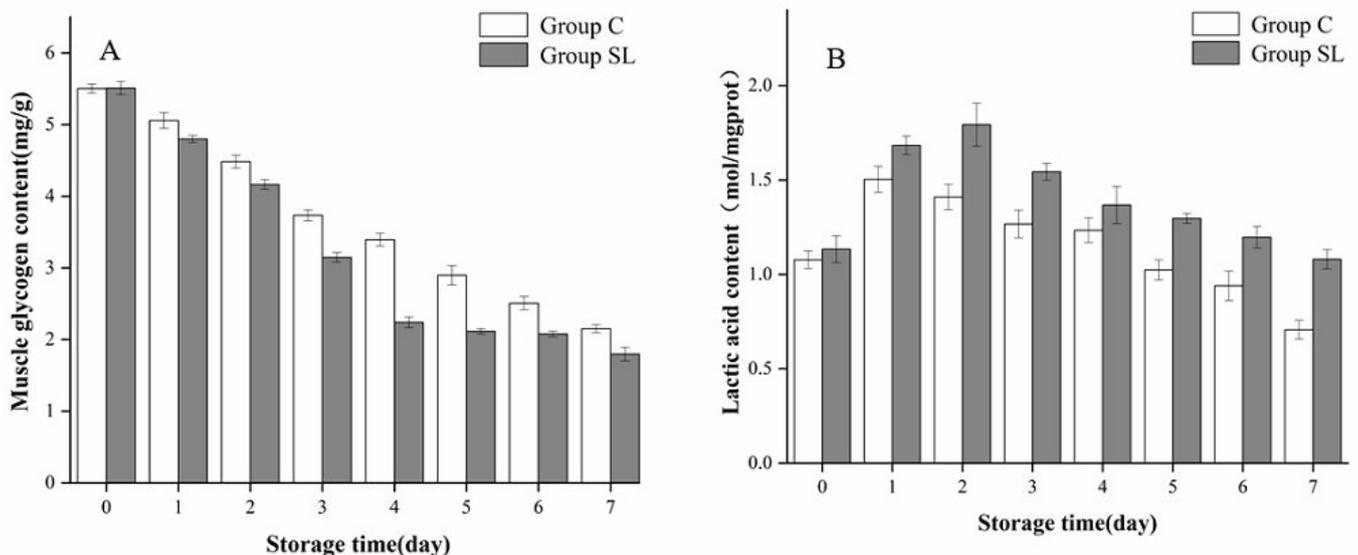


Figure 4. The effect of sodium lactate on the muscle glycogen content (A) and lactic acid content (B) of chilled beef during storage at 4 °C.

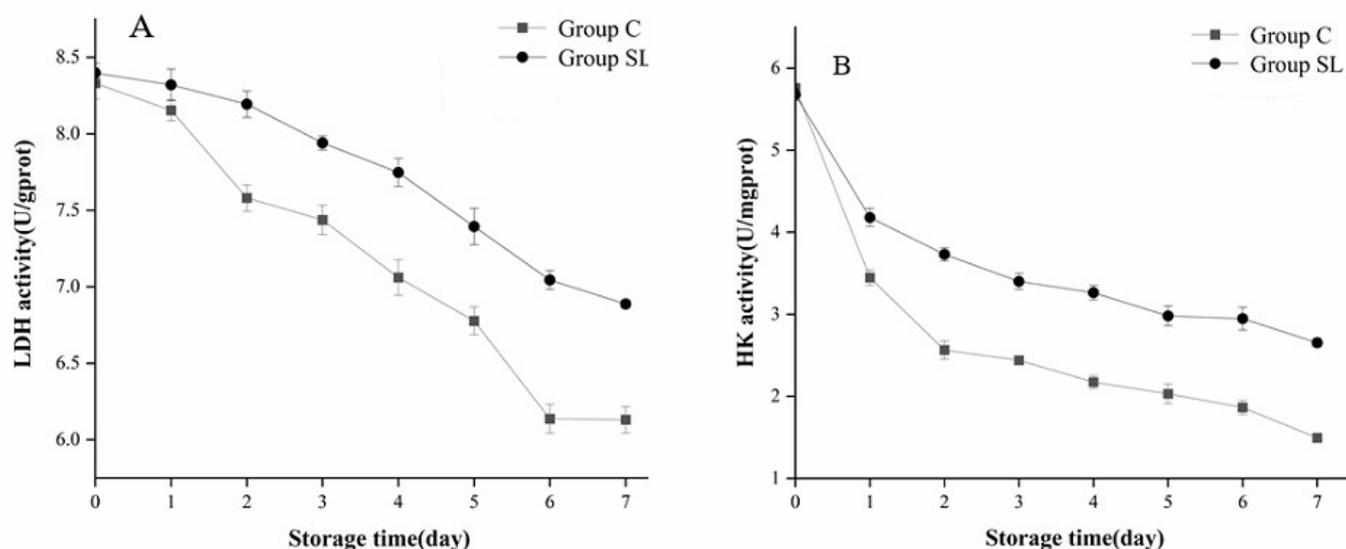


Figure 5. The effect of sodium lactate on the lactate dehydrogenase activity (A) and hexokinase activity (B) of chilled beef during storage at 4 °C.

hexokinase activity than that of Group C during chilled storage. The result also indicated that sodium lactate treatment had a positive relationship with glycolytic rate.

CH₃CHOHCOO⁻ from sodium lactate will form lactic acid (CH₃CHOHCOOH) which belongs to weak electric acid through combination with H⁺ (Wang et al., 2022b). Subsequently, CH₃CHOHCOOH is catalyzed to CH₃COCOOH by lactate dehydrogenase with the NADH production. The NADH is essential for the initiation of the reduction reaction of Fe⁺³-MetMb which is reduced to Fe⁺²-Mb by NADH oxidation via mitochondrial respiration (Biffin et al., 2019). High proportion of Fe⁺²-Mb will contribute to maintaining color stability of chilled beef. The Changes of NADH/NAD⁺ ratio in Group C and Group SL during the chilled storage are shown in Table 1. The initial NADH/NAD⁺ ratio was 0.56 in Group C and 0.57 in Group SL, respectively. As the storage extended, Group C had a significant higher ($p < 0.05$) of NADH/NAD⁺ ratio compared with Group SL which maintained at a stable level with a range from 0.54 to 0.68 during the whole chilled storage. The high NADH/NAD⁺ ratio indicates insufficient oxidation of NADH, resulting in a poor reduction effect (Holman et al., 2017). Therefore, a suggestion is reached that the addition of sodium lactate as the substrates of the reaction that lactic acid is catalyzed to pyruvic acid promotes NADH regeneration and replenishment for MetMb reduction system, resulting in an increase of Fe⁺²-Mb accumulation which is conducive to forming a characteristic bright pink red color of chilled beef.

Moreover, CH₃CHOHCOOH is partially ionized into CH₃CHOHCOO⁻ and releases a small amount H⁺ into muscle tissue, resulting in a slight reduction in pH value along with the H⁺ supplement. Thus, the pH is well regulated and maintains stability. The pH value plays a critical role in raw beef quality during storage, which can affect color appearance through influencing oxygen consumption and metmyoglobin reducing activity (Lishianawati et al., 2022). In this study, the fluctuation

Table 1. Effect of sodium lactate on the NADH/NAD⁺ ratio of beef samples during chilled storage at 4 °C for 7 days.

Samples	Storage time						
	1 d	2 d	3 d	4 d	5 d	6 d	7 d
Group C	0.56	0.84	1.75	1.91	1.98	1.72	1.68
Group SL	0.57	0.68	0.62	0.64	0.58	0.67	0.54

Table 2. Sensory score of group SL and group C during storage for 7 days.

	0 d	1 d	3 d	5 d	7 d
Group C	19.50±0.20	17.45±0.54	15.62±0.24	11.45±0.45	8.58±0.34
Group SL	19.60±0.30	19.20±0.62	18.56±0.36	17.60±0.36	16.65±0.45

of pH values in beef treated with sodium lactate was within a narrow range with a range from 5.68 to 5.91. In contrast, the fluctuation of pH values in the control was relatively larger. The result revealed that sodium lactate treatment effectively maintained pH stability of chilled beef, which is conducive to color stability of chilled beef.

3.4 The effect of sodium lactate on the sensory quality

The sensory evaluation is a scientific discipline to evaluate the changes of raw meat quality during storage (Paglarini et al., 2020; Vidal et al., 2020). The sensory assessment of Group SL and Group C are shown in Table 2. The Group SL had a significant higher ($p < 0.05$) sensory score than that of Group C. The sensory score of Group C was only 11.45 on the 5th day, indicating an unacceptable appearance, while the sensory score of Group SL still was 16.65 on the 7th day, suggesting an acceptable quality. These results are in agreement with the color value.

4 Conclusion

Sodium lactate usage exhibited better pH stability and color stability, which is conducive to maintaining the meat quality.

According to these results of glycolytic activity, the sodium lactate treatment had a positive relationship with glycolytic rate and promoted the increase of glycolysis rate. The increase of glycolysis rate will be conducive to increase of NADH production which is essential for the initiation of the reduction reaction of Fe⁺³-MetMb. The reduction of Fe⁺³-MetMb will be beneficial to maintain pink red color of chilled beef. Thus, Sodium lactate usage was beneficial for color stability via increase of glycolysis rate.

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