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Research Article

Study on color browning of lactic acid injection to yak meat

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Abstract: During the lactic acid tenderization process of yak meat, an observable phenomenon is the color browning. To delve into the underlying causes and devise solutions, this study meticulously injected varying concentrations and volumes of lactic acid into yak meat samples, the freshness and color appearance of the meat were assessed by measuring metmyoglobin (MetMb) content, color parameters, lipid oxidation levels, and conducting sensory evaluations. Furthermore, the mechanisms behind the color alterations were elucidated by quantifying the activity of MetMb reductase and lactate dehydrogenase (LDH). The results indicated that when lactic acid was introduced at a concentration of 0.4 mol/L, accompanied by an injection volume equivalent to 2.5% of the meat weight, yak meat underwent a marked browning. This transformation was primarily attributed to the diminished MetMb reductase activity. Under specific pH conditions, augmented mitochondrial respiration fosters the formation of MetMb, culminating in its substantial accumulation and subsequently altering the color of the yak meat. Conclusively, this research underscores that the combined factor of injecting lactic acid at a concentration of 0.4 mol/L and a volume corresponding to 2.5% of the meat weight serves as a crucial catalyst for the browning of yak meat, emphasizing the need for careful regulation of these parameters during the tenderization process.

Keywords: yak meat; lactic acid; color browning; metmyoglobin

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1 Introduction

Yak widely disseminates over Chinese plateau region. Chinese yak accounts for 95% of the world and occupies 1/6 of the number of Chinese cattle[1], yak meat has the advantages of rich nutrition and green health. As we know, due to various factors such as variety and living environment, beef is harder than other livestock meat and poultry meat. Yak meat is harder than ordinary beef, but beef tenderness is very valued, and is an important basis for consumers to choose and judge[2]. Among all dietary quality attributes, ordinary consumers currently consider the tenderness of meat to be one of the most important factors. Researchers have found that lactic acid solution has significant tenderization effect for meat. There have been many studies on the tenderization of meat by injection of lactic acid solution[3]. Lactate was an antimicrobial that also can extend fresh meat color life[4]. Researches assessing the mechanisms associated with color-stabilizing effect of lactic acid indicate that the regeneration of reduced nicotinamide adenine dinucleotide (NADH) via lactate dehydrogenase (LDH) activity can be used for enzymatic, non-enzymatic, and mitochondria mediated metmyoglobin (MetMb) reduction, studies have shown that a certain amount of lactic acid and lactate not only promote the improvement of meat color, but also affect the tenderness of meat[5]. Since lactic acid is present in humans and animals body, the use of lactic acid as a tenderizer does not pose any safety problems, it was a relatively green tenderizer. But during the test, after lactic acid injection, the beef color browning, and similar phenomena have occurred in other researches[6]. Because meat color plays an important role in meat quality to a certain extent, the problem brought about by the use of lactic acid tenderized for beef should not be ignored. It was necessary to find the cause of the problem and avoid it. As we know, myoglobin (Mb) is the primary sarcoplasmic protein that is the main reason that affects the color of beef[7], in the fresh meat stored and sold in different packaging systems, the Mb is present in one form of four redox states: deoxymyoglobin (DeoMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb) and metmyoglobin (MetMb), the oxidation of 3 kinds of Mb to trivalent iron will form brown MetMb.

The formation of MetMb results in the brown of fresh meat[8]. The beef color was determined by the predominant form of Mb on the surfaces[9]. Surface discoloration resulting from MetMb formation affects consumers' purchasing decisions[10]. When MetMb content on the beef surface accounts for 30%–40% of the total Mb, consumers will lose the purchase desire. So, the norm of MetMb content was very representative of meat color and consumer selectivity. According to the survey, the USA loses one billion dollars every year because of the deterioration of meat color. Studies have shown that unsuitable processing and storage temperatures affect meat color[11]. And pH has an important effect on the oxidation of Mb, which mainly affects the stability of meat color[12]. Other things such as packaging, antioxidants, and lipid oxidation all influence changes in meat color[13]. In general, numerous factors are influencing the change of meat color, the reason for the browning of beef after injection of lactic acid solution is not clear, but the adverse effects have always bothered enterprises and sellers.

The purpose of this study is to investigate the causes of color browning in the process of tenderizing yak meat using lactic acid, and further to investigate its browning mechanism. By measuring

the MetMb content, pH, visual colors brightness value (L^*), redness value (a^*), and yellowness value (b^*), MetMb reductase and LDH activities of yak meat after injection of different amounts of lactic acid to screen the key points leading to the browning of beef and explain the causes of browning of yaks, providing theoretical reference for the application of lactic acid solution in the tenderizing of meat products.

2 Materials and methods

2.1 Materials and reagent

Six Chinese female yaks living in Qinghai-Tibetan Plateau at the age of 24 months were slaughtered at a commercial slaughterhouse according to the requirements of national standards of China GB/T 19477–2018 *Operating procedure of livestock and poultry slaughtering–Cattle* in Ningxia Xiahua Meat Food Co., Ltd. (Zhongwei, China). A total of 50 kg yak shoulders were purchased from Ningxia Xiahua Meat Food Co., Ltd. (Zhongwei, China). The carcass (105–110 kg) was stored at –40 °C for 1 month. Lactic acid (food grade, 80%) provided by Henan Jindan Co., Ltd. (Zhengzhou, China). Horse heart Mb (≥ 95%) was purchased from Sigma Chemical Co., Ltd. (Saint Louis, USA). Syringe (10 ×, 1 mL), ethylenediaminetetraacetic acid (EDTA), NADH, and $K_4Fe(CN)_6$ were purchased from Jintu Co., Ltd. (Zhengzhou, China).

2.2 Preparation of lactic acid injection beef sample

Beef sample was cut into 1.5 cm × 3 cm × 5 cm steak shape in a frozen state, and thawed in an environment of 0–4 °C. Lactic acid solutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mol/L) were prepared and injected into the beef. The injection volume (mL) was 50% of the weight (g) of each piece of beef (each piece of meat weighs about 200 g). The blank control group needed not any treatment, all the samples should be protected from light and sealed 8 h, and the experimental group had consistent environmental conditions. Remove visible fat and connective tissue after thawing, noting that the injection in the direction of the muscle fibers could facilitate the diffusion of lactic acid. The injection method was as follows: the needle was pierced into the meat 2–3 cm, injected four times separately, and gently massage it so that the lactic acid solution could evenly distribute into the meat.

2.3 Determination of the MetMb content

The absorption spectra of MetMb were obtained using a UV-2101 spectrophotometer (Shimadzu, Japan) with cuvettes of 1 cm path length from 475 to 650 nm, after 5 g of meat was chopped, 10 mL of 0.04 mol/L phosphate buffer solution (pH 6.8) was added, homogenized for 60 to 90 s, centrifuged at 12 000 × *g* for 20 min at 4 °C to obtain a crude sarcoplasmic protein, and the absorbance at wavelength 525, 545, 565, and 572 nm was measured following the method described by Tang et al.[14]. The content of MetMb was calculated according to formula (1):

$$\text{MetMb content}\ (\%) = (-2.541R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100 \quad (1)$$

Where R_1, R_2, and R_3 were $A_{572\ nm}/A_{525\ nm}$, $A_{565\ nm}/A_{525\ nm}$, $A_{545\ nm}/A_{525\ nm}$, respectively.

2.4 Determination of pH

The 5.0 g of beef were homogenized in a blender (LA569, Joyoung Co., Ltd., Jinan, China) with 50.0 mL of 0.6 mol/L KCl solution, the inserting pH probe of a standard pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, China) was used to measure the pH of the mixture at room temperature (approximately 25 °C).

2.5 Determination of instrumental color parameters

The surface color of meat labeled L^*, a^*, and b^* of each beef sample was determined by Chroma Meter CR-400 colorimeter (Konica Minolta Co., Japan) with 0° viewing angle, 8 mm aperture, and D65 illuminator (Konica Minolta Sensing Incorporated, Osaka, Japan). The colorimeter was calibrated by a black and a white plate before use. The color parameters of each beef sample were determined after blooming for 30 min at 4 °C, each parameter of beef sample was read 3 times[15].

2.6 Determination of visual color

The sensory analysis was carried out through a sensory panel of 10 assessors, which consisting of 5 females and 5 males. They were selected for their sensory abilities and experience in sensory evaluation of meat products. Ten assessors were trained 3 preliminary sessions for the samples by the expert of meat science laboratory in Henan Agricultural University[16]. Use a nine-point hedonic scale, where 9 = 'like extremely', 8 = 'like very much', 7 = 'like moderately', 6 = 'like slightly', 5 = 'neither like nor dislike', 4 = 'dislike slightly', 3 = 'dislike moderately', 2 = 'dislike very much', 1 = 'dislike extremely'[17]. The color score obtained from sensory evaluation was used as the final score evaluation.

2.7 Determination of MetMb reductase activity (MRA)

The sample at –40 °C was thawed to a semi-frozen state at 4 °C, the fat and fascia of the epidermis were removed, and the meat was mashed. Accurately weighing 10 g, added 20 mL ice extract to the tissue disperser at 4 °C continuous homogenization for 1 min, then centrifuged at 10 200 r/min for 20 min, the supernatant was filtered through medium-speed qualitative filter paper to remove fat. OxyMb in the filtrate was oxidized to a slight excess of $K_4Fe(CN)_6$, and then dialyzed for 24 h with ice extract at 4 °C (dialysis bag has a molecular weight cutoff of 14 000 Da), in which the liquid was replaced 5 times to remove excess $K_4Fe(CN)_6$. After dialysis, the sample was centrifuged at 12 000 r/min for 20 min at 4 °C, and then taken the supernatant with pH 7.0, 2.0 mmol/L phosphate buffer. The solution was made up to 20 mL and stored at –80 °C.

The enzyme activity of beef muscle extracts was measured by the hand in absorbance at 580 nm of a beef MetMb solution. Assays were arrived out at 25 °C and cuvettes of 1 cm path length with 1.0 mL final reaction volumes. The assay mixture contained the following: 0.1 mL 5.0 mmol/L EDTA, 0.1 mL 5.0 mmol/L phosphate buffer (pH 5.65), 0.1 mL 3.0 mmol/L $K_4Fe(CN)_6$, 0.2 mL 0.75 mmol/L horse heart MetMb dissolved in 2.0 mmol/L phosphate buffer (pH 7.0), 0.1 mL deionized distilled water, 0.3 mL muscle extract, and 0.1 mL 2.0 mmol/L NADH. This mixture has a pH of 6.4. The final pH of the assay mixture was varied by altering the pH of the phosphate buffer. Blanks contained all additions except NADH. The cuvettes were placed in a thermostat-controlled Beckman DU-65 spectrophotometer (Beckman Co., USA) for 10 min to attain the desired temperature. The reaction was initiated with addition of NADH to the sample cuvette and followed at 580 nm which the difference in absorption between OxyMb and MetMb was maximal. The spectrophotometer recorded absorbance at 580 nm every 2 s. Molar absorptive difference at 580 nm of 12×10^3 was used to calculate the amount of OxyMb formed. One unit of MRA was defined that amount which would reduce 1 nmol

of MetMb per min. MRA was expressed as MetMb reduction amount/(min·g muscle) during the initial linear phase of the assay. These values were divided by the Mb content (mg Mb/g muscle) of the respective muscles to obtain MRA activity per 1 mg Mb[18]. MRA is calculated according to formula (2):

$$\text{MRA (U/g)} = \frac{\Delta A \times V_1 \times 10^9}{\varepsilon \times V_2 \times L \times m} \quad (2)$$

Where ΔA was the changes in absorbance after reaction per min; V_1 was the volume of the reaction system (mL); ε was the molar extinction coefficient (L/(cm·mol)); V_2 was the sample volume (mL); L was the cuvette light path (cm); m was the mass of the sample (g); 10^9 was the conversion factor (mol converted to nmol).

2.8 Determination of LDH activity

Activity of LDH was measured according to the method of Kucukgoz Gulec et al.[19]. Chopped muscle tissue (2.0 g) that did not contain any visible connective tissue was homogenized in 8 mL of 0.01 mol/L phosphate buffer (pH 7.5) for 30 s or until the muscle tissue disrupted completely. The homogenate was centrifuged at 13 800 × g for 30 min at 4 °C. Aliquots of supernatant (2 mL) were diluted with 0.01 mol/L phosphate buffer to yield a 200:1 sample dilution ratio. The diluted supernatant (0.05 mL) was added to the glass cuvette with 2.5 mL of Tris/NaCl/NADH (pH 7.2) and 0.5 mL of Tris/NaCl/pyruvate (pH 7.2). Activity of LDH was measured in duplicate by the continuous decrease in absorbance at 339 nm after 30 s and repeat readings every 30 s for 2.5 min. Decreased absorbance (decreased NADH) between 30 and 150 s was used to calculate LDH activity.

$$\text{LDH activity (U/mL)} = \frac{\Delta A}{\Delta T} \times 9.68 \times 10^3 \quad (3)$$

Where ΔA was the average change in absorbance after reaction 30 s; ΔT was reaction time (min).

2.9 Lipid oxidation analysis

Thiobarbituric acid reactive substances (TBARS) value was measured according to the procedure of Witte et al.[20]. After each beef sample was injected into lactic acid, 5 g of beef was blended with 25 mL 20 g/100 mL trichloroacetic acid (TCA) solution and 20 mL distilled water. Samples were homogenized using a tabletop blender (Dynamics Co., USA) for 1 min and filtered through Whatman (#1) filter paper (Yuling Filter Equipment Co., Ltd., Shanghai, China). The filtrate (1 mL) was mixed with 1 mL 2-thiobarbituric acid (TBA) solution (20 mmol/L), incubated at 25 °C for 20 h, and absorbance at 532 nm was measured using a UV-2101 PC spectrophotometer (Shimadzu Co., Japan). Blank samples consisted of 2 mL TCA solution (in distilled water, 1:1, V/V) and 2 mL TBA solution.

2.10 Statistical analysis

The Origin 6.1 (Origin Lab Co., Northampton, MA, USA) was used to analyze the data and draw the line chart. Each indicator measurement was repeated three times, and the values were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare means followed by Duncan's multiple range test using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Confidence levels were set at 95%, and statistical differences were considered as significant at $P < 0.05$.

3 Results and discussion

3.1 Effect of lactic acid injection concentration on meat color

The results showed that the MetMb content suddenly increased significantly ($P < 0.05$) to 29.97% at the lactic acid concentration of 0.4 mol/L, and the meat turned brown, corresponding to the color parameter (L^*, a^*, and b^*) changes in Table 1. The surface a^* is one of the most important characteristics closely relate to fresh meat quality, the loss of a^* is mainly due to the Mb oxidation which leads to the transformation of bright red OxyMb into grey MetMb. During chilled storage, although lactic acid concentration increased continuously, the MetMb content tended to be stable ($P > 0.05$). When lactic acid injection concentration was higher than 0.3 mol/L, there was no significant difference in pH ($P > 0.05$). According to the sensory evaluation, the color score was significantly lower than the previous score when the lactic acid injection concentration was 0.4 mol/L. In summary, the injection of 0.4 mol/L lactic acid could make meat color suddenly browning.

3.2 Effect of lactic acid injection volume on meat color

The lactic acid solution (0.4 mol/L) was injected into yak meat in 10%, 20%, 30%, 40%, 50% of meat weight respectively to explore the effect of lactic acid injection volume on the meat color browning. As shown in Table 2, although the pH was gradually decreased, but the MetMb content, L^*, a^*, and b^* were relatively stable, among them, the MetMb content remained consistently stable at approximately 30%, which illustrated by the color score. Hence, at a concentration of 0.4 mol/L, an injection volume exceeding 10% of the meat weight exhibited minimal influence on its coloration. Consequently, it is imperative to investigate the impact of injection volumes below 10% on the browning of meat color.

Table 1 Effect of different concentrations of lactic acid on MetMb content, pH, L^*, a^*, b^*, and color score of beef.

Injection concentration (mol/L)	MetMb content (%)	pH	L^*	a^*	b^*	Color score
0.0	19.92 ± 0.11^b	5.99 ± 0.01^a	46.11 ± 1.32^a	14.46 ± 0.48^a	14.98 ± 0.55^a	8.96 ± 0.20^a
0.1	21.32 ± 0.09^b	4.65 ± 0.08^b	44.79 ± 1.28^a	14.11 ± 0.11^a	14.79 ± 0.13^a	8.67 ± 0.48^a
0.2	20.82 ± 0.10^b	4.27 ± 0.05^c	45.12 ± 2.12^a	14.36 ± 0.58^a	14.89 ± 0.23^a	8.75 ± 0.61^a
0.3	21.41 ± 0.16^b	4.01 ± 0.01^d	44.68 ± 0.98^a	14.00 ± 0.36^a	14.67 ± 0.68^a	8.58 ± 0.58^a
0.4	29.97 ± 0.55^a	3.98 ± 0.06^d	38.12 ± 1.01^b	8.88 ± 0.26^b	8.53 ± 0.56^b	4.01 ± 1.71^b
0.5	27.75 ± 0.12^a	3.91 ± 0.01^d	39.95 ± 2.36^b	9.59 ± 0.48^b	8.95 ± 0.79^b	4.13 ± 1.23^b
0.6	28.22 ± 0.14^a	3.83 ± 0.02^d	39.01 ± 1.12^b	8.98 ± 0.33^b	8.68 ± 0.43^b	4.08 ± 1.45^b

Note: Different superscript letters within column indicate statistical significance between averages at $P < 0.05$.

Table 2 Effect of different injection volume (above 10%) of lactic acid on MetMb content, pH, L^*, a^*, b^*, and color score of beef.

Injection volume (%)	MetMb content (%)	pH	L^*	a^*	b^*	Color score
10	30.34 ± 0.22^{b}	4.83 ± 0.02^{a}	39.81 ± 1.64^{a}	9.29 ± 1.73^{a}	9.37 ± 1.40^{ab}	3.95 ± 0.98^{b}
20	31.87 ± 0.07^{a}	4.66 ± 0.03^{b}	39.18 ± 1.40^{a}	8.51 ± 0.71^{b}	10.05 ± 0.76^{a}	3.78 ± 1.38^{a}
30	29.47 ± 0.09^{c}	4.55 ± 0.03^{bc}	38.31 ± 2.15^{ab}	8.91 ± 0.82^{b}	8.81 ± 0.39^{b}	4.04 ± 1.11^{c}
40	32.29 ± 0.16^{a}	4.17 ± 0.04^{d}	37.39 ± 0.74^{b}	8.59 ± 0.36^{b}	9.07 ± 0.62^{ab}	3.53 ± 1.16^{a}
50	29.97 ± 0.55^{bc}	3.98 ± 0.06^{de}	38.12 ± 1.01^{b}	8.88 ± 0.26^{b}	8.53 ± 0.56^{b}	4.01 ± 1.71^{bc}

Note: Different superscript letters within column indicate statistical significance between averages at $P < 0.05$.

Table 3 Effect of different injection volume (below 10%) of lactic acid on MetMb content, pH, L^*, a^*, b^*, color score, and TBARS value of beef.

Injection volume (%)	MetMb content (%)	pH	L^*	a^*	b^*	Color score	TBARS value (mg/kg)
0.0	19.92 ± 0.11^{b}	5.99 ± 0.01^{a}	46.11 ± 1.32^{a}	14.46 ± 0.48^{a}	14.98 ± 0.55^{a}	8.96 ± 0.20^{a}	0.14 ± 0.01^{a}
1.0	21.99 ± 0.26^{b}	5.98 ± 0.11^{a}	44.23 ± 0.86^{a}	13.94 ± 0.61^{a}	14.42 ± 1.35^{a}	8.33 ± 1.49^{a}	0.13 ± 0.02^{b}
2.5	28.08 ± 0.11^{a}	5.62 ± 0.05^{ab}	39.18 ± 1.35^{b}	10.03 ± 1.03^{b}	10.11 ± 0.57^{b}	4.10 ± 1.47^{b}	0.12 ± 0.01^{c}
5.0	29.71 ± 0.12^{a}	5.60 ± 0.01^{bc}	39.26 ± 0.44^{b}	9.66 ± 1.00^{b}	9.86 ± 0.25^{b}	4.02 ± 1.35^{b}	0.12 ± 0.02^{c}
7.5	30.87 ± 0.13^{a}	5.48 ± 0.01^{c}	39.98 ± 0.74^{b}	9.35 ± 1.12^{b}	9.44 ± 0.59^{b}	3.90 ± 1.23^{b}	0.10 ± 0.01^{d}
10.0	30.34 ± 0.22^{a}	4.83 ± 0.02^{d}	39.81 ± 1.64^{b}	9.29 ± 1.73^{b}	9.37 ± 1.40^{b}	3.95 ± 0.98^{b}	0.09 ± 0.01^{e}

Note: Different superscript letters within column indicate statistical significance between averages at $P < 0.05$.

The lactic acid solution (0.4 mol/L) was injected into yak meat in 1.0%, 2.5%, 5.0%, 7.5%, 10.0% of meat weight respectively, as evident from Table 3, upon administering an injection volume of 1.0% of meat weight, the meat color remained unaltered and within normal parameters. When the injection volume increased to 2.5% of meat weight, the meat color turned browned, and the MetMb content tended to 30%, and then the MetMb content remained at about 30% with the increase of the injection volume. At this time, L^*, a^*, b^* and color score all decreased suddenly when the injection volume reached 2.5% of meat weight, and the color was unacceptable. The reduction in a^* over the length of the study may be attributed to a decrease in MRA. As meat lost its ability to reduce MetMb to OxyMb, the brownish color of MetMb began to appear on the surface of beef, and a^* decreased. The MetMb reductase was mainly present in mitochondria, with a small amount in microsomes. As shown in Figure 1, the MRA basically lower than 0.5 U/g, consistent with experimental conjecture, and observed loss of MRA was an explanation for loss of redness values[21]. The investigation into the mechanism underpinning the color stabilization of lactic acid revealed that the addition of lactic acid to beef can supplement NADH by increasing LDH activity, ultimately leading to more stable meat color[10]. However, by measuring LDH activity, as shown in Figure 1, it was found that the LDH activity was basically at a low level ($P > 0.05$) and exhibited insufficient activity to effectively facilitate NADH regeneration crucial for enzymatic reactions. According to the TBARS values, it can be seen that lactic acid has an inhibitory effect on lipid oxidation. The TBARS value of the control group was 0.14 mg/kg, which gradually decreased with the increase of the lactic acid injection volume, which could rule out the effect of lipid oxidation on the color of the test.

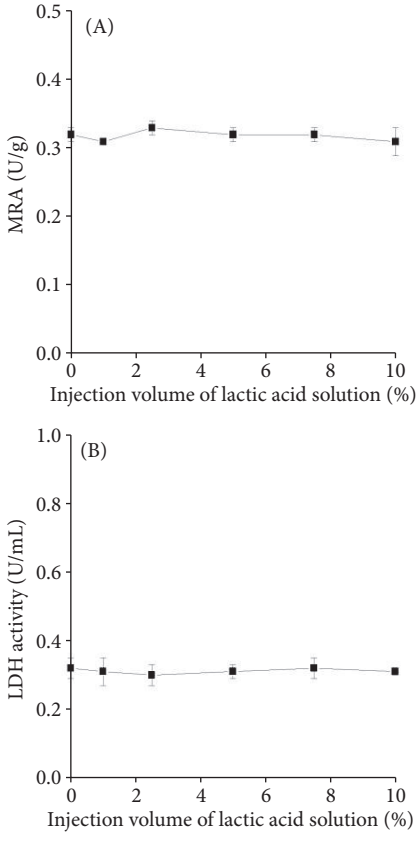

Figure 1 (A) MRA and (B) lactate dehydrogenase (LDH) activity under different injection volume. The injection volume of lactic acid solution (0.4 mol/L) was the percentage of meat weight.

In order to show the degree of meat color browning more clearly, several representative samples were selected for appearance

display, as depicted in Figure 2, the color of beef in Figures 2A, B, and D was normal and still retained the color of fresh meat, but the color of beef in Figures 2C, E, and F was similar with obvious browning, the corresponding MetMb content and color score also fit well with the color of the meat. It was finally known that the meat had a significant color browning at an injection concentration of 0.3 to 0.4 mol/L and volume of 1.0% to 2.5% of meat weight.

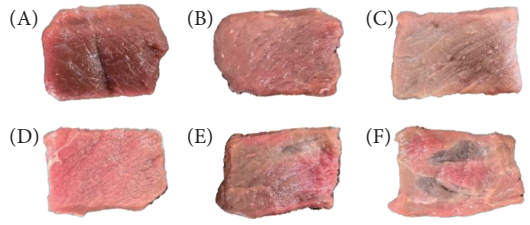

Figure 2 Comparison of beef color after injection of lactic acid. (A) Blank control (no injection); (B) Injection volume of 0.3 mol/L lactic acid solution: 50% of meat weight; (C) Injection volume of 0.4 mol/L lactic acid solution: 50% of meat weight; (D) Injection volume of 0.4 mol/L lactic acid solution: 1.0% of meat weight; (E) Injection volume of 0.4 mol/L lactic acid solution: 2.5% of meat weight; (F) Injection volume of 0.4 mol/L lactic acid solution: 5.0% of meat weight.

The injection concentration and volume of lactic acid are the key factors affecting meat color of the yak meat. One of the main reasons is pH, the alteration in pH significantly impacts the functionality of mitochondria, subsequently influencing the concentration of MetMb in yak meat. Specifically, at pH 5.6 (injection volume at 2.5% of meat weight), mitochondria undergo increased oxygen consumption, which facilitates the formation of DeoMb[22], which is then rapidly oxidized to MetMb[14]. The oxygen consumption capacity of mitochondria undergo a notable enhancement at a pH of 5.6. During this condition, DeoMb readily undergoes transformation into MetMb, with its oxidation rate surpassing that of OxyMb. Furthermore, under hypoxic conditions, DeoMb is prone to oxidation, swiftly converting into MetMb[23]. However, as the redox system balance has been destroyed, the formation of MetMb becomes irreversible, resulting in a significant increase in MetMb content. This inference was also fully demonstrated by the determination of MetMb reductase assays. According to the determination results, the MetMb reductase in yak meat was nearly inactivated. The main reason was that the MetMb reductase was gradually inactivated overtime after the yak were slaughtered. According to Figure 1, the MRA, MetMb reductase in yak meat gradually lost activity (MRA was less than 0.5 U/g when lactic acid (0.4 mol/L) injection volume was 0%–10% of meat weight) after nearly one month of storage. However, due to the continuous decrease in pH, mitochondrial respiration was inhibited, MetMb no longer increased rapidly, and eventually stabilized. Therefore, when the lactic acid concentration was 0.4 mol/L, with the injection amount exceeding 2.5% of meat weight, the meat color tended to be stable after browning. According to sensory evaluation, the color of the meat at this time was unacceptable. In conclusion, the experiment determined the key parameters of meat browning in yak meat after lactic acid injection, which used to prevent meat browning, thereby enabling beef to attain optimal tenderness while retaining a desirable meat color.

4 Conclusion

There can get a conclusion that in the case of the same injection volume, injecting 0.4 mol/L lactic acid solution could make the yak meat color suddenly brown. At this injection concentration, when the injection volume reached 2.5% of meat weight, the meat color will suddenly brown. However, if lactic acid injection volume was continued to increase, the color tended to be constant. Therefore, when using lactic acid to tenderize yak meat in practical applications, attention should be paid to the concentration and dosage of lactic acid. At the concentration of less than 0.4 mol/L, the injection volume of lactic acid solution less than 2.5% of meat weight can achieve the purpose of tenderizing and effectively control the brown color of meat.

Declaration of competing interest

The authors have no conflict of interest.

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