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ARTICLE

Effect of Succinate Sodium on the Metmyoglobin Reduction and Color Stability of Beef Patties

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In two experiments, the effect of succinate sodium on the metmyoglobin (MetMb) reduction and color stability of beef patties was investigated. In experiment 1, the ground-beef strip loins (longissimus dorsi muscle) were blended with different concentrations of succinate. Enhancing patties with 6 mM succinate significantly increased the MetMb-reducing ability and subsequent color stability during storage. In experiment 2, MetMb and different concentrations of succinate, lactate, and reduced nicotinamide adenine dinucleotide (NADH) were incubated with mitochondria, and their effect on meat MetMb reduction was investigated. Increasing the concentration of NADH and lactate increased MetMb reduction, but only succinate of 16 and 24 mM significantly decreased the relative MetMb percentage compared to other systems. This indicate that there are no significant differences between aerobic and anaerobic MetMb-reducing activities. In comparison to the systems of NADH−MetMb reduction (including the systems of lactate−MetMb reduction), the succinate−MetMb reduction systems are more stable and less affected by oxygen. More identification work is needed to obtain the more complete pathways on MetMb reduction.

KEYWORDS: Succinate; lactate; NADH; metmyoglobin (MetMb); beef color stability

INTRODUCTION

Myoglobin (Mb) is commonly found in three forms: deoxymyoglobin (DeoMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb), and the relative proportions of these determine the color of fresh meat. Each molecule of Mb contains a single iron atom that may bind one molecule of O_2 when the iron is in the ferrous state ($Mb-Fe^{2+}$). $Mb-Fe^{2+}$ undergoes spontaneous oxidation to the ferric state ($Mb-Fe^{3+}$) to form MetMb, which cannot bind O_2. DeoMb is purplish in color, and OxyMb is bright red in color, which is responsible for the consumer-preferred color of fresh meat (*1*) Interconversion among DeoMb, OxyMb, and MetMb is influenced by several factors including pH, temperature, relative humidity, partial oxygen pressure (p O_2), MetMb reducing activity, and lipid oxidation (*2*).

Because there is no appreciable accumulation of MetMb *in vivo* (*3*−*5*), a reductive system should be present. NADH−cytochrome b_5 MetMb reductase is the best characterized enzyme involved in the reduction of oxidized myoglobin. The major components required for the enzymatic reduction of MetMb by that system are the enzyme (NADH−cytochrome b_5 MetMb reductase), the intermediate (cytochrome b_5), and the cofactor NADH. Arihara et al. (*6*) further characterized MetMb reductase in subcellular fractions from bovine muscle. Using an immunoblotting technique, NADH−cytochrome b_5 reductase was found predominantly in the mitochondria fraction, but it was also detected at lower levels in the microsomal fraction. The function of NADH−cytochrome b_5 reductase is to transfer two electrons from NADH to two molecules of cytochrome b_5 (*7*). Reduced cytochrome b_5 then transfers the electrons to a variety of acceptors, including MetMb. Livingston et al. (*8*) provided evidence for a mechanism in which NADH−cytochrome b_5 reductase used NADH to reduce ferricytochrome b_5 to ferrocytochrome b_5. The ferrocytochrome b_5 then non-enzymatically reduced MetMb to ferrous Mb. They reported that cytochrome b_5 and MetMb bind to the enzyme in a one-to-one stoichiometry *in vitro*. Support for the contention that this enzyme was responsible for the reduction of MetMb *in vivo* was provided by Bailey and Driedzic (*9*).

Besides the NADH−cytochrome b_5 reductase in the MetMb reducing enzyme system, there exists other pathways that can reduce the oxidized myoglobin. Arihara et al. (*10*) demonstrated that the glycolytic pathway may play a significant role in the enzymatic metmyoglobin reduction, presumably by its supply of cytoplasmic NADH for the NADH−cytochrome b_5 reductase system in muscle. Intermediates of the citric acid cycle and glycolytic pathway, such as lactate and succinate, all have been reported to be active in the MetMb reduction. Kim et al. (*11*) proposed that lactate plays an indirect role in color stability by generating NADH, which is subsequently used to maintain reduced forms of myoglobin. Tang et al. (*12*) reported that both succinate and mitochondria were required for MetMb reduction by using succinate as an ideal substrate. The effects of succinate and lactate on lipid oxidation in meat products were also reported (*12*−*17*). Tretter et al. (*13*) found that succinate could prevent the permeability changes of mitochondria, proportionally to the inhibition of formation of thiobarbituric acid reactive material. However, Tang et al. (*12*, *14*) reported that no lipid oxidation was observed at either pH investigated in reaction systems. Seydim et al. (*15*) reported that the addition of sodium

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lactate delayed the oxidation. Maca et al. (*16*, *17*) also found that lipid oxidation was decreased with sodium lactate in vacuum-packaged cooked beef top rounds but lipid oxidation by thiobarbituric acid (TBA) was only slightly affected by treatment addition.

The involvement of mitochondria in MetMb reduction suggested by Giddings at an earlier time (*3*, *18*) suggested that mitochondria or submitochondrial particles could play a role in MetMb reduction in vacuum-packaged meat cuts by scavenging residual oxygen (hence, eliminating the potential for low oxygen-mediated myoglobin oxidation) or by supplying the meat tissue with the key reducing cofactor (NADH) generated by the reversal of electron transport. Results from other investigations support the postmortem generation of NADH (*6*, *19*, *20*).

Although succinate has been described as a "color stabilizer" in raw meat products, the effect of succinate on the relative MetMb percentage and the effect of succinate, lactate, and NADH on MetMb reduction when incubated with mitochondria are not reported. Therefore, the objectives of present study were to (1) determine the effect of sodium succinate on color stability of beef patties and (2) determine and compare the effect of different concentrations of lactate, succinate, and NADH on MetMb reduction when incubated with mitochondria.

MATERIALS AND METHODS

Experiment 1: Effects of Sodium Succinate on Instrumental Color Features and Relative MetMb Percentage in Beef Patties. *Reagents*. Equine myoglobin was obtained from Sigma (St. Louis, MO). All other chemicals and solvents used were of analytical grade and purchased from Lanyi Chemical Articles Co., Ltd. (Beijing, China).

Raw Materials and Processing. The animals were stunned by captive bolt and killed in a commercial plant in suburb Beijing following the industry practices. After dressing, the carcasses were held at 4 °C for 48 h. A total of 12 beef strip loins [longissimus dorsi (LD) muscle] were obtained on 2 days postmortem, and the average ultimate pH was 5.70 ± 0.05. LD was divided into six equal-length sections (which generated 72 sections in total) after removal of visible fat, and one of four treatments (succinate solution with 4, 6, and 8 mM and the control) was assigned randomly to each of the loin sections by using an incomplete block design, resulting in 18 treatment replications. All meat processing was performed at 4 °C. The sections were finely ground using a food processor (CombinMax600, German). Sodium succinate solution (4, 6, and 8 mM) was added during this process for postmortem measurements, and the patties were reground after the addition. From each samples, three 2.54 cm thick, 10 cm diameter patties were prepared. The samples were overwrapped with polyvinyl chloride film (23 °C, 60% RH, 350—400 $cm^3\ m^{-2}\ h^{-1}\ atm^{-1}$, Mitsui Chemical, Japan) and stored at 2 ± 0.5 °C in a cabinet (dark) for 2 h (reading taken as 0 day), 1, 2, 3, 4, and 5 days for color analysis. The pH of raw beef patties are as follows: control, 5.58; 4 mM succinate sodium, 5.62; 6 mM succinate sodium, 5.63; 8 mM succinate sodium, 5.64.

MetMb (%). Pattie samples (5 g) were homogenized in 25 mL ice-cold 40 mM phosphate buffer (pH 6.8) for 10 s using a superfine homogenizer (F6-10, Fluko, Shanghai, China) (13 500 rpm). The homogenate was allowed to stand for 1 h at 4 °C and centrifuged at 4500*g* for 30 min at 4 °C using a high-speed freezing centrifuge (GI-20G, Anke, Shanghai, China). The supernatant was filtered through a filter paper, and the absorbance was measured at 572, 565, 545, and 525 nm with a Unican UV4 spectrometer (Unican Ltd., China). The percentage of MetMb was determined as described by Krzywicki (*21*) using the formula:

$$\%\ \text{DeoMb} = \{0.369(A_{572}/A_{525}) + 1.140(A_{565}/A_{525}) - 0.941(A_{545}/A_{525}) + 0.015\} \times 100$$

$$\%\ \text{OxyMb} = \{0.882(A_{572}/A_{525}) - 1.267(A_{565}/A_{525}) + 0.809(A_{545}/A_{525}) - 0.361\} \times 100$$

$$\%\ \text{MetMb} = \{-2.514(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098\} \times 100$$

Color. The color of beef patties was determined using a reflectance colorimeter (WSC-S, Shanghai, China). The spectrophotometer was calibrated using black and white reference standards provided by the manufacturer. Values for CIE L^*, a^*, and b^* (illuminant A) were measured and used to calculate chroma $[(a^{*2} + b^{*2})^{1/2}]$. Patties were scanned at three different locations, and values were averaged for statistical analyses.

TBA Values. Lipid oxidation of treated beef patties were measured at 0, 1, 2, 3, 4, and 5 days. The thiobarbituric acid reactive substances (TBARS) assay was performed as described by Faustman et al. (*22*), with little modification. Pattie meat samples (10 g) were mixed with 45 mL of stock solution (25 mL of 20% TCA/20 mL of water), homogenized for 30 s with a superfine homogenizer (F6-10, Fluko, Shanghai, China), and centrifuged at 1000*g* for 20 min at 4 °C. The supernatant was filtered through a filter paper. The solution (3 mL of supernatant/3 mL of 0.02 M TBA) was heated in a boiling water bath (95−100 °C) for 30 min to develop the pink color. Samples were cooled under running tap water and centrifuged at 4500*g* for 25 min, and the absorbance of the supernatant was measured at 532 nm using a Unicam UV4 spectrometer (Unicam Ltd., China). TBARS were expressed as milligrams of malonaldehyde per kilogram of sample.

Experiment 2: Reduction of MetMb by Different Concentrations of NADH, Sodium Succinate, and Sodium Lactate. *Reagents*. Phenazine methsulphate (PMS), 2,6-DCPIP, NADH, 4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid (HEPES), and equine myoglobin were obtained from Sigma (St. Louis, MO). All other chemicals and solvents used were of analytical grade and purchased from Lanyi Chemical Articles Co., Ltd. (Beijing, China).

Bovine Mitochondria Isolation. The mitochondria preparation follows three simple steps: cell rupturing, centrifugation to remove large particles, and centrifugation to isolate mitochondria. Buffers and samples should be chilled when possible.

Mitochondria were isolated at 2 h postmortem from bovine cardiac muscle according to Smith (*23*), with minor modifications. An appropriate amount (about 25 g) of bovine cardiac muscle (five hearts were used for each replicate) was weighed out and washed twice with cold physiological saline. The bovine cardiac muscle was minced and placed in a prechilled superfine homogenizer (F6-10, Fluko, Shanghai, China) (13 500 rpm). Up to 250 mL of mitochondrial isolation buffer [100 mM sucrose, 10 mM Tris-HCl, and 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.3] was added. The homogenate was transferred to 50.0 mL eppendorf tubes. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was kept, and the pellet was discarded. The supernatant was transferred to new tubes and centrifuged at 12000*g* for 15 min at 4 °C. Each pellet was collected and washed by resuspending in 1.0 mL of mitochondrial isolation buffer at 12000*g* for 15 min at 4 °C. The pellets were collected, and the wash step was repeated. Finally, the pellets were combined and suspended in mitochondrial suspension buffer (250 mM sucrose and 10 mM Tris-HCl at pH 7.3), and the mitochondria was stored in liquid nitrogen until use. Before use, mitochondria were thawed in an ice bath (4 °C) and diluted by distilled deionized water for the protein content measurements and the activity. All steps were performed at 0−4 °C. The mitochondria protein content was determined by a biuret reaction.

Mitochondria Respiratory Chain Enzyme Activity. The activity of the mitochondria pellet resulting from differential centrifugation was detected by measuring the succinate dehydrogenase (SDH) activity, which was determined using a Unican UV4 spectrometer (Unican 119 Ltd., China).

The standard reaction mixtures at pH 7.3 and 25 °C contained one or more of the following according to Wang et al. (*24*) (**Table 1**): 1.5 mL of 10 mM phosphate buffer [pH 7.3, including 0.3 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, and 0.1% bovine serum albumin (BSA)], 1 mL of 150 mM sodium succinate, 0.1 mL of 10 mM PMS solution, 0.1 mL of 0.9 mM 2,6-DCPIP solution, and distilled deionized water to make the total reaction volume of the assay 3.0 mL. The reaction was initiated by adding the mitochondria with different protein contents and determined by following the change in absorbance at 600 nm. Blanks contained all of the additions, except mitochondria, which were replaced by deionized water. To measure the activity, absorbance at 600 nm was recorded every 10 s for 5 min in a spectrophotometer and the activity was calculated as the means of three replicates.

The SDH activity was calculated by the following formula: SDH is measured in units of min^{-1} and is calculated as SDH = $\Delta A/\Delta t$, where ΔA_{600} is the change in absorbance during the period where the rate of change is linear with time and Δt is the time between two measurements of the absorbance. SDH specific activity [unit (mg of protein)$^{-1}$ min^{-1}] = $\Delta A_{600}/(\Delta t \times 0.01)$/protein (mg/mL)/$0.2 \times 3.0$.

Equine MetMb Preparation. MetMb was prepared as follows: Commercial Mb (Sigma Co.) was dissolved in 2 mM phosphate buffer (pH 7.0) and chemically oxidized by an equimolar amount of $K_3Fe(CN)_6$, and the residual $K_3Fe(CN)_6$ was removed by dialysis for 24 h and changed every 4 h against 2 mM phosphate buffer (pH 7.0). Extraction was conducted in a cold room at 4 °C. The MetMb solution was stored in liquid nitrogen until use. Before use, MetMb was thawed in an ice bath (4 °C) and centrifuged at 10000*g* for 10 min at 4 °C. The MetMb concentration was adjusted to 0.75 mM with 2.0 mM phosphate buffer (pH 7.0).

Reduction of MetMb. Mitochondria (4.61 mg of mitochondria protein/mL, isolated 2 h postmortem) and Mb (0.15 mM) were combined with pH 7.3 (250 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.1% BSA, and 20 mM HEPES) buffers in the incubation chamber (25 °C) to a final volume of 3 mL, and the reaction was initiated by adding NADH (0, 0.5, 1, 1.5, and 2.5 mM), sodium succinate (0, 8, 16, 24, 32, and 64 mM), and sodium lactate (0, 50, 100, 150, 200, and 300 mM), respectively. At specific time points, samples were removed and centrifuged (15000*g*) for 3 min. The resulting supernatant was scanned at 572, 565, 545, and 525 nm with a Unican UV4 spectrometer (Unican Ltd., China). The percentage of DeoMb, OxyMb, and MetMb was calculated as described in experiment 1.

Table 1. Reduction of Succinate with a SDH System at 25 °C and pH 7.3 ($n = 3$)

solution components[a]	test 1 (mL)	test 2 (mL)
phosphate buffer (10 mM)	1.5	1.5
sodium succinate (150 mM)	1.0	1.0
PMS (10 mM)	0.1	0.1
2,6-DCPIP solution (0.9 mM)	0.1	0.1
deionized water (0.1 mL)	0.1	0.3
mitochondria	0.2	0
total	3.0	3.0

[a] PMS, phenazine methsulphate solution. Total assay volume = 3.0 mL. Blanks contained all of the additions except mitochondria, which were replaced by deionized water. All of the components were added with deionized distilled water to bring to a final volume.

Statistical Analysis. The design for experiment 1 was a split plot. In the whole plot (incomplete randomized block), 12 loins served as blocks and each of the six sections within a loin was an experimental unit to which treatments were applied randomly. This resulted in 18 replications of the four added treatments for the storage of 5 days. In the subplot, each of the three patties from a loin section was considered to be an experimental unit to which storage time (0, 1, 2, 3, 4, and 5 days) was assigned randomly.

Data were analyzed by using the SPSS 15.0. Type-3 tests of fixed effects for injection treatment, storage time, and their interaction were used to evaluate *F* test significance. Least-square means (LSMeans) were separated (*F* test, $p < 0.05$) by using least significant differences generated by the Diff option. Random error terms included loin for the incomplete-block portion and loin × treatment for the whole-plot error A. Residual unspecified error B was evaluated by using loin × treatment × storage time.

Experiment 2 was a completely randomized design replicated 3 times. LSMeans and standard errors (SEs) were used in software SPSS 15.0 to determine the effects of succinate, lactate, and NADH when incubated with mitochondria on MetMb reduction *in vitro*. The figure was created by SigmaPlot 10.0.

RESULTS AND DISCUSSION

Experiment 1: Effects of Sodium Succinate on Instrumental Color Features and Relative MetMb Percentage in Beef Patties. *Instrumental Color Features and MetMb (%)*. The effects of succinate sodium on instrumental color features and relative MetMb percentage in beef patties are presented in **Table 2**. The addition of succinate decreased the *L** value. Differences in *a** values were observed both between treatments and over the storage period. In a number of cases, stable *a** values were observed within patties from day 0 to 1. Ledward (*25*) noted that muscle myoglobin typically oxygenates very rapidly upon exposure to oxygen; however, muscle within 96 h postmortem has a relatively high OCR that can inhibit myoglobin from fully

Table 2. LSMeans for Instrumental Color of Beef LD Muscle Patties at Days 0 (Initial), 1, 2, 3, 4, and 5[a]

		treatments[b]			
trait	day	ck	s4	s6	s8
lightness (*L**)	0	47.60 ± 1.23 cx	43.36 ± 0.13 dy	41.06 ± 0.53 cy	46.38 ± 2.24 bx
	1	46.89 ± 0.89 cx	44.53 ± 0.77 cxy	42.69 ± 2.46 cy	45.41 ± 0.67 bx
	2	50.72 ± 0.76 abx	48.37 ± 0.47 by	48.2 ± 0.41 aby	49.37 ± 0.92 ay
	3	50.64 ± 0.66 abx	48.59 ± 0.38 by	48.91 ± 0.98 ay	49.1 ± 0.27 ay
	4	52.09 ± 0.69 ax	49.86 ± 0.63 ay	46.33 ± 0.5 bz	48.49 ± 0.24 az
	5	49.96 ± 0.46 bx	49.68 ± 0.11 ax	47.2 ± 0.22 abz	48.71 ± 0.1 ay
redness (*a**)	0	21.57 ± 0.19 ay	23.48 ± 0.71 ax	24.55 ± 0.73 ax	22.06 ± 0.65 ay
	1	21.37 ± 0.21 ay	23.35 ± 0.17 ax	23.93 ± 1.24 ax	20.23 ± 0.74 by
	2	19.69 ± 0.67 bx	19.19 ± 0.20 bxy	19.8 ± 0.27 bx	18.57 ± 0.22 cy
	3	18.89 ± 2.04 bx	18.38 ± 1.23 bcx	18.37 ± 2.47 bcx	18.40 ± 0.12 dx
	4	15.63 ± 0.56 cdz	16.90 ± 0.38 dy	18.58 ± 0.04 bcx	16.37 ± 0.28 dy
	5	14.28 ± 0.52 dz	15.13 ± 0.13 ey	17 ± 0.15 cx	14.57 ± 0.54 eyz
chrome[c]	0	29.84 ± 0.43 az	31.73 ± 0.94 by	34.04 ± 0.31 ax	32.35 ± 0.71 ay
	1	29.98 ± 0.94 az	33.23 ± 0.35 ax	33.88 ± 1.12 ax	31.16 ± 0.27 by
	2	28.26 ± 0.81 abx	25.64 ± 0.19 dz	27.1 ± 0.4 cy	25.74 ± 0.19 dz
	3	27.08 ± 2.81 bcx	25.4 ± 0.24 dx	26.25 ± 1.07 cdx	25.71 ± 0.22 dx
	4	22.08 ± 0.49 dz	24.58 ± 0.36 ey	26.08 ± 0.44 cdx	24.36 ± 0.17 ey
	5	21.33 ± 0.31 dz	23.54 ± 0.21 fy	25.67 ± 0.41 dx	23.96 ± 0.37 ey
MetMb (%)	0	15.59 ± 0.41 ex	17.92 ± 4.58 dx	15.09 ± 0.15 dx	16.36 ± 2.31 dx
	1	24.03 ± 0.49 dx	21.61 ± 2.93 cdx	21.19 ± 1.38 cx	22.32 ± 1.58 cx
	2	29.56 ± 0.6 cx	25.93 ± 3.54 bcx	27.10 ± 0.40 cy	25.74 ± 0.19 dz
	3	27.08 ± 2.81 bcx	25.4 ± 0.24 dx	26.87 ± 2.31 bx	24.59 ± 1.59 bcx
	4	30.08 ± 0.01 cx	27.19 ± 2.53 abcx	27.04 ± 0.84 bx	27.46 ± 0.79 abx
	5	33.34 ± 0.49 bx	30.67 ± 0.45 aby	29.18 ± 0.19 bz	29.03 ± 0.54 az

[a] Means within a column with different letters (a−f) are different ($p < 0.05$). Means in a row within a trait with different letters (x−z) are different ($p < 0.05$) ($n = 3$). [b] s4, 4 mM sodium succinate; s6, 6 mM sodium succinate; s8, 8 mM sodium succinate. [c] $(a^{*2} + b^{*2})^{1/2}$.

Table 3. Protein Concentration and the SDH Activity of the Mitochondrion at 48 h Postmortem[a]

treatment[b]	$\triangle A_{600}$[c]	SDH activity unit[d]	protein content (mg/mL)[e]	specific activity of SDH [unit (mg of protein)$^{-1}$ min^{-1}][f]
0	0	0	0	0
1	0.0029	17.10	10.08	25.45 ± 0.63 f
2	0.0016	9.30	6.03	23.14 ± 1.06 fg
3	0.0011	6.30	4.61	20.50 ± 1.38 g
4	0.0006	3.30	3.64	13.59 ± 1.75 h

[a] Means within a column with different letters (f−h) are different ($p < 0.05$). [b] The samples were mitochondria diluted by distilled deionized water. [c] ΔA_{600} is the change in absorbance during the period where the rate of change is linear with time. [d] SDH is measured in units of min^{-1} and is calculated as SDH = $\Delta A/\Delta t = \Delta A_{600}/(\Delta t \times 0.01)$, where Δt is the time between two measurements of the absorbance. [e] The mitochondria protein content was determined by the biuret reaction. [f] SDH specific activity [unit (mg of protein)$^{-1}$ min^{-1}] = $\Delta A_{600}/(\Delta t \times 0.01)$/protein (mg/mL)/0.2 × 3.0.

oxygenating. As storage time progressed, patties enhanced with s4 and s6 had higher a^* values (redness) and chroma ($p < 0.05$) than that of the control patties until the end of storage. Whatever the treatments, a^* and chroma all decrease from days 0 to 5 ($p < 0.05$); however, the s6 treatment has the most stable ($p < 0.05$) a^* and chroma during storage (days 4−5) and the s4 and s8 treatments had a slower rate of a^* decrease than the control.

MetMb (%) generally supports the instrumental color features. The relative MetMb percentage increased during the storage, and the control increased more significantly ($p < 0.05$) than the other treated samples after 3 days. The relative MetMb percentages in samples treated with s6 and s8 sodium succinate were the lowest at day 5.

Watts et al. (*26*) reported an increase in beef redness because of succinate addition. The addition of 100−200 mg of succinate/100 g of meat increased the rate of oxygen use in meat slurries to 3−9 times than that of the control. Succinate addition facilitated the rapid establishment of anaerobic conditions in succinate-treated samples, which effectively shortened and eliminated the lag period in MetMb reduction. Our result is consistent with these findings. Tretter et al. (*13*) found succinate, an inhibitor of mitochondrial lipid peroxidation that prevented and delayed most of the damage caused by the peroxidation on different mitochondrial structures and functions. However, we did not found the significant effect of succinate on the lipid oxidation of patties (results not shown).

Experiment 2: Reduction of MetMb by Different Concentrations of NADH, Sodium Succinate, and Sodium Lactate. *Measurement of Mitochondria Activities*. As one of the best studied enzymes in the Krebs cycle, succinate dehydrogenase (SDH; EC 1.3.99.1) is an important enzyme in cell energy metabolism, and its activity can reflect the status of the metabolism. To measure the activity of mitochondria, SDH activity was detected. The mitochondria were diluted, and the protein concentration was measured (**Table 3**). The results indicated that the SDH had the higher activity compared to the control. Further dilution decreased the protein content and SDH activity. In comparison to treatment 4, treatment 3 significantly increased the activity of SDH and has no significant difference compared to treatments 1 and 2. On the basis of the results, mitochondria protein content of 4.61 mg/mL was chosen for later experiments.

Reduction of MetMb (%). Significant time−succinate concentration interactions existed for the relative MetMb percentage in the model system ($p < 0.05$; **Figure 1A**). It is apparent from **Figure 1A** that, between 0 and 3 h of incubation at pH 7.3, succinate at concentrations of 8, 16, 24, 32, and 64 mM minimized OxyMb oxidation compared to controls and the succinate at concentrations of 16 and 24 mM had a more significant effect on MetMb reduction ($p < 0.05$). **Figure 1B** indicted that the level of MetMb reduction in samples of NADH with 1.5 mM was the same as the samples of succinate with 16 mM and the relative MetMb percentages significantly increased compared to the samples of NADH with 3 mM. The electrons from NADH to the intermediates including the MetMb can be responsible for the rapid MetMb reduction. This indicated that, while there are several pathways to reduce the MetMb and stabilize the meat color, as the intermediate, NADH is the main one in the progress of MetMb reduction (*6*, *26*, *27*). Lactate of 300 mM significantly decreased the relative MetMb percentage compared to the others ($p < 0.05$; **Figure 1C**). While both NADH and lactates increase the MetMb reduction ability during the first 2 h or so and then their reduction ability decreases gradually, succinates decrease the relative MetMb percentage during the incubation time of 3 h.

These results *in vitro* demonstrated that succinate addition can increase the color stability of the patties and decrease the relative MetMb percentage. Tang et al. (*14*) proposed that electrons become available for MetMb reduction at a site(s) between complexes III and IV. MetMb was reduced by a mitochondrial ETC-linked pathway. The reduction of MetMb could follow the pathway: succinate → complex II → ubiquinone → complex III → cytochrome c → OM cytochrome b_5 → MetMb. To prove the proposal, sodium succinate was used as a substrate and the results indicated that mitochondrial ETC-linked MetMb reduction increased with increased mitochondrial density and succinate concentration (*14*). Watts et al. (*26*) reported that succinate addition facilitated the rapid establishment of anaerobic conditions in succinate-treated samples, which effectively shortened and eliminated the lag period in MetMb reduction. However, our results indicated that only succinate of 16 and 24 mM significantly decreases the relative MetMb percentage compared to other systems. A reasonable explanation for the observation is that the electron release from the higher concentration succinte (32 and 64 mM) may band the oxygen, generate the superoxide radical, and then oxidize DeoMb and OxyMb, leading to the higher concentration of MetMb. The results demonstrated no significant differences between aerobic and anaerobic reduction of MetMb. This was in agreement with an earlier report by Echevarne et al. (*28*). The author reported no significant differences between aerobic and anaerobic MetMb-reducing activities for different fractions of four different beef muscles. Hagler et al. (*4*) showed that the measured rates of MetMb reduction using purified enzyme were identical in anaerobic and aerobic conditions. Lanier et al. (*29*) studied MetMb reduction in ground beef, beef slurries, and extracts held in air, nitrogen, or CO/air mixtures and found that significant MetMb reduction occurred under both aerobic and anaerobic conditions.

The MetMb reduction involved with the lactate has been reported (*11*, *15*−*17*, *30*−*34*). Kim et al. proposed that lactate plays an indirect role in color stability by generating NADH, which is subsequently used to maintain reduced forms of myoglobin (*11*). In addition to the ability of lactate to generate reducing equivalents via lactate dehydrogenase, Mancini and Ramanathan suggest that lactate may also be directly involved in myoglobin redox stability; the interaction between lactate and myoglobin might improve redox stability via nonheme protein

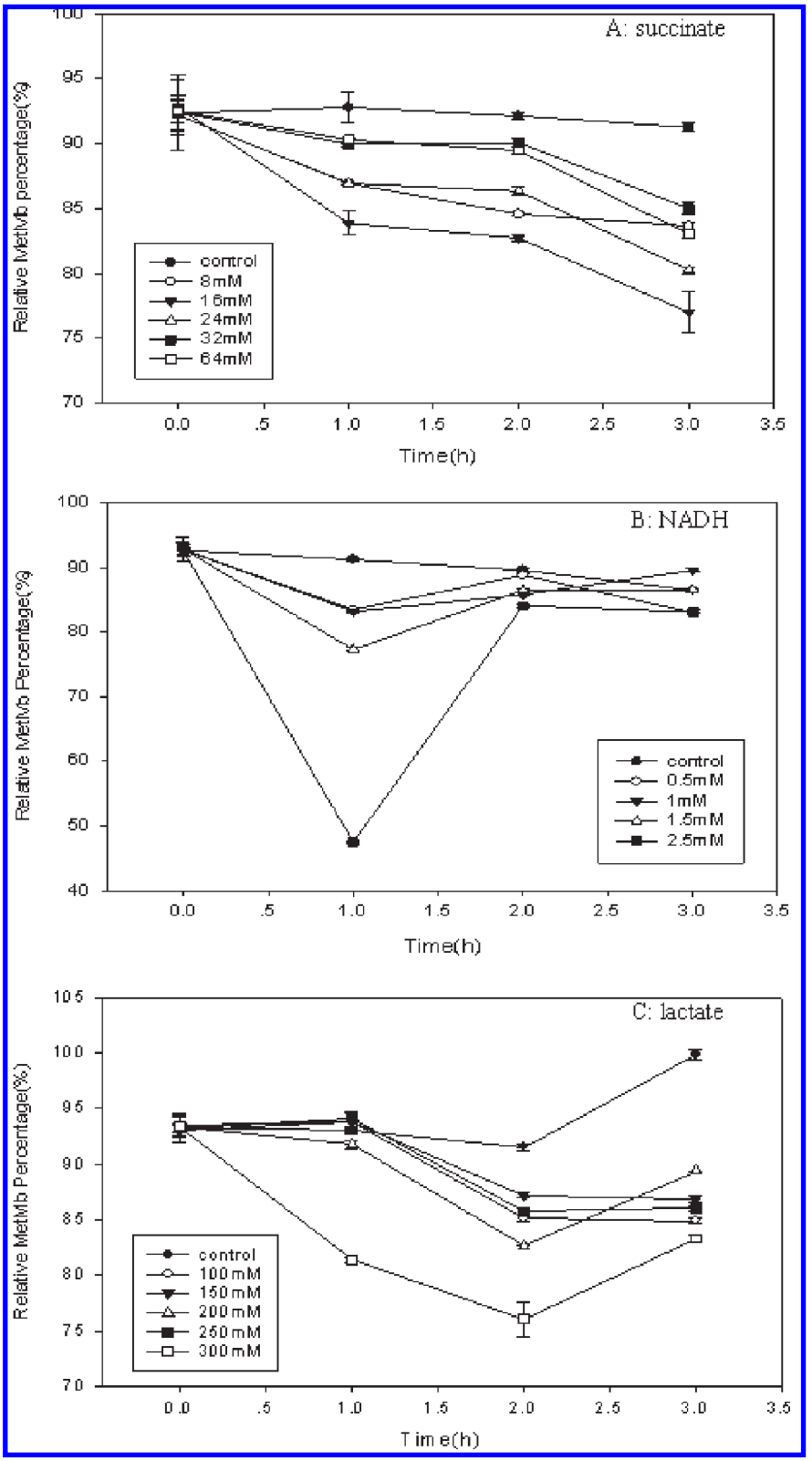

Figure 1. Effect of different concentrations of sodium succinate, NADH, and lactate on MetMb reduction (mitochondria, 4.61 mg/mL) at 25 °C and pH 7.3: (A) Effect of succinate on MetMb reduction, (B) effect of NADH on MetMb reduction, and (C) effect of lactate on MetMb reduction. Standard error bars are indicated.

adduction (*34*). Our results indicated that succinate, lactate, and NADH incubated with high-activity mitochondria all have the ability to reduce the MetMb. Increasing NADH and sodium lactate could increase MetMb reduction, and the MetMb reduction ability decreases significantly within 2 h. Only succinate with 16 and 24 mM significantly decrease the relative MetMb percentage compared to other systems, and succinate decreased the relative MetMb percentage during the incubation time of 3 h. This indicated that the succinate−MetMb reduction systems are more stable and less affected by oxygen compared to the systems of NADH−MetMb reduction when incubated with mitochondria. The different mechanisms of lactate and

succinate on MetMb reduction will be responsible for this phenomenon.

The present study investigated the effect of different concentrations of succinate on MetMb reduction by transferring the electron to MetMb by the intermediates in mitochondria rather than preventing the mitochondria lipid oxidation. The comparison of the effect of different concentrations of succinate, lactate, and NADH on MetMb reduction suggested that there are different pathways for the MetMb reduction. In comparison to the systems of NADH−MetMb reduction (including the systems of lactate−MetMb reduction), the succinate−MetMb reduction systems are more stable and less affected by oxygen when incubated with mitochondria. More identification work is needed to obtain the explanation of the more complete pathways on MetMb reduction. Perhaps this research will renew interest in clarifying the pathways of the MetMb reduction and the comparison between them in the specific environment, such as in mitochondria.

ABBREVIATIONS USED

MetMb, metmyoglobin; DeoMb, deoxymyoglobin; OxyMb, oxymyoglobin; TBARS, thiobarbituric acid reactive substances; EDTA, ethylenediaminetetraacetic acid; NADH, reduced nicotinamide adenine dinucleotide; s4, 4 mM sodium succinate; s6, 6 mM sodium succinate; s8, 8 mM sodium succinate; HEPES, 4-(2-hydroxyerhyl)piperazine-1-erhanesulfonic acid; BSA, bovine serum albumin; PMS, phenazine methsulphate; SDH, succinate dehydrogenase; 2,6-DCPIP, 2,6-dichlorophenol indophenol; LSMeans, least significant means; SE, standard error.

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