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Proteomics analysis to investigate the effect of sodium lactate on color stability of beef *longissimus lumborum* muscle during chilled s torage

Yongping WEN[1+], Yu KANG[1+], Lu ZHANG[1], Tian TIAN[2], Xinhui WANG[1*]

Abstract

Sodium lactate contribution on color stability of chilled beef was evaluated by proteomics analysis combined with the relative Mb content and NADH/ NAD^+ ratio. The sodium lactate exhibited a remarkable efficacy against discoloration of chilled beef and showed a desirable color appearance for 6 days, while the control showed an undesirable color appearance on the 3rd day. Based on the significance analysis of protein interaction network, 12 proteins responsible for color stability of chilled beef treated by sodium lactate have been identified, which participated mainly in redox systems, metabolic enzymes, oxidoreductase, structural proteins and heat shock proteins. The proteins were correlated with NADH regeneration. Thus, we suggest that the color stability promotion may attribute to the role of in the regeneration of NADH by sodium lactate treatment.

Keywords: chilled beef; discoloration; proteomics analysis; sodium lactate.

Practical Application: In this study, sodium lactate as preservation treatment was used in fresh beef under chilled storage and comparative efficacy of sodium lactate against discoloration and spoilage was systematically evaluated by proteomics analysis. Sodium lactate displayed a remarkable efficacy against discoloration and spoilage of fresh beef during storage at 4 °C, resulting in shelf life extension by 3-4 days. Based on the significance analysis of protein interaction network, 12 proteins responsible for color stability of chilled beef treated by sodium lactate have been identified, which participated mainly in redox systems, metabolic enzymes, oxidoreductase, structural proteins and heat shock proteins.

1 Introduction

Fresh beef is a large part of the human diet in many countries. However, due to its rich nutrition, fresh beef is an ideal substrate for the growth of spoilage microorganisms, resulting in a perishable product and a short shelf life (Liu et al., 2020). 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). Consequently, the hygiene and safety of the fresh beef is of major concern to consumers, processors, retailers and meat industries. Hence, it is essential to implement hygiene procedures and adequate preservation technologies to promote freshness of fresh beef to maintain its quality and safety.

Recently, the meat quality enhancement by preservatives usage during chilled storage has been received 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; Wang et al., 2022a; Tian et al., 2022). To date, although sodium lactate has been applied in sausage and manufactured meat to control food spoilage, sodium lactate is rarely used in fresh meat to freshness improvement. Additionally, three are few reports in detail of mechanistic insights on freshness promotion for meat by sodium lactate, especially for color stability.

With rapid development of "omics"techniques, proteomics analysis has been applied in meat color research successfully, to discover differential expression of proteins among color biomarkers (Zhang et al., 2018; Yang et al., 2018). Thus, in this study, sodium lactate was used as preservative for fresh beef under chilled storage, and the efficacy of sodium salt against discoloration was focused on. In order to understand the biochemical and structural mechanisms related to color stability promoted by sodium lactate treatment using high-throughput proteomic approaches. It was expected that the outcomes of this study would provide a comprehensive understanding of the effects of sodium lactate treatment on color stability improvement, and would extend the application of sodium lactate in chilled beef preservation.

2 Materials and methods

2.1 Sample processing

Fresh beef was obtained from a commercial processing facility located in Chengdu, Sichuan Province, China, within 2 h after slaughter, which demonstrated normal color and firmness, and had similar marbling scores with Slight 90. Then the fresh beef was immediately kept in an insulation ice box and transported

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[1] *Meat-Processing Application Key Laboratory of Sichuan Province, School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan, China*
[2] *Chengdu Agricultural College, Chengdu, Sichuan, China.*
*Corresponding author: wangxinhui19820319@163.com
[+] *Equal Contribution*

to laboratory within 1 h. Then the striploins were cut ans the *longissimus lumborum* muscle was separated into 42 samples for experiments. Each sample was fabricated into 10 cm × 10 cm × 1.5 cm with the weight of 100 g. Subsequently, all samples were equally divided into two groups. One group was sprayed by (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. In our preliminary experiments, a good color stability of sample was achieved by (3 g/L) spray. Therefore the concentration of sodium lactate was designated as 3 g/L in this experiment. After spray, all samples were immediately packed with polyethylene sterile bags and stored at 4 °C for 6 days for experiments. The pH, TVB-N content, Color a*values, weight loss, total bacterial counts, relative myoglobin content and NADH content were determined on samples aged for 1, 2, 3, 4, 5 and 6 d. The proteomic analysis was only conducted on the samples stored on the 3rd day. All experiments were conducted three times with 3 samples in each replicate.

2.2 pH measurement

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 the method described in Wang et al. (2015a). Before measurement, the pH probe was calibrated in buffers at pH 4.00 and 7.00 at room temperature. The pH probe was inserted directly into beef, and all measurements were performed in triplicate at each time point and an average was calculated.

2.3 TVB-N measurement

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

2.4 Redness color measurement

A bright red color is considered a positive attribute for freshness and superior quality of beef (Holman et al., 2016). The degree of redness ($a*$) of chilled beef is associated with the concentration of reduced MetMb or Mb or OxyMb (Wang et al., 2022b). Thus, the indicator $a*$ is of the greatest interest in the analysis. The $a*$ value of chilled beef was determined by using a colorimeter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China) according to the method described in Wang et al. (2015b). At each time point, the $a*$ value of each sample was determined at three random locations and then triplicate readings were averaged.

2.5 Weight loss measurement

At each time point, the weight of each sample was measured by Mettler Toledo ME104E electronic balance. Weight loss was calculated as the percentage of weight reduction with respect to the initial weight.

2.6 Total bacterial counts(TVC) determination

A 10 g of sample was aseptically removed into stomacher bags containing 90 mL sterile 0.1% peptone water, and then was homogenized in a blender. After mixing, a 10-fold dilution series was performed for microbiological analysis. The TVC counts were enumerated on plate count agar (PCA, Sangon Biotech Co. Ltd, Shanghai, China) and incubated at 37 °C for 48 h. The results of bacterial counts were expressed as $\log_{10}$ CFU/g sample.

2.7 Relative myoglobin content measurement

The spectrophotometer records reflectance values in the range of 360 to 740 nm at 10 nm intervals. The reflectance values (R) were calculated by integrations of the measurement at 473, 525, 572 and 700 nm, and the R was converted to reflex attenuance (A) according to the equation: A = log (1/R). Then the percentage of myoglobins, namely metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), was determined by the Equations 1, 2 and 3 as described by Wu et al. (2020).

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

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

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

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

2.8 The NADH/NAD⁺ ratio determination

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

2.9 Protein extraction

At each time point, the samples were frozen in liquid nitrogen, and then stored at -80 °C until protein extraction. 4 g frozen sample was homogenized in 10 mL cold protein extraction buffer (pH 8.0) containing 40 mM Tris, 2 mM EDTA and 2 M DTT by using a hand held homogenizer (Biodex, U.S.A). The homogenate was centrifuged at 15,000 g for 25 min at 4 °C. The supernatant was fractionated and stored at -80 °C for the subsequent analysis. The protein concentration was determined by BCA method as described in BCA protein assay kit (Qcbio Science &Technologies Co., Ltd, Shanghai, China). The same protein sample was used for proteomic analyses.

2.10 Trypsin digestion

A total of 100 μg protein was added to 10 mM dithiothreitol (DTT, Thermo). The mixture was incubated for 30 min at 56 °C. Then iodoacetamide (IAM, Thermo) was added to make the

final concentration of protein solution 11 mmol/L and incubated for 15 min at 25 °C in darkness. Dilute the urea concentration of the sample to less than 2 mol/L. The protein sample was digested with trypsin (Hangzhou Putai Biotechnology Co. Ltd, Hangzhou, China) according to the ratio of trypsin: protein of 1:50(m/m)overnight at 37 °C. The peptides were then desalted by C18 cartridge and freeze-dried in vacuum

2.11 HPLC-MS/MS analysis

The peptide mixture was separated by an ultra-high performance liquid chromatography (Waters, USA). Peptides were dissolved in Buffer A (0.1% (v/v) formic acid aqueous solution) and the column was balanced with Buffer A. The liquid gradient was set as follows: the linear gradient of Buffer B (0.1% (v/v) formic acid acetonitrile solution) was: 4%-6% for 0-2 min, 6%-25% for 2-70min, 25%-32% for;70-84 min, 32%-80% for 84-87 min, 80% for 87-90 min at a flow rate of 300 nL/min. After separation, the peptides were injected into capillary ion source for ionization, and then analyzed by timsTOF Pro mass spectrometer (Bruker, Germany) for mass spectrometry analysis. The mass spectrometer was operated in positive ion mode and the ion source voltage was set to 1.4 kV. The scanning range of secondary mass spectrometry was set to 100 -1700 m/Z. The data was acquired using a parallel cumulative serial fragmentation (PASEF) method. After a primary mass spectrum was collected, the PASEF method was used for 10 times to collect the secondary spectrum with precursor ion charge in the range of 0-5. The dynamic exclusion time of tandem mass spectrometry scanning was set to 24 s to avoid repeated scanning of precursor ions.

2.12 Protein identification and quantification

Secondary mass spectrometry data were retrieved using MaxQuant software version 1.6.6.0 (Thermo Fisher Scientific) and searched against the database Bos_taurus_9913_PR_20190915. fasta with 37, 948 protein sequences. In the database search, the trypsin was used as the cleavage enzyme allowing up to 2 missing cleavages. The minimum length of the peptide was set to 7 amino acid residues, following the first search of 40 ppm and main search of 40 ppm. The mass error tolerance of secondary fragment ions was set to 0.04 Da. Cysteine alkylation was specified as fixed modification, and methionine oxidation and protein N-terminal acetylation was specified as variable modifications. For protein identification, the false discovery rate (FDR) was adjusted to 1%.

2.13 Statistical analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) was used for KEGG pathway analysis of differentially expressed proteins. The detailed elucidation of physiological functions and biological pathways of these proteins were applied to further explore the impact of differentially expressed protein in cell physiological processes and discover internal relations between differentially expressed proteins. Three replicates were used for all samples and these results were expressed as mean ± standard deviation (SD). Duncan's multiple range test (p-value <0.05) was employed for the independence of error terms using the SPSS 15.0 statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussion

3.1 Quality traits of chilled samples

A bright red color 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 consumer-defined beef color acceptability, and when $a^* < 14.5$ the beef color is considered unacceptable (Holman et al., 2017). As shown in Table 1, the initial $a*$ value of Group C and Group SL was 29.39 and 30.32, respectively, suggesting a desirable color acceptability. Subsequently, the $a*$ value of Group C reduced rapidly along with the storage period extend, and the $a*$ value reduced to 11.88 on the 6th day and reached the rejection level ($a* < 14.5$), suggesting an unacceptable color (Holman et al., 2017). Correspondingly, Group SL maintained a relatively stable $a*$ value during the storage for 6 days in the in the range of 30.32-24.05, and stayed above this threshold ($a* > 14.5$) for the entire storage period.

The TVB-N level has been used as a direct quality indicator of meat freshness/deterioration of fresh meat in China (Zhang et al., 2009), and 15 mg/100 g of TVB-N content is set as the upper limit

Table 1. The pH value, TVB-N level, redness, weight loss and total plate count of Group C and Group SL during storage at 4 °C ± 1°C.

Quality parameters	Samples	Storage time (d)		
		1	3	6
pH	C	5.62 ± 0.22 bx	5.83 ± 0.18 ax	6.00 ± 0.14 ax
	SL	5.61 ± 0.12 bx	5.69 ± 0.16 ax	5.73 ± 0.08 ax
TVB-N	C	7.47 ± 0.56 ax	15.54 ± 0.46 ay	23.53 ± 0.78 bx
	SL	7.25 ± 0.45 ax	11.13 ± 0.85 by	17.85 ± 0.82 ax
Redness (a*)	C	29.39 ± 1.24 ax	17.57 ± 1.08 bx	11.88 ± 0.89 ax
	SL	30.32 ± 1.45ax	27.27 ± 1.68 cx	24.05 ± 1.43 by
Weight loss(%)	C	2.16 ± 0.22 bx	7.16 ± 0.12 by	10.40 ± 0.22 ax
	SL	1.57 ± 0.34 ax	6.20 ± 0.34 by	9.29 ± 0.24 cx
Total plate count (lg cfu/g)	C	3.45 ± 0.12 ax	7.27 ± 0.42 ax	8.78 ± 0.46 bx
	SL	2.08 ± 0.22 bx	4.86 ± 0.64 cy	6.85 ± 0.66 ax

Means within columns with differing letters (a, b, c) aresignificantly different at $p<0.05$. Means within rows with differing letters (x, y) are significantly different at $p < 0.05$.

for fresh level and reaches the threshold of acceptability according to the National Food Safety Standard of China (GB 2707-2016). The initial TVB-N level was 7.25 mg/100 g and 7.47 mg/100 g in Group C and Group SL, respectively, suggesting a good meat quality (Wang et al., 2021; Tian et al., 2017). Subsequently, the TVB-N level in Group C increased and reached the rejection level on the 3rd day. Correspondingly, the TVB-N level in Group SL reached the rejection level on the 6th day, displayed a longer shelf life than that of the control sample.

The weight loss was a critical factor in beef quality degradation. As shown in Table 1, Group C displayed a higher weight loss percentage compared with Group SL. Obviously, the sodium lactate treatment effectively inhibited weight loss, which can be explained by the inhibition of relevant protein hydrolysate activities by sodium lactate, resulting in a protein hydrolysis retardation.

The pH value plays a critical role in fresh beef quality, which could affect color stability through influencing oxygen consumption and metmyoglobin reducing activity, the water holding capacity and microbial growth (Mancini et al., 2010). The initial pH value in Group C and Group SL was 5.62 and 5.61 as shown in Table 1, respectively. During storage, the pH values in Group C as the control fluctuated violently, varying over a range of 5.62-6.00. In contrast, the pH value in Group SL maintained at a stable level with a range of 5.61 to 5.73, which was within the normal range during the whole storage. The results revealed that sodium lactate treatment effectively inhibited the increase of pH value, which was conducive to freshness maintenance, and was highly consistent with the results of redness (a∗) and water holding capacity.

The TVC counts have been recognized as the direct quality indicator of fresh meat, which is positively correlated with the spoilage process. Based on the International Commission on Microbiological Specifications for Foods (ICMSF), the value of 7 $\log_{10}$ CFU/g has been defined as a threshold of microorganism counts for good quality fresh meat. As shown in Table 1, the TVC counts in Group C reached to above 7.0 $\log_{10}$ CFU/g on the 3rd day. In contrast, the value of TVC counts in Group SL was significantly lower ($p < 0.05$) than that of Group C. Obviously, sodium lactate exhibited a good bacteriostatic efficacy.

3.2 Metmyoglobin reducing activity

Metmyoglobin reducing activity indicates the inherent ability to reduce metmyoglobin in muscles, which is crucial for meat color shelf life (Bazile et al., 2019). In this study, the state of myoglobin was monitored by its three redox forms, namely MetMb, OxyMb and DeoxyMb as shown in Figure 1. The initial proportion of OxyMb was 77% and 66% in Group C and Group SL, respectively. As display time extended, the proportion of OxyMb in Group C dropped significantly ($p > 0.05$) to 24% on the 5th day, which can result in a dark redness increased sharply. By contrast, the proportion of OxyMb in Group SL maintained a high level with 76% on the 5th day. These results revealed that sodium lactate usage had a significant effect ($p < 0.05$) on the state of myoglobin and was conducive to maintaining a high proportion of OxyMb. These results were well in agreement with

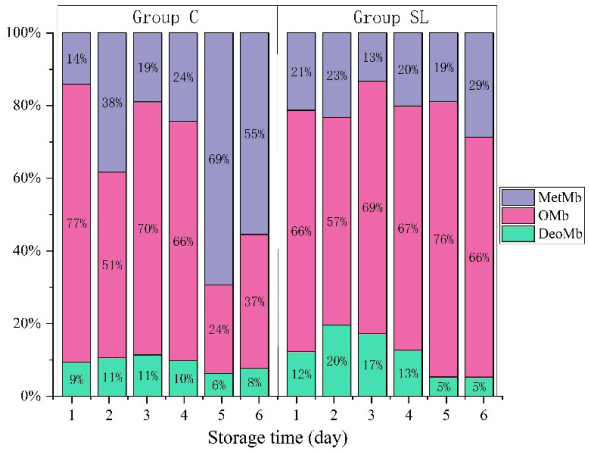

Figure 1. Effect of sodium lactate treatment on of relative content of three myoglobin forms of chilled beef samples.

the results of redness (*a*∗), which can partially explain the reason why the color appearance was more acceptable in Group SL.

3.3 The NADH/NAD⁺ ratio

The initial NADH/NAD⁺ ratio in Group C and Group SL was 0.59 and 0.58, respectively, as shown in Table 2. As the storage extended, the NADH/NAD⁺ ratio in Group C continued to rise, and reached a level approximately five times on the 3rd day compared with the initial NADH/NAD⁺ ratio. In contrast, NADH/NAD⁺ ratio in Group SL displayed a small reduction with 0.38 on the second day. Subsequently, NADH/NAD⁺ ratio in Group SL maintained at a stable level with a range of 0.34 to 0.37 until 6th day.

The NADH and NAD⁺ have been acknowledged as the primary redox carriers involved in metabolism. The high NADH/NAD⁺ ratio indicates insufficient oxidation of NADH, resulting in a poor reduction effect. Fe^{+3}-MetMb is reduced to Fe^{+2}-Mb by NADH oxidation via mitochondrial respiration. The high NADH/NAD⁺ ratio in Group C indicates insufficient reducing power. As a result, Fe^{+3}-MetMb was insufficiently restored to Fe^{+2}-Mb, resulting in a high proportion of MetMb and discoloration. Clearly, the sodium lactate usage was conducive to stabilizing the cellular NADH/NAD⁺ ratio, which is probably the main positive contribution against discoloration.

3.4 Protein identification and proteomic profile

In this study, the biggest difference of NADH/NAD⁺ ratio between the Group C and Group SL was on the 3rd day. Therefore, the Group C and Group SL stored for 3 days were sampled for proteomic analysis. A total of 1572 proteins were identified between the two samples, in which 126 proteins were significantly different between Group C and Group SL (Figure 2), according to a difference multiple of 1.2 times. Among these differentially expressed proteins identified in Group SL, 63 proteins were

Table 2. Effect of sodium lactate treatment on the NADH/NAD$^+$ ratio of chilled beef during storage at 4 °C ± 1°C.

	1d	2d	3d	4d	5d	6d
Group C	0.59 ± 0.07 ax	0.86 ± 0.05 bx	2.93 ± 0.08 cx	1.72 ± 0.03dx	1.86 ± 0.07ex	1.29 ± 0.02ax
Group SL	0.58 ± 0.05 ax	0.38 ± 0.03 by	0.35 ± 0.07cx	0.34 ± 0.04dy	0.38 ± 0.06 ex	0.37 ± 0.01 ax

Group C, the control sample; Group SL, sample treated with sodium lactate. Means within columns with differing letters (a, b, c, d, f) are significantly different at $p < 0.05$. Means within rows with differing letters (x, y) are significantly different at $p < 0.05$.

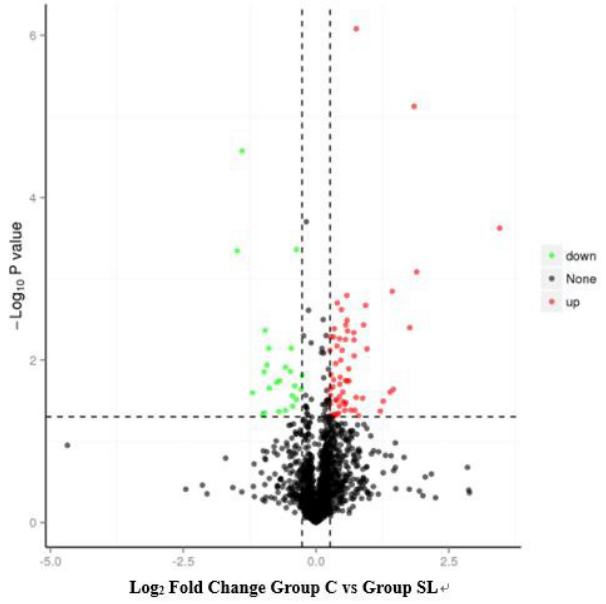

Figure 2. Volcano plots showing proteins identified between Group SL and Group C. The red dot is the significantly up-regulated, the green dot is the significantly down-regulated, and the gray point is no-significant differences.

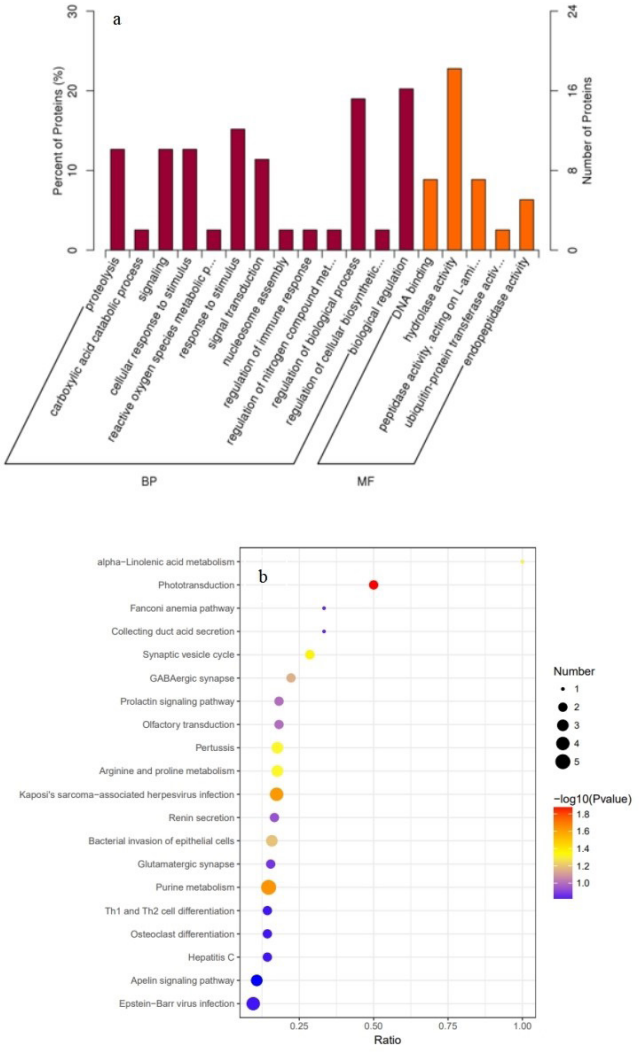

Figure 3. Gene Ontology (a) classification and KEGG pathway (b) of deferentially abundant proteins between in Group SL and Group C.

up-regulated and 39 proteins were down-regulated, compared with Group C.

Furthermore, to better visualize the differences in protein function between Group C and Group SL, the functional characteristics of proteins were analyzed by the Gene Ontology (GO) classification as shown in Figure 3a. The GO annotation indicated that these proteins could be categorized into two groups on the basis of functional activities, namely biological process (BP), accounting for 76.5%, and molecular function (MF), accounting for 33.5%. The majority of investigated proteins with different expression levels were associated with biological regulation (20%), regulation of biological processes (19%), response to stimulus (12%), signaling (12%) and proteolysis (12%) in biological processes. Meanwhile, the molecular function analysis displayed that the most of deferentially expressed proteins belonged to hydrolase activity, followed by peptidase activity and DNA binding.

Different proteins usually cooperate with each other to play their biological functions. To further analyze the crucial metabolic pathways enrichment in response to sodium lactate treatment, the KEGG pathway enrichment was performed as shown in Figure 3b. The deferentially abundant proteins were enriched into 20 pathways, and the most enriched pathways (number of proteins > 3) included purine metabolism, arginine and proline metabolism.

3.5 Relationship between deferentially abundant proteins and meat color traits in the late stage of storage

Based on the significance analysis of protein interaction network, 98 proteins with significant differences between Group C

Table 3. Significantly different proteins with Group SL compared with Group C.

Protein	Description	Gene	FC	Pvalue	UP/DOWN
Peroxidase system					
L8IFZ2	Superoxide dismutase [Cu-Zn]	E5288_WYG011986	0.437524868	0.025276048	down
L8IZ67	Glutaredoxin-3 (Fragment)	M91_00380	0.894881115	0.003693231	up
Metabolic enzyme					
L8I4Y3	Creatine kinase B-type (Fragment)	M91_04951	0.526115299	0.011570668	down
A0A6B0RV40	Pyruvate kinase	E5288_WYG019610	0.615468555	0.043051228	down
A0A6B0RWV8	Creatine kinase	E5288_WYG021711	1.255087609	0.005195976	up
Oxidoreductase					
L8IZY4	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7	E5288_WYG011646	1.314326778	0.00670572	up
L8IEL0	NAD(P)(+)--arginine ADP-ribosyltransferase (Fragment)	M91_16150	1.354482553	0.028251238	up
L8IGU7	Mitochondrial pyruvate carrier	M91_01088	0.560101163	0.033324756	up
Structural protein					
L8HY54	Myosin-13 (Fragment)	M91_13181	0.714943256	0.013808743	down
L8I8R7	Myomesin-3	M91_13276	1.636614507	0.004576276	up
L8IG28	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform (Fragment)	M91_16441	1.257338191	0.017447714	up
Other proteins					
L8I7M6	Heat shock protein beta-2	E5288_WYG001974	1.418047882	0.024876208	up

and Group SL stored for 3 days were selected. Pearson's correlation analysis was carried out between 98 proteins and muscle quality traits (color and water holding capacity). Among them, 12 proteins including 2 redox systems, 6 metabolic enzymes, 3 structural proteins and 1 other protein were correlated with meat color or water holding capacity (Table 3). The proteins with correlations could be potential biomarkers for meat color improvement by contribution of sodium lactate treatment in chilled beef.

The redox state of myoglobin is the key internal factor to maintain the color stability of meat. After slaughter, the myoglobin will present three different states of transition, namely MetMb, OxyMb and DeoxyMb. Once the relative content of MetMb reaches 50%, the meat color exhibits dark redness which is unacceptable meat color for consumers. Thus, MetMb reducing ability is crucial for meat color stability. The NADH has been acknowledged as the primary redox carriers involved in metabolism. The NADH regeneration plays an important role in the reduction of MetMb. The higher the accumulation of NADH, the higher the autoxidation rate of myoglobin. Therefore, the metabolism associated with NADH regeneration was indirectly related to meat color (Gagaoua et al., 2017), and the storage capacity and regeneration capacity of NADH were different due to different glycolysis potential, which would indirectly lead to differences in meat color. In this study, the expression of mitochondrial pyruvate carrier was up-regulated in Group SL. The mitochondrial pyruvate carrier transports pyruvate from the cytosol into the mitochondrial matrix, thereby gating the tricarboxylic acid (TCA) cycle, in which 3 molecules of NADH could be generated (Kiyimba et al., 2021) . Thus, the results revealed that the up-regulated expression of mitochondrial pyruvate carrier in Group will contribute to the regeneration ability of NADH, which plays an important role in the reduction of MetMb. Meanwhile, the up-regulated expression of NADH dehydrogenase was found in Group SL. The NADH dehydrogenase is the first enzyme complex in the respiratory chain, and it accepts electrons from NADH to create an electrochemical gradient across the inner mitochondrial membrane, which also plays an important role in the reduction of Fe^{3+}- MetMb.

Moreover, glycolysis potential was found to be negatively correlated with redness (a*) (Tao et al., 2021). Pyruvate kinase (PKM) is a rate limited glycolytic enzyme that irreversibly catalyzes the transfer of a phosphate group from phosphoenolpyruvate to an ADP molecule, competing with a pyruvate molecule and an ATP molecule (Wu et al., 2020). In this study, the down-regulated expression of pyruvate kinase was found in Group SL. The down-regulated expression of pyruvate kinase will directly lead to reduce glycolysis, which could be conducive to the appearance of redness.

The changes of muscle ultrastructure not only affected the muscle water migration, but also affected the extent of muscle light scattering. Myosin binding protein (MYBP) family, a class of myosin, plays an important role in the structure and function of striated muscle. It has been reported that myomesin-3 was positively correlated with the redness (a*) (Lang et al., 2021). In this study, the myomesin-3 exhibited up regulation in Group SL, suggesting a contribution to redness improvement by sodium lactate spray.

Additionally, heat shock proteins protect the correct conformation and biological function of protein after animal slaughter, which maintain intracellular homeostasis by acting as molecular chaperones in protein assembly, folding and unfolding,

and in the refolding of damaged proteins (Tao et al., 2021). Moreover, it has been reported that heat shock proteins could maintain the stability of meat color and water holding capacity (Wu et al., 2015; Yu et al., 2017). In this study, the up-regulated expression of heat shock protein beta-2 was detected in Group SL, which might be related to the color stability of Group SL.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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