Journal of Integrative Agriculture 2017, 16(11): 2646–2654

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RESEARCH ARTICLE

The effect of dehydrogenase enzyme activity in glycolysis on the colour stability of mutton during postmortem storage

XIN Jian-zeng[1, 2], LI Zheng[2], LI Xin[2], LI Meng[2], WANG Ying[2], YANG Fu-min[1], ZHANG De-quan[2]

[1] *College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P.R.China*
[2] *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*

Abstract

This study investigated the influence of activities of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase-B (LDH-B) on the colour stability of mutton. From 60 sheep (Bayannur mutton sheep), 15 *longissimus dorsi* (LD) muscles were selected on the basis of colour stability (R630/580 and a* value) during the storage and classified into three groups (5 for each group) as high colour stability (HCS), intermediate colour stability (ICS) and low colour stability (LCS). The activities of GAPDH and LDH-B, muscle colour attributes, nicotinamide adenine dinucleuotide (NADH) concentration and lactate concentration were measured. The samples in HCS had higher activities of GAPDH and LDH-B than the samples in the LCS, and the samples in the HCS group also possessed higher NADH and lower lactate concentration. The higher activity of dehydrogenase enzyme may result in higher NADH concentrations and colour stability in muscle tissue. The results suggest that the activity of GAPDH and LDH-B may also play a role in maintaining colour stability.

Keywords: mutton, meat colour, GAPDH, LDH-B, NADH

1. Introduction

Meat colour is one of the most important quality attributes which influence consumer purchasing decisions, because consumers often consider the cherry-red colour of red meat as an indicator of wholesomeness at the point of sale (Joseph *et al*. 2012). Discolouration of fresh meat will lead to rejection by consumers and it is estimated that the United States meat industry incurs more than one billion dollars in lost opportunity due to discolouration every year (Joseph *et al*. 2012; Suman and Joseph 2013). The metmyoglobin reducing system is considered as the most important factor that maintains the stability of meat colour during postmortem storage (Ledward *et al*. 1985; Kim *et al*. 2009a). Nicotinamide adenine dinucleuotide (NADH) is the ultimate reducing equivalent for metmyoglobin reduction of enzymes and non-enzymes, which have been shown to be vital for delaying the discolouration in postmortem muscle (Kim *et al*. 2006; Ramanathan *et al*. 2011).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase B (LDH-B) are important enzymes in muscle glycolysis which play a key role in forming NADH and maintaining the equilibrium of the NADH pool in cytoplasm (Barron *et al*. 1998; Li *et al*. 2015). Kim *et al.* (2006, 2009a, b) reported that addition of lactate could enhance the colour stability of meat through the replenishment

Received 27 December, 2016 Accepted 9 March, 2017
XIN Jian-zeng, E-mail: jianzeng77@sina.com; Correspondence YANG Fu-min, E-mail: yfumin@163.com; ZHANG De-quan, E-mail: dequan_zhang0118@126.com

doi: 10.1016/S2095-3119(16)61622-2

of NADH catalyzed by LDH-B activity. In addition, the colour stability tends to be higher in bovine *longissimus lumborum* (LL) muscles compared with *semimembranosus* (SM), and *psoas major* (PM) muscles, due to higher LDH-B (Kim *et al*. 2006, 2009a, b). Based on the above studies, LDH-B activity and its substrates are important factors for colour stability of fresh muscle because of their role in the regeneration of NADH. In a previous study, glyceraldehyde-3-phosphate, the substrate of GAPDH, was shown to reduce the metmyoglobin in beef ground (Saleh and Watts 1968).

Studies of the effects of GAPDH activity on meat colour are limited, and it is not clear whether the activity of GAPDH in muscles influences muscle colour stability. We hypothesized that the activity of GAPDH in meat with different colour stabilities is inconsistent, the objectives of the present study were to determine the GAPDH activity and other biochemical characteristics of colour stability of *longissimus dorsis* (LD) muscles, and to evaluate the potential use of GAPDH for regulating the meat colour stability.

2. Materials and methods

2.1. Raw materials and preparation

Sixty male sheep (Bayannur mutton sheep, 8 months of age) with an average carcass weight of (24.74±2.05) kg from a commercial plant (Inner Mongolia Grassland Hongbao Food Co. Ltd., China) were slaughtered following the industrial practice. At 30 min postmortem, both loins (LDs) were collected (yielding 120 loins in total). Each muscle was divided into 4 steaks and placed on the styrofoam trays, and wrapped with oxygen-permeable polyvinylchloride (PVC, 35×300 type, Nantong South Asia Plastic Film Co., Ltd., Nantong, Jiangsu, China; oxygen transmission rate=25 mL m^{-2} 24 h^{-1} 0.1 MPa^{-1}) film. The sample trays were stored in an industrial refrigerator at (4±2)°C and were taken out for analysis after 2 h, 6 h, and 1, 2, 4, 6 and 8 d storage, respectively. One steak was used for determination of colour and pH during storage. The other seven steaks were frozen in liquid nitrogen, and then stored at –80°C until used for biochemical analysis.

2.2. Colour measurement

The colour of the samples wrapped with PVC was measured through the PVC film after storage at 4°C for 2 h, 6 h, 1, 2, 4, 6, and 8 d. Colour was measured using a Minolta CM-600d spectrophotometer (Konica Minolta SensingInc., Osaka, Japan) with specular reflectance excluded, 8 mm diameter measuring aperture, illuminant D65, 10° standard observer and Commission Internationale de L'Eclairage (CIE) L^*, a^*, b^* colour scale. The average value of four measurements on the meat surface was used. The spectrophotometer recorded reflectance values in the range of 360 to 740 nm at 10 nm intervals. Reflectance values that were not directly measured by the instrument at specific wave lengths (474, 525 and 572 nm) were calculated by integrations. The percentage of myoglobins was determined as described by Hunt and King (2012) using the formulas:

$$\text{Metmyoglobin\%}=(1.395-\frac{A_{572}-A_{700}}{A_{525}-A_{700}})\times 100$$

$$\text{Deoxymyoglobin\%}=(2.375-\frac{A_{473}-A_{700}}{A_{525}-A_{700}})\times 100$$

$$\text{Oxymyoglobin\%}=100\%-(\text{MMb\%}+\text{DMb\%})$$

Where, reflex attenuance (A)=log(1/R), A_{473}, A_{525}, A_{572} and A_{700} is the reflex attenuance at 473, 525 and 700 nm, respectively, and R is reflectance.

The ratio of reflectance at 630 and 580 nm (R630/580) was calculated as an indirect estimate of surface colour stability, a greater ratio indicates a lesser amount of metmyoglobin brown discolouration and thus higher colour stability.

The colour of steaks from sixty carcasses was ranked based on the R630/580 ratio and a^* value from d 4 to 8. From this ranking, five high colour-stable (HCS, R630/580, (2.74±0.13)), five intermediate colour-stable (ICS, R630/580, (2.21±0.22)) and five low colour-stable (LCS, R630/580, (1.98±0.18)) steaks were identified to examine the molecular basis of animal-to-animal variation in colour stability.

2.3. pH value

The pH was measured by inserting the glass calomel probe of the pH meter (Testo205 pH meter, Lenzkirch, Germany) directly into the raw sample. The device was calibrated with three buffers (pH 4, 7 and 10). All measurements were made in triplicate and an average was calculated.

2.4. GAPDH activity

GAPDH was extracted from samples using the method described by Baibai *et al*. (2007) with slight modifications. All steps were carried out on ice during the extraction. The muscle sample (approximately 2 g, fresh weight) was ground and homogenized using an IKA Ultra-Turrax homogenizer T10 basic S25 (IKA, Germany) in 25 mmol Tris-HCl buffer (pH 7.5), containing 5 mmol EDTA, and 10 mmol 2-mercaptoethanol at a ratio of 4 mL g^{-1} fresh tissue. The supernatant (soluble protein fraction) obtained after centrifugation at 15 000×g for 45 min was considered as the extracted enzyme.

The GAPDH activity of samples was measured with a ScienCell colourimetric GAPDH Assay Kit (Catalog # 8148). It was determined spectrophotometrically by monitoring NADH generation at 340 nm.