Food Chemistry 138 (2013) 96–100

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Apoptosis during postmortem conditioning and its relationship to duck meat quality

Muhan Zhang [a,1], Daoying Wang [a,1], Wei Huang [a], Fang Liu [a], Yongzhi Zhu [a], Weimin Xu [a,*], Jinxuan Cao [b]

[a] Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China
[b] College of Life Science and Biotechnology, Ningbo University, Ningbo 315211, PR China

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form 19 October 2012
Accepted 31 October 2012
Available online 10 November 2012

Keywords:
Apoptosis
Meat quality
Postmortem conditioning
Duck

ABSTRACT

The aim of this work was to examine the relationship of skeletal muscle apoptosis and postmortem development of meat quality. Colour, cooking loss, myofibril fragmentation index (MFI) and shear force of duck breast and thigh meat postmortem were measured, and changes of positive nuclei were assessed with Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphophate nick end-labelling method (TUNEL). Correlation analysis revealed that apoptosis were positively correlated with colour (L^*, a^* and b^*), cooking loss and MFI ($P < 0.05$), while it is negatively correlated with shear force ($P < 0.05$). Our results indicate the growing level of duck skeletal muscle cell apoptosis was associated with the postmortem development of meat quality traits such as meat colour, water holding capacity and tenderness.

1. Introduction

Colour, tenderness and water holding capacity of meat are important attributes for customer satisfaction that influences their buying decisions and perception of freshness of the meat product. Understanding the factors and mechanisms involved in the development of meat quality is a major concern for producers and customers. The major eating qualities of meat are developed during muscle ageing process and a number of complex biochemical reactions are engaged in this process with the influence of various factors (Ouali et al., 2006). Evidence has shown that the first step of the conversion of muscle to meat is the programmed cell death or apoptosis with its subsequent induced structural changes, and caspases are the main enzymes responsible for apoptosis (Ouali et al., 2007).

Apoptosis often happens with the cleavage of cytoskeleton proteins and cellular components, shrinkage of muscle cells and loss of membrane asymmetry etc., leading to the changes of meat quality traits (Ouali et al., 2007). However, there were quite a few studies that correlate the apoptosis with meat quality and the actual processes of meat quality development are far from being understood. Kemp, Bardsley, and Parr (2006) and Kemp, Parr, Bardsley, and Buttery (2006) found the caspase activities during postmortem conditioning were negatively correlated with shear force in porcine muscle. Cao et al. (2010) detected the apoptotic nuclei and caspase activities in bovine muscle, and implied the role of apoptosis and caspase on postmortem development of meat quality. The relationship of apoptosis and meat quality has been discussed in many reviews (Herrera-Mendez, Becila, Boudjella, & Ouali, 2006; Ouali et al., 2006, 2007). It was suggested that the effects of cell death on the cellular structures and proteins may explain some observations reported in postmortem meat (Ouali et al., 2006).

This study was designed to detect the postmortem apoptosis process in duck muscle and explore their relationship with postmortem meat quality changes such as colour, cooking loss, MFI and shear force during ageing process.

2. Materials and methods

2.1. Samples

Five ducks with ages from 4 to 5 months and weights approximately 2 kg were slaughtered following commercial practices in a processing plant ($n = 5$). The breast and thigh meat were removed from each duck carcasses immediately after exsanguination and trimmed of all subcutaneous fat and connective tissue to make up a breast and thigh meat sample respectively, and then packed individually in the polyester bags in the chiller at 4 °C. Two portions were cut from each breast and thigh meat samples at the sampling points of 0.5 h, 4 h, 8 h and 12 h after slaughter. A portion was stored individually at −20 °C for meat colour, cooking loss and shear force measurements, and a portion was frozen rapidly in liquid nitrogen for MFI and histological analysis. All measurements were performed in triplicate.

2.2. Meat quality measurements

The frozen samples were thawed overnight at 4 °C. The surface colour (CIE L^*, a^*, b^*) of duck breast and thigh meat samples were

* Corresponding author. Tel./fax: +86 25 84390065.
E-mail address: weiminxu2002@yahoo.com.cn (W. Xu).
[1] Both authors contributed equally to this work.

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.142

measured using a Chromameter (CR 400, Minolta, Japan). The chromameter was calibrated using a standard white ceramic tile before measuring each sample.

To measure the cooking loss of the samples, duck breast and thigh meat were weighed, and then placed individually in the polyester bags and boiled to a water bath temperature of 80 °C for 20 min (Rammouz, Babile, & Fernandez, 2004). Then the samples were cooled to the internal temperature of room temperature, surface dried and weighed again. Cooking loss was determined by expressing cooked sample weight as a percentage of precooked samples weight.

After measurements of cooking loss, the same muscles were then used for the determination of shear force. Shear force measurements were performed according to Cavitt, Meullenet, Xiong, and Owens (2005) using a texture analyzer (TVT-300XP, TexVol Instruments, Viken, Sweden) equipped with a razor blade with a height of 24 mm and a width of 8.9 mm. Muscle strips were cut across the fibre axis. The crosshead speed was set at 2 mm/s, and the test was triggered by a 10 g contact force.

Myofibril fragmentation index (MFI) was determined by the method of Hopkins with slight modifications (Hopkins, Martin, & Gilmour, 2004). Muscle tissue was pulverized in liquid nitrogen, and 0.5 g of powdered tissue was homogenised for 1 min in 30 mL 25 mM phosphate buffer (0.1 M Potassium Chloride, 1 mM EDTA, pH 7.0). The suspension was filtered to remove connective tissue, and residue was washed with 10 ml 25 mM phosphate buffer. Then filtrate was centrifuged at 1000*g* for 15 min at 4 °C, the precipitate was resuspended in 10 mL phosphate buffer and centrifuged again. This step was repeated twice more and the pellet was suspended in buffer solution. The protein concentration was diluted to 0.5 mg/mL and measured spectrophotometrically at 540 nm (UV 6100). MFI was calculated by multiplying readings with 150.

2.3. Detection of apoptotic nuclei

The assays were conducted as described by Cao et al. (2010). Muscle tissues from liquid nitrogen were cut into 10 μm sections with a cryostat, then immediately mounted onto slides. The sections were then fixed in 4% paraformaldehyde, blocked in 3% H_2O_2 in 100% methanol and immersed into 0.1% Triton X-100 and 0.1% sodium nitrate for 20 min. Subsequently they were washed with 1X PBS (137 mM Sodium Chloride, 2.7 mM Potassium Chloride, 4.3 mM Disodium Chloride, 1.4 mM Monopotassium Phosphate, pH 7.4). After drying, sections were blocked with goat antiserum for 30 min and washed with 1X PBS (137 mM Sodium Chloride, 2.7 mM Potassium Chloride, 4.3 mM Disodium Chloride, 1.4 mM Monopotassium Phosphate, pH 7.4) for 30 min. Then TUNEL reaction mix was added in a recommended 1:9 ratio, and the sections were incubated for 60 min at 37 °C in a humidified chamber in the dark. Negative control was incubated with label solution without terminal transferase instead of TUNEL reaction mix. Positive control was incubated with DNase I with the concentration of 5.1 unit/mL (Cat.# M6101, RQ1 RNAase-free DNAase, Promega) for 10 min at room temperature before adding TUNEL reaction mix. Samples were analysed in a drop of glycerol under a fluorescence microscope (Olympus IX71, Japan). Positive nuclei were counted on at least 3 different fields (×200) of each sample. Apoptosis was quantified by the number of positive nuclei per muscle cell.

2.4. Statistic analyses

Statistical analysis of the difference was performed using one-way analysis of variance (ANOVA) by SPSS 18.0. Significance among the groups was determined with Duncan's multiple range test. The significance level was settled at $P < 0.05$. Correlation coefficients were generated using the Pearson's Correlation Coefficient option of SPSS 18.0.

3. Results and discussion

At 0.5 h there was almost no positive nucleus in the meat sample, the number increased gradually with the extension of postmortem time as shown in TUNEL photograph (Figs. 4 and 5). The positive nuclei raised significantly from 0.5 to 8 h ($P < 0.01$), while it is not significant from 8 to 12 h. At 8 h postmortem, the positive nuclei in thigh meat was significantly higher than that of breast meat ($P < 0.01$). The increased positive nuclei were also associated with shrinkage of cells and reduction in cellular volume. Guignot, Vignon, and Monin (1993) found that extracellular space reached its maximum value approximately 10 h post-mortem. The ultimate pH of duck was reported to be reached at 12 h postmortem (Wang et al., 2010). It might be inferred that apoptosis reached its ultimate point approximately 8–12 h postmortem in duck muscle.

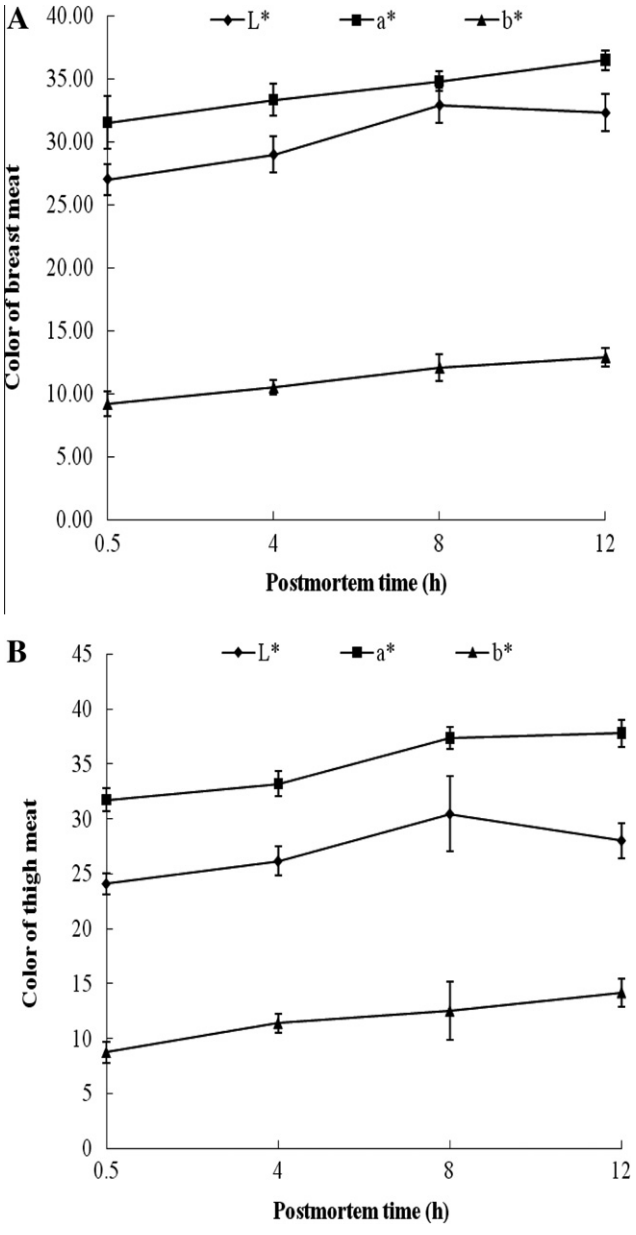

Fig. 1. CIE* colour of breast (A) and thigh (B) meat during postmortem ageing.