Apoptosis during postmortem conditioning and its relationship to duck meat quality

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A B S T R A C T
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-labeling 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.

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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 Burtley (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
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 poly-ester 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% H2O2 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 post-mortem 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.
These results were different from those in bull muscles, while the apoptotic nuclei increased from 0.5 h to 7 d and on the seventh day it was significantly different in three muscle types (Cao et al., 2010). Kemp, Bardsley et al. (2006) and Kemp, Parr et al. (2006) also reported the caspases levels in porcine muscles, and distinct in apoptosis rates in different fibre types. The apoptosis is determined by animal species and fibre types that the entire process of apoptosis can take hours or even days; however, there were few studies on apoptosis in poultry meat during ageing process. Our results showed the rapid apoptosis process in duck muscle from 0.5 h to 12 h, and it was associated with postmortem meat quality.

As shown in Fig. 1, lightness ($L^*$) of breast and thigh meat increased significantly from 0.5 h to 8 h ($P < 0.05$) and declined from 8 h to 12 h. Redness ($a^*$) of breast and thigh meat kept increasing from 0.5 to 12 h after postmortem ($P < 0.05$). Yellowness ($b^*$) elevated significantly from 0.5 to 8 h in both breast and thigh meat ($P < 0.05$), and no marked change was found afterwards.

Meat colour is an important quality trait of meat products; it is highly correlated with the metmyoglobin formation, muscle fibre type and water distribution (Froning, 1995; Livingston & Brown, 1981). As the oxygen from air comes into contact with the exposed meat surfaces, the myoglobin is oxygenated to oxy-myoglobin and gives meat a bright red colour. With the extension of storage time, oxymyoglobin is oxidised and changed to metmyoglobin and contribute to the decreased level of meat brightness (Bekhit & Faustman, 2005; McMillin, 2008; Renerre & Labas, 1987). Our results were in accordance with these processes that brightness of duck meat initially increased and then declined, while the redness consistently increased. It was demonstrated that mitochondrial electron transport chain and mitochondrial oxygen consumption played important roles in myoglobin ferrous form and meat colour. When cells undergo apoptosis, cyt C is released from mitochondrial and the electron transfer was interrupted, leading to the formation of brown pigment metmyoglobin (Lanari & Cassens, 1991; Tang, Faustman, Mancini, Seyfert, & Hunt, 2005, 2006).

The cooking loss of breast meat increased significantly in the range of 0.5–8 h ($P < 0.05$) (Fig. 2), while in thigh meat the cooking loss increased significantly from 0.5 h to 12 h ($P < 0.05$).

MFI of breast and thigh meat increased significantly in 12 h postmortem ($P < 0.05$) (Fig. 3). The shear force of both breast and thigh meat went down during 12 h postmortem ($P < 0.05$), and the shear force of breast meat is significantly lower than that of thigh meat ($P < 0.05$).

The correlation of cooking loss, MFI, and shear force with apoptosis is shown in Table 1. The apoptotic nuclei between 0.5 h and 12 h were positively correlated with meat colour, cooking loss and MFI, and negatively correlated with shear force in both breast and thigh meat.

MFI is a useful indicator of the extent of proteolysis indicating both rupture of the I-band and breakage of intermyofibrils linkages (Volpelli, Failla, & Sepulcri, 2005; Volpelli, Valusso, & Morgante, 2003). Meat tenderization during postmortem ageing is mainly enzymatic and involves proteolytic process. Evidence has shown the proceeding of apoptosis process was associated with increased caspase activation and expression levels, which acted on skeletal proteins, consequently the MFI was increased and tenderness was improved (Adams, Gielen, Hambrecht, & Schuler, 2001; Kemp, Parr et al., 2006; Liu & Ahearn, 2001; Liu, Lyon, Windhan, Lyon, &
Savage, 2004). Caspases could also have a role in tenderization by modulating the calpain system, largely through cleaving the calpain specific inhibitor calpastatin (Porn-Ares, Samali, & Orrenius, 1998). Laville et al. (2009) studied the proteome changes during meat ageing in tough and tender beef and suggested apoptosis was important for beef ageing and tenderization.

Proteolysis could also result in muscle cell shrinkage and mobilisation of water to the extracellular space, giving rise to the variation in water holding capacity. Trump and Berezesky (1995) proposed that apoptosis is very important for the determination of water holding capacity because apoptotic cells dissociate from others. It was generally believed that the decrease of muscle pH postmortem led to the decreased protein charges and reduced water holding capacity, however, it was unexplained that the extracellular space was increased immediately after slaughter while pH was stable. The rapid apoptosis process may provide the explanation of the early changes of water holding capacity (Ouali et al., 2007).

Our study demonstrated that apoptosis occurred promptly after slaughter and it was highly correlated with meat colour, water holding capacity and tenderness. Between 8 h and 12 h, the apoptosis slowed down and meat quality did not significantly change, suggesting the ageing process was accomplished. This work is an attempt to establish a link between apoptosis and meat quality during duck meat ageing process; further efforts are required for

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Fig. 4. TUNEL photographs of apoptotic nuclei in breast (A–D), thigh (E–H) muscles and negative control (I).

Fig. 5. Time course of apoptosis in breast and thigh muscles during ageing at 0.5–12 h postmortem shown as counts of positive nuclei per muscle fibre.
the mediators of apoptosis process and subsequent meat quality improvements.

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References


Table 1
Correlation coefficients (r) and probabilities of significance (P) between apoptosis and meat quality parameters.

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Cooking loss</th>
<th>MFI</th>
<th>Shear force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis of breast meat</td>
<td>0.800*</td>
<td>0.847*</td>
<td>0.878**</td>
<td>0.556*</td>
<td>0.683**</td>
<td>-0.784**</td>
</tr>
<tr>
<td>Apoptosis of thigh meat</td>
<td>0.781**</td>
<td>0.851*</td>
<td>0.568**</td>
<td>0.848**</td>
<td>0.629**</td>
<td>-0.653**</td>
</tr>
</tbody>
</table>

* P < 0.05.
** P < 0.001.

The table shows the correlation coefficients (r) and probabilities of significance (P) between apoptosis and meat quality parameters. The data indicate a strong positive correlation between apoptosis and meat quality, suggesting that apoptosis may be a mediator of meat quality improvements.