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The effect of $CaCl_2$ marination on the tenderizing pathway of goose meat during conditioning

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ABSTRACT

In order to figure out the effect of $CaCl_2$ on the tenderizing pathway of goose meat, breast muscles of thirty-two Eastern Zhejiang White Geese were divided into three treatments: the control, 150 and 300 mM $CaCl_2$. Shear force, myofibrillar fraction index (MFI), actin filaments and F-actin, G-actin and tropomodulins (Tmods) levels were investigated during 168 h. Results showed that 300 mM treatment had lower shear force at 48, 96 and 168 h and higher MFI at 24, 48, 96 and 168 h than the control. The rate of actin filaments disruption, the decrease of F-actin, the degradation of Tmods, the increase of G-actin in 300 mM treatment was faster than 150 mM treatment; the rate in the control was the slowest among treatments. $CaCl_2$ accelerated the transformation of F-actin into G-actin. We concluded that $CaCl_2$ tenderized goose meat by depolymerizing actin filaments and cleaving Tmods.

1. Introduction

The palatability of meat is influenced by flavor, juiciness, tenderness, cooking temperature and consumers' preferences (Bowers, Craig, Kropf, & Tucker, 1987; Christensen et al., 2012; Gagaoua et al., 2016). Among them, tenderness is regarded as the most important meat quality trait (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). A large number of studies have shown that calcium ion treatments had positive effect on meat tenderization during conditioning by the following mechanisms: one is that high concentration of calcium ion as a crucial signal factor leads to activation of calpain, which resulted in the degradation of cytoskeletal protein (Geesink & Koohmaraie, 1999; Ouali et al., 2013; Sentandreu, Coulis, & Ouali, 2002); the other one is that the direct effect of calcium ion strength, including repulsion between electric charge, leads to the mechanical fracture of the *Z*-disk (Gerelt, Ikeuchi, Nishiumi, & Suzuki, 2002). The degradation of these structural proteins such as titin, nebulin and desmin was related to the loss of myofibrillar integrity, which improved meat tenderness (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995).

However, cytoskeletal proteins such as titin, nebulin and desmin account for limited percentage of the myofibril, while the main body parts of the myofibril included the thick filament composed of myosin and the thin filament composed of actin (Clark, Mcelhinny, Beckerle, & Gregorio, 2002). Based on that, we assumed that the disorder of actin filament by calcium ion could be one of the most direct reasons of meat tenderization. Actin exists as a dynamic equilibrium mixture of monomeric globular actin (G-actin) and filamentous actin (F-actin) in muscle cells (Pollard & Cooper, 2009). The *Z*-disc had function to stable actin filament during contraction (Sequeira, Nijenkamp, Regan, & Velden, 2014). The disruption of *Z*-disk integrity by calcium ion during conditioning is possible to cause the depolymerization of actin filament. In our previous study, we demonstrated that the direct depolymerizing progress of actin filaments was correlated with myofibrillar fraction positively (Zhou, Wang, Pan, Sun, & Cao, 2016). Up to now, the possible involvement of calcium ion in the tenderization by the depolymerization of F-actin has not been evaluated.

It was reported that calcium ion activated intra-cellular calpains in poultry meat (Chang & Chou, 2012). Tropomodulins (Tmods) was a potential substrate of calpains in muscle tissue (Gokhin, Tierney, Sui, Sacco, & Fowler, 2014). Almenarqueralt, Lee, Conley, Ribas, and Fowler (1999) indicated that Tmods had a key role in the stability of actin filaments as their pointed end capping proteins. It was not known that whether calcium ion could weaken actin filaments by hydrolyzing Tmods. No literature is available on the mechanism of depolymerization of actin filaments after $CaCl_2$ marination during postmortem conditioning.

In order to clarify the possible pathway of calcium ion in the tenderization by the depolymerization of F-actin, the present study was to evaluate the influence of $CaCl_2$ treatments on the change of shear force, myofibrillar fraction and Tmods levels, the actin filaments morphology, and the equilibrium of G-actin and F-actin contents during

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conditioning.

2. Materials and methods

2.1. Treatments and sampling

Thirty-two Eastern Zhejiang White male Geese of 75 days old with living weight 4205 ± 85 g were slaughtered by cutting off the neck from a local processing plant with no stress according to the permission of Ningbo University Animal Welfare Committee. After 15 min of animal exsanguinations, 46 geese breast muscles were cut into 184 strips (2 × 1 × 1 cm for each) parallel to fiber direction (every breast muscle almost was cut in 4 strips); 18 geese breast muscles were cut into 72 cubes (0.8 cm^3) parallel to fiber direction (every breast muscle almost was cut in 4 cubes). 72 cubes and 184 strips were cut from 64 breast muscles of 32 geese. The 60 cubes and 180 strips with standard sizes, straight fiber and visible connective tissue were selected from all of cubes and strips and then divided into three equal groups randomly. 20 cubes and 60 strips for each group. Three groups were treated with distilled water, 150 and 300 mM $CaCl_2$ respectively (Koohmaraie, Crouse, & Mersmann, 1989). One group was marinated in distilled water at 4 °C for 0.5 h as the control. The other two groups were marinated in 150 and 300 mM $CaCl_2$ (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at 4 °C for 0.5 h as 150 and 300 mM $CaCl_2$ treatment, respectively. At the end of marination, 5 cubes and 10 strips from each treatment were drained and obtained immediately as the defined samples at 0 h. Then, the rest of cubes and strips were drained, transferred to plastic trays, enclosed with a low-density polyethylene plastic film (oxygen, carbon dioxide and water vapor transmission rates of the film were 14.483 cm^3 / (m^2 × 24 h × atm), 63.683 cm^3 / (m^2 × 24 h × atm) and 54 g / (m^2 × 24 h), respectively) and stored at 4 °C for 168 h. At each stored point (8, 24, 48, 96 and 168 h), 10 strips from each group were taken immediately. 5 shear force samples (1 × 1 × 1 cm) were made from 10 strips parallel to fiber direction in each of point in three treatments and stored at − 25 °C; the rest of 5 strips in each point in three treatments were taken immediately and frozen in liquid nitrogen and stored at − 80 °C until being used for F-actin, G-actin, Tmods and myofibril fragmentation index determination. The 5 cubes of each group at 0, 24, 96 and 168 h were also taken and frozen in liquid nitrogen for cryosections respectively and stored at − 80 °C until being analyzed. An explanatory graph of the sampling method was shown in Supplementary.

2.2. Shear force measurement

Shear force measurement was done as described by Marino et al. (2013). Briefly, the fiber direction of the strips used for analysis was parallel to the direction of the muscle fibers. Frozen strips were individually placed in plastic bags and cooked in a water bath at 85 °C until the internal temperature of samples reached 75 °C with a K-thermocouple (Shanghai Xinghua Instrument Factory, China). The peak force values (kg) were measured by placing the cooked samples perpendicular to the longitudinal axis of the muscle fibers under Warner-Bratzler shear blade on XL1155 equipment (Xielikeji Co. Ltd., Herbin, China). Each sample was sheared perpendicular to the fiber at 100 mm/min cross-head speed. The average peak force values of the 5 measurements for each sample were considered as shear force values.

2.3. The measurement of MFI

The measurement of MFI was done based on a standard protocols as described previously by Hopkins, Martin, and Gilmour (2004) with slight modification. In our protocols, the centrifuge time of filtrates was set as 15 min according to a study in chicken (Wilhelm, Maganhini, Hernández-Blazquez, Ida, & Shimokomaki, 2010). Samples (0.5 g) avoiding any visible fat or connective tissue were taken and homogenized in 10 mL of ice cold buffer (0.1 M KCl, 1 mM EDTA, 1 mM sodium azide (NaN_3), 7 mM KH_2PO_4 and 18 mM K_2HPO_4, pH 7.0) at 4 °C with a DY89-I high speed homogenizer (Scientzco., Ningbo, China) for 3 × 10 s at 10,000 rpm. After the homogenate was completed, the myofibrillar suspensions were filtered through mesh strainers (Culina, UK) with 1 mm^2 holes into the 50 mL centrifuge tubes to remove the connective tissue. The suspensions with 10 mL of cold buffer were filtrated through the mesh strainers with 1 mm^2 holes again. The filtrates were centrifuged with a refrigerated centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Changsha, China) at 1000 g for 20 min at 4 °C. The precipitation of myofibril was resuspended in a cold 10 mL buffer, vortexed and centrifuged again. This process was repeated twice and the precipitation finally resuspended in a cold 10 mL buffer. A bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, MA) was used to determine the protein content of supernatant. The absorption was measured at 562 nm using with a 96-Well Plate Reader M200 (Tecan, Austria). A bovine serum albumin standard curve was used. Suspensions were diluted with buffer to a final protein concentration of 0.5 mg/mL. The diluted suspensions were poured into a cuvette and mixed. The absorbance measured immediately at 540 nm using a 96-Well Plate Reader M200. The mean of five absorbance readings was multiplied by 150 which was the MFI.

2.4. Morphological analysis for actin filaments

The histology analysis was done as described previously by Gurniak et al. (2014) with slight modification. Each cube was cut into 8 μm sections with a Leica Frigicut cryostat (Leica, Nussloch, Germany). The slices were attached to Apes coated slides. The cryosections were fixed in 4% paraformaldehyde with 10 mM PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) at room temperature for 1 h. Then the cryosections were washed in 10 mM PBS for 15 min. Then they were permeabilized with 0.2% Triton X-100 (Beijing Sdarbio Science & Technology Co., Ltd., Beijing, China) in 10 mM PBS for 7 min and washed in 10 mM PBS for 15 min again. The cryosections were incubated in 0.1 μM FITC-phalloidin (Molecular Probes, cytoskeleton, Inc) for 1 h and washed in 10 mM PBS for 15 min. Actin filaments were observed by IX71 fluorescence microscope (Olympus, Japan).

2.5. Extraction and determination of F-actin

The F-actin content was determined as described originally by Cano, Lauffenburger, and Zigmond (1991), but modified by Zhou et al. (2016). Briefly, 0.5 g of samples were homogenized in 5 mL of lysis buffer by DY89-I high speed homogenizer (Scientz co., Ningbo, China) for 3 × 10 s at 10,000 rpm while cooled in ice. The homogenate was incubated in 200 μL of 0.6 μM FITC-phalloidin at 4 °C for 2 h. The samples were centrifuged at 80,000 g for 60 min at 4 °C in ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). The pellet was extracted in 1 mL of methanol for 24 h. The fluorescence of extracted solution was continuously monitored at 540 nm as excitation wavelengths and at 575 nm as emission wavelengths by using a 96-Well Black Plate Reader M200 (Tecan, Austria) at room temperature. The intensity of fluorescence per-gram of muscle tissue was expressed as arbitrary unit (a.u.).

2.6. Extraction of G-actin

The G-actin was extracted as described originally by Cano et al. (1991), but modified by Zhou et al. (2016). Briefly, 1.0 g of muscle samples were homogenized with 8 mL of lysis buffer by a DY89-I high speed homogenizer (Scientz co., Ningbo, China) at 10,000 rpm for 3 × 10 s while cooled in ice. The homogenate was centrifuged at 6000 g for 20 min at 4 °C with a refrigerated centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Changsha, China). Suspensions were diluted with lysis buffer to the same protein concentration

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