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Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle

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ABSTRACT

Changes in Warner–Bratzler shear force, cooking loss, protein solubility and microstructure of duck breast muscle cooked to 40, 50, 60, 70, 80, 90 and 95 °C were determined. Warner–Bratzler shear force was found to increase in two separate phases from internal temperature of 40–50 °C and again from 60 to 95 °C ($P < 0.05$), with a decrease from 50 to 60 °C. With increasing internal temperature cooking loss gradually increased while protein solubility significantly decreased ($P < 0.05$). Two significant decreases in fiber diameter were observed in samples cooked to internal temperatures of 40–50 °C ($P < 0.01$) and 70–80 °C ($P < 0.01$). The sarcomere length decreased for the most part with increased internal temperature from ambient (raw) to 95 °C, but with a noticeable increase between 50 and 60 °C ($P < 0.01$). It was found that the amount of protein solubility and shrinkage of sarcomere significantly correlated with tenderness of duck meat during cooking process.

1. Introduction

Tenderness is thought to be the most important eating quality characteristic (Barbanti & Pasquini, 2005; Caine, Aalhus, Best, Dugan, & Jeremiah, 2003; Destefanis, Brugiapaglia, Barge, & Dal Molin, 2008; Mutungi, Purslow, & Warkup, 1995; Van Oeckel, Warnants, & Boucqui, 1999; Voges et al., 2007), and the most important factor dictating poultry meat quality and consumer acceptability (Lee, Owens, & Meullenet, 2009). Tenderness can be determined by a trained panel or physical methods, with Warner–Bratzler (W–B) shear force being the most widely used method (Combes, Lepetit, Darche, & Lebas, 2004).

Cooking of meat products is essential to achieve a palatable and safe product (Tornberg, 2005), and cooking has a large effect on meat toughness (Christensen, Purslow, & Larsen, 2000). Thermal processing in meat and poultry strongly influences texture, protein changes and cooking yield (Wattanachant, Benjakul, & Ledward, 2005). Davey and Gilbert (1974) defined cooking as the heating of meat to a sufficiently high temperature to denature proteins. Mechanical properties of meat are known to be affected by the myofibrillar, sarcoplasmic and connective tissue proteins (mainly collagen). During heating process, different meat proteins denature, and they cause structural changes in the meat, such as the destruction of cell membranes, shrinkage of meat fibers, aggregation and gel formation of myofibrillar and sarcoplasmic proteins and shrinkage and solubilize of the connective tissue (Tornberg, 2005).

Electron microscope is useful in revealing the details of the structural changes of muscles subjected to a variety of treatments (Palka & Daun, 1999). Electron microscope offers a direct view of how meat structure changes when cooked to different internal temperatures. Muscle fiber diameter and sarcomere length, as observed with scanning electron microscopy (SEM) and transmission electron microscopy (TEM), are closely related to flesh firmness (Kong, Tang, Lin, & Rasco, 2008; Palka, 1999; Palka & Daun, 1999; Wattanachant et al., 2005).

Factors affecting meat tenderness during heating have been investigated by many researchers. It is widely accepted that heat solubilizes collagen (connective tissue) that results in tenderization, whereas heat denatures myofibrillar proteins that results in toughening. These heat-induced changes are time and temperature dependent, and the net effect of this toughening or tenderization depends on cooking conditions (Obuz, Dikeman, & Loughin, 2003).

Cooked duck products are well accepted by consumers in China and Southeast Asia due to their delicate flavor and texture (Xu, Xu, Zhou, Wang, & Li, 2008). In Nanjing city alone, about thirty million ducks are consumed annually (Liu, Xu, & Zhou, 2007), and the consumption is still increasing. To our knowledge, the influence of final cooked temperature on tenderness, the solubility of proteins and microstructure of duck meat are unknown. Therefore the

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objective of this study was to evaluate the changes in tenderness of duck breast muscle during cooking, and to determine the solubility of major muscle proteins and microstructural changes on the textural properties.

2. Materials and methods

2.1. Preparation of samples

A total of 48 skinless, de-boned breast fillets (pectoralis major) of Cherry Valley ducks were obtained from a local continuous processing plant after slaughter. Samples were weighted, subsequently placed into plastic bags individually and vacuum-sealed, packed on ice, and transported to the laboratory. The average weight of breast fillet was 116 ± 12 g. Breast fillets were subjected to 24 h aging in a refrigerator (4 ± 1 °C) prior to further study. Samples were cooked in plastic bags individually in a water bath kettle set at 100 °C. The samples were stopped cooking when the internal meat temperature reached desired final temperature (40, 50, 60, 70, 80, 90, 95 °C). Internal meat temperature was monitored throughout the cooking process with a thermocouple that was inserted into the geometric center of a meat sample. After cooking, the cooked meat samples were cooled in tap water and blotted with filter papers; then the cooking loss, Warner–Bratzler shear force and microstructure were measured. Samples for protein solubility and soluble collagen were stored at −20 °C until being analyzed.

2.2. Warner–Bratzler shear force

Six rectangular shaped samples (1 × 1 × 5 cm) were removed from the raw and all cooked samples parallel to the muscle fiber. Samples were analyzed on a TA-XT2 texture analyzer (Stable Micro Systems, Godalming, UK) with a Warner–Bratzler blade (code HDP/BS, Stable Micro Systems). Shear force was measured perpendicular to the axis of muscle fibers in six replicates for each treatment. The instrument settings were: pre-test speed: 2 mm/s; test speed 2 mm/s; post test speed: 10 mm/s; distance: 30 mm; trigger force: 10 g. The results were expressed in kg.

2.3. Cooking loss

Cooking losses were calculated from differences in the weight of muscle samples before and after cooking process.

2.4. Protein solubility

The solubility of sarcoplasmic and total (sarcoplasmic and myofibrillar) proteins of raw and cooked meat samples were determined according to the procedure of Joo, Kauffman, Kim, and Park (1999) with slight modification. To extract the sarcoplasmic protein, 1 g sample was minced and homogenized in 10 mL of ice-cold 0.025 mol/L potassium phosphate buffer (pH 7.2) with a ULTRA-TURRAX (IKA, Deutschland, Germany) setting at 6500 rpm. The sample was then left on a shaker at 4 °C for 12 h before centrifuged at 1500 × g for 20 min. Protein concentration in the supernatant was determined by the Biuret method (Slater, 1986, p. 296). To extract total soluble protein, a 0.5 g meat sample was homogenized in 10 mL ice-cold 1.1 mol/L potassium iodide in 0.1 mol/L phosphate buffer (pH 7.2). The same procedures for homogenization, shaking, centrifugation, and protein determination were used as described above. Myofibrillar protein concentrations were obtained by difference between total and sarcoplasmic protein solubility. The protein solubility was expressed as a percentage of the soluble protein in the sample (w/w, wet basis).

2.5. Collagen solubility

Soluble collagen was extracted according to the method of Kong et al. (2008) and Palka (1999) with some modifications. Two grams muscle samples were homogenized with 6 mL of Ringer's solution (32.8 mmol/L, NaCl, 1.5 mmol/L KCl and 0.5 mmol/L $CaCl_2$). The homogenates were heated at 77 °C for 70 min, and centrifuged (4000 × g, 30 min). The sediments were mixed with 4 mL of Ringer's solution and centrifuged again. The supernatant solutions were combined. The sediments and supernatants were hydrolyzed with 6 mol/L HCl at 110 °C for 24 h. The hydroxyproline content in the hydrolysate was analyzed by the method of Bergman and Loxley (1963) and converted to collagen content using the factor of 7.25 (Liu, Nishimura, & Takahashi, 1996). Total collagen content was the sum of the collagen content in the sediment and supernatant. Soluble collagen was expressed as percent of the total collagen.

2.6. Microstructural analysis

Samples from raw and cooked duck muscle were selected for microstructure examination using SEM and TEM.

The procedure for SEM analysis was as follows. Pieces (1 × 1 × 0.5 cm) were excised from raw and cooked muscle samples and fixed in 2.5 mL/100 mL glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3) at room temperature. The specimens were then rinsed with 0.1 mol/L phosphate buffer (pH 7.3) and dehydrated in 50, 70, 80, and 90 mL/100 mL ethanol, respectively for 15 min in each solution and 30 min in absolute ethanol three times. The specimens were freeze-dried and mounted on aluminum stubs and coated with gold for examination and photographing using a SEM. The micrographs were taken at magnification of 150×, 500×, and 2500× to examine overall microstructural change, perimysium and endomysium connective tissue, respectively. Four micrographs at magnification of 1000× were also taken from each sample to determine the fiber diameter. Procedure for TEM analysis was as follows. Pieces (1 × 1 × 2 mm) were excised from raw and cooked muscle samples and fixed in 2.5 mL/100 mL glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.3) followed by a secondary fixation with 20 g/L osmium tetroxide at room temperature. The specimens were then dehydrated in 50, 70 80, and 90 mL/100 mL ethanol, respectively for 15 min in each solution and 30 min in absolute ethanol three times. The samples were embedded in epoxy resin (Durcupan) and the resin was allowed to cure at 70 °C for 24–48 h. The cured resin blocks were cut in an ultra cut ultra-microtome. The ultra cuts were stained using a solution of 4 mL/100 mL uranyl acetate in ethanol for 10 min followed by an aqueous solution of Reynolds' lead (7 min). Four micrographs at magnification of 2500× were taken for each sample. The sarcomere length was determined from the micrographs.

2.7. Statistical analysis

Data were evaluated statistically as a one-way ANOVA using the SPSS 18.0. The significant differences between heat treatment means were analyzed by Duncan's multiple range tests. The Pearson correlation coefficient was estimated for variables.

3. Results and discussion

3.1. Changes in Warner–Bratzler shear force

Changes in the Warner–Bratzler (W–B) shear force value of duck breast muscle with final cooked temperature are shown in Table 1. Toughness increased in two separated phases from internal meat temperature of 40–50 °C and again from internal meat temperature of 60–95 °C ($P < 0.05$) with a decrease of W–B shear