

CHAPTER 2

MATERIALS AND METHODS

1. Study on quality characteristics of Thai indigenous chicken muscles as influenced by age and rearing system

1.1 Raw materials

Mix-sex Thai indigenous chickens (Kaidang, *Gallus domesticus*) aged 16, 18 and 20 weeks raised under the intensive farming system and extensive farming system which life weights 1.8-2.0 kg were obtained 20 birds of each age from farm in Department of Animal Science, Faculty of Natural Resources, Prince of Songkla University. All chickens were killed by the method mentioned in Wattanachant *et al.* (2004) by conventional neck cut, bled for 2 min, scalded at 60°C for 2 min, plucked in a rotary-drum picker for 30 s and eviscerated. *Pectoralis major* and *biceps femoris* muscles were dissected from the carcasses after chilling at 4°C for 24 h. The skin was removed and the muscles were trimmed of obvious fat and connective tissue. Muscle samples from each of 10 birds of each age were stored at 4°C within 2 days for cooking loss, colour, shear force values and muscle structure determinations. The muscle samples from each of 10 birds were minced, placed in plastic bags and stored frozen (-20°C) until used for chemical analysis.

1.2 Physical analyses

1.2.1 Determination of cooking loss

Cooking loss was calculated from differences in the weight of raw and cooked muscle strips (Wattanachant *et al.*, 2005a).

1.2.2 Determination of colour

The colour of muscles in the anterior and posterior locations was determined using a Hunterlab colourimeter and reported as the complete International Commission on Illumination (CIE) system colour profile of L*, redness (a*) and yellowness (b*) (Wattanachant *et al.*, 2005a).

1.2.3 Shear force analysis

Muscle samples, raw and cooked were cut to size of 1.0 x 2.0 x 0.5 cm for shear analysis using the Texture Analyzer (TA-XT2i, Stable MicroSystem, Godalming, Surrey UK.) equipped with Warner-Bratzer shear apparatus (Wattanachant *et al.*, 2004). The operating parameters consisted of a cross-head speed of 2 mm/s and a 25-kg load cell. The shear force perpendicular to the axis of muscle fibers was measured. The peak of the shear force profile was regarded as the shear force value.

1.2.4 Thermal denaturation

Thermal denaturation of muscle proteins was studied using Differential Scanning Colorimetry (DSC). The samples (15-20 mg) were accurately weighed into aluminum pans and sealed. The samples were scanned at 10°C/min over the range of 20-100°C using ice water as the cooling medium. The empty pan was used as the reference. Total denaturation enthalpy (ΔH) was calculated by measuring the area in the DSC thermogram. The maximum transition temperature (T_{max}) was estimated from the thermogram.

1.3 Microstructure of muscle

The microstructure of muscle samples was determined using a scanning electron microscope (SEM) according to Wattanachant *et al.* (2005b) which modified method of Palka and Daun (1999). Pieces (1 x 1 x 0.5 cm) were excised from *pectoralis major* and *biceps femoris* muscles and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 2 h at room temperature. The specimens were then rinsed with distilled water and dehydrated in a serial solution of 25%, 50%, 70%, 95% and absolute ethanol (twice), for 1 h in each solution. The samples were cut in liquid nitrogen using a razor blade. The fragments of dried specimens were mounted on aluminum stubs and coated with gold. The specimens were examined and photographed in a JSM 5200 scanning electron microscope (JEOL, Ltd., Akishima, Japan). The micrographs and video prints were taken at magnification of 500x for transverse sections and 10,000x for longitudinal ones. The area of muscle fibres and the length of sarcomeres were measured in video prints, using a special morphometric facility. Three videoprints from each sample were taken for transverse sections and 10 measurements of fibre area on each were made (n = 30). The fibre diameter was calculated from the fibre area. Three videoprints from each

sample were taken for longitudinal sections and 10 measurements of sarcomere length on each were made (n=30).

1.4 Chemical analyses

1.4.1 Proximate analysis

Pectoralis major and *biceps femoris* were determined for moisture, protein, ash and fat contents according to AOAC method (AOAC, 1999). The values were expressed as percentage (wet weight basis).

1.4.2 Determination of total collagen content

Analysis for total collagen and soluble collagen were conducted as described by Liu *et al.* (1996). Finely ground muscle (1 g) was hydrolyzed with 10 ml of 6 M HCl at 110°C for 24 h. The hydrolysate was clarified with activated carbon, filter, neutralized with 10 M and 1 M NaOH and diluted with distilled water to a final volume of 100 ml. The hydroxyproline content in the hydrolysate was determined by the procedure of Bergman and Loxley (1963) and convert to collagen content using the factor 7.25. The collagen content was expressed as milligrams of collagen per gram of muscle. Soluble collagen was extracted according to the method of Liu *et al.* (1996).

1.4.3 Determination of soluble collagen content

Muscle sample (2 g) was homogenized with 8 ml of 25% Ringer's solution. The homogenates were heated at 77°C for 70 min and centrifuged at 2,300 x g at 4°C for 30 min. The extraction was repeated twice. Supernatants were pooled. The sediments and supernatants were hydrolyzed with 6 M HCl at 110°C for 24 h. The collagen content of the sediments and supernatant were determined as described previously. The amount of heat-soluble collagen was expressed as a percentage of the total collagen (collagen content in sediment plus that in the supernatant).

2. Effect of heating under Tom Yum soup condition on chemical composition and physical properties of Thai indigenous chicken, spent hen and broiler

2.1 Material and preparation

2.1.1 Raw materials

Thai indigenous chickens (Kaidang, *Gallus domesticus*) selected the chicken age provided the most toughness and low soluble collagen in muscle from the result of Part 1, spent hen (*H&M Brown Nick*) aged 52 weeks and commercial broiler (*CP707*) aged 38 days of similar life weight (1.8 - 2.0 kg) were obtained 30 birds of each birds of each from farm in the Department of Animal Science. All chickens were killed and prepared as mentioned in item 1.1 Part 1.

2.1.2 Tom Yum soup preparation

Tom Yum soup was prepared according to the method of Siripongvutikorn (2004). Its were consisted of lemon grass, galangal, red onion, garlic, kaffir lime leave, chili, coriander root, garcinia, salt and sugar. The acid condition of the soup was performed by dried garcinia. All ingredients were minced and boiled in water for 5 min. The soup was filtered through thin layer cloth to separate the solid parts. The pH of Tom Yum soup was in range 2.8-3.0.

2.1.3 Cooking under Tom Yum soup condition

Pectoralis major and *biceps femoris* muscle samples of all chickens were cut to size of 1.5 x 3.0 x 0.5 cm and precooked in water at 80°C for 10 min. The precooked muscles were weighed and cooked in Tom Yum soup with the ratio 1:2 at 95°C for 20 min. The muscles in Tom Yum soup were cooled to room temperature and kept at 4°C for 24 h before physical and chemical properties analysis. The influence of Tom Yum soup on chicken muscles was studied comparatively to cooking in water and acid solution (0.1 % citric acid, pH 2.8).

2.1.4 Thermal processing under Tom Yum soup condition

Pectoralis major and *biceps femoris* muscle of the most toughness with low soluble collagen chicken selected from item 2.1.3 were prepared and precooked as mentioned above in 2.1.3. The precooked muscles were weighted and packed in retort pouch size 8.5 x 15 cm (10 pieces per pouch). Tom Yum soup was filled in the ratio 1:2 (w/w) for chicken muscle per soup. The pouch of samples was sealed and processed in over pressure steam retort (FMC Food

Tech, German) at 116°C and 121°C to an F_0 value of 6.0. The samples were cooled to room temperature and kept for 24 h before analyses.

2.2 Physical Analyses

2.2.1 Determination of cooking loss

Cooking losses were determined as mentioned in Part 1 item 1.2.1.

2.2.2 Determination of colour

The colour of muscles in the anterior and posterior locations was determined as mentioned in Part 1 item 1.2.2

2.2.3 Shear force analysis

Muscle samples, raw and cooked, were determined as mentioned in Part 1 item 1.2.3.

2.2.4 Thermal denaturation

Thermal denaturation of raw muscle proteins were determined as mentioned in Part 1 item 1.2.4.

2.3 Microstructure of muscle

The microstructure of muscle samples was determined as mentioned in Part 1 item 1.3.

2.4 Chemical analyses

2.4.1 pH

The pH of raw and cooked chicken muscles, Tom Yum soup and cut out pH of product were determined. The pH of muscles was determined by homogenizing the muscle sample with distilled water at the ratio 1:5 (w/v). The homogenate was subjected to pH measurement using pH meter.

2.4.2 Proximate composition of raw muscle

Pectoralis major and *biceps femoris* muscle of all chickens were determined as mentioned in Part 1 item 1.4.1.

2.4.3 Total collagen and soluble collagen content

Analysis for total collagen and soluble collagen were determined as mentioned in Part 1 item 1.4.2 and 1.4.3.

2.5 Sensory evaluation

Sensory evaluation was carried out by thirty panelists comprising post-graduate students and technicians from the Department of Food Technology, Prince of Songkla University. The panelists evaluated the preferences in colour, toughness and juiciness of each sample using a nine-point hedonic scale, ranging from “1- dislike extremely” to “9 like extremely”.

3. Study on quality changes of thermal processed Tom Yum spent hen muscles during storage

3.1 Material and preparation

Pectoralis major and *biceps femoris* muscle of spent hen were prepared, precooked and cooked at 116°C to obtain F_0 value of 6.0 as mention in above 2.1.3 and 4. The samples were cooled to room temperature and kept at room temperature (25-30°C) for six months. During storage, Tom Yum spent hen were randomly sample at 0, 0.5, 1, 2, 4 and 6 months, respectively for analyses.

3.2 Physical analyses

3.2.1 Determination of weight loss

Samples were drained for 2 min before weighing the muscles. Weight losses were evaluated as precooked muscle before thermal process and after thermal process until reaching storage time.

3.2.2 Determination of colour

The colour of muscles in the anterior and posterior locations was determined as mentioned in Part 1 item 1.2.2.

3.2.3 Shear force analysis

Muscle samples in Tom Yum soup were determined as mentioned in Part 1 item 1.2.3.

3.2.4 Fiber diameter

The microstructure of muscle samples was determined using a scanning electron microscope (SEM) according to Wattanachant *et al.* (2005b) which modified method of Palka and Daun (1999). Pieces (1 x 1 x 0.5 cm) were excised from *pectoralis major* and *biceps femoris* muscles and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 2 h at room

temperature. The specimens were then rinsed with distilled water and dehydrated in a serial solution of 25%, 50%, 70%, 95% and absolute ethanol (twice), for 1 h in each solution. The samples were cut in liquid nitrogen using a razor blade. The fragments of dried specimens were mount on aluminum stubs and coated with gold. The specimens were examined and photographed in a on a JSM 5200 scanning electron microscope (JEOL, Ltd., Akishima, Japan). The micrographs and video prints were taken at magnification of 500x for transverse sections. The area of muscle fibres were measure in video prints, using a special morphometric facility. Three videoprints from each sample were taken for transverse sections. The fibre diameter was calculated from the fibre area.

3.3 Chemical analyses

3.3.1 pH

The pH of muscle samples was determined as mentioned in Part 2 item 2.4.1.

3.3.2 Total collagen and soluble collagen content

Analysis for total collagen and soluble collagen were determined as mentioned in Part 1 item 1.4.2 and 1.4.3.

3.4 Sensory evaluation

Sensory evaluation was carried out as mentioned in Part 2 item 2.5.

4. Statistical analysis

A factorials designs were use in the section 1. The data were analyses as a 2 x 3 factorial (two systems x three ages) with five replicates. Following factors were investigated to determine the effect of rearing system and age on meat quality. Complete randomized design (CRD) was used for other sections. Data were evaluated statistically as a one-way ANOVA using the SPSS 11.0. Significant differences between treatment means were analyzed by Duncan's multiple range test (DMRT) (Steel & Torrie, 1980).