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Thermal effects on chicken and salmon muscles: Tenderness, cook loss, area shrinkage, collagen solubility and microstructure

Fanbin Kong[a], Juming Tang[a,*], Mengshi Lin[b], Barbara Rasco[c]

[a]Department of Biological Systems Engineering 6120, Washington State University, P.O.Box 646120, Pullman, WA 99164-6120, USA
[b]Food Science Program, University of Missouri, Columbia, MO 65211-5160, USA
[c]Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376, USA

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Abstract

The objective of this study was to gain insight into the mechanisms underlying heating-induced tenderness in muscle food products by comparing tenderness changes in chicken breast (*Pectoralis major*) and salmon (*Oncorhynchus gorbuscha*) during high-temperature treatment. Relationships among changes in chicken breast and pink salmon muscle were investigated for tenderness, cook loss, area shrinkage, collagen solubility and microstructure. Small white muscle samples (D 30 mm × H 6 mm) cut from pink salmon fillets and chicken breast were sealed in small aluminum containers (internal dimension: D 35 mm × H 6 mm) and heated in an oil bath at 121.1 °C for different time intervals up to 2 h to simulate various thermal process durations. The changes in salmon tenderness had 4 phases (rapid toughening, rapid tenderizing, slow toughening and slow tenderizing), while that of the chicken breast only had 2 phases (rapid tenderizing and slow tenderizing). Twenty minutes was found to be a critical heating time in which >85% collagen was solubilized and shear force reached a minimum. Cook loss and area shrinkage were significantly ($P<0.05$) correlated with shear force change for both the salmon and chicken, while collagen solubility was only significant for the chicken.

Keywords: Pink salmon; Chicken breast; Thermal processing; Collagen; Tenderness

1. Introduction

Thermally processed shelf-stable fish and chicken are an important food source, but they must be tender to achieve consumer acceptance. Meat tenderness is defined by the ease of mastication, which involves initial penetration by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Lawrie, 1998). Shear force value has often been used as an objective measurement of meat tenderness (Kong, Tang, Rasco, Crapo, & Smiley, 2007). Major proteins in muscle foods include myofibrillar proteins (myosin and actin), connective tissue proteins (mainly collagen) and sarcoplasmic proteins. The effect of heat on these proteins has a major influence on the resulting texture of the cooked meat, including denaturation, dissociation of myofibrillar proteins, transversal and longitudinal shrinkage of meat fibers, aggregation and gel formation of sarcoplasmic proteins and solubilization of connective tissue (Kong, Tang, Rasco, Crapo, & Smiley, 2007; Murphy & Marks, 2000; Ofstad, Kidman, Myklebust, & Hermansson, 1993; Tornberg, 2005; Wattanachant, Benjakul, & Ledward, 2005a, b).

Factors affecting changes to meat tenderness during heating have been investigated by many researchers. Solubilization of connective tissue improved meat tenderness, while heat-denaturation of myofibrillar proteins generally caused toughening (Harris & Shorthose, 1988). Collagen characteristics, mainly content and solubility, determined the contribution of connective tissue to meat toughness. Shrinking of connective tissue exerted pressure on the aqueous solution in the extracellular void and expelled water, and the cook loss was connected to

*Corresponding author.
E-mail addresses: fanbin_kong@mail.wsu.edu (F. Kong), jtang@wsu.edu (J. Tang), linme@missouri.edu (M. Lin), rasco@wsu.edu (B. Rasco).

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tenderness and rigidity of tissue (Dunajski, 1979; Palka & Daun, 1999). Muscle fiber diameter and sarcomere length, as observed with scanning electron microscopy (SEM) and transmission electron microscopy (TEM), are closely related to flesh firmness (Hatae, Yoshimatsu, & Matsumoto, 1990; Hurling, Rodell, & Hunt, 1996; Sigurgisladottir, Sigurdardottir, Ingvarsdottir, Torrissen, & Hafsteinsson, 2001; Wattanachant et al., 2005a, b). Denatured and aggregated sarcoplasmic proteins are also believed to contribute to firmness by forming coagulated interstitial material that obstructs or impedes fiber movement (Hatae et al., 1990).

A previous study by the authors (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007; Kong, Tang, Lin, & Rasco, 2007) showed that the shear force of pink salmon dorsal muscle during high-temperature thermal processing has 2 peaks over a 2 h treatment time at 121.1 °C, which were divided into 4 phases: (1) rapid toughening, when the shear force increased from raw muscle to the first peak; (2) rapid tenderization, when the shear force decreased from the first peak to a minimum value; (3) slow toughening, when the shear force increased again to a second peak; and (4) slow tenderization, when the tissue gradually became soft with prolonged heat. The 4 phases of shear force are due to a combined effect of different reactions (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007).

It would be meaningful to examine if other types of meat exhibit a similar phenomenon during high-temperature thermal processing, thus providing insights into the mechanisms behind these changes. Chicken breast muscle was selected for the comparison, because it is somewhat similar to salmon dorsal muscle in that both are white muscle and contain lower collagen content than mammalian muscle tissue. However, compared with chicken breast muscle, fish contains lower amounts of collagen; the collagen is also significantly less cross-linked and appears to be degraded more readily when heated (Bracho & Haard, 1990). In this study, the shear force of chicken breast muscle was compared with that of salmon subjected to the same heating conditions. The objective was to investigate and compare tenderness changes in chicken breast and salmon during high-temperature processing, relating changes in cook loss, area shrinkage, collagen and microstructure. The results could provide insight into the mechanisms underlying heating-induced tenderness in muscle-food products during thermal processes for low-acid (pH > 4.5) shelf-stable products.

2. Material and methods

2.1. Materials

Pacific pink salmon (*Oncorhynchus gorbuscha*) used for the study were female, from the same catch harvested in August 2005 near Kodiak, Alaska. The fish were 1350 ± 100 g in weight, 370 ± 10 mm in length and 120 ± 10 mm in width, having similar color, firmness, odor and overall appearance. The fish were gutted, frozen, packaged in plastic bags and stored (−31 °C), shipped overnight under cold storage (with gel ice pack) to Washington State University in Pullman, WA, and then stored in a freezer (−35 °C) until used in June 2006.

Fresh breasts (*Pectoralis major*, 180–250 g each) of Washington State-grown broiler chicken were acquired from a local processing plant 3 days after the birds were sacrificed. The chicken breasts were frozen in a freezer (−35 °C) overnight before testing.

2.2. Sampling and heating

To reduce the influence of slow heat transfer in heat treatments on the meat texture and cooking properties, small samples were used to reduce come-up time and improve heating uniformity (Kong, Tang, Rasco, Crapo, & Smiley, 2007). To facilitate sampling, the frozen fish and chicken breast were partly thawed in a 4 °C refrigerator for 2 and 1 h, respectively, so that the muscle was softened enough to be cut easily using a slicer. The sampling and heating of fish fillets and chicken breast followed the methods described in Kong, Tang, Rasco, Crapo, and Smiley (2007). In brief, small disk-shaped samples (6 mm in thickness and 30 mm in diameter) were taken from the white muscle in the dorsal area of the salmon or the middle of the chicken breast muscle using an electrical food slicer (Model 632, Chef'Choice Int., Germany) and a 30 mm diameter corer. They were then hermetically sealed in custom-designed cylindrical aluminum test cells of 35 mm inner diameter, 6 mm inner height, and 2 mm wall thickness (Kong, Tang, Rasco, Crapo, & Smiley, 2007). Prior to sealing, a 0.1 mm diameter copper-constanstan thermocouple (Type-T) was inserted through a rubber gland in the lid of the container to measure the temperature at the geometrical center of the sample. Samples were then placed in a 121.1 °C oil bath (Model HAAKE W13, Thermo Electron Corp., Germany) using glycerol as the heating medium. During heating, signals from the thermocouple junctions were transferred to a computer equipped with a DLZe-type data logger (DELTA-T Devices, Cambridge, England, UK). It took 2.5–3 min for sample center temperatures to reach within 1 °C of the target treatment temperature. The final sample temperature was between 121.1 ± 0.3 °C. Several aluminum disks were introduced into oil bath at the same time, and removed at specified time intervals. After heating, the sample test cells were immediately placed in ice upon removal from the oil bath. Since the sample temperature dropped to below 20 °C within 0.5 min, the thermal effect of the cooling step on product quality was considered to be insignificant. After cooling, the samples were removed from test cells, dried with a filter paper, weighed on an analytical balance (Ohaus Analytical Plus, Pine Brook, NJ) sealed in air-tight containers and stored in a cooler (4 °C) for further analysis.

2.3. Experimental design

In a traditional retorting process, the packaged salmon meat is typically heated at around 120 °C for various time periods ranging from 20 to 90 min, depending upon package size and shape. Therefore, in this study, the samples were heated up to 2 h to reflect industrial practice. Samples taken from chicken and fish were heated at 121.1 °C for 2.5, 10, 20, 30, 90 and 120 min. Six sample replicates were used for each experimental treatment. After heating, the physical properties (shear force, cook loss and area shrinkage) were determined on each sample. Representative samples were selected for SEM and TEM microscopy as described later. Samples subjected to the same heating conditions were pooled, homogenized in a blender and used for collagen and moisture analysis.

2.4. Physical analysis

Cook loss was calculated as the percent weight reduction of the cooked sample compared with the raw sample.

A multiple thin-bladed Kramer-type texture fixture (MTB) developed in Kong, Tang, Rasco, Crapo, and Smiley (2007) was used to determine the shear force of the raw and heated samples. Compared with a standard Kramer shear cell, the MTB used much thinner blades (0.5 mm vs. 3 mm), which reduced compression when cutting through the samples and led to more accurate and consistent results (Kong, Tang, Rasco, Crapo, & Smiley, 2007). The cell was fitted to a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a load cell of 5 kg. Before shear force measurement, the raw and heated samples were allowed to equilibrate to room temperature, and then placed on a support base so that the muscle fibers were perpendicular to the blades. The traveling speed of the blades was 1 mm/s. Force-time graphs were recorded by a computer and analyzed using the Texture Expert for Windows (Version 1.15, Stable Micro Systems Ltd., London, UK), while shear force was measured as the peak height in the force-time profile.

A computer vision system (CVS) described in Pandit, Tang, Liu, and Pitts (2007) and Kong, Tang, Rasco, Crapo, and Smiley (2007) was used to capture images of the fresh and cooked samples. Sample area was determined using Vision IMAQ Builder image processing software Version 6.1 (National Instruments, Austin, TX). The area shrinkage ratio was calculated as the percent area reduction of cooked compared with the corresponding raw sample.

Longitudinal and transverse shrinkage ratios were determined as the percent reduction in the cooked sample length along and across the muscle fiber, respectively, compared with the raw sample. Maximum lengths of the raw and cooked samples radially and axially (Fig. 1) were measured using the Vision IMAQ Builder image processing software, and the shrinkage ratios were

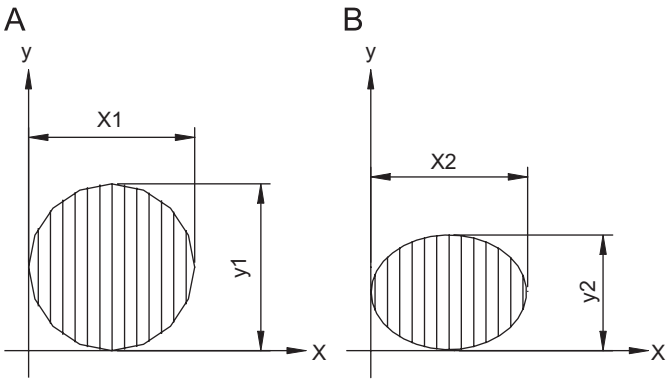

Fig. 1. Sample shape for raw and cooked salmon and chicken muscle. The parallel lines indicate muscle fiber. A: raw sample; B: cooked sample.

calculated as follows:

$$\text{Transverse shrinkage ratio} = \frac{x1 - x2}{x1} \times 100\%, \quad (1)$$

$$\text{Longitudinal shrinkage ratio} = \frac{y1 - y2}{y1} \times 100\%, \quad (2)$$

where $x1$ and $x2$ were the diameters of the raw and cooked sample disks across the muscle fibers, respectively, and $y1$ and $y2$ were the diameters of the raw and cooked sample disks along the muscle fibers, respectively.

Moisture content was determined by vacuum-drying a sample (3–5 g) at 65 °C to constant weight (Hart & Fisher, 1971).

2.5. Collagen

Total collagen (based on hydroxyproline content) was determined as described in Wattanachant, Benjakul, and Ledward (2004). Specifically, ground muscle samples (ca. 0.5 g) were hydrolyzed in 25 mL of 6 mol/L HCl at 110 °C for 24 h. The hydrolyzed solutions were decolorized by activated charcoal and vacuum filtered through Whatman #2 filter paper. The filtrate was neutralized with 10 mol equivalent/L NaOH, then diluted to 100 mL with distilled water. The hydroxyproline content in the hydrolysate was determined according to the procedure described in Bergman and Loxley (1963). The absorbance at 560 nm of samples and hydroxyproline standards was determined using a diode array spectrophotometer (Hewlett Packard 8452A). Hydroxyproline content was extrapolated from the standard curve and converted to collagen content using the following formula: collagen (mg/g sample) = hydroxyproline (mg/g sample) × 11.42 (salmon) (Eckhoff, Aidos, Hemre, & Lie, 1998) or 7.25 (for chicken) (Wattanachant et al., 2004).

Soluble collagen for chicken was extracted according to the method of Wattanachant et al. (2004): 2 g muscle samples were homogenized with 8 mL of 25% Ringer's solution (32.8 mmol/L NaCl, 1.5 mmol/L KCl, and 0.5 mmol/L $CaCl_2$). The 2300 g homogenates were heated at 77 °C for 70 min and centrifuged for 30 min at 4 °C. The

extraction was repeated twice, with supernatants combined. The sediment and supernatants were then hydrolyzed with 6 mol/L HCl at 110 °C for 24 h. The collagen content of the sediments and supernatants were determined separately, with total collagen content as the sum of the collagen content in the sediment plus that in the supernatant. The amount of heat-soluble collagen was expressed as a percentage of the total collagen.

As fish collagen can be degraded more readily when heated (Bracho & Haard, 1990), soluble salmon collagen was determined using a different method in which all operations were performed in a 4 °C room (Eckhoff et al., 1998). The samples were subjected to preliminary extraction with a cold 0.1 mol equivalent/L NaOH solution to remove non-collagen proteins. The alkali extraction procedures included homogenizing muscle with 10 volumes (v/w) 0.1 mol equivalent/L NaOH, followed by centrifugation at 10,000 *g* for 20 min. The residue was added to 20 volumes of NaOH solution, stirred overnight and centrifuged at 10,000 *g* for 20 min. The NaOH addition and centrifugation steps were repeated 3 times. The final precipitate was washed with distilled water before centrifugation. Ten volumes (v/w) of 0.5 mol/L acetic acid were added to the residue. The mixture was stirred for 2 days and centrifuged again at 10,000 *g* for 20 min, followed by collection of the supernatant and used as the acid-soluble collagen fraction (ASC). Pepsin-soluble collagen (PSC) was rendered soluble by limited digestion with porcine pepsin (Sigma-Aldrich, St. Louis, MO) at an enzyme:substrate ratio of 1:20 (w/w, wet weight) in 0.5 mol/L acetic acid. Digestion was performed at 4 °C for 2 days before another centrifugation at 10,000 *g* for 20 min. The final supernatant was the PSC, and the insoluble matter was the insoluble collagen (ISC). The collagen content of the ASC, PSC and ISC fractions were determined separately using the procedure described in Bergman and Loxley (1963), with total collagen content as the sum of the collagen content in ASC, PSC and ISC fractions. The amount of collagen in each fraction was expressed as a percentage of the total collagen.

2.6. Microscopy examination

Samples from raw and cooked salmon and chicken muscle (20 and 120 min heating) were selected for microstructure examination using SEM and TEM. Pieces ($2 \times 2 \times 2$ mm) were excised from raw and cooked samples, and placed in a fixative containing 2.5% glutaraldehyde/2% paraformaldehyde in a 0.1 mol/L phosphate buffer overnight at 4 °C. The specimens were then rinsed in phosphate buffer, post-fixed in 1% osmium tetroxide in phosphate buffer and dehydrated in a serial ethanol solution containing 30%, 50%, 70%, 95% and 100% ethanol for 15 min in each solution.

For SEM analysis, the ethanol in the dehydrated samples was removed with 2 changes of 100% acetone at 10 min for each, then another 10 min with a mixture of acetone and hexamethyldisilizane (HMDS) (1:1) followed by 2 changes of 100% HMDS. The HMDS was air-dried overnight in a fume hood. The samples were cut along and perpendicular to muscle fibers using a razor blade to produce longitudinal and transverse sections. The specimen fragments were then mounted on aluminum stubs, coated with gold and examined and photographed in a Hitachi S-570 SEM (Hitachi, Japan) using an accelerating voltage of 25 KV. Macrographs were taken at a magnification of 200 × for transverse sections and 10,000 × for longitudinal ones. Pictures of the transverse and longitudinal sections were analyzed using the Vision IMAQ Builder image processing software to characterize fiber diameter and sarcomere length. The total area of the 6–10 fiber bundles containing 10–20 muscle fibers each were added up, and the fiber diameters calculated from the fiber cross-section area. Sarcomere length was measured from randomly chosen muscle myofibers that contained ca. 100 sarcomeres.

For TEM analysis, the ethanol in the dehydrated samples was replaced with a mixture of acetone and ethanol (1:1) followed by 100% acetone. The samples were infiltrated with a mixture of acetone and Spur resin (1 h) followed by 100% Spurs overnight before being polymerized at 70 °C for 24–48 h. The embedded material was sectioned (gold interference color) to around 80–100 nm using a Reichert–Jung ultratome, mounted on copper grids and stained using a solution of 4% uranyl acetate in ethanol for 10 min followed by an aqueous solution of Reynolds' lead (7 min). The stained material was then observed in a JEOL JEM1200EX-II TEM using an accelerating voltage of 100 k.

2.7. Statistical analysis

The data for quality attributes of raw and heated samples were compared using analysis of variance (ANOVA) and the general linear model procedure of the SAS System for Windows V8.01 (SAS Institute Inc., Cary, NC). Differences between group means were analyzed by Duncan's multiple-range test. Statistical significance was set at $P<0.05$. Correlation analysis between cook loss, shear force, area shrinkage and collagen solubility was conducted using the SAS program and the result expressed in a linear correlation coefficient matrix.

3. Results

3.1. Shear force

The effects of heat treatment on the measured shear force of chicken breast and salmon fillets are shown in Fig. 2. For the raw muscle, the shear force of the chicken breast sample (127 N) was much higher than that for the salmon muscle sample (38 N). For both samples, changes in shear force were large in the first 20 min of heating and moderate thereafter. Shear force in the salmon experienced the 4-phase changes of salmon fillets during

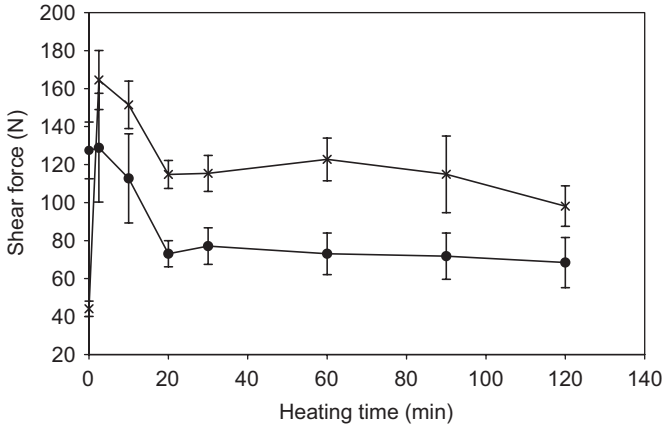

Fig. 2. Shear force of raw and cooked chicken and salmon samples after heating at 121.1 °C for different time periods. Bars indicate the standard deviation for 6 determinations. (●) chicken; (×) salmon.

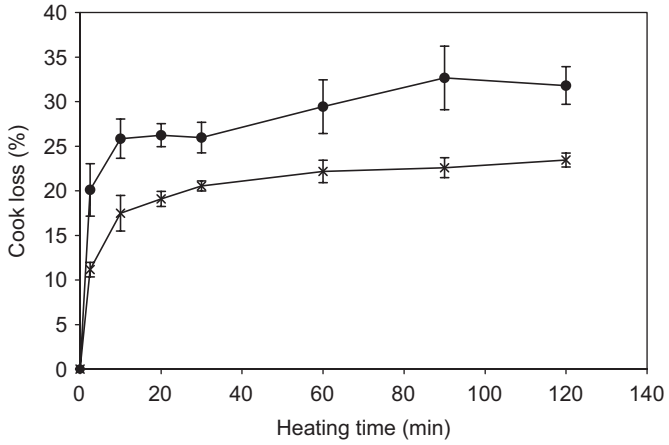

Fig. 3. Cook loss of salmon and chicken breast muscle after heating at 121.1 °C for different time periods. Bars indicate the standard deviation for 6 determinations. (●) chicken; (×) salmon.

high-temperature thermal processing as reported previously (Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007; Kong, Tang, Lin, & Rasco, 2007). Specifically, the salmon muscle shear force rapidly increased from 38 N in the raw samples to a maximum of 165 N after 2.5 min heating at 121 °C (rapid toughening), then quickly decreased to a minimum of 115 N after 20 min (rapid tenderizing). After that, it increased again to 124 N after 1 h of heating (slow toughening). The shear force then decreased consistently and reached 98 N after 2 h of heating (slow tenderizing). The shear force change in the chicken, however, showed only 2 phases, namely rapid tenderizing during which the shear force decreased from 127 N to 73 N (20 min), and slow tenderizing in which the shear force gradually ($P > 0.05$) decreased from 73 N to 68 N in 2 h.

Both fish and chicken samples had a minimum shear force values after about 20 min; thereafter the shear force changed slowly, indicating that 20 min heating was a critical cooking time. Although the raw pink salmon tissue was more tender than the chicken breast, after heating, the cooked salmon was consistently tougher.

3.2. Cook loss

Fig. 3 compares the cook loss of the chicken breast and salmon fillet samples. After heating, the cook loss increased rapidly to reach a plateau, which was higher for the chicken breast (30.6%) than salmon (23.4%). Most cook loss occurred within the first 20 min, which was 26.2% and 19.1% for chicken and salmon, respectively. An additional cook loss of 4.4% and 4.3% occurred after the first 20 min of heating, representing 14.4% and 18.4% of total cook loss for the chicken and salmon, respectively. Most cook loss was water. After 2 h of heating at 121 °C, the moisture in the chicken and salmon samples decreased from 73.43% (wet basis) and 74.15% in the raw muscle to 67.12% and 68.90%, respectively.

3.3. Area shrinkage ratio

Fig. 4 shows area shrinkage for both salmon and chicken during heating at 121.1 °C over the 2 h heating period. Similar to cook loss, the muscle shrank rapidly during the first 20 min, followed by a plateau. The area shrinkage ratio at the end of heating was much higher in the chicken (37.3%) than the salmon (23.6%). Around 34% and 19% shrinkage for chicken and salmon, respectively, occurred in the first 20 min. An additional 3.3% and 4.6% shrinkage, representing 8.8% and 19.5% of the total area shrinkage ratio in the chicken and salmon, respectively, occurred after the first 20 min of heating.

Fig. 5 shows changes in longitudinal and transverse shrinkage ratios for both salmon and chicken muscle samples, indicating the shrinkage mainly occurred along the muscle fiber. For example, chicken had a 27% and 17% shrinkage in the longitudinal and transverse directions, respectively, after 2 h of heating at 121.1 °C. For salmon, 2 h of heating at 121.1 °C caused 20% longitudinal shrinkage, but only 2% transverse shrinkage. These results suggest shrinkage parallel to the fiber direction is more significant for salmon than for chicken muscle following cooking.

3.4. Collagen

Total collagen contents were 5.13 ± 0.22 and 2.60 ± 0.13 g/kg (wet basis) in raw chicken and salmon, respectively. The soluble collagen content in raw salmon was 5.1%, 84.8% and 10.1% in ASC, PSC and ISC fractions, respectively (Table 1). The total soluble collagen content (ASC + PSC) in salmon was 89.9%, which was much higher than that in chicken breast (36.1%), indicating that chicken collagen is richer in cross-linkages, and therefore of greater stability. The results confirm that salmon, like other fish species, have a unique collagen with fewer cross-links and higher solubility in dilute acid

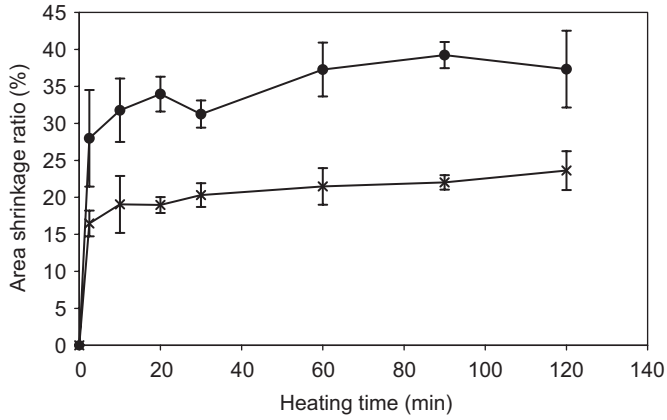

Fig. 4. Area shrinkage ratio of salmon and chicken breast muscle after heating at 121.1 °C for different time periods. Bars indicate the standard deviation for 6 determinations. (●) chicken; (×) salmon.

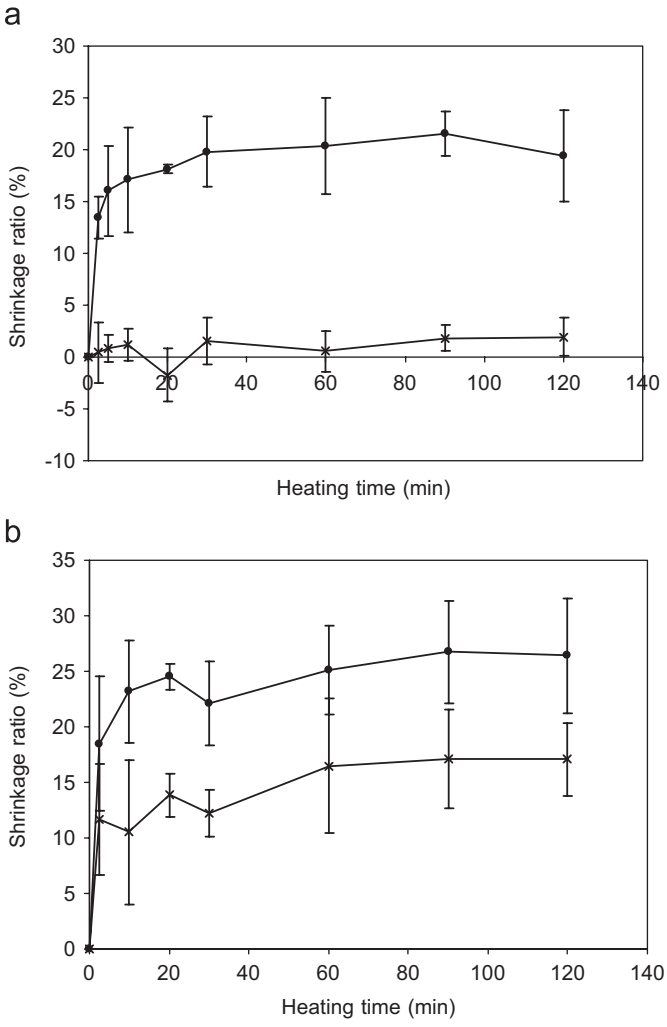

Fig. 5. Shrinkage ratio at longitudinal and transverse directions in pink salmon (a) and chicken breast (b) after heating at 121.1 °C for different time periods. Bars indicate the standard deviation for 6 determinations. (●) longitudinal shrinkage ratio; (×) transverse shrinkage ratio.

compared with the collagen in warm-blooded terrestrial animals (Bracho & Haard, 1990; Aidos, Lie, & Espe, 1999). Similarly, Eckhoff et al. (1998) reported the collagen content of Atlantic salmon at 2.9 g/kg, and the ASC, PSC and ISC at 6%, 93% and 1%, respectively. In contrast, Wattanachant et al. (2004) reported 5.09 and 3.86 g/kg collagen content and 22.2% and 31.4% collagen solubility for Thai indigenous and commercial broiler chicken breast muscles.

The collagen solubility significantly increased with heating time for both chicken and salmon, and most changes occurred within the first 20 min (Table 1). The soluble collagen content in chicken muscle increased rapidly from 36.1% in the raw tissue to reach a plateau (82.6–86.1%) at 20 min, indicating that most collagen had been solubilized and gelatinized after 20 min of heating, with only a small proportion of the fiber matrix remaining (14–18%). For salmon muscle, heating caused reductions in the ASC and ISC fractions, but an increase in the PSC. The total soluble collagen (ASC + PSC) had a slight increase (from 89.9% to 93.3%) over the entire heating time. During the first 20 min of heating at 121 °C, the ASC decreased from 5.1 to 1.6%, ISC decreased from 10.1 to 8.4%, but PSC increased from 84.8 to 90%. The significant increase in the PSC fraction indicates an increase in the proportion of non-helicoid regions vs. the helicoid ones as a result of heat denaturation of the helicoid regions (Suárez, Abad, Ruiz-Cara, Estrada, & García-Gallego, 2005).

3.5. Microscopy examination

The changes in microstructure of raw and cooked muscle (20 and 120 min heating) for both chicken breast and salmon fillet are presented in Figs. 6–8. On the transverse sections (Fig. 6), gaps between muscle fibers were visible in cooked samples due to solubilization and gelation of collagen (perimysium and endomysium). The fiber diameters of raw and cooked salmon and chicken muscle are shown in Table 2. A small increase (ca. 9%) was observed in the fiber diameter of chicken, from 49.7 μm in the raw tissue to 54 μm after 2 h heating. A more significant increase was shown in the salmon muscle, in which the fiber diameter increased from 72.1 μm in the raw tissue to 127 μm after 2 h heating, corresponding to a 76% increase. The swelling of fish muscle fiber can be clearly seen when comparing Fig. 6A, C and E. The average diameter of white fibers in raw chicken muscle has been reported from 32.6 to 68.2 μm (Wattanachant et al., 2005a).

In the raw salmon and chicken muscle, myofibrils were closely packed and covered by a thin, thread-like pericellular layer surrounding the compact cells (Fig. 7A and B). After cooking, the collageneous tissue denatured and melted, muscle cells ruptured, the sarcomere shrunk and the extracellular space and intracellular cavities and canals increased (Fig. 7C–F). Granulates of protein aggregates appeared in the extracellular space (Fig. 7C–F). After

Table 1
Soluble collagen content (expressed as a percentage of total collagen) in samples of chicken breast and salmon fillet heated at 121.1 °C for different times

Heating time (min)	Chicken breast (%)	Salmon dorsal muscle			
		ASC (%)	PSC (%)	ISC (%)	Total soluble (ASC + PSC) (%)
0 (raw)	36.1 ± 0.5^{d}	5.13 ± 0.29^{a}	84.8 ± 1.1^{b}	10.1 ± 0.6^{a}	89.9 ± 1.3^{a}
10	69.0 ± 0.9^{c}	2.02 ± 0.62^{b}	88.7 ± 1.8^{a}	9.24 ± 1.08^{ab}	90.8 ± 1.1^{a}
20	82.6 ± 2.2^{b}	1.62 ± 0.39^{b}	90.0 ± 1.8^{a}	8.37 ± 0.76^{b}	91.6 ± 2.6^{a}
60	84.2 ± 1.0^{ab}	1.81 ± 0.12^{b}	91.9 ± 0.3^{a}	6.25 ± 0.72^{c}	93.8 ± 0.2^{a}
120	86.1 ± 1.3^{a}	1.91 ± 0.46^{b}	91.4 ± 1.7^{a}	6.70 ± 0.15^{c}	93.3 ± 2.2^{a}

ASC: Acid-soluble collagen; PSC: pepsin-soluble collagen; ISC: insoluble collagen content.
Different letters in the same column indicate significant differences ($P < 0.05$). Values are means ($n = 2$) ± standard deviation (SD).

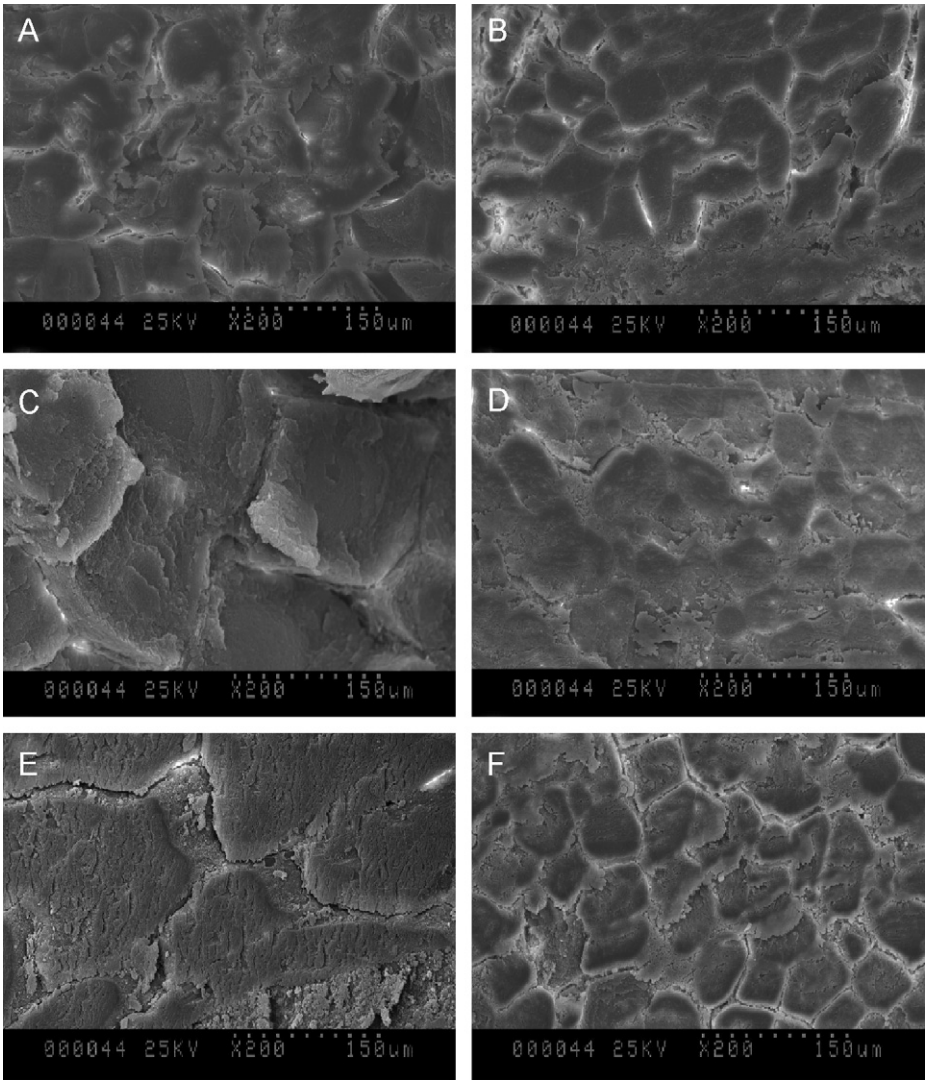

Fig. 6. SEM (200 ×) images showing transverse section of raw and cooked muscle after heating at 121.1 °C for different time periods. The horizontal dotted bars indicate 150 μm. A, C, E: raw, 20 and 120 min cooked salmon muscle; B, D, F: raw, 20 and 120 min cooked chicken muscle.

120 min heating at 121.1 °C, myofibrils retained their peripheral ribbon-like shape (Fig. 7E and F). Sarcomere lengths for the raw salmon and chicken muscle were 1.60 and 1.36 μm, respectively, but were reduced to 1.08 and 1.11 μm after 120 min of heating, corresponding to a 32 and 24% shortening, respectively (Table 2). Most sarcomere shortening occurred within the first 20 min of heating. Sigurgisladottir et al. (2001) reported sarcomere length in raw fish muscle between 1.5 and 2.2 μm depending upon the fish species. Wattanachant et al. (2005b) reported

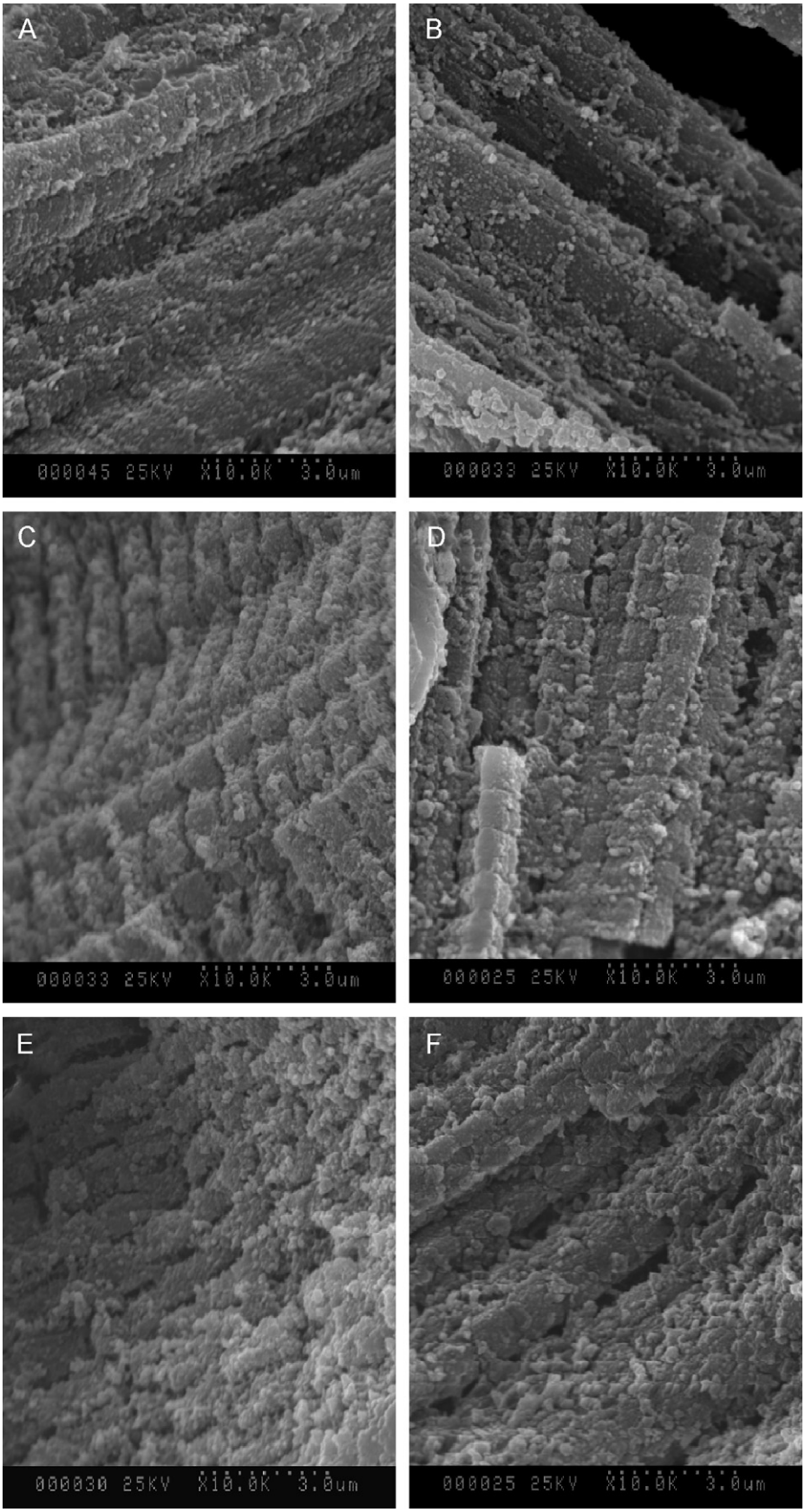

Fig. 7. SEM (10,000 ×) images showing myofibers of raw and cooked muscle after heating at 121.1 °C for different time periods. The horizontal dotted bars indicate 3 μm. A, C, E: raw, 20 and 120 min cooked salmon muscle; B, D, F: raw, 20 and 120 min cooked chicken muscle.

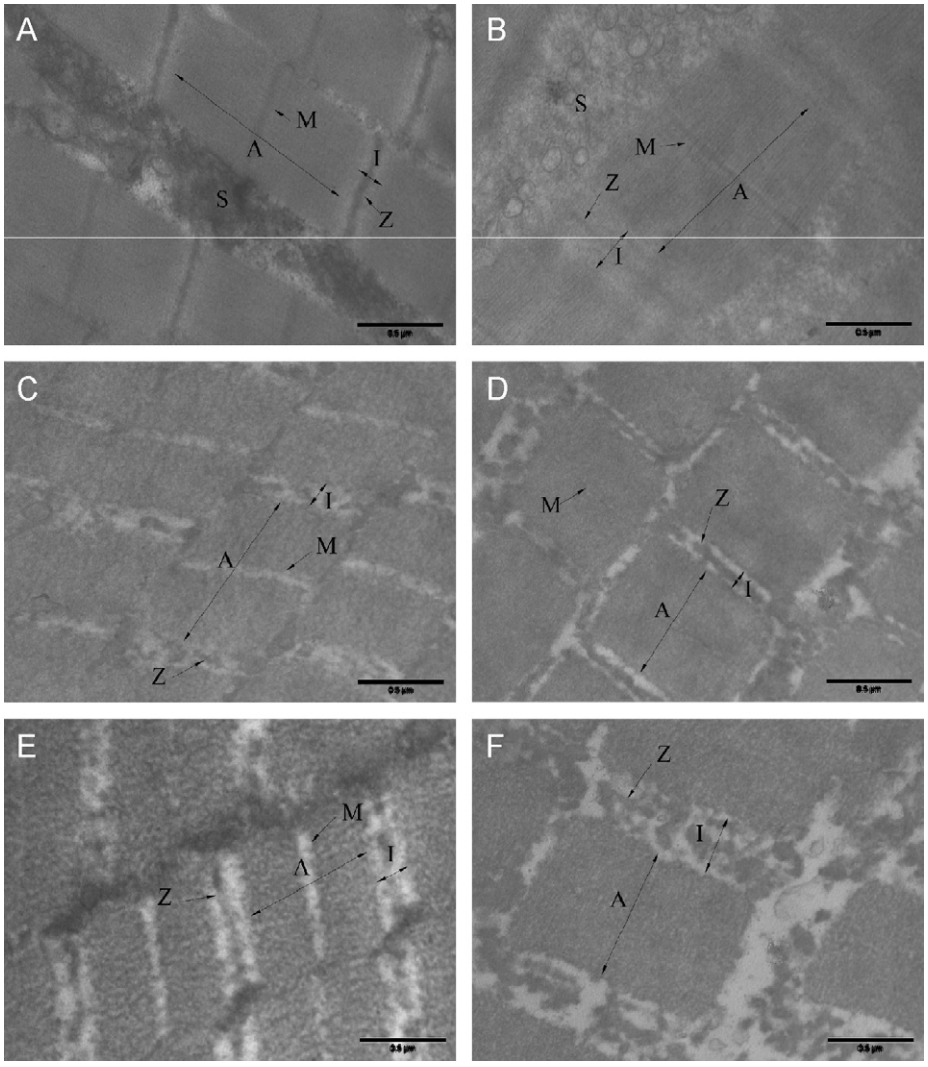

Fig. 8. TEM (50,000 ×) image showing sarcomere of raw and cooked muscle after heating at 121.1 °C for different time periods. Bars indicate 0.5 μm. A, C, E: raw, 20 and 120 min cooked salmon muscle; B, D, F: raw, 20 and 120 min cooked chicken muscle. The black letters A, I, M, Z indicate A band, I band, M line, and Z line, respectively. The black letters indicates sarcoplasmic reticulum.

Table 2
Fiber diameter and sarcomere length in chicken breast and salmon fillet samples heated at 121.1 °C for different times[a]

Heating time (min)	Fiber diameter (μm)		Sarcomere length (μm)	
	Salmon	Chicken breast	Salmon	Chicken breast
0 (raw)	72.14 ± 8.48^{b}	49.68 ± 3.92^{b}	1.60 ± 0.07^{a}	1.36 ± 0.08^{a}
20	101.2 ± 10.3^{a}	60.95 ± 9.23^{a}	1.09 ± 0.13^{b}	0.99 ± 0.03^{c}
120	127.0 ± 24.7^{a}	53.98 ± 8.96^{ab}	1.08 ± 0.05^{b}	1.11 ± 0.10^{b}

[a]Different letters in the same column indicate significant differences ($P < 0.05$). Values are means ($n = 100$) ± standard deviation (SD).

sarcomere lengths between 1.56 and 1.64 μm for the raw muscle of Thai indigenous and commercial broiler chicken, and 0.99 to 1.35 μm after cooking at 80 °C for 10 min.

The TEM images for longitudinal sections of salmon and chicken muscle are shown in Fig. 8. Figs. 8A and B indicates that the ultrastructure of the fresh muscle has intact sarcomeres with clear A and I bands and M and Z lines, and sarcoplasmic reticulum surrounds the sarcomeres. The actin and myosin can also be distinguished. After heating, denaturation and aggregation of myosin and actin caused sarcomere shrinkage (Fig. 8C–F) and the cooked meat to appear grainy. As a result of shrinkage of myosin and actin, the I band was enlarged and A band shrunk, producing gaps and discontinuity between sarcomeres. After 120 min heating at 121.1 °C, the typical structure of the sarcomere was still recognizable and the

A and I bands and Z lines still visible (Fig. 8E and F). A large gap was formed along the M line in the sarcomere of salmon (Fig. 8C and E), while this was not obvious in the chicken breast (Fig. 8D and F). Coagulated sarcoplasmic proteins can be seen in both the inter- and intracellular spaces. Ofstad et al. (1993) showed that aggregated sarcoplasmic proteins and collagen can form a gel that glues the fibers and fiber bundles together, holding water and/or plugging the intercellular capillaries to prevent water from being released. The stability of cooked meat relies on the gel network formed with the melted collagen, denatured and aggregated myofibrillar proteins and sarcoplasmic proteins (Tornberg, 2005).

4. Discussion

Compared with the salmon, the cooked chicken samples had lower moisture content (67.12% vs. 68.90%, wet basis), higher insoluble collagen (Table 1), a higher shrinkage ratio (Fig. 4) and smaller fiber diameter (Table 2). Although these properties are correlated with tougher meat (Dunajski, 1979; Tornberg, 1996), in our study, the cooked chicken was consistently more tender (ca. 40 N lower in shear force) than the cooked fish muscle. This was likely caused by the different properties of the myofibrillar proteins and the greater sarcomere shortening in salmon (32%) than chicken (24%) during heating, which contributed to a larger shearing resistance in salmon muscle. According to Palka and Daun (1999) and Wattanachant et al. (2005b), sarcomere contraction and shortening are positively correlated with meat toughness.

The change in sarcomere length resulted in longitudinal shrinkage, whereas that in fiber diameter was more related to transverse shrinkage. The shortening of sarcomere lengths in salmon and chicken reached between 24 and 32% after 120 min heating at 121.1 °C (Table 2), which was in good agreement with the longitudinal shrinkage ratio of the samples (20–27%) (Fig. 5). On the other hand, the same length of heating resulted in a fiber diameter increase in both chicken (9%) and salmon muscle (76%), while they shrunk transversely by 17 and 2%, respectively (Table 2 and Fig. 5). The simultaneous occurrence of the increase in fiber diameter and transverse shrinkage for both chicken and salmon indicates that the shrinkage of collagen, particularly perimysium, might be the main reason for the transverse shrinkage. Perimysium constitutes some 90% of intramuscular connective tissue, and is believed to be the main factor affecting the contribution of connective tissue to toughness (Light, Champion, Voyle, & Bailey, 1985).

Fig. 5 and Table 2 show much more shrinkage of salmon muscle along the fiber than transversally compared with the chicken muscle. According to Lepetit, Grajales, and Favier (2000), the extent of muscle fiber deformation during cooking depends on the compression stress applied by collagen fibers and the resistance of the muscle fibers to compression, while the compression force applied by collagen networks on muscle fiber bundles depends upon the amount of collagen present and its thermal solubility. Compared with chicken breast, cooked salmon muscle contains a lower content of collagen with higher solubility, and the muscle fibers have higher resistance, resulting in more parallel shrinkage and transverse expansion along the fibers. Similarly, Wattanachant et al. (2005a) found the shrinkage in cooked broiler muscle more parallel to the fibers than transversally compared with that in Thai indigenous chicken muscle.

The shear force for the raw salmon muscle (38 N) tested for this study was much higher than that in the raw chicken breast (127 N), probably as a result of lower collagen content (2.60 vs. 5.13 g/kg wet tissue) and higher collagen solubility (Table 1) in the raw salmon muscle. After cooking, the apparent shear force change in salmon and chicken was a combined effect of different reactions: toughening caused by denaturation and aggregation of the proteins and the subsequent shrinkage and dehydration, and a tenderizing effect caused by solubilization and gelation of collagen. These changes were quantitatively characterized by measuring cook loss, area shrinkage ratio and collagen solubility in the cooked muscle.

Correlation analyses were conducted to evaluate the relationships among different variables including shear force, cook loss, collagen solubility and shrinkage ratios in the area and longitudinal and transverse directions. Except for shear force, the data used for correlation analyses of other variables covered the entire heating time from 0 to 2 h. Heating in the first 2.5 min was non-isothermal, and denatured most proteins, which had a rapid toughening effect on the salmon muscle. To simplify the correlation analysis of shear force, data from 2.5 min to 2 h of heating were used (excluding that for raw muscle), which corresponded to an isothermal heating period. Tables 3 and 4 show the correlation matrix results for both chicken and salmon muscle.

From 2.5 min to 2 h of heating, the shear force of chicken breast muscle was negatively correlated with cook loss ($r = -0.82$), the area shrinkage ratio ($r = -0.80$) and collagen solubility ($r = -0.99$) (Table 3). The shear force of salmon muscle was negatively correlated with cook loss ($r = -0.91$) and the area shrinkage ratio ($r = -0.89$), but was not significantly correlated with ISC, ASC or PSC (Table 4). These results confirm that collagen is relatively unimportant in fish after muscle texture cooking compared with that in warm-blooded animals (Hatae, Tobimatsu, Takeyama, & Matsumoto, 1986). Following cooking, the muscle fibers themselves provide the main resistance to mastication (Dunajski, 1979).

This study showed that cook loss and area shrinkage were highly correlated with collagen solubility (Tables 3 and 4). For chicken, collagen solubility significantly affected cook loss ($r = 0.97$) and area shrinkage ratio ($r = 0.98$). For salmon, both cook loss and the area shrinkage ratio were positively affected by PSC ($r = 0.97$), but negatively

Table 3
Correlations among shear force, cook loss, shrinkage ratio and collagen solubility for chicken breast[a]

	Shear force	Cook loss	A-shrinkage	T-shrinkage	L-shrinkage	Collagen solubility
Shear force	1					
Cook loss	−0.8161*	1				
A-shrinkage	−0.8028*	0.9922****	1			
T-shrinkage	−0.7679*	0.9732****	0.9753****	1		
L-shrinkage	−0.8227*	0.9919****	0.9951****	0.9609***	1	
Collagen solubility	−0.9946**	0.9739**	0.9789**	0.9941***	0.97012**	1

*$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$; ns = no significant difference.
[a]A-shrinkage: area shrinkage ratio; T-shrinkage: transverse shrinkage ratio; L-shrinkage: longitudinal shrinkage ratio.

Table 4
Correlations among shear force, cook loss, shrinkage ratio and collagen solubility for salmon[a]

	Shear force	Cook loss	A-shrinkage	T-shrinkage	L-shrinkage	PSC	ASC	ISC
Shear force	1							
Cook loss	−0.9118**	1						
A-shrinkage	−0.8869**	0.9667****	1					
T-shrinkage	ns	ns	ns	1				
L-shrinkage	−0.8297*	0.9746****	0.9812****	ns	1			
PSC	ns	0.9731**	0.9504*	ns	0.9535*	1		
ASC	ns	−0.9649**	−0.9696**	ns	−0.9877**	−0.9074*	1	
ISC	ns	ns	ns	ns	ns	−0.9251*	ns	1

[a]A-shrinkage: area shrinkage ratio; T-shrinkage: transverse shrinkage ratio; L-shrinkage: longitudinal shrinkage ratio. *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$; ns = no significant difference.

correlated with ASC ($r = -0.96$). The higher cook loss and area shrinkage ratio in chicken (Figs. 3 and 4) might be explained by its higher amount of collagen with more cross-links that compelled more water out of the cooked muscle.

Tables 3 and 4 also indicate that the shear force of chicken was negatively correlated with both the longitudinal ($r = -0.82$) and transverse ($r = -0.77$) shrinkage ratios, while that of salmon was only significantly correlated with the longitudinal ($r = -0.83$) shrinkage ratio. For both chicken and salmon, cook loss was positively correlated ($r = 0.97$) with the area shrinkage ratio. These results confirm the significant correlations among cook loss, collagen solubility and shear force reported in the literature (Ofstad, Kidman, & Hermansson, 1996; Murphy & Marks, 2000; Sigurgisladottir et al., 2001; Kong, Tang, Rasco, & Crapo, 2007; Kong, Tang, Rasco, Crapo, & Smiley, 2007).

The different shear forces we found in chicken and salmon muscle samples during heating were related to changes in cook loss, shrinkage and collagen solubility. For chicken muscle, as a result of the high content of strongly cross-linked collagen and low-shearing resistance of denatured muscle fibers, the rapid solubilization of collagen (from 36 to 82.6% within the first 20 min of heating) dominated, so the net effect was a rapid tenderizing (Fig. 2). At 20 min, most of the solubilization of collagen, cook loss and area shrinkage were completed, so prolonged heating (from 20 min to 2 h) did not cause significant change in the shear force values for chicken.

The initial rapid toughening phase for the salmon muscle was characterized by a more profound toughening than tenderization after heating because of low collagen content, few cross-bonds and high-shearing resistance of the denatured muscle fibers. After anywhere from 2.5 to 5 min of heating, most proteins in the salmon were denatured and the gelation and melting of collagen dominated, resulting in a rapid tenderizing phase (Fig. 2). The third phase (slow toughening from 20 to 60 min), during which shear force slightly increased, resulted from substantial dehydration and area shrinkage. As stated earlier, compared with chicken, salmon had a higher proportion of shrinkage (19.5% vs. 8.8% of total area shrinkage ratio) and cook loss (18.4% vs. 14.4% of total cook loss) after 20 min, which could explain its toughening. However, after 60 min of heating, muscle disintegration and fragmentation softened the texture of both the salmon and chicken (Fig. 2).

The critical heating time for both chicken and salmon muscle was found to be 20 min, as it marked the endpoint of the rapid tenderizing phase; thereafter the shear force (Fig. 2), collagen solubility (Table 1), cook loss (Figs. 3) and area shrinkage (Fig. 4) did not change much. After 20 min, the denatured sarcoplasmic and myofibrillar proteins and melted collagen formed an aggregate gel that reduced cook loss (Ofstad et al., 1993; Tornberg, 2005). The 20 min critical heating time also implies that short heat exposure can yield tender products, and that heating beyond this time does not improve texture, and in the case

of salmon, can cause texture to worsen. Studies have reported that combining hot water heating with microwave heating can be used to reduce the thermal process times for prepackaged foods (Guan, Plotka, Clark, & Tang, 2002, 2003), and this strategy may be important for thermal processing of fish.

More work remains to be done. The chicken and fish samples used in this study were both frozen and thawed before cooking treatments, since it was not possible to conduct this study with fresh pink salmon. Freezing and thawing, the rate of freezing, storage period and temperature of freezing all affected product texture and structure, primarily through ice crystal damage to the muscle and through biochemical changes such as lipid peroxidation that occurs during frozen storage of muscle tissue. Further study is needed to clarify the impact of freezing and thawing adopted in the experiment on the observed textural and structural changes in the meat samples. Moreover, fat content could significantly influence meat texture, which was not covered in this study.

5. Conclusion

Raw salmon muscle is more tender than chicken due to lower collagen content and fewer collagen cross-linkages, but after cooking it can be tougher as indicated by a higher shearing resistance of the denatured muscle fibers. At a heating time of 20 min, shear force was lowest with the dominant tenderizing effect caused by collagen solubilization. The difference in the shear force between chicken and salmon muscle during heating was a combined effect of various interactions quantitatively characterized by cook loss, area shrinkage and collagen solubility. A significant increase in fiber diameter and decrease in sarcomere length were obtained for both salmon and chicken muscle during high-temperature thermal processing, with salmon muscle exhibiting more shrinkage parallel to the muscle fiber than chicken.

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