Chemical Compositions, Properties and Structure of Muscle Affecting Textural Characteristics of Meat from Thai Indigenous Chicken and Broiler

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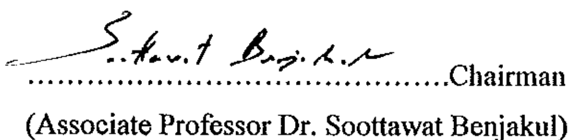
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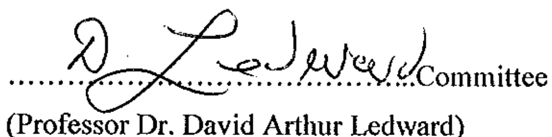
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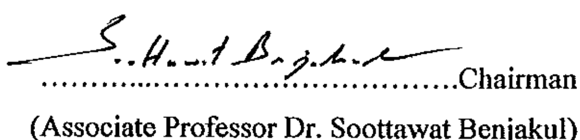
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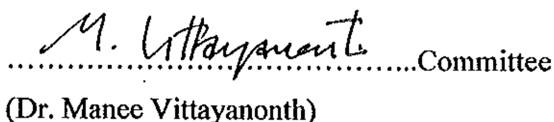
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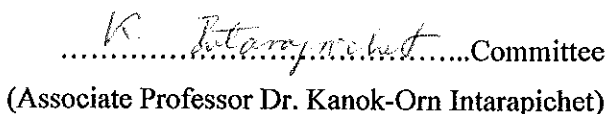
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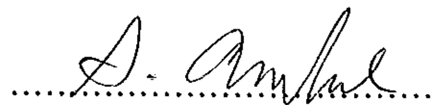
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ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมี โครงสร้าง และคุณสมบัติของกล้ามเนื้อที่มีผลต่อลักษณะเนื้อสัมผัสของเนื้อไก่พื้นเมืองไทยและไก่กระทง
ผู้เขียน	นางเสาวคนธ์ วัฒนจันทร์
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บทคัดย่อ

การศึกษาไก่พื้นเมืองไทยในเชิงเปรียบเทียบกับไก่กระทง โดยทำการประเมินคุณภาพกล้ามเนื้อสะโพก (*Biceps femoris*) และเนื้อหน้าอก (*Pectoralis*) ในด้านองค์ประกอบทางเคมี คุณสมบัติทางเคมีและกายภาพ โครงสร้างทางจุลภาค การเปลี่ยนแปลงภายหลังการตาย และคุณลักษณะทางความร้อน พบว่ากล้ามเนื้อไก่พื้นเมืองมีปริมาณโปรตีนสูงกว่า แต่มีปริมาณไขมันและเถ้าต่ำกว่ากล้ามเนื้อไก่กระทง (P<0.001) กล้ามเนื้อไก่พื้นเมืองมีไมโอไฟบริลาร์โปรตีนต่ำกว่า แต่มีปริมาณสโตรมาโปรตีนสูงกว่าเมื่อเปรียบเทียบกับไก่กระทง (P<0.05) กล้ามเนื้อไก่พื้นเมืองมีปริมาณกรดกลูตามิกสูงกว่ากล้ามเนื้อไก่กระทง (P<0.05) กล้ามเนื้อไก่พื้นเมืองมีปริมาณกรดไขมันอิ่มตัวสูงกว่า และมีปริมาณกรดไขมันไม่อิ่มตัวชนิดพอลิต่ำกว่าไก่กระทง (P<0.05) ปริมาณคอ ลลาเจนทั้งหมดในกล้ามเนื้อไก่พื้นเมืองมีปริมาณสูงกว่า แต่มีปริมาณคอลลาเจนที่ละลายได้ต่ำกว่าเมื่อเปรียบเทียบกับกล้ามเนื้อไก่กระทง (P<0.001) สำหรับค่าแรงตัดผ่านเนื้อของไก่พื้นเมืองทั้งในรูปเนื้อดิบและสุกมีค่าสูงกว่ากล้ามเนื้อไก่กระทง (P<0.001) กล้ามเนื้อไก่พื้นเมืองมีเนื้อเยื่อเกี่ยวพันชั้นเพอริไมเซียมหนากว่าและมีเส้นผ่านศูนย์กลางของเส้นใยกล้ามเนื้อใหญ่กว่ากล้ามเนื้อไก่กระทง (P<0.05) ความยาวของซาร์โคเมียร์ในเส้นใยกล้ามเนื้อดิบของไก่ทั้งสองสายพันธุ์มีค่าเฉลี่ยอยู่ในช่วง 1.56 ถึง 1.64 ไมโครเมตร

การศึกษาการเปลี่ยนแปลงภายหลังการตายของกล้ามเนื้อส่วนอก (*Pectoralis*) ของไก่พื้นเมืองและไก่กระทงในระหว่างการบ่มซากที่ระยะเวลา 0 2 4 6 24 48 และ 72 ชั่วโมง ที่อุณหภูมิ 4 องศาเซลเซียส พบว่าไก่พื้นเมืองมีการลดลงของค่าความเป็นกรด-ด่างในกล้ามเนื้อช้ากว่า และมีปริมาณกรดแลคติกสูงกว่าเมื่อเปรียบเทียบกับกล้ามเนื้อของไก่กระทง โดยมีค่าความเป็นกรด-ด่างสุดท้ายเท่ากับ 5.85 และ 5.90 สำหรับกล้ามเนื้อไก่พื้นเมืองและไก่กระทงตามลำดับ กล้ามเนื้อไก่กระทงมีอัตราการย่อยสลายของโปรตีนสูงกว่ากล้ามเนื้อไก่พื้นเมือง ซึ่งแสดงจากการเพิ่มของปริมาณเปปไทด์ที่ละลายในสารละลายกรดไตรคลอโรอะซิติก โดยเฉพาะในช่วงต้นของการบ่มภาย

หลังการตาย ($P<0.01$) อย่างไรก็ตามไม่พบการการเปลี่ยนแปลงของปริมาณคอลลาเจนที่ละลายได้ในกล้ามเนื้อของไก่ทั้งสองสายพันธุ์ในระยะเวลาบ่มซาก การย่อยสลายของโปรตีนที่ก่อให้เกิดความนุ่มของกล้ามเนื้อต้องการเวลาในการบ่มซากภายหลังการตายที่อุณหภูมิ 4 องศาเซลเซียส อย่างน้อย 4 และ 6 ชั่วโมง สำหรับไก่กระทงและไก่พื้นเมืองตามลำดับ

การประเมินการเปลี่ยนแปลงของเนื้อสัมผัส โครงสร้างจุลภาค และสมบัติบางประการของกล้ามเนื้อส่วนอก (*Pectoralis*) ของไก่พื้นเมืองและไก่กระทงที่ทำให้สุกที่อุณหภูมิต่างๆ พบว่าค่าแรงตัดผ่านเนื้อมีการเปลี่ยนแปลง 2 ระยะที่ช่วงอุณหภูมิ 50 ถึง 60 องศาเซลเซียส และ 80 ถึง 100 องศาเซลเซียส ตัวอย่างเนื้อที่ให้ความร้อนจนมีอุณหภูมิภายในเท่ากับ 60 องศาเซลเซียส มีเส้นผ่านศูนย์กลางของเส้นใยกล้ามเนื้อลดลง ($P<0.05$) ส่วนความยาวของซาร์โคเมียร์ลดลงเมื่ออุณหภูมิการให้ความร้อนเพิ่มขึ้น การสูญเสียน้ำเมื่อให้ความร้อนของกล้ามเนื้อไก่พื้นเมืองเพิ่มขึ้นอย่างเห็นได้ชัดในช่วงอุณหภูมิ 80 ถึง 100 องศาเซลเซียส ซึ่งพบว่าสูงกว่าในกล้ามเนื้อไก่กระทง ($P<0.001$) ค่าความสว่าง (L^*) และค่าความเหลือง (b^*) ของกล้ามเนื้อไก่ทั้งสองสายพันธุ์เพิ่มขึ้นเมื่ออุณหภูมิของการให้ความร้อนเพิ่มขึ้นจาก 50 ถึง 70 องศาเซลเซียส ($P<0.05$) เมื่ออุณหภูมิการให้ความร้อนในกล้ามเนื้อทั้งสองสายพันธุ์เพิ่มขึ้น พบว่าการละลายของโปรตีนลดลง แต่ปริมาณคอลลาเจนที่ละลายได้เพิ่มขึ้น ($P<0.05$) ผลการศึกษาชี้ให้เห็นว่าการละลายของโปรตีนมีความสัมพันธ์สูงกับเนื้อสัมผัสของกล้ามเนื้อไก่กระทง ขณะที่ความยาวของซาร์โคเมียร์และการละลายของคอลลาเจนเป็นปัจจัยที่สำคัญที่มีผลต่อค่าการสูญเสียน้ำขณะทำให้สุกและเนื้อสัมผัสของกล้ามเนื้อไก่พื้นเมือง

จากการศึกษาการเปลี่ยนแปลงของเนื้อเยื่อเกี่ยวพันในกล้ามเนื้อของไก่ทั้งสองสายพันธุ์ในระหว่างกระบวนการให้ความร้อน โดยใช้เทคนิค Differential Scanning Calorimetry พบว่าไม่มีความแตกต่างในด้านความคงทนต่อความร้อนระหว่างเนื้อเยื่อดิบของไก่พื้นเมืองและไก่กระทง อย่างไรก็ตามความแตกต่างระหว่างเนื้อเยื่อไก่ทั้งสองสายพันธุ์สามารถตรวจพบได้หลังจากผ่านกระบวนการให้ความร้อน อุณหภูมิที่ให้ความร้อนต่ำกว่า 70 องศาเซลเซียส มีผลเพิ่มความคงทนต่อความร้อนของเนื้อเยื่อเกี่ยวพัน ความร้อนที่อุณหภูมิ 70 องศาเซลเซียส เป็นเวลา 20 นาที ทำให้เนื้อเยื่อเกี่ยวพันเสียสภาพธรรมชาติอย่างสมบูรณ์และคืนสภาพกลับบางส่วนในขณะทำเย็น ที่อุณหภูมิ 80 และ 90 องศาเซลเซียส เนื้อเยื่อเกี่ยวพันของกล้ามเนื้อไก่กระทงและไก่พื้นเมืองเสียสภาพธรรมชาติจนไม่สามารถคืนสภาพกลับได้ นอกจากนี้ไม่พบความแตกต่างของรูปแบบโปรตีนระหว่างเนื้อเยื่อเกี่ยวพันในกล้ามเนื้อของไก่กระทงและไก่พื้นเมืองที่ผ่านการให้ความร้อน

Thesis Title	Chemical Compositions, Properties and Structure of Muscle Affecting Textural Characteristics of Meat from Thai Indigenous Chicken and Broiler
Author	Mrs. Saowakon Wattanachant
Major Program	Food Technology
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Abstract

Chemical composition, physical properties, microstructure, post-mortem changes and thermal characteristics of Thai indigenous chicken (TIC) and broiler *Biceps femoris* and *Pectoralis* muscles were compared. TIC muscles contained higher protein contents but lower fat and ash contents, compared to broiler muscles (*P*<0.001). TIC muscles had lower myofibrillar protein but higher stromal protein contents than broiler muscle (*P*<0.05). The greater glutamic acid content was found in the TIC muscles (*P*<0.05). The TIC muscles contained more saturated and less polyunsaturated fatty acids than the broiler muscles. The total collagen contents of TIC muscles were higher than those found in broiler muscles (*P*<0.001). However, soluble collagen contents were lower in TIC muscles. The muscles from both breeds had similar color values. The shear values of TIC muscles either raw or cooked were higher than those of broiler muscles (*P*<0.001). TIC muscles had thicker perimysium and greater fiber diameter than those of the broiler (*P*<0.05). The means of sarcomere lengths of the raw muscles from both breeds were in the ranges of 1.56 - 1.64 μm.

Post-mortem changes of *Pectoralis* muscle of TIC and broiler were studied during carcass ageing for 0, 2, 4, 6, 24, 48, and 72 h at 4°C. TIC muscle had a slower pH decline and contained higher lactic acid content, compared to broiler muscle. The ultimate pH for TIC and broiler muscle were 5.85 and 5.90, respectively. Broiler muscle had higher degree of proteolysis than indigenous muscle as evidenced by an increase in TCA-soluble peptides especially early

post-mortem (P<0.01). However, no change in soluble collagen in muscle of both breeds was observed during the ageing period. Improvement of tenderness of muscle by proteolysis required at least 4 h and 6 h post-mortem ageing at 4°C for broiler and TIC carcasses, respectively.

Changes in texture, microstructure and some properties of TIC and broiler *Pectoralis* muscle stripes cooked at different temperatures were evaluated. The change in shear value of both chicken muscles was observed in two steps: 50-60°C and 80-100°C. A significant decrease in fiber diameter was obtained in samples heated to internal temperature of 60°C. Sarcomere length decreased with increasing temperature. Cooking losses of TIC muscles increased markedly in the temperature ranges of 80-100°C and were higher than those of the broiler (P <0.001). Lightness (L*) and yellowness (b*) of both chicken muscles increased significantly with increasing temperature ranging from 50 to 70°C. Solubility of protein decreased, whereas soluble collagen contents of both chicken muscles increased when heating temperature increased (P<0.05). The result indicated that solubility had high correlation with the texture of broiler muscle while sarcomere length and collagen solubility were very important factors influencing the cooking loss and texture of cooked TIC muscle.

Changes in intramuscular connective tissue (IMCT) of muscles from both breeds during thermal processing were investigated. DSC study revealed that no differences in thermal stability between native IMCT of broiler and TIC was observed. However, after thermal processing, differences in IMCT between both breeds were detectable. Heating temperature lower than 70°C resulted in the increased thermal stability of chicken IMCT. With heating at 70°C for 20 min, IMCT samples was fully denatured and partially refolded during cooling. With heating at the temperature of 80°C and 90°C, broiler and indigenous IMCT could not be renatured. With heat treatment, no marked difference in protein patterns between broiler and indigenous IMCT was observed.

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Saowakon Wattanachant

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Chapter 1

INTRODUCTION

Introduction

In Thailand, production of animal for consumption spreads economically and tends to increase in the future. Chicken is produced mostly 78.49% of total animal production in all parts of Thailand (Table 1). Jittangsomboon (2000) reported that production would be increased to 860 million chickens with 56.5% for domestic consumption and 43.5% for export. Thailand exports chicken in form of frozen and processed chicken meat to Australia, Hong Kong, Southern Korea, Netherlands and USA. The export value was reported to increase from year 1999 to 2000 (Katenil, 2000). The data obtained clearly indicates that chicken is one of the most important commercial animals of food industry of Thailand.

Table 1. Number of livestock and poultry raised in all parts of Thailand in year 1996

Animal type	Middle	Northeast	North	South	Total	Percent
Beef cattle	1,242,521	2,477,834	1,272,189	891,985	5,854,592	3.18
Dairy cattle	182,467	68,527	18,840	6,511	276,345	0.15
Buffalo	153,892	2,258,494	236,686	70,602	2,719,674	1.48
Swine	5,322,550	1,360,637	1,194,921	819,779	8,707,887	4.78
Goat	9,659	1,582	10,892	75,671	97,804	0.05
Sheep	8,734	1,009	2,211	30,389	42,343	0.02
Duck	11,591,646	5,933,781	1,836,174	2,203,036	21,924,637	11.90
Chicken	69,963,645	37,506,727	23,028,677	14,080,379	144,579,428	78.49
Total	88,485,114	49,608,591	27,600,590	18,178,352	184,202,710	100.00
Percent	48.04	26.93	14.98	9.87	100.00	

Source: Dept. of Livestock development (1996)

There are many chicken breeds in Thailand. Among those breeds, broiler and Thai indigenous chicken are commercially produced for consumption. Both are different in growth rate. Broiler has faster growth rate than the latter. Broiler aged 38-45 days has 1.2-2.0 kg live weight, while Thai indigenous chicken aged 4-5 months will obtain the same live weight. However, the indigenous chicken can be raised with the low production cost. Farmers generally simply raise them in free range with any organic feed or supplement with the concentrated pellet. Moreover, its meat has unique taste and texture and has been regarded as a great delicacy and becomes very popular among Thai consumers. It is also an alternative for consumers preferring low fat and antibiotic-free white meat. This leads to a higher price, approximately two or three times higher than that of commercial broilers (Chotsangkad and Kongrattananun, 1999). A similar phenomenon is also seen in Hong Kong, Southern China and Japan (Ding *et al.*, 1999). Leoytarakul and Pimkumhlai (1999) reported that the consumption of Thai indigenous chicken meat has a trend to increase from 5 kg/man/year in 1997 to 12.5 kg/man/year in 2001.

The indigenous chicken generally has slower growth rate than the commercial broiler when raised under the same commercial condition. In addition, they have traits of fighting cocks including strong and firmer muscles. This possibly contributes to the differences in the meat properties and quality between both chickens. Generally, indigenous chicken possesses a firm texture and much more flavour, particularly after cooking, compared to commercial broiler. This is postulated to be due to the differences in muscle structure arrangement and meat compositions, especially protein and lipid. However, no information on chemical compositions, properties and structure of the indigenous chicken muscle has been reported. The information gained from this research will be beneficial for the improvement of chicken meat with the dominant characteristics like indigenous chicken in term of breeding, production as well as processing.

Literature Review

Many researches in Thailand have been focused on the development of indigenous chicken production and quality of its carcass (Panja, 1998; Chomchai *et al.*, 1998; Chotsangkad and Kongrattananun, 1999; Leoytarakul and Pimkumhlai, 1999; Jaturasitha, 2002). However, the quality of indigenous chicken carcass from those researches was evaluated by comparing only its proximate chemical compositions. The quality of meat in term of textural characteristic, microstructure and chemical composition of muscle protein is very important for comparison of meat from different breeds (Chomchai *et al.*, 1998).

Chemical composition and structure of muscle

Muscle contains water, protein, lipid (fat), carbohydrate, mineral (ash), organic extractives, and nucleic acids in variable amounts upon the forms in which they are present and the changes occurring as the muscle reaches maturity, as shown in Table 2. Muscle proteins can be divided into three main groups: (1) sarcoplasmic, (2) myofibrillar, and (3) connective tissue proteins based on their solubility characteristics. The sarcoplasmic proteins are those muscle proteins that are soluble in water or dilute (<50 mM) salt solutions. Their name comes from the fact that these proteins are located in the sarcoplasm, the fluid bathing the muscle cells or fibres. They comprise about 5.5% of typical adult mammalian muscle, and are somewhat higher in the muscles of young immature animals (Table 3). The myofibrillar proteins are muscle proteins that are soluble in concentrated salt solution, especially in 0.6 M KCl. They make up approximately 11.5% of the total protein in muscles. The connective tissue proteins are structural components that vary in abundance and density. Connective tissue is composed of three major components: cells, the fibrous intercellular substances and the amorphous intercellular substances. The fibrous components include three types of fibers, collagenous, reticular, and elastic fibers, which each have their own characteristic chemical composition and staining specificity. All of the

fibrous substances are complex proteins formed from long chains of amino acid joined together by peptide linkages. They are relatively insoluble in neutral solvents; thus, they exist as formed fibers in the internal fluid environment of the body.

Table 2. Proximate composition of muscle, forms present, and changes during development

Constituent	Type of Tissue	Range (%)	Forms Present	Changes during Development
Water	Adult skeletal muscle	70-78	Immobilized and free, mainly in association with the proteins	Maximum in embryo, decreases with age and with fat content
Protein	Adult skeletal muscle	15-22	Sarcoplasmic, myofibrillar, and stromal proteins	Decreases from embryonic to adult state and with fat content
Lipid	Total fat in adult skeletal muscle	1-13	Triacylglycerides, phosphoglycerides, glycolipids, proteolipids, and depot fat	Highly variable, increases during development, inversely related to water content
Lipid	Intracellular fat in muscle cells	0.5-3.0	Mainly in membranes as glycolipids and proteolipids, some as fat droplets in sarcoplasm	Increases slightly up to adulthood, low and relatively constant
Carbohydrate	Adult skeletal muscle	1-2	Glycogen, monosaccharides and other metabolic intermediates, glycolipids, and acid mucopolysaccharides	Increases slightly from embryonic to adult states, relative constant
Minerals	Adult skeletal muscle	1-2	Constituents of extracellular and intracellular fluids, also may be bound to tissue	Increases with age up to maturity
Vitamins	Adult skeletal muscle	μg% range	Largely found bound as coenzymes or constituents of tissue	Increases from embryonic state to birth, may vary with levels in diet
Nitrogenous nonprotein extractives	Adult skeletal muscle	1.5-1.8	Free amino acids, creatine, carnosine, aserine, glutathione, and various hormones	Increases slightly from embryonic to adult, largely because of decrease in water content

Source: Pearson and Young (1989)

Table 3. Chemical composition of typical adult mammalian muscle

Components	Wet % weight
Water	75.0
Protein	19.0
Sarcoplasmic	(5.5)
Myofibrillar	(11.5)
Connective tissue	(2.0)
Lipid	2.5
Carbohydrate	1.2
Soluble non protein substances	2.3
Nitrogenous substances	(1.65)
Minerals	(0.85)
Total soluble phosphorus	(0.20)
Potassium	(0.35)
Sodium	(0.05)
Magnesium	(0.02)
Calcium, Zinc, trace metals	(0.23)
Vitamins	Trace

Source: adapted from Lawrie (1991)

Muscle proteins tend to be hydrophillic and bind 300-600 g of water per 100 g of protein (Pearson and Young, 1989). The major part of this water is present as free molecules within the muscle fibers and associated connective tissue. Hamm (1963) (cited in Pearson and Young, 1989) reported that 70% of the water content of fresh meat is bound by the myofibrils, 20% by the sarcoplasm, and 10% by the connective tissue. Thus the myofibrillar proteins appear to be the main water-binding components in muscle.

Myofibrillar proteins are the structural proteins that make up the myofibrils, which contain the basic structural unit responsible for contraction in the living animal. The myofibrillar proteins are also involved in the development of rigor mortis following death. Not all of myofibrillar proteins are believed to be directly involved in the series of events occurring during contraction but may further subdivided into three subgroups:

(1) The major contractile proteins, which include only actin and myosin;

(2) The regulatory proteins, which play an important function in initiation and control of contraction; and

(3) The cytoskeletal or scaffold proteins, which provide structural support and may function in keeping the myofibrils in alignment or register.

The various myofibrillar proteins by groups and gives their proportions relative to the total protein in the myofibril are presented in Table 4. The location of individual proteins of the myofibril is indicated by fine structure of single sarcomere, unit of muscle fiber structure as shown in Fig 1.

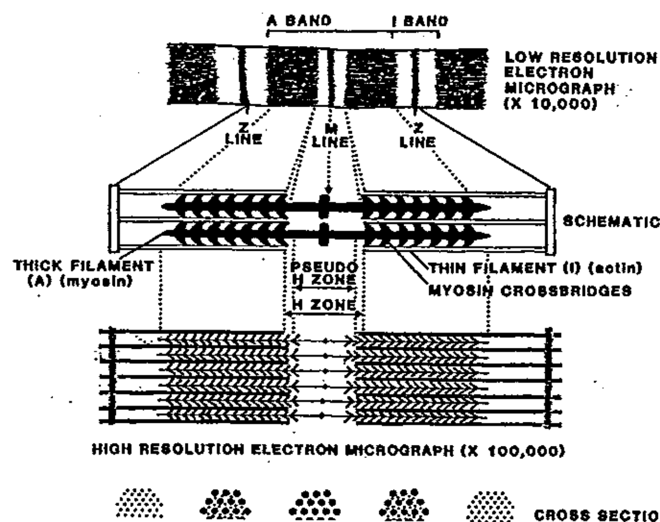

Fig 1. Fine structure of a single sarcomere along with portions of two adjacent sacomeres

Source: Pearson and Young (1989)

Table 4. Relative percentages of total myofibrillar protein accounted for by individual proteins of the myofibril

Protein	% of total myofibrillar protein	Location in sarcomere	Major function
Major contractile proteins			
Myosin	50	A-band thick filaments	Contraction
Actin	20	Thin filaments	Contraction
Regulatory proteins			
Tropomyosin	3	Thin filaments	Regulates contraction
Troponin (complex)	4.5	Thin filaments	Regulates contraction
α-Actinin	1	Z-disk	Cements thin filament to Z-filament in Z-disk
β-Actinin	<0.01	A-band end of thin filament	Regulates length of thin filaments
γ-Actinin	<0.01	Thin filament	Inhibits G-actin
EU-actinin	0.3	Z-disk	Polymerization, interacts with actin and α-actinin to contribute to Z-disk density
Cytoskeletal proteins			
Titin (connectin)	5-8	Throughout sarcomere	Hold thick filaments in leteral register
N_2-line protein (nebulin)	3	I-band	Binds and holds titin in lateral resister (?)
C-protein	1.5	Thick filament	Binds myosin molecules in the thick filaments
Myomesin (M-protein)	0.5	M-line in center of A-band	Binds myosin molecules in the thick filaments
Desmin (skeletin)	<0.2	Z-disk	Transversely links myofibrils in Z-disks
Filamin	0.1	Z-disk	Transversely links myofibrils in Z-disks
Vimentin	0.1	Z-disk	Links Z-disks at periphery (?)
Synemin	0.1	Z-disk	Association with desmin and vimentin (?)
X-protein (55,000 dalton component)	0.2	Z-disk	Binds Z-disk filaments
I-protein	0.1	Thick filament (except center)	Inhibits Mg^{2+}-ATPase activity of actomyosin in absence of Ca^{2+}
F-protein	0.1	Thick filament	Binds to thick filaments but binding is inhibited by C-protein
Creatine kinase	0.1	M-line	Binds to M-protein

Sources: Pearson and Young (1989)

Muscle fiber is composed of smaller subunits called myofibrils. Each myofibril contains a number of smaller long thin filaments called myofilaments, which can be divided into thick and thin filaments. On examination of striated muscle with the light microscope, alternating light and dark bands are apparent (Pearson and Young, 1989). The A-bands are so called because they are anisotropic (birefringent), while the I-bands are isotropic under polarized light. Since the A-bands and I bands of neighboring myofibrils tend to lie adjacent to their counterparts, the entire muscle has a cross-banded appearance. The dark-staining A-bands contain the thick or myosin filaments with some overlapping by the thin filaments at each end of the A-band. On the other hand, the I-bands, which stain less intensely, are made up of the thin filaments on each side of the narrow but heavily staining Z-band. The repeating structural and functional unit in muscle contraction is the sarcomere or the unit lying between two adjoining Z-bands in the same muscle fiber. Fig 1 shows a diagram of the fine structure of a single relaxed sarcomere and part of two longitudinally adjoining sarcomeres in both longitudinal and cross section. The presence of the longitudinally oriented myofibrils also imparts longitudinal striations to skeletal muscle, although they are broken up at regular intervals by the cross-banding pattern. The longitudinal arrangement of the myofibrils is necessary for contraction, in which the sarcomeres shorten.

Skeletal muscle is made up of a grouping of muscle fibers surrounded and supported by connective tissue. This is shown in Fig 2, which illustrates the arrangement of the muscle fibers and the supporting connective tissues. The epimysium comprises the heavy sheath of connective tissue surrounding an entire muscle. The muscle is divided into tertiary and secondary fiber bundles that are surrounded by a finer connective tissue layer called perimysium. The perimysium then divides into an even smaller and finer connective tissue strand to surround the primary muscle fiber bundles. Within the primary bundle, a still finer connective tissue subdivision, the endomysium, envelopes each individual

muscle fiber. The endomysium is in close proximity to the sarcolemma or cell membrane that surround and provides a barrier to the sarcoplasm within each muscle fiber; however, they are distinctly separated structures. Each muscle fiber or cell is long, cylindrical, and multinucleate, with the nuclei normally being on the surface of the cell just underneath the sarcolemma. Skeletal muscle fiber vary from 1 to 40 mm in length (Pearson and Young, 1989) and approximately 1 μm in diameter (Romans *et al.*, 1994).

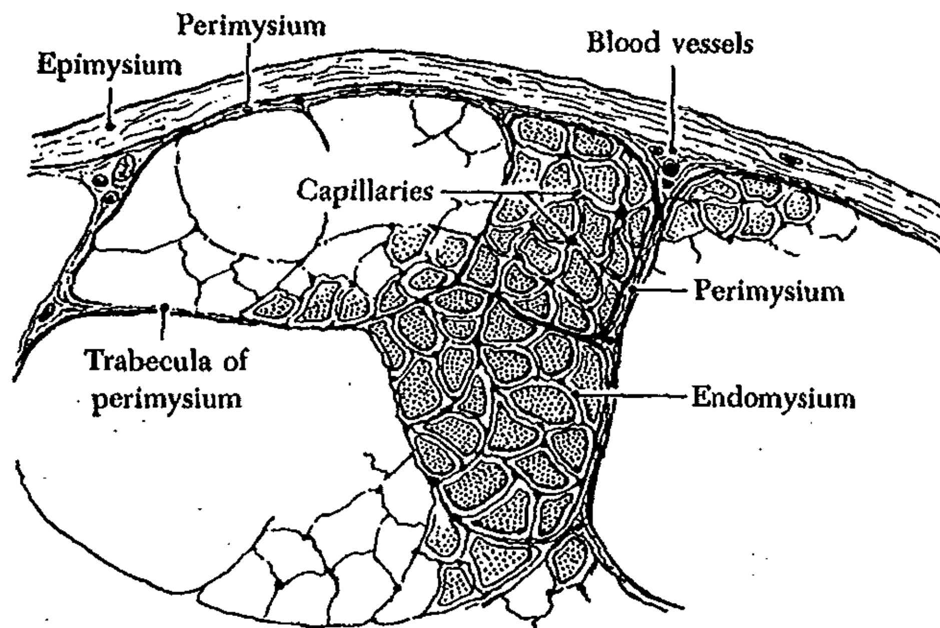

Fig 2. Diagram of a muscle in cross section showing the arrangement of the connective tissue into epimysium, perimysium, and endomysium in relationship to the muscle fibers.

Source: Ham (1965) as cited by Pearson and Young (1989)

Quality characteristics of muscle foods

Quality characteristics of meat are influenced by various intrinsic and extrinsic factors, such as muscle structure and its chemical composition, antemortem stress, as well as postmortem handling and storage condition as concluded by Xiong *et al.* (1999) in Table 5.

Table 5. Quality characteristics of muscle foods

Attribute	Responsible agent	Intrinsic factor	Extrinsic factor
Color	Myoglobin, hemoglobin	Animal age, species, fiber type, muscle pH	Oxidation, antioxidants (vitamins E&C, Se), NaCl, nitrite, packaging, storage condition (temperature&time)
Flavor	Volatiles, lipids, amino acids, nucleotides, carbohydrates	Species, muscle type, marbling	Feeding regime, oxidation, cooking condition (method, temperature), Mailard reaction
Tenderness	Muscle fibrils, connective tissue	Fiber diameter, amount and type (cross-links) of collagen, actomyosin cross-links, endogenous proteases	Postmortem glycolysis, aging (time and temperature), electric stimulation, calcium injection, plant proteases, high pressure, mechanical fiber disruption
Juiciness	Proteins and their matrix	Muscle pH, actomyosin cross-links, interfilamental spaces	NaCl, polyphosphates, water-binding agents (gums, starch, proteins), tumbling, massaging
Texture	Proteins, lipids	Muscle type, fiber diameter, fat, protein functionality (protein-protein, protein-lipid and protein-water interactions)	Protein extraction, conditions favoring gelation and emulsification (NaCl, pH, chopping, cooking rate and end temperature)

Source: Xiong *et al.* (1999); Fletcher (1999a)

The quality attributes that influence the acceptability of various meat, poultry and fishery products, should be consider how they may differ. Main factors contributing to the eating quality of poultry meat include appearance (color), texture and flavor (Northcutt, 1997).

Color

Color of raw poultry meat is critical for consumer selection whereas color of the cooked meat is critical for final evaluation (Fletcher, 1999a). The major contributing factors to poultry meat color are myoglobin content, the chemical state and reactions of the myoglobin, and meat pH.

- Myoglobin

In the live animal, myoglobin functions to temporarily bind oxygen, bridging the gap between oxygen bound to hemoglobin in blood, and chemically reduced oxygen bound to hydrogen (as water) produced by respiration in mitochondria. The porphyrin ring structure held in the confines of the myoglobin protein accounts for four of the six coordination sites available on the iron atom (Fig 3). These four sites are the nitrogen atoms of the porphyrin's pyrrole groups. A fifth coordination site is a strategically placed histidine molecule resident in the globular protein. The sixth coordination site is available for binding oxygen or other small molecule that qualifies. Binding at the sixth site is largely responsible for the various colors of meat, mainly red, but also purple, brown, and other colors (Young and West, 2001).

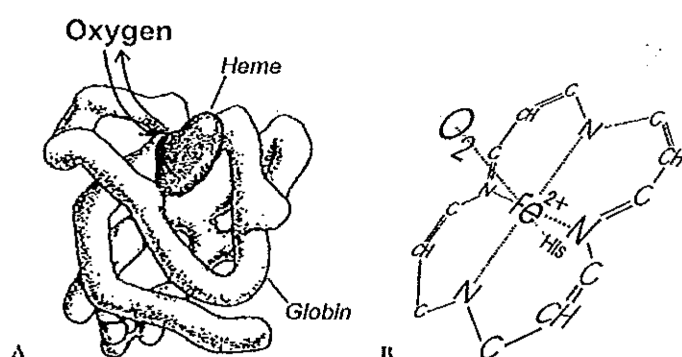

Fig 3. (a) A schematic view of myoglobin showing heme (a porphyrin ring plus iron) nestled in the globin, the protein part of the molecule. (b) A closeup of heme showing iron (Fe^{2+}) coordinated to four nitrogens of the porphyrin ring (incompletely drawn) plus a histidine residue in the globin protein. The sixth site is available for oxygen binding.

Source: Young and West (2001)

Myoglobin content has been shown to be primarily related to species, muscle type and age of the animal (Miller, 1994; Fletcher, 1999a; Young and West, 2001). The concentration of myoglobin differs from species to species as shown in Table 6. Within an animal, different muscles often have different concentrations of myoglobin, generally reflecting their role in the animal. Muscles involved in sustained repetitive action, like breathing, contain higher concentrations of myoglobin than muscles used less often (Young and West, 2001). As shown in Table 7, the *pectoralis* muscle of chicken, used for power take-offs, is paler than leg muscles.

Table 6. Myoglobin in the muscles of several species or breeds (mature animals)

Species	Approximate conc. (mg/g)	Reference
Cattle (breed unstated)[a]	2-5	Hunt and Hedrick (1977)
Sheep (Dorset)[b]	3-7	Ledward and Shorthose (1971)
Pig (Hampshire)	3-6	Topel *et al.* (1966)
Wild pig	Higher than for domestic pigs	Rahelic and Puac (1980)
Chicken	0.1-5	Nishida and Nishida (1985)
Tuna[c]	0.5-1	Brown (1962)
Human[a]	4.4-5.2	Moller and Sylven (1981)
Whale (*Hyperoodon rostratus*)	60	Scholander (1940)
Dolphins	50-72	Dolar *et al.* (1999)
Seal (*Phoca vitulina*)	80	Robinson (1939)

[a] For a range of muscles. [b] Longissimus. [c] Light muscle.

Source: Young and West (2001)

The relationship of animal species, muscle type and animal age on meat myoglobin content and visual color was presented by Miller (1994). White meat from 8-week-old poultry had the lowest myoglobin content (0.01 mg/g meat) compared to 26-week-old male poultry white meat (0.1 mg/g meat), young turkey white meat (0.12 mg/g), 8-week poultry dark meat (0.4 mg/g), 26-week male poultry dark meat (1.5 mg/g), 24-week male turkey dark meat (1.5 mg/g)

and compared to 5-month-old pork (0.30 mg/g), young lamb (2.50 mg/g), dark meat fish species (5.3-24.4 mg/g), white meat fish species (0.30-1.0 mg/g), 3-year-old beef (4.6 mg/g), and old beef (16-20 mg/g).

Table 7. Concentration of myoglobin (mg/g) within muscles of chicken

Muscle	Myoglobin concentration (mg/g)
Pectoralis	~0.1
Vastus lateralis	2.8
Vastus intermedius	5.0
Biceps femoris	0.7
Rectus femoris	2.5
Gizzard	19.0

Source: Nishida and Nishida (1985)

- Muscle pH

Muscle pH has been shown to be primarily related to the biochemical state of the muscle at time of slaughter and following rigor mortis development, affects both the light reflectance properties of the meat as well as the chemical reactions of the myoglobin (Fletcher, 1999a). Muscle pH and meat color are highly correlated. In a survey of five commercial broiler processing plants, breast meat colors were found to range with lightness values (L*) from 43.1 to 48.8 with a strong negative correlation with muscle pH (Fletcher, 1995). As mentioned by Fletcher (1999a,b), higher muscle pH is associated with darker meat whereas lower muscle pH values are associated with lighter meat. In the extremes, high pH meat is often characterised as being dark, firm and dry (DFD) and the lighter meat as being pale, soft and exudative (PSE). The effect of pH on meat color is complex. One effect, as noted earlier, is that many of the haem-associated reactions are pH dependent. In addition, muscle pH affects the water binding nature of the proteins and therefore directly affects the physical structure

of the meat and its light reflecting properties. Also, pH affects enzymatic activity of the mitochondrial system thereby altering the oxygen availability for haem reactivity (Fletcher, 1999a).

Flavor

Flavor is one of the main eating quality attributes which, together with appearance, and texture. Farmer (1999) stated that most research on the chemistry of flavor formation in poultry has been conducted on chicken (*Gallus domesticus*). Substances contributing to flavor can be divided into aroma compounds and taste compounds. Taste compounds are non-volatile or water-soluble substances with taste or tactile properties. In contrast to the taste compounds in chicken, aroma compounds are largely formed during the cooking process. However, fresh meat is characterised by two distinct types of flavors: 'species flavors' and 'meat flavor'. Species-specific flavors are due generally to fat-soluble substances, including aromatic compounds and short-chain fatty acids and their derivatives, while meaty flavor is derived from water-soluble compounds especially nitrogen-containing molecules, including degradation products and derivatives from high-energy nucleotides such as inosine monophosphate, IMP (Xiong *et al.*, 1999). Fujimura *et al.* (1995, 1996) cited in Farmer (1999) analysed the water-soluble components of cooked chicken extract and recombined the listed amino acids, metabolites of adenosine triphosphate and inorganic ions to simulate the sensory properties of the chicken extract. Only inosine monophosphate, glutamic acid and potassium ions were found to have a detectable effect on the taste.

Factors which might affect flavour include the production factors of age, sex, genotype, diet and stocking density, the method of slaughter, the postslaughter factors of time of evisceration, time and temperature of chilling and storage, and finally, cooking method. All of these factors could, in theory, affect either the composition of the raw meat, and therefore the availability of

precursors, or the progress of flavour-forming reactions during cooking (Farmer, 1999). Some researchers have reported flavour differences for genotypes of differing growth rate and have variously attributed these to differences in age (Farmer, 1999).

Texture

The most important quality attribute of chicken meat is texture (Smith and Fletcher, 1988; Dransfield, 1994; Chrystall, 1994). For intact meat, texture of muscle refers to the definition and fineness of muscle fibers and the amount and distribution of fat in the muscle. In this case, meat texture is determined by the age of the animal, the type of muscle, gender, and the growth condition (Xiong *et al.*, 1999). Tenderness is a quality attribute uniquely important to meat texture. The word tenderness is often used in reciprocation of its antonym, toughness. The role of tenderness in meat quality varies with species. Tenderness of meat may be simply defined as the ease of teeth to cut meat fibers during mastication. For intact or noncomminuted meat, tenderness or toughness is determined by two groups of meat components: the connective tissues and the muscle fibers (Xiong *et al.*, 1999). There are many factors considered to influence meat texture such as intramuscular fat (Miller, 1994; Fernandez *et al.*, 1999), intramuscular connective tissue (Liu *et al.*, 1996), muscle fiber types (Ozawa *et al.*, 2000), myofibrillar structure (Palka and Daun, 1999), post-mortem carcass aging temperature and time (Dunn *et al.*, 1993; Dunn *et al.*, 2000).

Factors influencing meat texture

Intramuscular fat

Meat fat content has been highly related to quality as fat content has been shown to affect flavour, juiciness, and tenderness of meat (Miller, 1994). Documentation of the role of intramuscular fat in meat palatability has been extensively studied. The study of Fernandez *et al.* (1999) in pig meat showed that

the acceptation of marbling and flavour increase according to the increment of intramuscular fat content. While toughness of muscle *longissimus lumborum* decrease as level of intramuscular fat increase up to 3.5%.

Intramuscular connective tissue

Connective tissue is a fibrous structure composed primarily of collagen fibrils. There are three types of connective tissue in meat: epimysium, perimysium and endomysium. Since epimysium is normally removed when cooked meat is consumed, only the intramuscular connective tissue, perimysium and endomysium, present a realistic toughness problem to meat and meat product (Xiong *et al.*, 1999). Intramuscular connective tissue (IMCT) is an important constituent when the physical properties of meat are considered, although the amount of collagen and elastin in muscle is low (Palka, 1999). The amount, composition, and arrangement of IMCT directly affect the texture of meat (Bailey and Light, 1989; Lawrie, 1991; Liu *et al.*, 1996). Total amount of connective tissue from different muscles of the same animal are different (Miller, 1994; Liu *et al.*, 1996). The age of an animal can influence the quantity of intramuscular collagen, which could contribute to the difference in tenderness among those muscles (El, 1995; Liu *et al.*, 1996). So far the role of connective tissue on meat tenderness has been elucidated (Nishimura *et al.*, 1995; Liu *et al.*, 1995; Liu *et al.*, 1996; Nakamura *et al.*, 2003; Brooks and Savell, 2004). The specific influence of intramuscular connective tissues depends on their thickness, i.e., the amount of collagen present, as well as the density and type of cross-linkages between collagen fibrils (Xiong *et al.*, 1999). Liu *et al.* (1996) found that collagen content and thickness of perimysium connective tissue affected toughness of chicken muscle (Liu *et al.*, 1996). The collagen content and its property are important because it contributes significantly to toughness in muscle. Some of the collagen is soluble in neutral salt solution, some is soluble in acid, and some is insoluble. The properties of collagen are dependent on its structure and composition with change as animal age. Foegeding and Lanier

(1996) mentioned that the amount of some amino acids in collagen could affect collagen thermal stability. The collagen that contains small concentration of hydroxyproline and proline denature at lower temperatures than do those with large concentration. The molecule of collagen can be dissociated and form the α, β, and γ components which are monomers, dimers and trimers of α chains, respectively (Pearson and Young, 1989). The covalent crosslinks are involved the β and γ components. As animals age increase, collagen crosslinks are converted from a reducible form to a more stable nonreducible form (Pearson and Young, 1989; Foegeding and Lanier, 1996). Cross-links stabilize the collagen molecule and impart tensile strength to the connective tissue, which is necessary to resist the physical force place on these structural elements. This may partially explain why meat from older animals is tougher than that from younger animals, even though muscles from younger animals generally contain more collagen (Pearson and Young, 1989; Foegeding and Lanier, 1996; Nakamura *et al.*, 1975). This fact also suggests that collagen molecules of intramuscular connective tissue changes to a structure of higher heat resistant through the formation of cross-linkages (Nakamura *et al.*, 1975). As cross-linking of collagen increase it becomes less soluble in a variety of solvents, such as salt and acid solutions. The crosslinking extent can be measured by determination of the thermal solubility of collagen or by differential scanning calorimetry (Torrescano *et al.*, 2003).

Muscle fibers

The essential structure unit of all muscles is the fiber. Fibers may attain a length of 34 cm but only 10-100 μm in diameter. The diameters of muscle fibers differ from one muscle to another and between species, breeds and sexes. Lawrie (1991) stated that the size of the muscle fiber bundles determines the texture of the muscle. However, there is an indirect correlation between muscle fiber diameter and tenderness. Muscle fiber can be classified into two types, red and

white fibers, based on their histological and biological differences. Red fibers are rich in mitochondria, respiratory enzymes and myoglobin and have wide Z-lines. They tend to operate over long periods without rest. White fibers are poor in mitochondria and have small amounts of respiratory enzymes and myoglobin. They have larger diameter and narrow Z-lines as compared to red fibers. White fibers tend to operate in short fast bursts with frequent periods of rest. The characteristics of fiber types may be influenced by both inherited, and environmental conditions such as breed, sex, age and feeding practice (Lawrie, 1991; Ozawa *et al.*, 2000). The report stated by Ozawa *et al.* (2000) shows that the white muscle fiber content was negatively correlated ($P<0.05$) with shear value while at the same time red muscle fiber content was positively correlated ($P<0.05$) to this value. Sarcomere length (distance between adjacent Z-discs) is used as a measure of muscle contraction and is highly correlated with tenderness of prerigor and rigor meat (Lyon and Buhr, 1999). The peak in resistance for shear data are observed at 35-40% sarcomere shortening (1.4 μm) when actin filaments touch opposite Z-discs and myosin filaments penetrate Z-discs (Lyon and Buhr, 1999; Foegeding and Lanier, 1996). Lower resistance to shearing and greater tenderization are associated with longer and shorter sarcomeres. However, in post-rigor meat, wide ranges of sarcomere lengths among bovine muscles were observed but had weak correlation with the shear value (Torrescano *et al.*, 2003).

Weakening of muscle during post-mortem ageing

Takahashi (1996) mentioned that tenderness of meat is the sum total of the mechanical strength of skeletal muscle tissue and its weakening during post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animal and fowl. Post-mortem ageing to store intact carcasses at refrigerated temperatures prior to deboning or cooking consists of two phases (Walker *et al.*, 1995). The first is rigor mortis development, in which post-mortem muscle cell metabolism gradually shifts from aerobic to

anaerobic pathways, adenosine triphosphate (ATP) is depleted, and actomyosin is formed. In broiler breast muscle, this process requires 4 h and if meat is cut-up and deboned prior to this time it is toughened (Stewart *et al.*, 1984; Lyon *et al.*, 1985; Dawson *et al.*, 1987). The second phase, termed rigor mortis resolution, involves structural degradation of muscle protein and results in improved meat tenderness (Lawrie, 1991). To obtain meat of high quality, post-mortem ageing of meat at around 4°C for a certain period is required. Ageing periods are usually more than 10 days, 5-6 days and 0.5-1 days for beef, pork and chicken, respectively (Takahashi, 1996). As reported by Takahashi (1996), the tenderization of each meat occurs in two steps, a rapid phase first and slow phase thereafter. The rapid increase in tenderness are mainly due to the structural weakening of myofibrils and intermediate filaments, and slow process is caused chiefly by the structural weakening of intramuscular connective tissue. The major factor responsible for weakening in skeletal muscle is degradation of muscle proteins such as Z-disk proteins, actomyosin, connectin, nebulin and desmin (Takahashi, 1996). The structural weakening of intramuscular connective tissue was found to be due to the denaturation of three dimension networks of collagen fibrils in endomysium and perimysium (Nishimura *et al.*, 1995; Takahashi, 1996).

The degree to which meat becomes tender is affected by post-mortem proteolysis. There has been a debate about the specific proteases responsible for meat tenderisation during post-mortem ageing. Several arguments supporting, or opposing, a single role or synergistic action of the calpain/calpastatin or the cathepsin/cystatin systems in meat tenderisation have been put forward (Ouali, 1990; Walker *et al.*, 1995; Wiklund *et al.*, 1997). However, there is overwhelming evidence in support of the former system as the primary mechanism of post-mortem proteolysis (Koohmaraie, 1992; Alvarado and Sams, 2000). In most mammalian and avian tissues, the calpain proteolytic system consists of two isoforms (μ and m) of a neutral (pH) calcium-dependent protease

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(Northcutt *et al.*, 1998). However, differences in an initial level of those proteolytic enzymes have been reported among muscles from different chicken strains with different growth rates (Johari *et al.*, 1993; Schreurs *et al.*, 1995). Meat tenderisation is also a multifactorial process dependent on a number of biological (e.g. species, age, sex and muscle type) and environmental factors (nutrition, ante-mortem stress, slaughter and chilling conditions and ageing) (Wiklund *et al.*, 1997). Meat from animal in different species or breeds exhibits different the changing rate of structural weakening of muscle and degradation of muscle protein, leading to differences in its textural properties.

Decrease in pH of muscle caused by the accumulation of lactic acid is generally determined as a measure of rigor mortis development (Cavitt and Sams, 2003). The ultimate pH is very important since it affects the texture quality of meat, the water-holding capacity, the resistance to growth of microorganisms, and the color (Foegeding and Lanier, 1996). Differences in the extent of the myofibrillar proteolysis among muscles or species can be demonstrated using the myofibrillar fragmentation index, the intensity of low molecular weight proteins or peptides and the solubility of protein (Alvarado and Sams, 2000; Walker *et al.*, 1995; Wiklund *et al.*, 1997; Young *et al.*, 1980; Morrissey *et al.*, 1993). The proteolysis of intramuscular connective tissue during post-mortem ageing can be indicated by disintegrating of endomysium and perimysium, collagen solubility and heat stability of collagen (Liu *et al.*, 1995; Nishimura *et al.*, 1995; Wiklund *et al.*, 1997; Mills *et al.*, 1989; Berge *et al.*, 2001). Change in texture of muscle related to the post-mortem proteolysis is usually monitored by shear value (Cavitt and Sams, 2003).

Thermal process

Thermal processing has a large effect on meat toughness. Heating muscle food results in chemical and physical changes that will effect the texture, palatability, and consumer acceptance of the final product (Califano *et al.*, 1997).

The principle proteins responsible for meat texture include stromal (mostly collagen) and myofibrillar proteins (Dawson *et al.*, 1991; Califano *et al.*, 1997). According to Bailey and Light (1989), during the cooking of meat there is first an increase in toughness between 40 and 50°C owing to the beginning of denaturation of myofibrillar proteins, a further increase between 60 and 70°C because of shrinkage of intramuscular collagen at 65°C, and a third increase in the range 70-90°C when shrinkage and dehydration of the actomyosin occurs. However, Christensen *et al.* (2000) studied the effect of heating temperature on changes in whole meat, single muscle and perimysium connective tissue of beef *semitendinosus* muscle and found that the changes were divided into two phases. The initial rise in meat toughness was due to thermal shrinkage of intramuscular connective tissue at temperatures between 40-50°C. The second rise in meat toughness could be due to heat denaturation of myofibrillar proteins at temperature above 60°C. The difference in results later might be because Christensen *et al.* studied single fibers. Palka and Daun (1999) found that tenderizing of beef *semitendinosus* muscle was occurred when processed at the high temperature of 121°C. In contrast, unacceptable effects on textural characteristics including toughening, drying, and loss of particulate shape, were observed after high temperature processing of chicken breast meat (Dawson *et al.*, 1991). The different results obtained from the different specie muscles may be caused by the difference in muscle type, structure and compositions such as intramuscular collagen. The increment in collagen content and collagen cross-linking in meat (often associated with older animals and specific muscle types) will increase the toughness of cooked meat (Dawson *et al.*, 1991; Rochdi *et al.*, 2000).

Type and maturity of collagen affect the structure of connective tissue and consequence to their thermal stability. According to Rochdi *et al.* (2000), the influence of type and maturity of collagen on their thermal stability were elucidated by the variation of their thermal solubility as a function of heating

time. Changes in collagen solubility with heating temperature could affect the textural and water-binding properties of the product (Eilert and Mandigo, 1993). Heat denaturation of collagen is accomplished by unfolding of the triple helix results in dramatic changes in the physical and chemical properties (Pearson and Young, 1989). Cooking of muscle from several species of animal caused the changes in the structure, solubility and mechanical properties of the intramuscular connective tissue, mainly owing to the denaturation of collagen (Palka, 1999, Christensen *et al.*, 2000; Lepetit *et al.*, 2000;. Mizuta *et al.*, 1999). Changes in thermal properties of extracted intramuscular connective tissue (IMCT) under various conditions were studies on beef IMCT (Rochdi *et al.*, 2000; Akta and Kaya, 2001; Akta, 2003).

Information related to texture of chicken meat

As discussed previously, the texture of muscle could be affected by composition, structure and properties of muscle fiber and intramuscular connective tissue, which changed under various conditions. Muscle structure can be elucidated by using microscope, laser diffraction, transmission electron microscope or scanning electron microscope to reveal details of the structure of muscle fibers both original and subjected to a variety of treatments (Jones *et al.*, 1976; Sundell *et al.*, 1986; Young *et al.*, 1990; Liu *et al.*, 1996; Palka and Daun, 1999). The morphology of muscle structures both original or changes after treatments can be related to meat tenderness (Jones *et al.*, 1976; 1977). The muscle structure may vary, depending on muscle types, species and breed of animal, which contribute to the differences in texture of muscles. Most studies on the composition, properties and structure of the chicken meat have focused on the muscles with high commercial value especially from breast and thigh muscles (Smith and Fletcher, 1988; Smith *et al.*, 1992; 1993; Ding *et al.*, 1999). From previous report, *Pectoralis m.* (breast muscle) and *Biceps femoris m.* (thigh

muscle) can be represented for tender and tough muscle as related to their shear values (Liu *et al.*, 1996). The anatomy of chicken muscles is shown in Fig 4.

Liu *et al.* (1996) reported that the thickness of perimysium varied depending on the muscle types of chicken and had high correlation with the shear value. Furthermore, muscle fiber types and diameter in chicken, goose and duck were compared and found to determine their meat quality (Kiessling, 1977; Smith *et al.*, 1993). Smith *et al.* (1992; 1993) compared structure and chemical composition of *Pectoralis M.* between Pekin duckling and broiler. It was found that both meats showed the different chemical compositions, muscle fiber type and area, muscle color and collagen content, resulting in difference in tenderness of meat from both species. Ding *et al* (1999) identified chicken meat species from either minced or whole carcasses of broiler or local chickens by using the NIR spectroscopic technique. The spectroscopic classification was supported by physical and chemical properties of meat samples, which showed significant differences in collagen and fat contents and pH and chromatic values between the two breeds of chicken. Information on changes of chicken muscle during post-mortem ageing of carcass is also needed for processors to determine carcass quality for further process. So far, post-mortem ageing of broiler muscle in several aspects has been reported (Stewart et al., 1984; Lyon *et al.*, 1985; Dawson *et al.*, 1987; Savenije *et al.*, 2002; Alvarado and Sams, 2000; Northcutt *et al.*, 2001). The indigenous and broiler chickens are consumed approximately at the same commercial live weight. However, indigenous chicken generally has different behavior and slower growth rate than the commercial broiler and this may contribute to the differences in the properties of their meats. Nevertheless, no information about chemical composition, physical and chemical properties, microstructure, post-mortem changes and thermal characteristics of Thai indigenous chicken has been published.

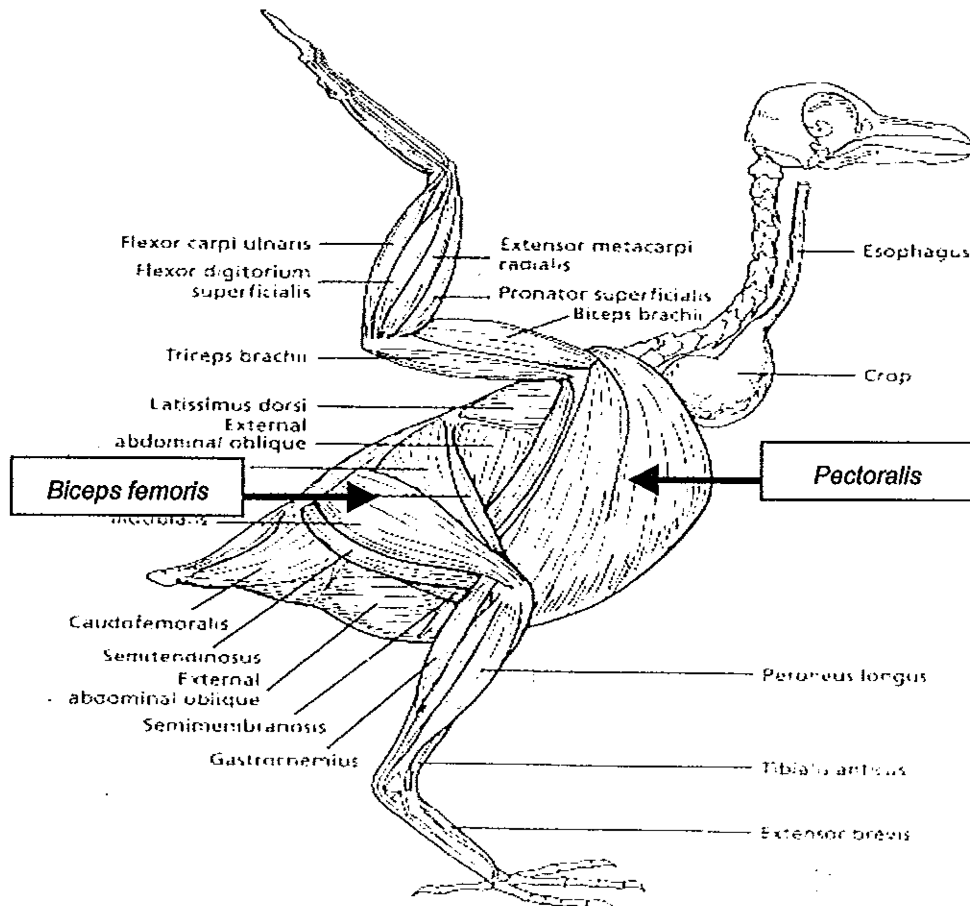

Fig 4. Anatomy of chicken muscles

Source: Graaff and Crawley (1995)

Objectives

1. To determine the textural characteristic, chemical composition and microstructure of muscle from Thai indigenous chicken and broiler.
2. To investigate the changes in textural characteristic, muscle structure and proteolysis of Thai indigenous chicken meat during post-mortem ageing.
3. To study the effect of heating on the changes in texture, muscle structure and muscle protein properties of Thai indigenous chicken meat.

Expected Outcomes

1. To understand the relation between structure and chemical composition of muscle and textural characteristic of chicken meat. The data obtained can be used as basic knowledge for the improvement of chicken breeding, production, and processing.
2. To know the factors affecting texture of chicken meat. Subsequently, the utilization of Thai indigenous chicken meat in the food industry will be maximized.

Scope of the Research

To fully understand the differences in texture between the Thai indigenous chicken and broiler meats, it was necessary to characterize both chicken meats in term of microstructure of muscle, chemical compositions and their properties. Chickens raised under commercial condition in the local farm were used throughout this study. The age and live weight of chicken used were equivalent to those available in the market.

Chapter 2

MATERIALS AND METHODS

1. Study on chemical compositions, muscle structure and textural characteristic of meat from Thai indigenous chicken and broiler

1.1 Sample characteristics

Thai indigenous chickens (*Gallus domesticus*) used throughout this study had traits of fighting cocks with black feather, red face, yellow tarsus and pea crest either male or female. They were raised under the scavenging of backyard farming system. Commercial broilers (*CP707*) were raised under intensive system using commercial broiler diet. Both breeds aged 16 weeks and 38 days, respectively, of similar liveweights (1.5 ± 0.2 kg) were obtained from a local farm in Songkhla, Thailand. The characteristics of both breeds are shown in Fig 31 (see Appendix).

1.2 Sample preparation

Thirty six mixed-sex Thai indigenous chickens aged 16 weeks and 36 mixed-sex commercial broilers aged 38 days of similar liveweights (1.5 ± 0.2 kg) were killed 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. The muscle samples from each of four birds of each breed were stored at 4°C until analysed for cooking loss, color, shear force values perimysium thickness, muscle structure and thermal transition within two days (used totally 24 birds for each breed). The muscle samples from each of four birds of each breed were minced, placed in plastic bags and stored frozen (-20°C) until used for chemical analyses (used totally 12 birds for each breed).

1.3 pH of muscle and proximate analysis

The pH of the muscles was determined 24 h post-mortem by homogenizing the muscle samples with distilled water at a ratio of 1:5 (w/v). The homogenate was subjected to pH measurement using a combined glass electrode pH meter (Sartorious model PB-20, Goettingen, Germany).

Proximate analysis: moisture, protein, fat and ash were determined as follows: moisture, oven method (AOAC, 1999); protein, Kjeldahl method (AOAC, 1999); fat, Soxhlet apparatus method (AOAC, 1999); and ash, furnace 600°C (AOAC, 1999).

1.4 Determination of protein composition

The protein components in muscle samples were fractionated according to the method of Hashimoto *et al.* (1979) with a slight modification. Samples (10 g) were extracted with 10 volumes of solution A (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.5) using homogeniser Ultra Turrax T25 (IKA Labortechnik, Germany) at a speed of 11,000 rpm for 1 min. The homogenate was centrifuged at 5,000xg for 15 min. The extraction was repeated twice. The supernatants were combined and mixed with cold 50% TCA to a final concentration of 10%. The resulting precipitate was collected by centrifugation at 5,000xg for 15 min (the sarcoplasmic protein fraction). The filtrate was the non-protein nitrogen (NPN) fraction. The pellet was extracted with 10 volumes of solution B (0.45M KCl, 15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.5) using homogeniser at 11,000 rpm for 1 min and centrifuged at 5,000xg for 15 min. The extraction was repeated twice. The supernatants were combined (the myofibrillar protein fraction). The pellet obtained was extracted with 10 volumes of 0.1M NaOH with continuous stirring overnight. The mixture was centrifuged and supernatant was the alkali-soluble protein fraction. The final residual was used as stroma protein fraction. The nitrogen content of all protein and non-protein fractions was determined by the Kjeldahl method (AOAC, 1999). All protein fractions

were analysed by SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (1970).

1.5 SDS-PAGE analysis

Samples (3 g) were homogenised in 5% (w/v) SDS (27 ml) at 11,000 rpm for 1 min with a homogeniser. The mixture was incubated at 85°C for 1 h. the extract was then centrifuged at 6,100xg for 10 min. The protein content of the supernatant was analysed according to the Biuret method (Robinson and Hodgen, 1940). SDS-PAGE was carried out by the method of Laemmli (1970). The supernatants were mixed at a ratio of 1:1 (v/v) with the SDS-PAGE sample buffer containing 1% β-ME and boiled for 3 min. the samples (20 μg) were loaded on the gel made of 4% stacking and 10% separating gels and then subjected to electrophoresis using a mini-Protein II cell Bio-Rad apparatus (Bio-Rad Laboratoies Ltd, USA). After electrophoresis, the gels were stained with 0.02% Coomassie Brilliant Blue R-250 in 50% methanol and 7.5% acetic acid and destained with 50% methanol and 7.5% acetic acid for 30 min followed by destaining with 5% methanol and 7.5% acetic acid for 24 h.

1.6 Determination of total collagen content

Total collagen content was determined after acid hydrolysis as described by Palka (1999). Finely ground muscle (500 mg) was hydrolyzed with 25 ml of 6 M HCl at 110°C for 24 hr. The hydrolysate was clarified with active carbon, filtered and neutralized with 10 M and 1 M NaOH, and diluted with distilled water to a final volume of 250 ml. The hydroxyproline content in the hydrolysate was determined by the procedure of Bergman and Loxley (1963) and converted to collagen content using the factor 7.25. The collagen content was expressed as mg of collagen per g of muscle.

1.7 Determination of soluble collagen content

Soluble collagen was extracted according to the method of Liu *et al.* (1996). Muscle samples (2 g) were homogenized with 8 ml of 25% Ringer's solution.

The homogenates were heated for 70 min at 77°C and centrifuged for 30 min at 2,300xg at 4°C. The extraction was repeated twice. The supernatants obtained were combined. The sediment and supernatants were hydrolyzed with 6 M HCl at 110°C for 24 hr. The collagen content of the sediments and supernatants 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).

1.8 Determination of myoglobin content

Myoglobin content in raw muscle samples was measured by spectrophotometric method (Geileskey *et al.*, 1998). Muscle samples approximately 5 g were homogenised at 16,000 rpm using Ultra Turrax T25 (IKA Labortechnik, Germany) to a fine slurry in 12 ml of 0.1 M phosphate buffer (pH 6.5) for 20 s. The slurry was centrifuged at 5,000xg for 15 min and the supernatant was filtered into a 25 ml volumetric flask through glass wool. The insoluble residue was homogenised in a further 12 ml of phosphate buffer for 20 s, centrifuged and the supernatant again was filtered into the volumetric flask. The extract was made up to volume and stored at 4°C. The myoglobin in the extract was determined by measuring the absorbance at 525 nm. The concentration was calculated according to the equation mentioned by Eder (1996): total myoglobin (mmol/l) = $0.132A_{525}$. The myoglobin content was expressed as mg per g of muscle using molecular weight of 16,110 (Gomez-Basauri and Regenstein, 1992).

1.9 Fatty acid determination

The fatty acid composition of the muscle samples were determined after extraction of the fat by the method of Bligh and Dryer (1959). The fatty acids present in the extracted lipids were transformed into their methyl esters (Metcalfe *et al.*, 1961). Fatty acid methyl esters were determined by gas chromatography (AOCS, 1991).

1.10 Amino acid determination

The amino acid profile of the muscle samples was determined by the method of Liu *et al.* (1995) using high performance liquid chromatography (HPLC: Waters, Water Corp., MA, USA).

1.11 Determination of muscle color

The color of the whole muscle samples in the anterior and posterior locations was determined in four replicates of each of four birds (n=16) using a Hunterlab colorimeter (Hunterlab ColorFlex model with Universal software, Hunterlab, USA) and reported in the complete International Commission on Illumination (CIE) system color profile of L*, redness (a*), and yellowness (b*).

1.12 Determination of cooking loss

Cooking loss of muscle samples was determined in four replicates of each of four birds (n=16) followed the method of Murphy and Marks (2000). Small pieces (1.5x3.0x0.5 cm) of each muscle from each chicken were cut, put in tightly sealed plastic bag and cooked in a water bath at 80°C for 10 min. After cooking, the samples were cooled to room temperature using running water. The muscle samples were removed from the container, blotted with filter paper, and weighed to determine the cooking loss as percentage of initial weight (w/w, wet basis). The cooked muscle samples were kept at 4°C for shear analyses.

1.13 Shear analysis

Muscle samples, raw and cooked, were cut to size 1.0x2.0x0.5 cm for shear analysis using the Texture Analyzer (TA-XT2i, Texture Expert Version 1.17; Stable Micro System, Godalming Surrey, England) equipped with a Warner-Bratzler shear apparatus (Dawson *et al.*, 1991). 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 in four

replicates for each of four birds, for both muscle types. The peak of the shear force profile was regarded as the shear force value.

1.14 Determination of perimysium thickness

Perimysium thickness were determined using the Picro-Sirius Red polarisation (PSRP) method as described by Liu *et al.* (1996). Raw muscle samples (0.5x0.5x1.0 cm) were cut out from each muscle type of each breed and frozen quickly in liquid nitrogen. Transverse sections (4 μm in thickness) of the frozen muscle were cut in a cryostat at -20°C. The sections were placed in acetone for 60 min and then fixed in picro-formalin fixative containing 5% formalin, 90% ethanol and saturated picric acid for 10 min at room temperature. The sections were rinsed in 90% ethanol and under gently running water for 1 min and 10 min, respectively. They were then stained with Picro-Sirius Red solution containing 0.1% Sirius Red F3BA and saturated picric acid for 60 min, transferred into 0.01M HCl solution for 5 min, and rinsed in distilled water for 1 min. They were dehydrated in three changes of absolute ethanol, cleared in two changes of xylene, mounted in Perma-Fluor Permanent Aqueous mounting medium and examined under a light microscope (Olympus BH-2, Olympus Co., Tokyo). The thickness of the perimysium was measured at intervals of 5 mm along single perimysia on 2 micrographs obtained from each of four birds, for both muscle types and breeds. The mean thickness was estimated from the measured values (n = 40).

1.15 Determination of microstructure

Muscle structures of samples were determined in raw and cooked meat using scanning electron microscope (SEM) according to the procedure of Palka and Daun (1999) with slight modification. To prepare cooked muscles, half of the muscles from each of 4 birds of each breed were cut to approximately 3 x 3 x 1.5 cm pieces for *Pectoralis m.* and approximately whole pieces for *Biceps femoris m.* to weigh approximately 10±0.5 g. The small pieces of each muscle

were put in a tightly sealed plastic bags and cooked in a water bath at 80°C for 10 min. The cooked muscles were then cooled in running water. Another half of muscle samples was analyzed raw. The raw and cooked muscles from each breed and muscle type were stored at 4°C and their microstructures were determined within 24 hours.

Pieces (1x1x0.5 cm) were excised from raw and cooked muscle samples and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.3 for 2 h at room temperature. The specimens were then rinsed with distilled water and dehydrated in graded ethanol solutions with serial concentration of 25, 50, 70, 95% and absolute ethanol (twice). The dehydration was conducted 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 SEM (JSM-5800LV, JEOL Scanning microscope), using an accelerating voltage of 5 or 10 kV. The micrographs and videoprints were taken at a magnification of x500 (5 kV) for transverse sections and x10,000 (10 kV) for longitudinal ones. The areas of muscle fibers and the length of sarcomeres were measured on videoprints, using a special morphometric facility. Two videoprints from each of four birds of each breed and muscle type sample were taken and ten measurements of fiber area and five measurements of sarcomere length on each were made, n = 80 and n = 40, respectively. The fiber diameter was calculated from the fiber area. The micrographs and videoprints for transverse sections of endomysium and perimysium from raw and cooked muscle specimens of each breed and muscle types were taken at magnification of x1,000 (5kV).

1.16 Determination of thermal transition

Raw chicken muscles from each breed and muscle type were ground and stored at 4°C for 3 days before thermal analysis. The average moisture content of muscle samples before analysis was in the range of 75.2 –75.9%.

For thermal analysis, samples were scanned in a differential scanning calorimeter (DSC: Perkin Elmer DSC7), calibrated with indium for heat flow, temperature, and enthalpy. Each muscle sample was exactly weighed (approximately 20 mg) into an aluminium pan and hermetically sealed. The heating rate of the DSC scans was 5°C/min over a range of 25-120°C. Empty aluminium pans were used as the reference and for baseline corrections. The onset temperature (T_o), temperature of peak transition (T_p) and enthalpy of transition (ΔH) were determined from typical thermograms. The determination was done in duplicate for each of four birds of each breed and muscle type, n=8.

2. Changes in structure and chemical composition of chicken muscle during post-mortem ageing

2.1 Source and storage of muscle samples

The thirty mixed-sex Thai indigenous chickens (*Gallus domesticus*) aged 16 weeks and commercial broilers (*CP707*) aged 38 days of similar liveweights (1.5 ± 0.2 kg) were obtained from a local farm in Songkhla, Thailand. The chickens were killed as previous description in item 1.2. *Pectoralis major* muscles from left side of the four carcasses were dissected immediately after slaughter and after ageing at 4°C for 2, 4, 6, 24, 48 and 72 h (Northcutt *et al.*, 2001). After removing the skin and trimming of obvious fat and connective tissue, each muscle was divided into 2 parts as shown in Fig 32 (see Appendix). The first part of muscle from each of four carcasses was cut to five small pieces (1.0 x 2.0 x 0.5 cm), n = 20, for shear analysis at each ageing time. The second part from each of four carcasses was minced, packed in plastic bag, frozen immediately in an air-blast freezer and stored at –20°C until used for chemical analysis.

2.2 pH determination

pH was determined on *Pectoralis* muscle in the right side of 30 carcasses at 0, 2, 4, 6, 24, 48 and 72 h post-mortem ageing at 4°C. pH was measured using a portable ISFET Model ARGUS pH-meter with a insertion-electrode probe (Red-Line LanceFET, Sentron, the Netherlands).

2.3 Determination of lactic acid content

Lactic acid was determined using a titration method (AOAC, 1999). Muscle sample (5 g) were homogenized with 50 ml of distilled water at a speed of 11,000 rpm for 1 min using Ultra Turrax T25 (IKA Labortechnik, Germany). The homogenate was centrifuged at 5,000xg for 15 min. The supernatant was filtered through a filter paper (Whatman No.4). The filtrate was titrated with the standardised 0.1M NaOH using phenolpthalene as the end point indicator. The total acid in the sample was expressed as the percentage of lactic acid in the muscle. The determination was performed in duplicate for each of four carcasses (n = 8) at each ageing time.

2.4 Determination of TCA-soluble peptides

The extent of proteolysis was monitored by the method of Morrissey *et al.* (1993). Muscle samples (3 g) were homogenized with 27 ml of cold 5% TCA using Ultra Turrax T25 (IKA Labortechnik, Germany) at 11,000 rpm for 1 min. The mixture was incubated at 4°C for 15 min and centrifuged at 8,000xg for 10 min. TCA-soluble peptides in the supernatant was measured according to the method of Lowry *et al.* (1951) and expressed as micromole of tyrosine per g muscle. Duplicate determination was performed for each of four carcasses (n = 8) for each ageing time.

2.5 Determination of soluble collagen content

Soluble collagen was extracted according to the method of Liu *et al.* (1996) as described previously. The determination was performed in duplicate for each of four carcasses (n = 8) at each ageing time.

2.6 Shear analysis

The pieces of muscle samples sized 1.0x2.0x0.5 cm were subjected to shear analysis using the Texture Analyzer (TA-XT2i, Texture expert Vision 1.17, Stable MicroSystem, Godalming, Surrey, UK) equipped with a Warner-Bratzler shear apparatus. 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 in five replicates for each of four carcasses, for both breeds (n = 20). The peak of the shear force profile was regarded as the shear force value.

3. Effect of heat treatment on changes in structure and protein properties of chicken muscle

3.1 Sample preparation

Twenty mixed-sex Thai indigenous chickens (*Gallus domesticus*) aged 16 weeks and commercial broilers (*CP707*) aged 38 days of similar liveweights (1.5 ± 0.2 kg) obtained from a local farm in Songkhla Thailand, were killed as previous mention in item 1.2. *Pectoralis major* 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. Breast muscles (*Pectoralis major*) from both broiler and indigenous chicken were subjected to 24 h aging at 4°C prior to further study. The muscles were cut to the size of 2.0x2.0x6.0 cm. The muscle strips were individually weighed, packed into tightly sealed plastic bag, and stored at 4°C for 24 h. Samples were heated in boiling water to the following internal temperatures: 50, 60, 70, 80, 90 and 100°C. Copper-constantan thermocouples were used for temperature measurement of samples and water bath environment. Heat penetration time to obtain the internal temperature designated was recorded. After heating, the samples were chilled with cold water to about 10°C and stored at 4°C until analysis.

3.2 Changes in textural characteristic and muscle structure

3.2.1 Textural measurement

The textural measurements of raw and cooked muscle was made using a TA-XT2 Texture Analyzer. Muscle samples were cut approximately to a dimension of 1x2x0.5 cm. Shear value was measured at room temperature as described in 2.6. The shear force perpendicular to the axis of muscle fibers was measured in twenty replicates for each treatment of both chicken breeds. The peak of the shear force profile was regarded as the shear force value.

3.2.2 Microstructure of muscle

Muscle structures of samples were determined using scanning electron microscope (SEM) according to the procedure of Palka and Daun (1999) with a slight modification as described in.1.14. The areas of muscle fibers and the length of sarcomeres were measured on videoprints, using a special morphometric facility. Four videoprints from each sample were taken for transverse sections and ten measurements of fiber area on each were made, n = 40. The fiber diameter was calculated from the fiber area. Three videoprints from each sample were taken for longitudinal sections and ten measurements of sarcomere length on each were made, n = 30.

3.3 Changes in physical properties of muscle

3.3.1 Cooking loss

Cooking losses were calculated from differences in the weight of raw and cooked muscle strips (Murphy and Marks, 2000). The measurements were conducted in seven replications.

3.3.2 Color

The color of muscle samples in the anterior and posterior locations was determined in seven replications of each sample (n=14) using a Hunterlab colorimeter and reported in the complete International Commission on

Illumination (CIE) system color profile of L*, redness (a*), and yellowness (b*).

3.4 Changes of muscle protein

3.4.1 Solubility of protein

Solubility of raw and heated samples were determined in different solvents according to the procedure of Roussel and Cheftel (1990) with some modifications. Solvents used included 0.6 M KCl (S1); 20 mM Tris, pH 8.0 (S2); 20 mM Tris, pH 8.0 containing 1% (w/v) SDS (S3); 20 mM Tris, pH 8.0 containing 1% (w/v) SDS, 8 M urea (S4); 20 mM Tris, pH 8.0 containing 1% (w/v) SDS, 8 M urea, and 2% (v/v) β-mercaptoethanol (S5), and 0.5 M NaOH (S6). A sample (2 g) was added to 20 ml of the solvent and agitated with a magnetic stirrer for 4 h at room temperature. S5 was heated in the water bath at 100°C for 2 min before agitation. All samples were centrifuged at 12,100xg for 30 min. To the supernatant (4 ml) was added cold 50% (w/v) TCA to a final concentration of 10%. Samples were kept at 4°C for 18 h and then centrifuged at 2,500xg for 20 min. The precipitate was solubilised in 0.5 M NaOH. Protein contents were determined by the Biuret method (Robinson and Hodgen, 1940) using BSA as a standard. Solubility was expressed as the percentage of total protein extracted by 0.5 M NaOH.

3.4.2 SDS-PAGE analysis

Raw and cooked muscle samples (3 g) were homogenised in 5% (w/v) SDS (27 ml) at 11,000 rpm for 60 sec with a homogeniser. Five percent (w/v) SDS was solubilised in exudates from cooked muscle samples. All mixtures were incubated at 85°C for 1 h. the extract was then centrifuged at 6,100xg for 10 min. The protein content of the supernatant was analysed according to the Biuret method (Robinson and Hodgen, 1940). SDS-PAGE was carried out by the method of Laemmli (1970) as described in 1.4.

3.4.3 Soluble collagen

Soluble collagen in heated and unheated samples was extracted according to the method of Eilert and Mandigo (1993) with a slight modification. Muscle samples (2 g) were homogenized with 8 ml of 25% Ringer's solution (32.75 mM NaCl, 1.5 mM KCl, 0.5 mM $CaCl_2$). The homogenates were heated for 15 min at 50°C and centrifuged for 30 min at 2,300xg. The supernatant solution was decanted, and the pellet was suspended with the same solution and was recentrifuged. The supernatant solutions were combined. The sediment and supernatants were hydrolyzed with 6 M 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 *et al.*, 1996). The amount of heat soluble collagen was expressed as a percentage of the total collagen (collagen content in sediment plus that in the supernatant).

3.5 Denaturation of connective tissue

3.5.1 Preparation of intramuscular connective tissue (IMCT)

The IMCT (perimysium, endomysium) were prepared from breast muscle (*Pectoralis major*) and thigh muscle (*Biceps femorsis*) of Thai indigenous chicken (*Gallus domesticus*) aged 16 weeks and commercial broilers (*CP707*) aged 38 days of similar liveweights (1.5 ± 0.2 kg) by the method of Akta and Kaya (2001). The IMCT fraction was prepared from the diced muscles (free of tendon and epimysium). The diced muscle samples (30 g) were homogenized in a buffer consisting of 0.1 M potassium chloride and 0.02 M potassium dihydrogen phosphate pH 6.1 for 10 s at maximum speed using an Ultra Turrax T25 (IKA Labortechnik, Germany) homogenizer. The connective tissue adhering to the homogenizer blades and the homogenate were filtered through a sieve of mesh size 25. The connective tissue was resuspensed in buffer and rehomogenized for 10 s. This procedure was repeated three times.

The samples were defatted with acetone and dialyzed against the distilled water for 9 h at 4°C, lyophilised and stored at 4°C until required for analysis. For DSC study the samples were rehydrated with the deionised distilled water at a ratio of 1:5 (wt./vol.) for 24 h at 4°C before analysis.

3.5.2 Heat treatment of intramuscular connective tissue

Lyophilised IMCT from *Pectoralis* muscle of indigenous and broiler chickens were rehydrated by adding the deionised distilled water using the sample/water ratio of 1:5 (w/v) and the mixture were left for 24 h at 4°C. The rehydrated samples (approximately 1.4 g) were weighed into the eppendrop microtube. Then the samples were heated in water bath at temperatures of 50, 60, 70, 80, 90 and 100°C for 20 min. After heating, the samples were cooled with water (10°C) and stored at 4°C until analysis.

3.5.3 Determination of hydroxyproline and collagen content

Lyophilised IMCT from muscles of both chicken breeds (50 mg) were hydrolyzed with 10 ml of 6 M HCl at 110°C for 24 h. The hydrolysates were clarified with activated carbon and neutralised with 10 M or 1 M NaOH. The volume was then adjusted to 100 ml with distilled water. Hydroxyproline contents in the hydrolysates were determined using the method of Bergman and Loxley (1963) and expressed as the percentage of dry weight sample. Collagen content was expressed as mg collagen per g of sample (dry wt.), using hydroxyproline conversion values of 7.25 (Liu *et al.*, 1996).

3.5.4 Differential scanning calorimetry

The endothermal transition of unheated and heated intramuscular connective tissues were determined using Differential Scanning Calorimetry (Perkin Elmer DSC7). Samples (20 mg, wet wt.) were heated from 30 to 100°C at a rate of 10°C/min and an empty aluminium pan (Perkin-Elmer part No. B014-3021) was used as a reference. For temperature and heat flow calibration, Indium standard (T_p: 159.83°C, ΔH 28.17 J/g) was used. T_{onset}, T_{peak} and

denaturation enthalpy (ΔH) of all samples were determined from typical thermograms.

3.5.5 Determination of soluble collagen content

Soluble collagen in heated and unheated IMCT was analysed using the method of Eilert and Mandigo (1993) with a slight modification. Samples (approximately 100 mg) were placed in the eppendrop microtube and 1 ml of 25% Ringer's solution was added. The mixtures were stirred with a Rotamixer and then heated in a water bath at 50°C for 15 min. The samples were then centrifuged at 9,500xg. The supernatant was collected. The 25% Ringer's solution (0.5 ml) were added to the pellet, then stirred with the Rotamixer and centrifuged at the same rate. The supernatants were combined and subjected to collagen analysis as previously described. The content of heat soluble collagen was expressed as a percentage of the total collagen content. The analysis was performed in six determinations.

3.5.6 SDS-PAGE analysis

IMCT from broiler *Biceps femoris* and *Pectoralis* muscles and indigenous *Biceps femoris* and *Pectoralis* muscles were solubilized in 0.02 M sodium phosphate buffer (pH 7.2) containing 1% SDS and 3.5 M urea (40 mg of IMCT in 10 ml of buffer). The mixtures were homogenized and allowed to stand at 4°C for 4 days. The soluble fraction from unheated and heated intramuscular connective tissue of broiler and indigenous *Pectoralis* muscles were solubilized in the same manner as with IMCT. The homogenates were centrifuged at 8,000xg for 20 min at 4°C. SDS-PAGE was performed by the method of Laemmli (1970) using 5% running gel and 3.5% stacking gel. Gels were stained overnight with 0.08% Coomasie Brilliant Blue G-250, 20% (v/v) methanol, 8% ammonium sulphate and 1.57% orthophosphoric acid and destained a few times with 25% (v/v) methanol (Neuhoff *et al.*, 1988). Molecular weights were estimated by comparing mobilities of protein in the

samples with mobilities of high-molecular-weight standards (Sigma Aldrich Co Ltd, UK) and purified Type III collagen, acid soluble from calf skin (Sigma Aldrich Co Ltd, UK).

4. Statistical analysis

A Completely Randomized Design was used to determine the effect of ageing time and heating temperature on chemical compositions, physical and chemical properties, textural characteristics, microstructure and thermal properties of muscle and intramuscular connective tissues. A 2 x 2 factorial design was used to investigate the effect of breed (Thai indigenous and broiler chicken) and muscle type (*Pectoralis* and *Biceps femoris*) on DSC parameters of the native intramuscular connective tissues. Data was subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's Multiple Range Test (DMRT), whereas Paired sample T-test was applied to analyze the significant difference of means between both breeds (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows version 10.0: SPSS Inc.).

Chapter 3

RESULTS AND DISCUSSION

Compositions, Color and Texture of Thai Indigenous and Broiler Chicken Muscles

1. Proximate composition

The proximate composition, pH and color values of Thai indigenous and broiler chicken *Pectoralis* and *Biceps femoris* muscles are presented in Table 8. Indigenous chicken muscles contained significantly higher percentages of protein, but lower fat and ash contents than those of broiler muscles ($P<0.001$). For moisture content, there was no difference between indigenous and broiler *Pectoralis* muscles ($P>0.05$). However, indigenous chicken *Biceps femoris* muscle contained less moisture than that of the broiler ($P<0.001$). *Biceps femoris* muscle was lower in protein content ($P<0.05$) but higher in moisture ($P<0.05$), fat ($P<0.05$) and pH value ($P<0.01$) as compared to *Pectoralis* muscle. The results were in agreement with those reported by Ding *et al.* (1999). Protein content of broiler *Pectoralis* muscle was in the range 22.6 to 24.7% (Smith *et al.*, 1993; Ding *et al.*, 1999; Qiao *et al.*, 2002). The protein contents found in this study were lower than those previously reported, probably due to differences in the ages of the birds used.

2. Collagen content

As shown in Table 8, indigenous chicken muscles contained larger total collagen but less soluble collagen than those of the broiler muscles ($P<0.001$). Significantly higher total collagen contents of Chinese local chicken compared with broiler muscles has been reported at 4.4 and 6.7 mg/g respectively for broiler and local chicken breast meats and 10.2 and 11.7 mg/g respectively for broiler and local chicken thigh meats (Ding *et al.*, 1999). Differences in the

Table 8. Chemical composition, pH and C.I.E. values of chicken muscles from broiler and Thai indigenous chickens

	Broiler	Indigenous	Significance
Pectoralis m.			
Protein (%)	20.59 ± 0.26	22.05 ± 0.62	***
Fat (%)	0.68 ± 0.06	0.37 ± 0.14	***
Ash (%)	1.10 ± 0.01	1.03 ± 0.04	***
Moisture (%)	74.87 ± 0.46	74.88 ± 0.61	ns
Collagen (mg/g muscle)	3.86 ± 0.24	5.09 ± 0.69	***
Soluble collagen (% of total collagen)	31.38 ± 2.66	22.16 ± 2.05	***
Myoglobin content (mg/g muscle)	5.59 ± 0.67	3.45 ± 1.07	***
pH	5.97 ± 0.09	5.80 ± 0.14	*
L*	52.51 ± 4.09	54.32 ± 3.61	ns
a*	-1.18 ± 0.60	-1.09 ± 0.58	ns
b*	6.96 ± 2.57	6.53 ± 3.91	ns
Biceps femoris m.			
Protein (%)	19.08 ± 0.23	20.42 ± 0.27	***
Fat (%)	0.81 ± 0.09	0.58 ± 0.06	***
Ash (%)	1.06 ± 0.02	0.97 ± 0.03	***
Moisture (%)	77.22 ± 0.51	75.97 ± 0.40	***
Collagen (mg/g muscle)	8.70 ± 1.28	12.85 ± 1.04	***
Soluble collagen (% of total collagen)	33.87 ± 1.03	26.04 ± 2.05	***
Myoglobin content (mg/g muscle)	4.70 ± 0.80	4.69 ± 1.70	ns
pH	6.04 ± 0.12	5.85 ± 0.11	**
L*	47.12 ± 2.01	49.57 ± 4.82	ns
a*	2.21 ± 1.19	2.14 ± 1.49	ns
b*	7.77 ± 1.55	6.05 ± 3.23	ns

Data are presented as mean ± standard deviation.
N = 8 for chemical compositions, n = 16 for C.I.E. values
Significant differences between broiler and indigenous chickens were determined by t-test: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns = no significant difference.

collagen contents between the two breeds could be attributed to differences in the age of the birds at the time of slaughter (Dawson *et al.*, 1991). It has also been shown that the heat solubility of collagen decreases with increased collagen crosslinking and crosslinking increases as the animal ages (Pearson and Young, 1989; Foegeding and Lanier, 1996). From the result, therefore, the older indigenous chickens had higher total with a more highly crosslinked collagen as indicated by the lower soluble collagen content, compared to the younger broilers. Liu *et al.* (1996) studied the collagen content and heat soluble collagen of six muscles of the Rhode Island Red chickens, aged 30 weeks. They found that the *Biceps femoris* muscle contained the most total and heat soluble collagen, whereas the *Pectoralis* muscle was low in these characteristics.

3. Myoglobin content and color

Myoglobin content was observed higher in broiler *Pectoralis* muscle than that of indigenous muscle ($P<0.001$) (Table 8). However, there was non-significant difference in myoglobin content of *Biceps femoris* muscle between both breeds ($P>0.05$). This result was not in agreement with Miller (1994) who reported that the content of myoglobin increased with increasing age of poultry meat and Ledward and Shorthose (1971) who stated that the differences in growth rate affected the different myoglobin contents in lamb. The *Biceps femoris* muscle of indigenous chicken contained higher myoglobin contents than that of *Pectoralis* muscle whereas non-significant differences were obtained between both muscle types of broiler chicken. The different result obtained was probably due to the differences in the role of muscle in the animal. Muscles involved in sustained repetitive action, like breathing, contain higher concentrations of myoglobin than muscles used less often (Young and West, 2001). Myoglobin content of chicken muscles found in this study was in the ranges (0.1-5 mg/g muscle) as also reported by Nishida and Nishida (1985).

The indigenous chicken *Pectoralis* and *Biceps femoris* muscle had not different in L*, a* and b* values compared to those of broiler muscle ($P>0.05$). However, the *Biceps femoris* muscle of both chickens had lower L* but higher a* values ($P<0.001$) than that of the *Pectoralis* muscles. This was probably related to the significant differences in muscle pH and myoglobin content between the muscle types. Muscle pH and meat color are highly correlated. Higher muscle pH is associated with darker meat than that of lower pH (Fletcher, 1999a,b).

4. Composition of protein and SDS-PAGE pattern

Protein compositions of chicken muscles from both breeds are shown in Table 9. The indigenous chicken muscle had lower myofibrillar and sarcoplasmic protein fractions ($P<0.05$) but much higher stroma and alkali-soluble protein fractions ($P<0.05$) compared with that of broiler muscles. The *Biceps femoris* muscle had significantly lower sarcoplasmic protein fraction ($P<0.05$) but had much higher stroma protein compared to *Pectolaris* muscle ($P<0.05$). The older age and lower muscle pH might contribute to the lower content of myofibrillar and sarcoplasmic proteins in Thai indigenous chicken muscles. At low pH muscle resulted in denaturation/precipitation and adherence of part of the sarcoplasmic protein fraction to the surface of myofibrils and reduced solubility of myofibrilar proteins (Flores *et al.*, 2000). The difference in muscle protein compositions between both breeds and muscle types might be attributed to the difference in properties and texture of their meats. The protein compositions in chicken breast muscle were generally reported that myofibrillar, sarcoplasmic, and stromal proteins comprised ~56.2, 42.3 and 1.5% of the total protein (Lan *et al.*, 1995). In addition, Lawrie (1991) and Murphy *et al.* (1998) reported that the myofibrillar and sarcoplasmic proteins comprised ~60 and 30% of the total muscle protein, respectively. The results obtained from *Pectoralis* muscle of both breeds were not consistent with those of previous reports. Different results found in this study were likely due to the sources of the muscles (Murphy *et al.*, 1998)

Table 9. Protein composition of chicken muscles (mg N/g muscle)

Composition	Broiler		Indigenous chicken	
	Biceps femoris m.	*Pectoralis m.*	*Biceps femoris m.*	*Pectoralis m.*
Non-protein N	3.13 ± 0.74[a]	4.91 ± 0.37[b]	4.40 ± 0.57[b]	3.00 ± 0.55[a]
<u>Protein N</u>				
myofibrillar	15.50 ± 0.17[c]	13.17 ± 0.16[b]	11.58 ± 0.49[a]	12.36 ± 0.30[ab]
	(52.93 ± 0.88)	(43.45 ± 0.66)	(37.91 ± 1.16)	(36.30 ± 1.34)
sarcoplasmic	10.64 ± 0.54[b]	14.80 ± 0.16[c]	6.91 ± 0.06[a]	15.16 ± 0.29[c]
	(36.30 ± 0.86)	(48.83 ± 0.35)	(22.64 ± 0.45)	(44.49 ± 0.28)
stroma	1.18 ± 0.16[b]	0.55 ± 0.03[a]	3.96 ± 0.32[c]	1.25 ± 0.03[b]
	(4.02 ± 0.42)	(1.81 ± 0.09)	(12.96 ± 1.19)	(3.67 ± 0.04)
Alkali-soluble	1.98 ± 0.06[a]	1.79 ± 0.07[a]	8.09 ± 0.24[c]	5.30 ± 0.42[b]
	(6.75 ± 0.40)	(5.91 ± 0.21)	(26.50 ± 0.48)	(15.54 ± 1.02)

Data are presented as mean ± standard deviation, n = 3. Data in parenthesis are calculated as mean percent of total protein nitrogen.

[a-c]Means with differing superscripts in the same row are significantly different ($P<0.05$).

and the influence of frozen storage of muscle samples (Hashimoto *et al.*, 1979). The increase of sarcoplasmic fraction during frozen storage might have resulted from decomposition of myofibrillar proteins such as myosin and troponin (Hashimoto *et al.*, 1979).

SDS-PAGE of chicken muscles from both breeds is presented in Fig 5. The protein bands obtained from SDS-PAGE were compared with standard molecular weights and published data (Xiong and Breakke, 1989; Claeys *et al.*, 1995). The electrophoretic patterns of muscles from both breeds were similar. However, differences between muscle types were observed. The *Pectolaris* muscle had more α–actinin, tropomyosins, troponin T and myosin light chain 1 compared with the *Biceps femoris* muscle. The electrophoretic patterns of myofibrillar protein fractions showed two major bands as myosin heavy chain and actin at 205 k and 45 k, respectively (Fig 6). However, the *Biceps femoris* muscle from Thai indigenous chicken was a lower density in actin band. The SDS-PAGE patterns of sarcoplasmic protein fractions were similar between both breeds (Fig 7). The pattern of alkali-soluble fractions (Fig 8) was close to that of myofibrillar fractions. The fractions from Thai indigenous chicken muscles were denser in myosin heavy chain, actin and troponin especially for the *Biceps femoris* muscle. This might be attributed to the lower solubility of the myofibrillar protein of Thai indigenous chicken muscles.

5. Amino acid composition

The amino acid composition of *Pectoralis* and *Biceps femoris* muscles of both chickens is presented in Table 10. Both muscles were very high in glutamic acid, arginine, leucine, aspartic acid and lysine. However, no significant differences in amino acid compositions were observed between broiler and indigenous chicken muscles, with the exception of glutamic acid. Indigenous chicken muscles contained slightly higher glutamic acid contents than broiler muscles ($P<0.05$). Glutamic acid was found to have a detectable effect on the

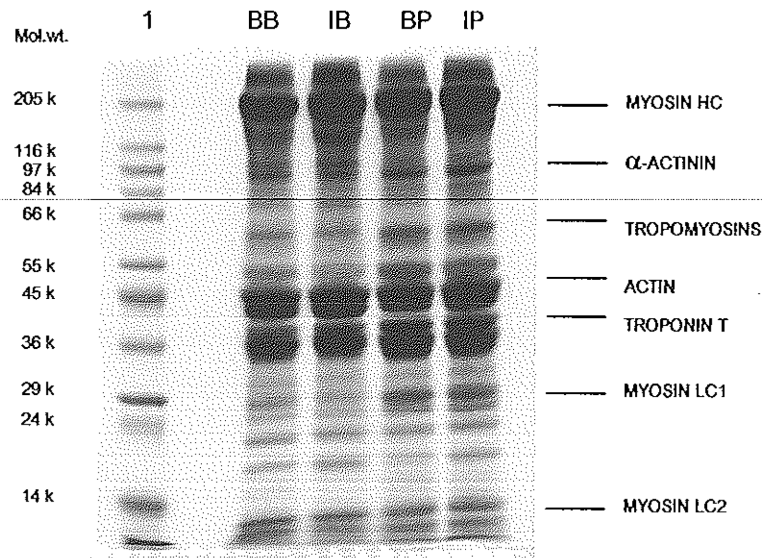

Fig 5. SDS-PAGE patterns of muscles from broiler and Thai indigenous chicken (1= molecular weight standard, BB= *Biceps femoris* muscle from broiler, IB= *Biceps femoris* muscle from indigenous chicken, BP= *Pectoralis* muscle from broiler and IP= *Pectoralis* muscle from indigenous chicken)

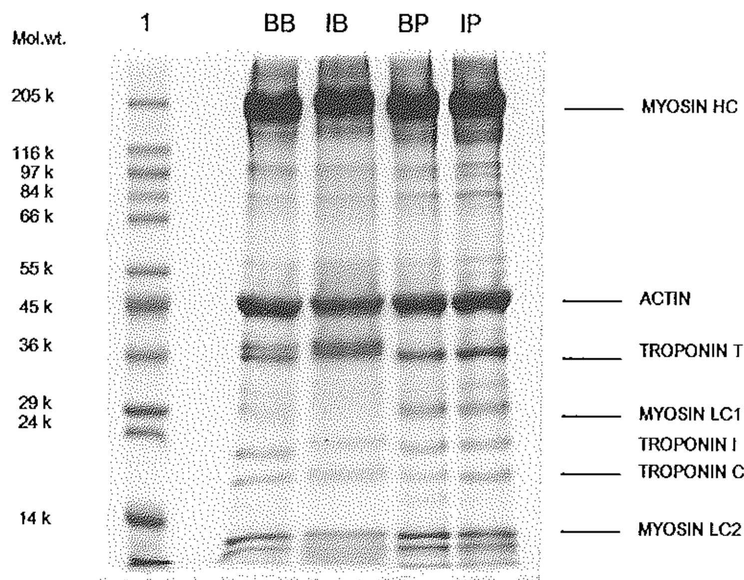

Fig 6. SDS-PAGE patterns of myofibrillar protein fractions of broiler and Thai indigenous chicken muscles (1= molecular weight standard, BB= *Biceps femoris* muscle from broiler, IB= *Biceps femoris* muscle from indigenous chicken, BP= *Pectoralis* muscle from broiler and IP= *Pectoralis* muscle from indigenous chicken)

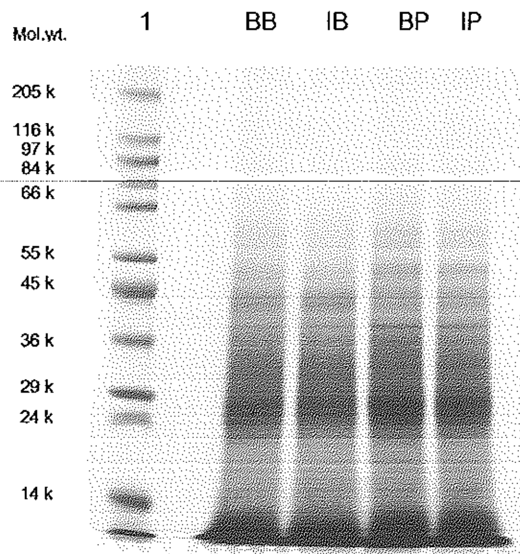

Fig 7. SDS-PAGE patterns of sarcoplasmic protein fractions of broiler and Thai indigenous chicken muscles (1= molecular weight standard, BB= *Biceps femoris* muscle from broiler, IB= *Biceps femoris* muscle from indigenous chicken, BP= *Pectoralis* muscle from broiler and IP= *Pectoralis* muscle from indigenous chicken)

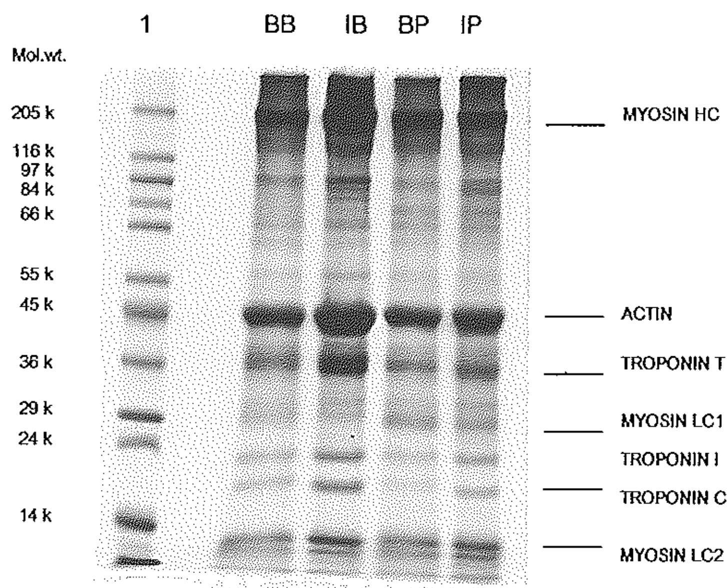

Fig 8. SDS-PAGE patterns of alkali soluble protein fractions of broiler and Thai indigenous chicken muscles (1= molecular weight standard, BB= *Biceps femoris* muscle from broiler, IB= *Bicep femoris* muscle from indigenous chicken, BP= *Pectoralis* muscle from broiler and IP= *Pectoralis* muscle from indigenous chicken)

Table 10. Amino acid composition of chicken muscles (g/100g dry muscle)

Amino acid	Broiler		Indigenous chicken	
type	*Biceps fermoris m.*	*Pectoralis m.*	*Biceps fermoris m.*	*Pectoralis m.*
ASP	3.48 ± 0.23	3.64 ± 0.12	3.34 ± 0.03	3.68 ± 0.07
SER	2.44 ± 0.06	2.38 ± 0.09	2.36 ± 0.09	2.46 ± 0.13
GLU*	6.33 ± 0.35	6.35 ± 0.22	6.63 ± 0.08	6.54 ± 0.16
GLY	2.95 ± 0.09	2.70 ± 0.06	2.29 ± 0.64	2.83 ± 0.17
HIS	2.49 ± 0.02	2.90 ± 0.03	2.75 ± 0.74	2.85 ± 0.14
ARG	4.75 ± 0.05	4.39 ± 0.13	4.48 ± 0.25	4.58 ± 0.27
THR	2.96 ± 0.02	3.02 ± 0.08	2.87 ± 0.09	3.12 ± 0.17
ALA	2.66 ± 0.22	2.80 ± 0.08	2.60 ± 0.10	2.80 ± 0.08
PRO	2.03 ± 0.11	1.93 ± 0.06	2.05 ± 0.17	1.98 ± 0.10
CYS	0.31 ± 0.00	0.31 ± 0.01	0.30 ± 0.01	0.34 ± 0.01
TYR	2.90 ± 0.00	3.03 ± 0.04	2.85 ± 0.05	3.10 ± 0.18
VAL	2.08 ± 0.05	2.16 ± 0.03	2.04 ± 0.08	2.20 ± 0.05
MET	1.83 ± 0.04	1.88 ± 0.05	1.81 ± 0.06	1.93 ± 0.08
LYS	3.16 ± 0.54	3.41 ± 0.10	3.15 ± 0.10	3.35 ± 0.04
ILE	2.29 ± 0.09	2.41 ± 0.08	2.26 ± 0.12	2.45 ± 0.12
LEU	4.19 ± 0.11	4.29 ± 0.09	4.11 ± 0.18	4.39 ± 0.13
PHE	2.94 ± 0.03	3.01 ± 0.03	2.89 ± 0.08	3.07 ± 0.19

Data are presented as mean ± standard deviation.
*Significant difference between broiler and indigenous chickens as determined by t-test ($P<0.05$).

taste of chicken meat and this may contribute to the differences in flavor between the meats (Farmer, 1999).

6. Fatty acid composition

The fatty acid composition of indigenous and broiler chicken muscles is shown in Table 11. Indigenous chicken muscles (*Pectoralis* and *Biceps femoris*) contained a higher percentage of saturated fatty acids ($P<0.05$) and a lower percentage of polyunsaturated fatty acids ($P<0.05$) as compared with broiler chicken muscles. There was no significant difference between the muscles for total monounsaturated fatty acids. The fatty acid profile of the broiler chicken muscles was similar to previous reports (Smith *et al.*, 1993; Alasnier *et al.*, 2000; Qiao *et al.*, 2002; Cherian *et al.*, 2002). However, no report on the fatty acid profiles of indigenous chicken muscles was found. The fatty acid profile of the indigenous chicken muscles in this study was different from that of the broilers. Some fatty acids such as C10:0 and C24:1 were found only in indigenous chicken muscles whereas C18:3, C20:2, and C20:3 were found only in broiler chicken muscles. This was possibly caused by the differences in feed diets between the breeds (Cherian *et al.*, 2002). The different fatty acid composition of muscle probably affects the lipid stability and taste. However, there are some reports indicating that although the chicken received the same feed diet, differences in such meat component as unsaturated fatty acid were observed. These were probably due to differences in eating behavior between breeds. The indigenous chickens tend to scratch while eating and were observed to pick up feed particles more selectively than the broiler (Van Marle-Koster and Webb, 2000).

7. Textural characteristic

The shear force values of the *Pectoralis* and *Biceps femoris* muscles of both chickens are shown in Table 12. The shear values of the indigenous chicken muscles, either raw or cooked, were significantly higher compared to the broiler

Table 11. Fatty acid composition (% of total fatty acids) of chicken muscles from broiler and Thai indigenous chicken

Fatty acid	Broiler		Indigenous chicken		Difference[1]
	Biceps femoris m.	*Pectoralis m.*	*Biceps femoris m.*	*Pectoralis m.*	Indigenous-Broiler
C8:0	0.18	0.21	0.21	0.74	0.28^{ns}
C10:0	0.00	0.00	0.11	0.16	0.13*
C12:0	0.37	0.36	2.74	1.85	1.93*
C14:0	0.95	0.87	3.06	2.31	1.77*
C16:0	32.65	31.82	32.04	33.01	0.29^{ns}
C17:0	0.29	0.21	0.32	0.33	0.07^{ns}
C18:0	14.97	14.56	25.61	22.72	9.40*
C20:0	0.21	0.23	0.42	0.38	0.18*
C22:0	0.26	0.31	0.63	0.57	0.32*
C24:0	0.13	0.17	0.42	0.56	0.34*
Saturated	**50.01**	**48.76**	**65.55**	**62.64**	**14.71***
C16:1	3.44	3.33	1.47	1.24	-2.03**
C18:1	35.13	37.76	26.35	31.25	-7.65^{ns}
C20:1	0.68	0.74	0.74	0.41	-0.14^{ns}
C24:1	0.00	0.00	0.21	0.20	0.20***
Monounsaturated	**39.25**	**41.82**	**28.77**	**33.09**	**-9.60^{ns}**
C18:2 n-6	8.86	7.63	4.74	3.36	-4.19*
C18:3 n-3	0.13	0.16	0.00	0.00	-0.14*
C20:2 n-6	0.27	0.32	0.00	0.00	-0.29**
C20:3 n-3 n-6	0.14	0.16	0.00	0.00	-0.15***
C20:4 n-6 n-3	0.56	0.45	0.21	0.27	-0.26^{ns}
C20:5 n-3	0.40	0.34	0.21	0.16	-0.18*
C22:6 n-3	0.38	0.37	0.53	0.47	0.12*
Polyunsaturated	**10.74**	**9.42**	**5.69**	**4.26**	**-5.10***

Significant differences between broiler and indigenous chickens were determined by t-test: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns = non significant difference.

[1] Difference in mean values of the two muscles for the indigenous and broiler chickens.

muscles. The *Biceps femoris* muscle has been reported to be the toughest while *Pectoralis* is the most tender chicken muscle (Liu *et al.*, 1996). As expected, the *Biceps femoris* muscle showed higher shear value than the *Pectoralis* muscle for both breeds ($P<0.05$). However, after cooking no differences in shear values were observed between the two muscles ($P>0.05$). For broiler chicken muscles, the shear values decreased after cooking. Different results were found in the cooked indigenous chicken muscles. The cooked indigenous *Biceps femoris* muscle was not significantly different in shear value to its raw counterpart while cooking the indigenous *Pectoralis* muscle gave rise to a large increase in shear value. This was probably due to the difference in total and soluble collagen contents between the muscles (Table 8). In the younger, broiler chickens the less crosslinked collagen will melt (denature) on heating giving rise much soluble collagen and concomitant of loss of texture. However, in the older, indigenous birds on heat denaturation the more highly crosslinked collagen will remain insoluble and shrink, effectively squeezing the heat denatured myofibrillar gel, leading to loss of moisture and a tougher texture. This was supported by the increased cooking losses seen in the muscles from the indigenous birds (Table 13). However, the microstructures of indigenous and broiler chicken muscles should be further studied to fully understand the differences in texture.

Table 12. Shear force values (kg) of chicken muscles from broiler and Thai indigenous chickens (TIC)

		Breed		Significance
Condition	Muscle	Broiler	Indigenous chicken	(T-test)
Raw	*Biceps femoris m.*	2.89 ± 0.52^{c}	5.20 ± 0.81^{c}	***
	Pectoralis m.	1.20 ± 0.30^{b}	1.78 ± 1.08^{a}	*
Cooked	*Biceps femoris m.*	0.77 ± 0.18^{a}	4.67 ± 1.09^{bc}	***
	Pectoralis m.	0.78 ± 0.23^{a}	4.09 ± 1.61^{b}	***

[a-c]Means with differing superscripts in the same column are significantly different ($P<0.05$). n = 16
Significant differences between broiler and indigenous chickens were determined by t-test: * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns = non significant difference.

Table 13. Cooking loss of chicken muscles from broiler and Thai indigenous chickens

Breed	Muscle	Cooking loss (%)
Broiler	*Biceps fermoris m.*	15.74 ± 3.90[a]
	Pectoralis m.	19.93 ± 2.38[b]
Indigenous chicken	*Biceps fermoris m.*	28.54 ± 3.10[d]
	Pectoralis m.	23.00 ± 1.83[c]

[a-d]Means with differing superscripts in the same column are significantly different (P<0.05). n = 16

Microstructure and Thermal Characteristic of Thai Indigenous and Broiler Chicken Muscles

1. Perimysium thickness

The thicknesses of perimysium of Thai indigenous and broiler chicken *Pectoralis* and *Biceps femoris* muscles are shown in Table 14. The perimysium of indigenous chicken muscles were thicker than those of broiler muscles (P <0.05). The perimysium of *Pectoralis* muscle was thinner than those of the *Biceps femoris* muscle (P<0.05). Thick perimysium in indigenous chicken muscles was coincidental with higher collagen contents and shear values of this breed muscle as previously reported by Wattanachant *et al.* (2004). Significantly high correlation ($r^2 = 0.95$) between the thickness of perimysium and shear value have been reported by Liu *et al.* (1996). In this study, perimysium thickness of the muscles correlated well with the magnitude of the shear values of the muscles, indigenous *Biceps femoris* (5.20 kg), broiler *Biceps femoris* (2.89 kg), indigenous *Pectoralis* (1.78 kg), and broiler *Pectoralis* (1.20 kg). The differences in the thickness of perimysium and the collagen contents between the two breeds may be attributed to differences in the age of the birds (Dawson *et al.*, 1991). However, with increasing animal maturity, the total collagen content of muscle does not increase (Dransfield, 1994) and does not correlate significantly with the

tenderness of meat (Nakamura *et al.*, 1975). Therefore, the difference between the breeds might be primarily due to genetic factors influencing the perimysium thickness and collagen content, resulting in the differences in textural properties.

Table 14. Thickness of perimysium of chicken muscles from broiler and Thai indigenous chickens

Breed	Muscle	Thickness of perimysium (μm)
Broiler	*Biceps femoris m.*	9.93 ± 2.28^{c}
	Pectoralis m.	3.87 ± 1.32^{a}
Thai indigenous	*Biceps femoris m.*	14.20 ± 3.45^{d}
	Pectoralis m.	7.10 ± 2.56^{b}

Data are presented as mean ± standard deviation. n = 40
$^{a-d}$Means with differing superscripts in the same column are significantly different (P<0.05).

2. Thermal denaturation

The onset (T_o) and peak (T_p) temperatures of protein denaturation were determined for the chicken muscles from both breeds and muscle types (Table 15). The enthalpies of protein denaturation of chicken muscles from both breeds are also presented in Table 16. Five endothermic peaks at 54.9, 61.7, 65.4, 70.6, and 76.1°C were obtained for broiler *Pectolaris* muscle, while only 3 endothermic peaks were found for broiler *Biceps femoris* muscle and Thai indigenous *Biceps femoris* and *Pectolaris* muscles at temperature range of 53.5-56.6, 60.7-62.6, and 74.9-76.9°C. Chicken breast muscle of 7 weeks old chicken broilers have been reported to have 5 endothermic peaks at 57, 62, 67, 72 and 78 °C, while their thigh muscle had 3 transitions at 59.6, 65.6 and 75.8°C (Kijowski and Mast, 1988). Murphy *et al.* (1998) found 3 transitions at 52-57, 67-72, and 76-83°C for ground and formed chicken breast patties. The difference in numbers

Table 15. Transformation temperature of chicken muscles from broiler and Thai indigenous chickens

Breed	Muscle	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5	
		T_o (°C)	T_p (°C)	T_o (°C)	T_p (°C)	T_o (°C)	T_p (°C)	T_o (°C)	T_p (°C)	T_o (°C)	T_p (°C)
Broiler	*Biceps femoris m.*	53.34^{c} (± 0.29)	56.65^{c} (± 0.29)	60.46^{d} (± 0.36)	62.62^{c} (± 0.34)					70.99^{a} (± 0.86)	74.90^{a} (± 0.29)
	Pectoralis m.	49.19^{ab} (± 0.82)	54.88^{b} (± 0.38)	59.92^{c} (± 0.31)	61.66^{b} (± 0.36)	63.83 (± 0.28)	65.37 (± 0.15)	68.78 (± 0.15)	70.63 (± 0.22)	72.97^{b} (± 0.66)	76.14^{b} (± 0.11)
Indigenous	*Biceps femoris m.*	49.57^{b} (± 0.73)	54.78^{b} (± 0.86)	58.72^{b} (± 0.22)	61.93^{b} (± 0.84)					73.01^{b} (± 0.77)	76.90^{c} (± 0.52)
	Pectoralis m.	48.62^{a} (± 0.36)	53.55^{a} (± 0.30)	57.78^{a} (± 0.24)	60.70^{a} (± 0.40)					73.48^{b} (± 0.58)	75.94^{b} (± 0.43)

Data are presented as mean ± standard deviation. n = 8, [a-d]Means with differing superscripts in the same column are significantly different ($P<0.05$).

Table 16. Enthalpy of denaturation (ΔH) of chicken muscles from broiler and Thai indigenous chickens

Breed	Muscle	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
		ΔH (J/g muscle[1])	ΔH (J/g muscle[1])	ΔH (J/g muscle[1])	ΔH (J/g muscle[1])	ΔH (J/g muscle[1])
Broiler	*Biceps femoris.*	0.698 ± 0.138^{d}	0.286 ± 0.124^{c}			0.314 ± 0.108^{b}
	Pectoralis m.	0.461 ± 0.045^{c}	0.053 ± 0.012^{a}	0.030 ± 0.005	0.036 ± 0.004	0.200 ± 0.043^{a}
Indigenous	*Biceps femoris.*	0.165 ± 0.027^{a}	0.325 ± 0.041^{c}			0.616 ± 0.148^{c}
	Pectoralis m.	0.349 ± 0.087^{b}	0.205 ± 0.067^{b}			0.223 ± 0.042^{ab}

Data are presented as mean ± standard deviation. n = 8, [1]wet basis, [a-d]Means with differing superscripts in the same column are significantly different ($P<0.05$).

of peaks could be due to the differences in source of raw material, type, age, sex and storage of chicken meat (Murphy *et al.*, 1998; Xiong and Brekke, 1989). Stabursvik and Martens (1980) concluded that greater differences were found between red and white muscle than between muscle from different animal species. However, the differences between the breast muscle thermoprofile, with a higher proportion of white muscle fibers, and the thigh muscle thermoprofile, with more red fibers, was not clear (Kijowski and Mast, 1988). The myofibrillar proteins have been reported to exhibit two transitions at T_p of 34.99 and 54.14°C, with ΔH's of 0.44 and 0.33 J/g, respectively (Murphy *et al.*, 1998). In this study found only one peak corresponding to myofibrillar protein in chicken muscles from both breeds (peak No.1, Table 15-16). Myofibrillar proteins in broiler chicken muscles showed higher T_o, T_p (peak No.1, Table 15) and greater enthalpy of denaturation compared with those in Thai indigenous chicken muscles ($P<0.05$) (Table 16). For the stromal proteins in broiler and Thai indigenous chicken muscles, one transition (peak No.2) was observed at peak temperature range of 60.7-62.6°C which were lower than previous reports at 63°C by Xiong *et al.* (1987), 65.5°C by Kijowski and Mast (1988), and 64.2°C by Murphy *et al.* (1998). For sarcoplasmic proteins of chicken breast muscle, three transitions have been observed at peak temperature of 62, 67, and 72°C (Wang and Smith, 1994), and 64.2, 71.9 and 78.4°C (Murphy *et al.*, 1998). For Thai indigenous chicken muscles, only one peak (peak No.5) was observed with T_p of 75.9 and 76.9°C for *Biceps femoris* and *Pectoralis* muscles, respectively. Three transitions (peak No.3,4,5) were obtained only in broiler *Pectolaris* muscle with T_p of 63.8, 65.4 and 76.1°C. These peaks were most likely belonging to sarcoplasmic proteins. For the same muscle tested, Thai indigenous chicken muscles exhibited significantly lower enthalpies for peak No.1 but higher enthalpies for peak No.2, compared with broiler muscles. The results suggested that stroma in Thai indigenous chicken muscle was less susceptible to thermal denaturation than that in broiler. This might be associated with the firmer

structure of indigenous chicken stroma, resulting in the tougher texture of this breed.

3. Fiber diameter and sarcomere length

The results of quantitative structural measurements of raw and cooked Thai indigenous and broiler chicken *Pectoralis* and *Biceps femoris* muscles are shown in Table 17. In raw muscles, the fiber diameter of Thai indigenous chicken muscles were larger than those of the broiler ($P<0.05$). The *Biceps femoris* muscle of broiler chickens had smaller fiber diameter than the *Pectoralis* muscle ($P<0.05$) while the opposite results were observed in the indigenous chicken muscles. The average diameter of chicken white fibers has been reported to be 68.2 μm (Kiessling, 1977), 38-46 μm (Smith and Fletcher, 1988) and 32.6 μm (Smith *et al.*, 1993). These differences in muscle fiber diameter were possibly due to the differences in age, rate of rigor onset and degree of sarcomere shortening (Smith and Fletcher, 1988). After cooking, however, the equivalent muscles from both breeds did not differ in the fiber diameters. The fiber diameter of cooked broiler muscles increased by 28.4% for *Biceps femoris* muscle ($P<0.001$) and 12.4% for *Pectoralis* muscle ($P<0.01$) compared to their raw counterpart. Conversely, the fiber diameter of the *Biceps femoris* Thai indigenous chicken muscle decreased 13.9% ($P<0.001$) after cooking, while that of *Pectoralis* muscle did not change ($P>0.05$) after cooking. The mean sarcomere lengths of the raw muscles from both breeds were similar although those of the indigenous *Biceps femoris* muscle were slightly shorter than those of the others ($P<0.05$). The sarcomere length of both muscle types from both breeds were in the range reported by Young *et al.* (1990). The sarcomere length of all muscles decreased after cooking ($P<0.05$) as shown in Table 17. The sarcomere lengths of the cooked muscles decreased by 42.5% for broiler *Biceps femoris* muscle, 19.5% for broiler *Pectoralis* muscle, 21.8% for indigenous chicken *Biceps femoris* muscle, and 16.1% for indigenous chicken *Pectoralis* muscle. These

Table 17. Fiber diameter and sarcomere length of chicken muscles from broiler and Thai indigenous chickens

Breed	Muscle	Fiber diameter[1] (μm)		Significance[3]	Sarcomere length[2] (μm)		Significance[3]
		raw	cooked	(t-test)	raw	cooked	(t-test)
Broiler	*Bicep femoris m.*	20.4 ± 2.40^{a}	26.2 ± 4.36^{a}	***	1.60 ± 0.14^{bc}	0.92 ± 0.09^{a}	***
	Pectoralis m.	26.6 ± 6.43^{b}	29.9 ± 6.46^{c}	**	1.64 ± 0.12^{c}	1.32 ± 0.12^{c}	***
Thai indigenous	*Bicep femoris m.*	31.7 ± 7.21^{d}	27.3 ± 3.26^{ab}	***	1.56 ± 0.15^{a}	1.22 ± 0.15^{b}	***
	Pectoralis m.	28.9 ± 5.95^{c}	28.8 ± 5.14^{bc}	ns	1.61 ± 0.16^{bc}	1.35 ± 0.14^{c}	***

Data are presented as mean ± standard deviation. [1]n = 80, [2]n = 40

[a-d]Means with differing superscripts in the same column are significantly different (P<0.05).

[3]Significant differences between raw and cooked samples were determined by t-test: * P<0.05; ** P<0.01; *** P<0.001; ns = non significant difference.

results indicated that the shrinkage found in cooked broiler muscles was more parallel to the fiber direction than the transverse direction compared to shrinkage of the indigenous chicken muscles. This was probably caused by the difference in content and thermal properties of the myofibrillar protein and collagen between the breeds as evidenced by the different thermal transitions between the breeds (Table 15 and 16). As mentioned by Lepetit *et al.* (2000), the extent of muscle fiber deformation during cooking depends on both the compression stress applied by collagen fibers and the muscle fibers resistance to compression. The compression forces applied by collagen networks on muscle fiber bundles depends on the amount of collagen and also on its thermal solubility, as the greater the thermal solubility of collagen the less its force of thermal contraction (Lepetit *et al.*, 2000). The broiler muscles contained higher myofibrillar protein contents with higher denaturation temperature and enthalpies (Table 15 and 16), but contained lower collagen contents with higher thermal solubility (Wattanachant *et al.*, 2004). This resulted in more resistance to compression and less force of thermal contraction from collagen fibers, so that the broiler muscles shrank in parallel and expanded in transverse to the fiber direction. Thai indigenous chicken muscles contained lower myofibrillar protein content, with lower denaturation temperatures but contained higher collagen contents with less thermal solubility than broiler muscle, leading to more force of thermal contraction in both directions.

4. Microstructure

The qualitative changes in the microstructure of raw and cooked *Bicep femoris* and *Pectoralis* muscles from both breeds are presented in Figs 9 and 10. On longitudinal sections (Fig 9), shrinkage of the sarcomeres was clearly seen for cooked *Biceps femoris* muscles of broiler and indigenous chickens. Cooking also caused weakening and loss of structure of the Z-disks in cooked broiler *Pectoralis* (Fig 9D) and *Biceps femoris* muscles (Fig 9B). The sarcomeres of the cooked indigenous chicken muscles (Fig 9F and 9H) were more compact with

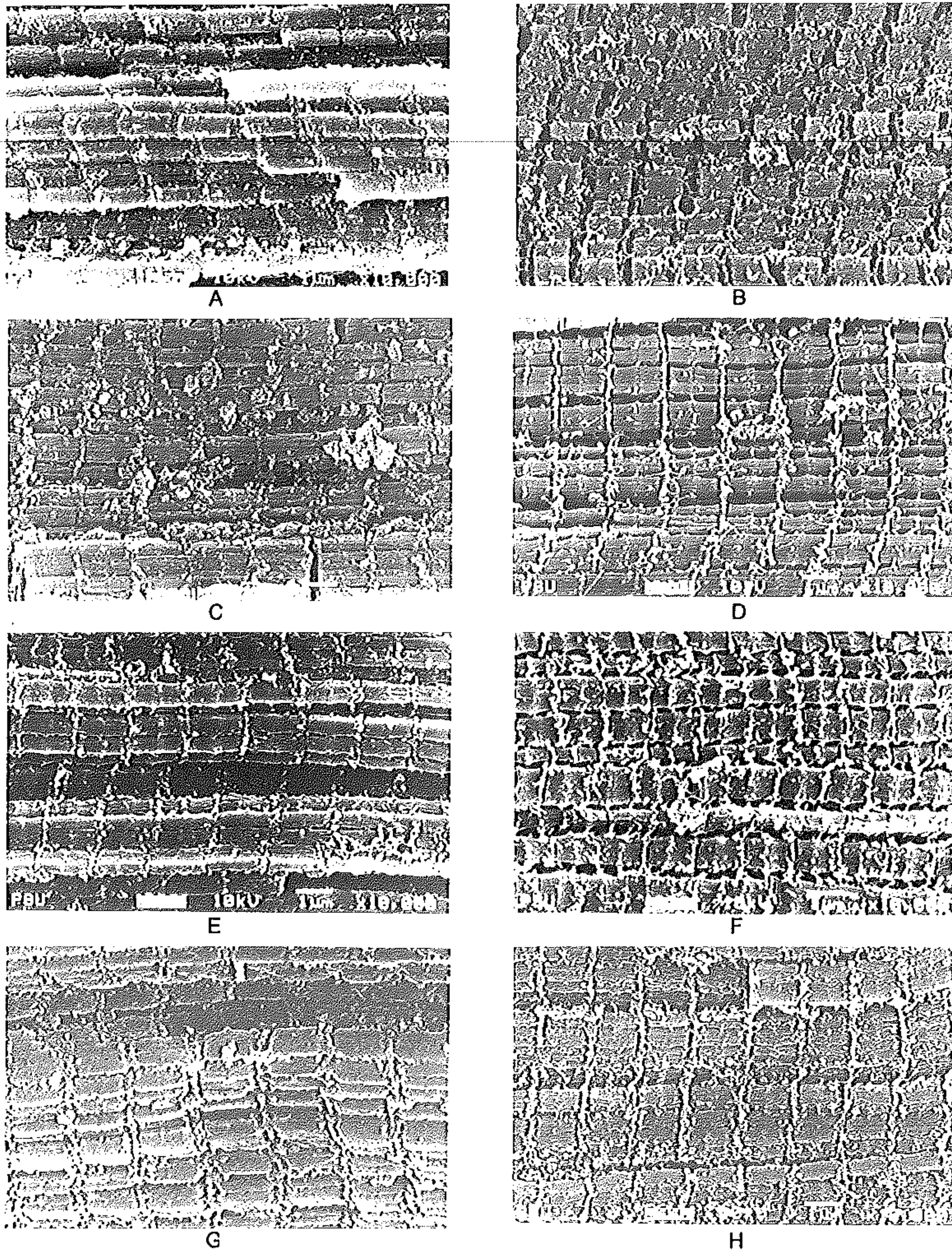

Fig 9. SEM micrographs of longitudinal sections of chicken muscles; *Biceps fermoris* muscle of broiler, raw (A) and cooked (B), *Pectoralis* muscle of broiler, raw (C) and cooked (D), *Biceps fermoris* muscle of Thai indigenous chicken, raw (E) and cooked (F), *Pectoralis* muscle of Thai indigenous chicken, raw (G) and cooked (H).

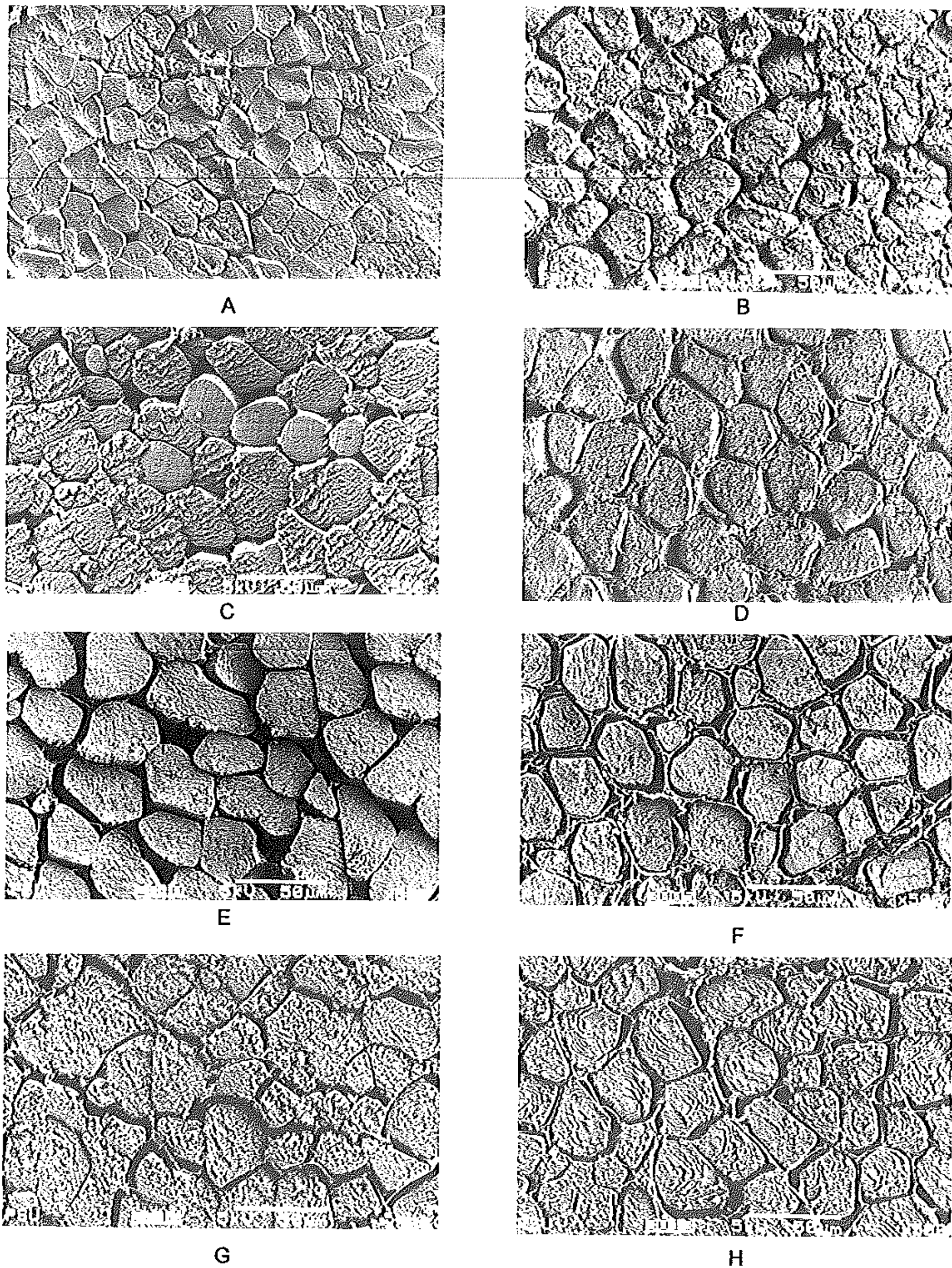

Fig 10. SEM micrographs of transverse sections of chicken muscles; *Biceps fermoris* muscle of broiler, raw (A) and cooked (B), *Pectoralis* muscle of broiler, raw (C) and cooked (D), *Biceps fermoris* muscle of Thai indigenous chicken, raw (E) and cooked (F), *Pectoralis* muscle of Thai indigenous chicken, raw (G) and cooked (H).

little loss of the Z-disks compared with the broiler muscles. This was probably because the Z-disk structures of the indigenous chicken muscles was more heat stable than those of the broiler muscles. This might contribute to the firmer structure of the cooked indigenous chicken muscles. These observations might partially explain the previous observation that the shear values of the broiler muscles decreased after cooking while the shear values of the indigenous chicken muscles increased after processing under the same condition (Wattanachant *et al.*, 2004). However, differences in the nature of the collagen structures were probably of more importance, as suggested previously

For the transverse section (Fig 10), the fibers in the raw broiler muscles had smaller diameters and were more compact than those of the indigenous chicken muscles. After cooking, denaturation and melting of the endomysium together with the denatured myofibrils resulted in swelling of the fibers of the broiler chicken muscles (Fig 10B and 10D). For the cooked indigenous chicken muscles, however, the muscle fibers were separated from the sheaths of the endomysium (Fig 10F and 10H). This difference was possibly caused by the differences in crosslinked collagen contents between the breeds (Wattanachant *et al.*, 2004). For the indigenous chicken muscles, more highly crosslinked collagens remained insoluble and shrank during heat denaturation, effectively squeezing the heat denatured myofibrils, resulting in loss of moisture and decreased fiber diameter. These microstructural changes could result in increasing toughness in the cooked indigenous chicken muscles. For the broiler muscles, heating might cause a softening of connective tissue caused by conversion of collagen to gelatin (Larick and Turner, 1992). Lan *et al.* (1995) suggested that collagen was the predominant stromal protein in chicken breast muscle. Therefore, the DSC result of peak No.2 indicated that the collagen began to shrink in which an endothermic transition was observed in the temperature range 57.8 to 62.6°C for *Biceps femoris* and *Pectoralis* muscles of broiler and

indigenous chickens (Table 15) and was converted to gelatin at 80°C (Larick and Turner, 1992).

The changes in the structure of the intramuscular connective tissues of broiler and indigenous chicken muscles after cooking are shown in Fig 11. The perimysium of broiler *Biceps femoris* and *Pectoralis* muscles lost their wavy sheet structures and melted to produce a soft compact texture after cooking at 80°C (Fig 11A-D). However, the structure of the perimysium and endomysium of the indigenous chicken muscles were changed only slightly after cooking under the same conditions (Fig 11E-H). The wavy sheets of perimysium and the sheaths of the endomysium were retained with only slight disintegration. The indigenous chicken muscles had low content of myofibrillar protein with less resistance to thermal denaturation but they had thicker perimysium and more highly crosslinked collagen, which were associated with heat stability.

Post-mortem Changes of Thai Indigenous and Broiler Chicken *Pectoralis* Muscle during Ageing

1. Changes in muscle pH

Changes in pH during post-mortem ageing of Thai indigenous and broiler chicken *Pectoralis* muscles are shown in Fig 12. The significant decreases in muscle pH ($P<0.05$) were observed during the first 6 h for Thai indigenous chicken muscle and the first 2 h for broiler muscle. Thereafter, no marked changes in pH were observed up to 72 h of ageing. The ageing time to obtain the ultimate pH for broiler carcasses has been reported to be at least 4 h postchill at 4°C (Walker *et al.*, 1995; Northcutt *et al.*, 2001; Savenije *et al.*, 2002). Muscle pH was used to measure lactic acid accumulation in the muscle to monitor post-mortem metabolism (Lawrie, 1991). The different metabolic rates for the rapid decrease in muscle pH found in this study might be caused by the different rates of fall in post-mortem temperature and glycolysis (Lawrie, 1991). It is known

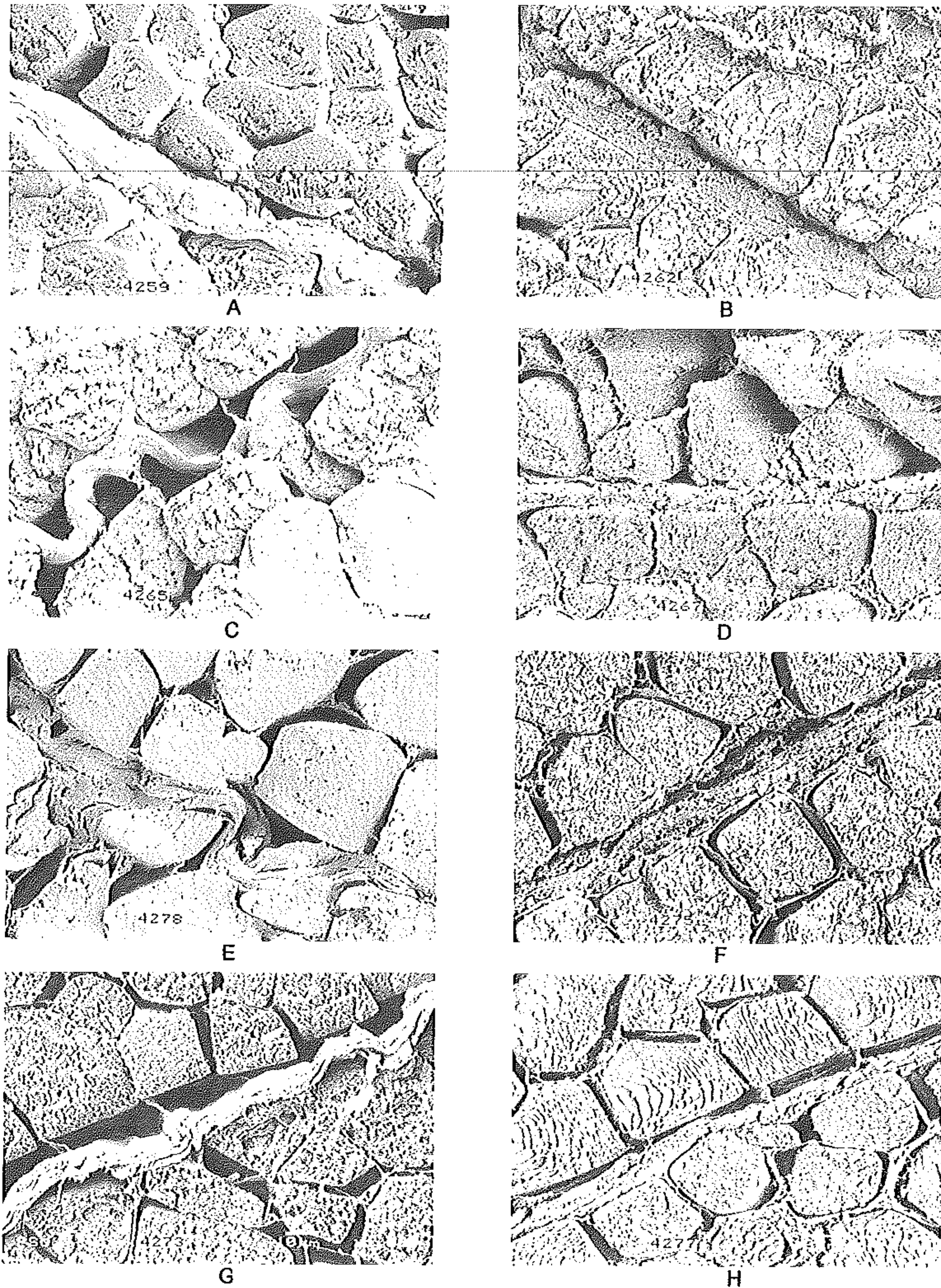

Fig 11. SEM micrographs of intramuscular connective tissue of chicken muscles; *Biceps fermoris* muscle of broiler, raw (A) and cooked (B), *Pectoralis* muscle of broiler, raw (C) and cooked (D), *Biceps fermoris* muscle of Thai indigenous chicken, raw (E) and cooked (F), *Pectoralis* muscle of Thai indigenous chicken, raw (G) and cooked (H).

that the rate of post-mortem glycolysis tends to be higher in muscles which are slow to cool (Lawrie, 1991). The ultimate pH for Thai indigenous and broiler muscle were 5.85 and 5.90, respectively. This result was in agreement with those reported by Wattanachant *et al.* (2004), Savenije *et al.* (2002) and Jaturasitha *et al.* (2002). The higher ultimate pH in muscle of broiler chicken ($P<0.01$) was probably due to it being slightly more sensitive to stress than the indigenous one. Glycogen is depleted by several stress conditions including exercise, fasting, hot and cold temperatures and fear (Foegeding and Lanier, 1996; Jaturasitha, 2000). This accelerated glycolysis before death led to a low glycogen content and hence high pH value in meat. However, the effect was small and might also reflect a genetic difference.

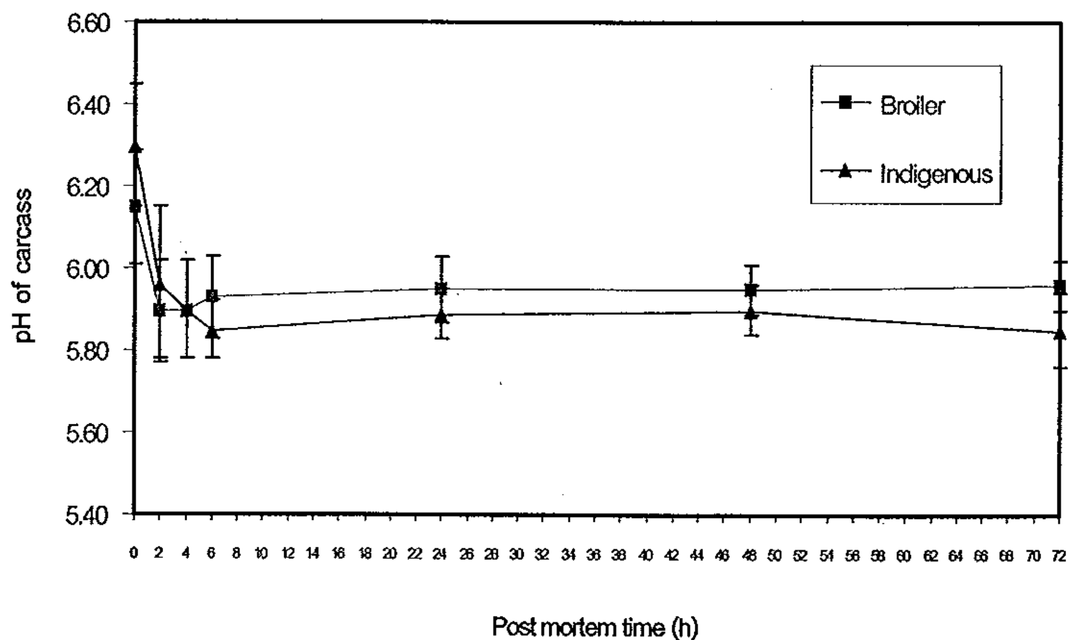

Fig 12. Change in pH of broiler and indigenous chicken *Pectoralis* muscle during post-mortem ageing at 4°C

Note: Bars indicate standard deviation from thirty determinations.

2. Lactic acid accumulation

Lactic acid accumulation in chicken muscle during post-mortem ageing is presented in Fig 13. The significant increase in lactic acid accumulation ($P<0.05$) was observed during 4 h post-mortem for broiler muscle and during 24 h post-mortem for Thai indigenous chicken muscle. The higher content of lactic acid in Thai indigenous chicken muscle compared to broiler muscle ($P<0.001$) correlated well with the lower pH as discussed previously. However, the increased lactic acid concentrations did not mirror the rate of pH fall. This was possibly due to the buffering capacity of muscle. Generally, white muscles have relatively large contents of carnosine and serine, which probably function as buffering agents in the muscle cell (Foegeding and Lanier, 1996).

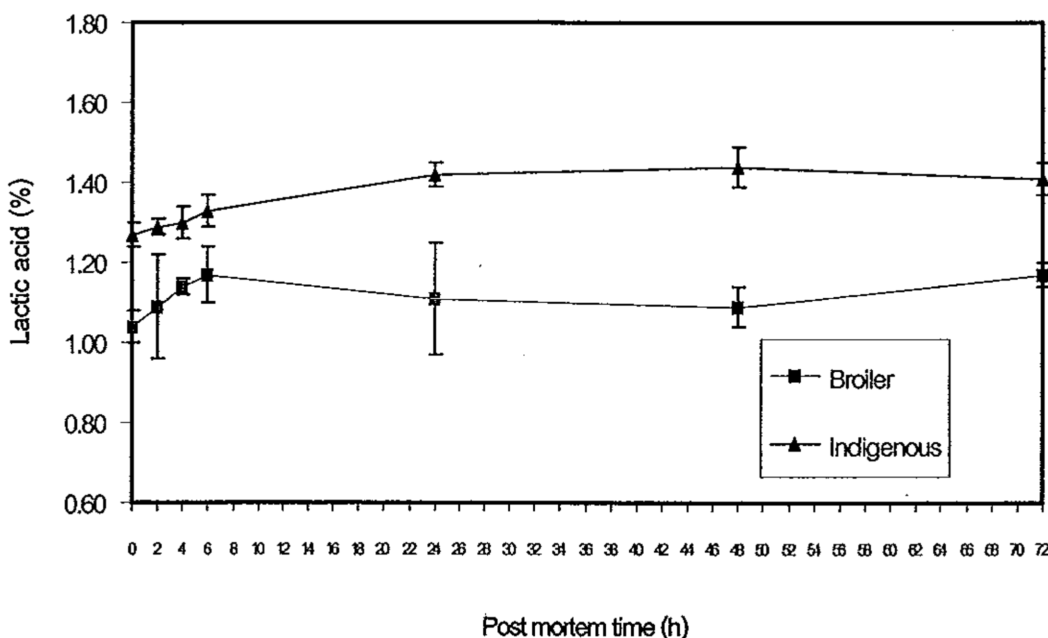

Fig 13. Change in lactic acid content of broiler and indigenous chicken *Pectoralis* muscle during post-mortem ageing at 4°C

Note: Bars indicate standard deviation from eight determinations.

3. Degradation of muscle proteins

Degradation of the myofibrillar and sarcoplasmic proteins resulted in an increase in peptides and free amino acids, leading to increased meat tenderness during ageing. Proteolysis in muscle of both chicken breeds during post-mortem ageing was observed as indicated by the increase in TCA-soluble peptides (Fig 14). TCA-soluble peptides of broiler muscle increased rapidly at 2 h of post-mortem ageing ($P<0.05$) and a gradual increase ($P<0.05$) was subsequently observed throughout ageing. For Thai indigenous chicken muscle, TCA-soluble peptides increased non-significantly ($P>0.05$) until 6 h of ageing and gradually increased thereafter ($P<0.05$). A higher correlation between proteolysis and ageing time (R^2 = 0.99) was observed for Thai indigenous chicken muscle, compared with that observed in broiler muscle (R^2 = 0.90). Broiler muscle had a higher degree of proteolysis than indigenous muscle, especially early post-mortem ($P<0.01$). Two groups of endogenous proteases in skeletal muscle, the calpains and the cathepsins, have been implicated in the degradation of the myofibrillar protein matrix (Ouali, 1990; Koohmaraie, 1992; Walker *et al.*, 1995). However, post-mortem ageing-induced tenderness is associated with decreases in calpain but not cathepsin enzyme levels (Lyon and Buhr, 1999). Veeramuthu and Sams (1999) reported that μ–calpain is an unstable enzyme and is inactivated by either autolysis or intermolecular rearrangement during post-mortem storage of *Pectoralis* muscle of broiler. In contrast, m-calpain activity slightly declined during 24 h of ageing. The difference in proteolysis between the chicken breeds was possibly due to the difference in initial level of calpains, cathepsin and calpain inhibitor (calpastatin). The calpastatins have been reported to prevent the degradation of Z-line proteins (Koohmaraie *et al.*, 1986). Alvarado and Sams (2000) found that chicken muscle had less initial calpastatin than duck muscle, and that there was greater loss of this inhibitor during post-mortem. In addition, calpastatin activity in breast muscle of turkey was slightly affected by age (Northcutt *et al.*, 1998). However, the effect of age on calpastatin activity in

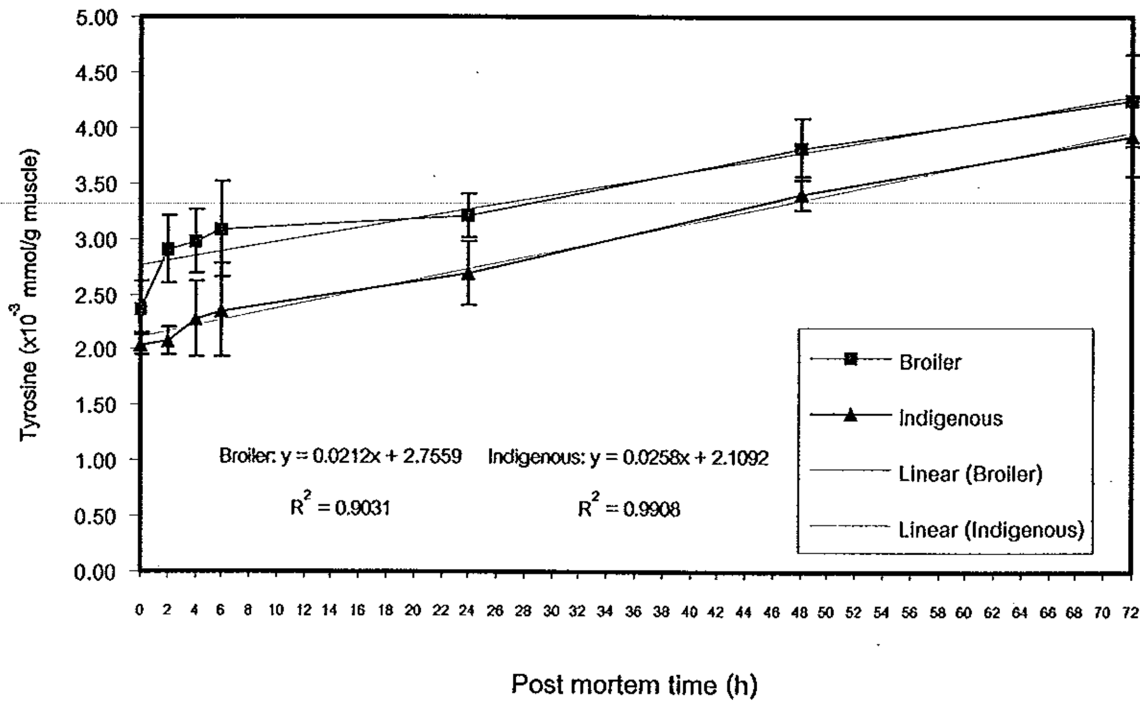

Fig 14. Change in TCA-soluble peptides of broiler and indigenous chicken *Pectoralis* muscle during post-mortem ageing at 4°C. Bars indicate standard deviation from eight determinations

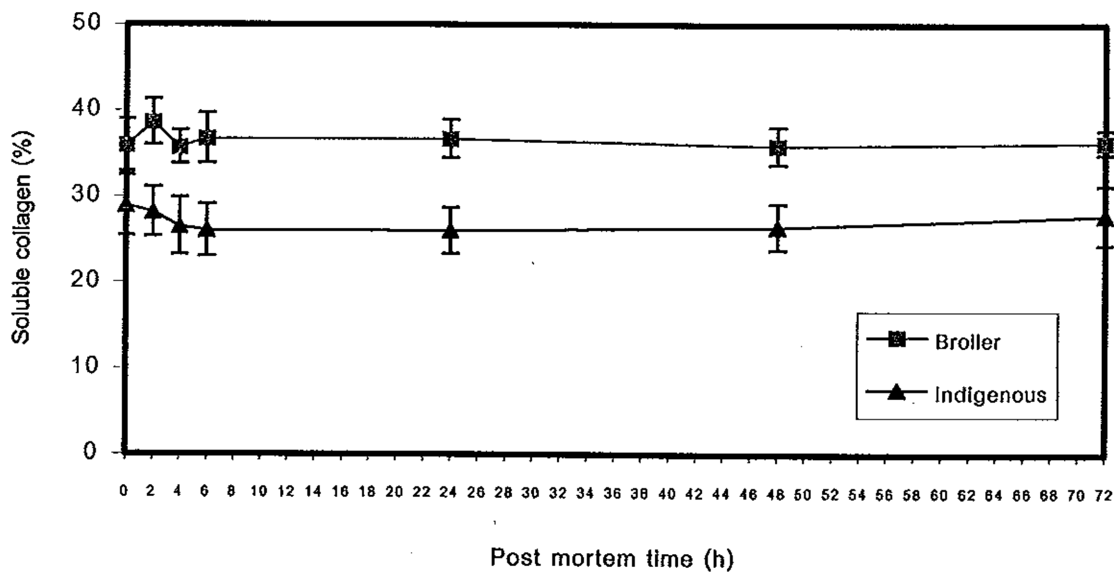

Fig 15. Change in soluble collagen of broiler and indigenous chicken *Pectoralis* muscle during post-mortem ageing at 4°C. Bars indicate standard deviation from eight determinations.

broiler and Thai indigenous muscle still is not clear. Furthermore, the significantly lower pH in Thai indigenous chicken muscle after 4 h of post-mortem ageing, compared with broiler muscle (Fig 12) could lower the activity of calpains, leading to the lower rate of proteolysis.

Soluble collagen in muscle of both chicken breeds during post-mortem ageing is depicted in Fig 15. No change in soluble collagen was observed within 72 h post-mortem ageing ($P>0.05$). However, Liu *et al.* (1994; 1995) observed a change in structure of intramuscular connective tissue at 12 h post-mortem in chicken muscle. For bovine muscle, the collagen solubility increased at first 8 h (Mills *et al.*, 1989) and doubled from 5 days to 12 days post-mortem ageing (Palka, 2003). The significantly lower soluble collagen in Thai indigenous chicken muscle compared to that of the broiler ($P<0.001$) was presumably due to the higher content of crosslinked collagen in the older chicken.

4. Changes in texture of muscle

The change in texture of *Pectoralis* muscle from both chickens during post-mortem ageing was monitored by the change in Warner-Bratzler shear value (Fig 16). The shear value increased to a maximum at 2 h of post-mortem ageing due to rigor mortis development. A rapid decrease in shear value was observed at 4 h of post-mortem ageing in broiler muscle. Thereafter, the shear value of broiler muscle decreased slightly ($P<0.05$) although no change in shear value occurred upon deboning after the first 4 h of on-carcass ageing in normal broiler has been reported (Walker *et al.*, 1995). The decrease in shear value after 4 h post-mortem found in this study was similar to that seen in a previous report (Hirschler and Sams, 1994). The shear value of Thai indigenous chicken muscle significantly decreased at 6 h of post-mortem ageing ($P<0.05$) which was later than that seen for the broiler muscle ($P<0.001$). The difference in shear value decline between the muscles of the two breeds might be due to the difference in the degree of proteolysis as discussed previously. Shear values during post-

mortem ageing of broiler and Thai indigenous chicken muscle was highly correlated with TCA-soluble peptides and lactic acid accumulation (Table 18). However, the different correlations between shear value and the other muscle parameters probably reflects the differing factors affecting each of these parameters. Although pH, soluble collagen and shear value are all related to the muscle structure and post-mortem metabolism, other integral factors might be involved in post-mortem changes.

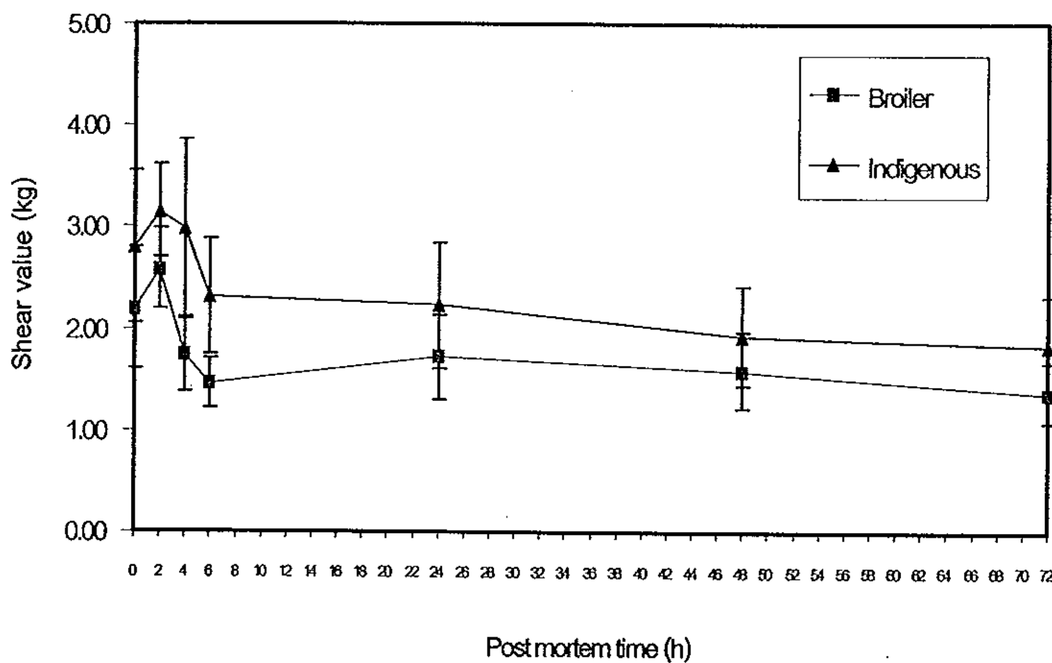

Fig 16 Change in shear value of broiler and indigenous chicken *Pectoralis* muscle during post-mortem ageing at 4°C

Note: Bars indicate standard deviation from twenty determinations.

Table 18. Correlation coefficients values (R^2) and standard error (SE) between some variables and shear value of Thai indigenous and broiler chicken *Pectoralis* muscle ageing at 4°C for 72 h.

Dependent variable	Independent variable	Type of model	Broiler		Thai indigenous	
			R^2	SE	R^2	SE
Shear value	TCA-solublepeptides	Linear	0.46	0.35	0.76	0.28
		Exponential	0.51	0.17	0.82	0.10
	Soluble collagen	Linear	0.39	0.37	0.14	0.53
		Exponential	0.32	0.20	0.11	0.22
	Lactic acid	Linear	0.50	0.33	0.78	0.27
		Exponential	0.55	0.16	0.79	0.11
	pH	Linear	0.04	0.46	0.19	0.51
		Exponential	0.05	0.24	0.20	0.21

Effect of Heat Treatment on Changes in Texture, Structure and Properties of Thai indigenous and Broiler Chicken Muscles

The heat penetration time of broiler and indigenous *Pectoralis* muscle stripes was recorded during heating the samples as shown in Table 19. The indigenous *Pectolaris* muscle stripes took a longer time to reach the end point temperature than the broiler muscle stripes. This might be due to the thicker perimysium and firmer muscle of the former as mentioned in previous part (Table 14).

1. Changes in textural characteristic and muscle structure

The effects of heat treatment on shear value of broiler and indigenous chicken muscles are shown in Fig 17. The indigenous chicken muscles showed more toughness, than that of the broiler at all temperatures tested ($P<0.001$). The change in shear value of both chicken muscles was similar and could be divided into two steps. A significant increase in shear value was found at 60°C for broiler muscle and it then slightly increased up to 80°C ($P>0.05$). At temperature higher

Table 19. Heat penetration of broiler and indigenous *Pectoralis* muscle samples

Temperature (°C)	Time to end point temperature (min)	
	Indigenous	Broiler
50	2.27 ± 0.09	2.00 ± 0.06
60	3.00 ± 0.16	2.83 ± 0.02
70	3.85 ± 0.24	3.23 ± 0.14
80	5.17 ± 0.07	4.83 ± 0.04
90	8.17 ± 0.15	5.27 ± 0.11
100	12.00 ± 0.37	8.45 ± 0.07

Note: -Data are presented as mean ± standard deviation. n = 2
-Heating medium: boiling water bath (100°C)
-Muscle strip size 2.0x2.0x6.0 cm packed individually in tightly sealed plastic bag.

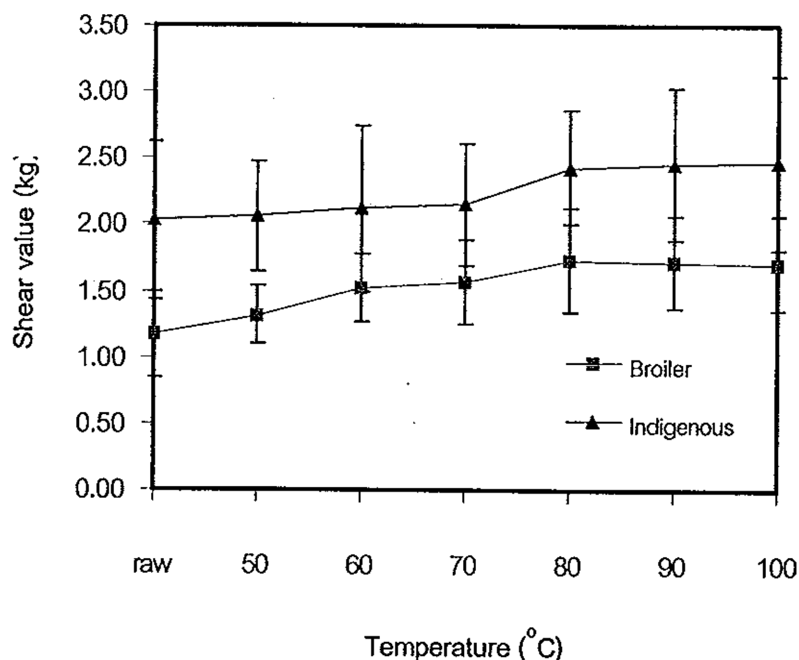

Fig 17. Effect of heating temperature on shear value of broiler and indigenous *Pectoralis* muscle

Note: Bars indicate standard deviation from twenty determinations.

than 80°C, shear value of broiler muscle tended to decrease (P>0.05). Shear value of indigenous chicken muscles slightly increased (P<0.05) when heated from 50 to 70°C and significantly increased at 80°C (P<0.05). No changes in shear value of the indigenous chicken muscles were observed when heated at 90-100°C (P>0.05). The increase in shear value with heating up to 80°C might be due to the combination effect of the denaturation of myofibrillar proteins, the shrinkage of intramuscular collagen, as well as the shrinkage and dehydration of the actomyosin (Bailey and Light, 1989).

The changes in microstructure of raw and cooked *Pectoralis* muscles from both chicken breeds are presented in Fig 18 and 19. On the transverse sections (Fig 18), the gaps between muscle fibers were visible in raw and cooked samples at 50°C. With increasing temperature, the structure of chicken meat became denser and more compact in fiber arrangements at 60 to 70°C, especially for the indigenous chicken muscle. This structure change resulted in increase shear value. The denaturation and melting of perimysial and endomysial together with the denaturation of myofibrils were observed in the range of 80-100°C. This result contributed to meat tenderness as postulated by no changes in shear value after 80°C. On the longitudinal sections (Fig 19), very slight changes were observed in the temperature range 50-60°C. However, shrinkage of the sarcomere was obviously seen with increasing heating temperature. Weakening and melting of connective tissue with loss in the structure of Z-disks occurred at temperature ranges of 80-100°C for broiler muscles. Differently, the sarcomeres of cooked indigenous chicken muscles were more closely compact with remaining Z-disks with increasing temperature up to 90°C and the structure of Z-disks of indigenous muscle still remained with heat treatment at 100°C. The result suggested that the Z-disks structure of the indigenous chicken muscles were stronger than those of the broiler muscles.

Microstructural measurements of raw and cooked Thai indigenous and broiler chicken muscles are presented in Fig 20 and 21. A statistically significant

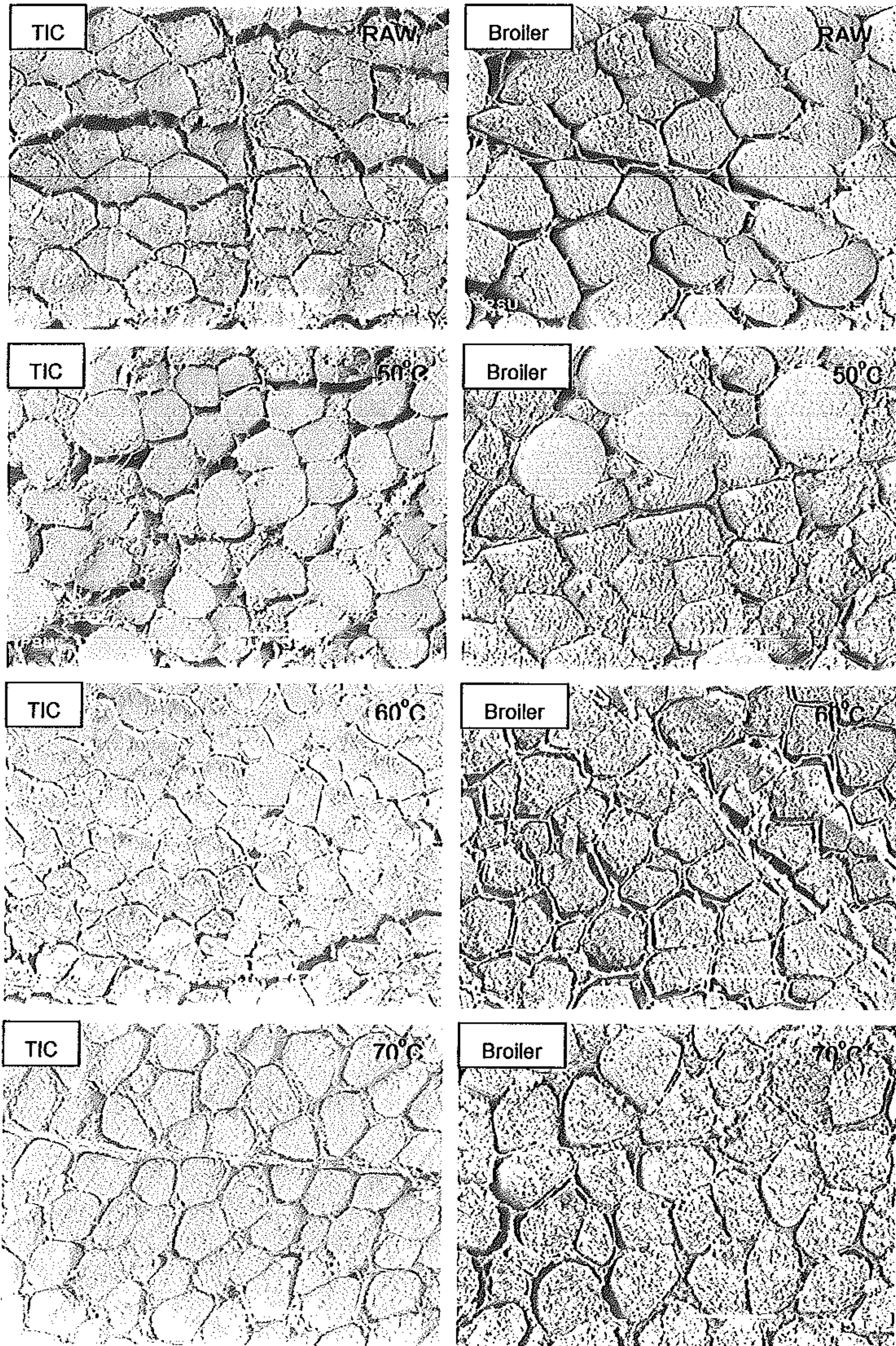

Fig 18. SEM micrographs of transverse sections of raw and cooked broiler and indigenous *Pectoralis* muscle

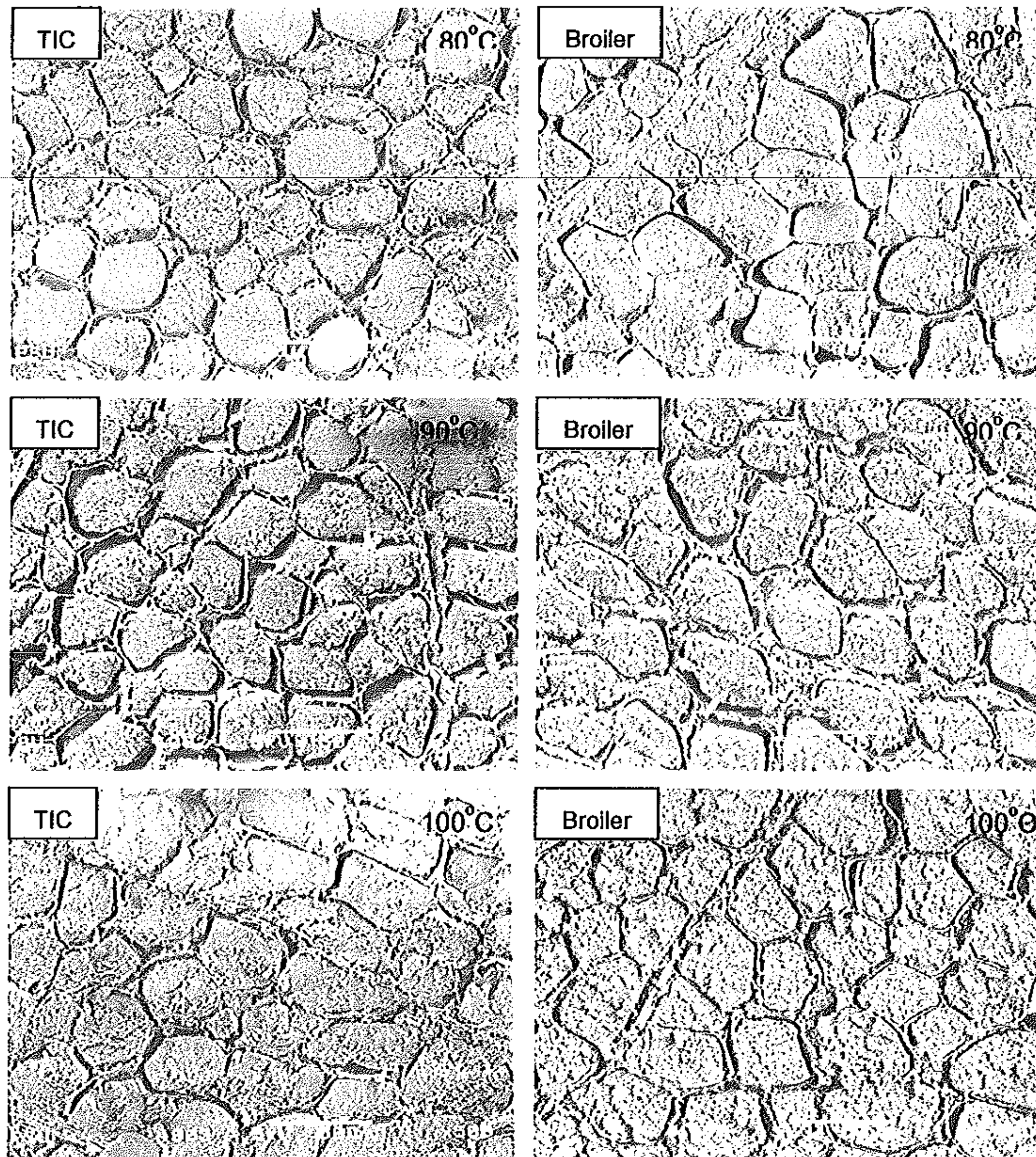

Fig 18. (Cont.) SEM micrographs of transverse sections of raw and cooked broiler and indigenous *Pectoralis* muscle

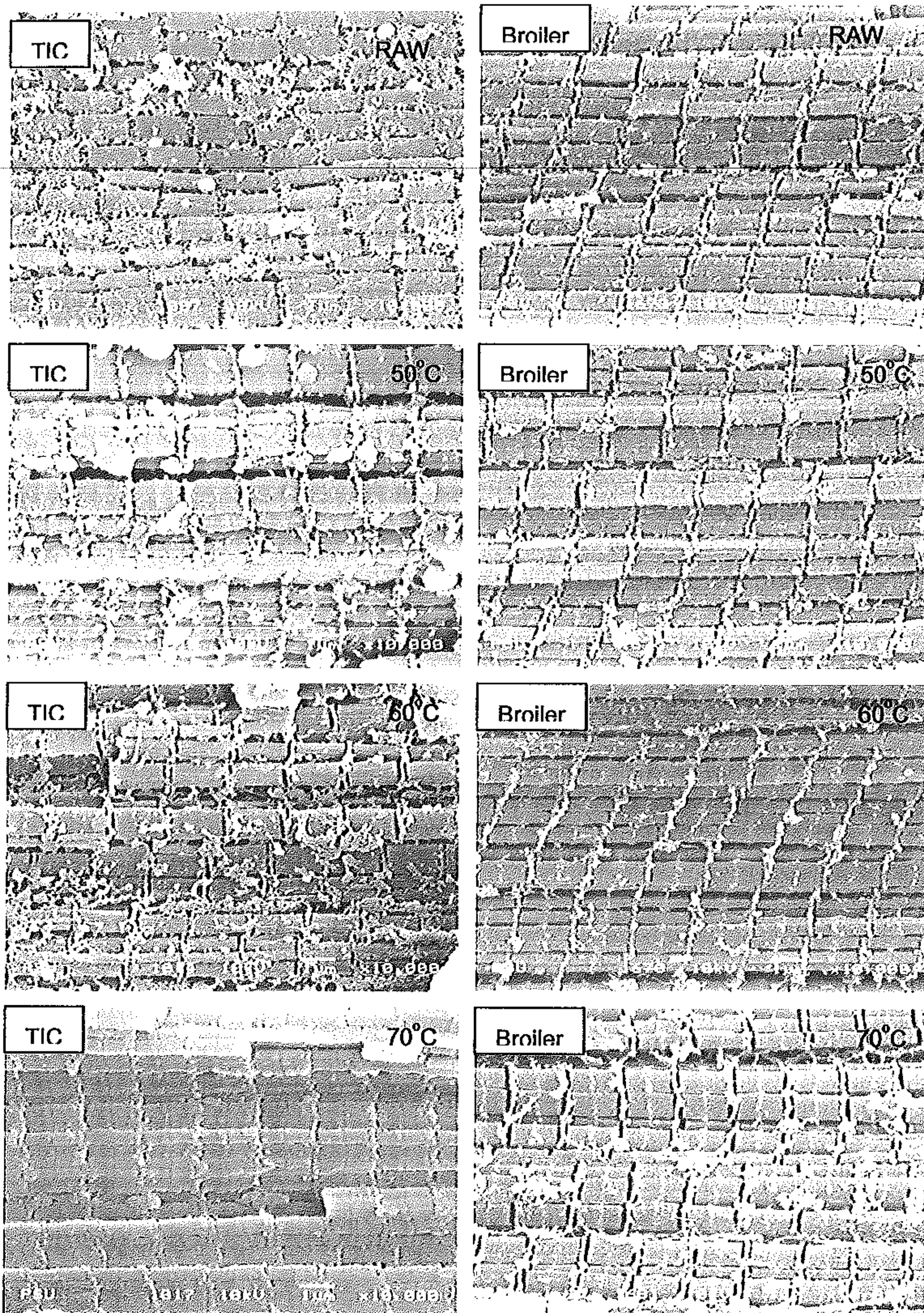

Fig 19. SEM micrographs of longitudinal sections of raw and cooked broiler and indigenous *Pectoralis* muscle

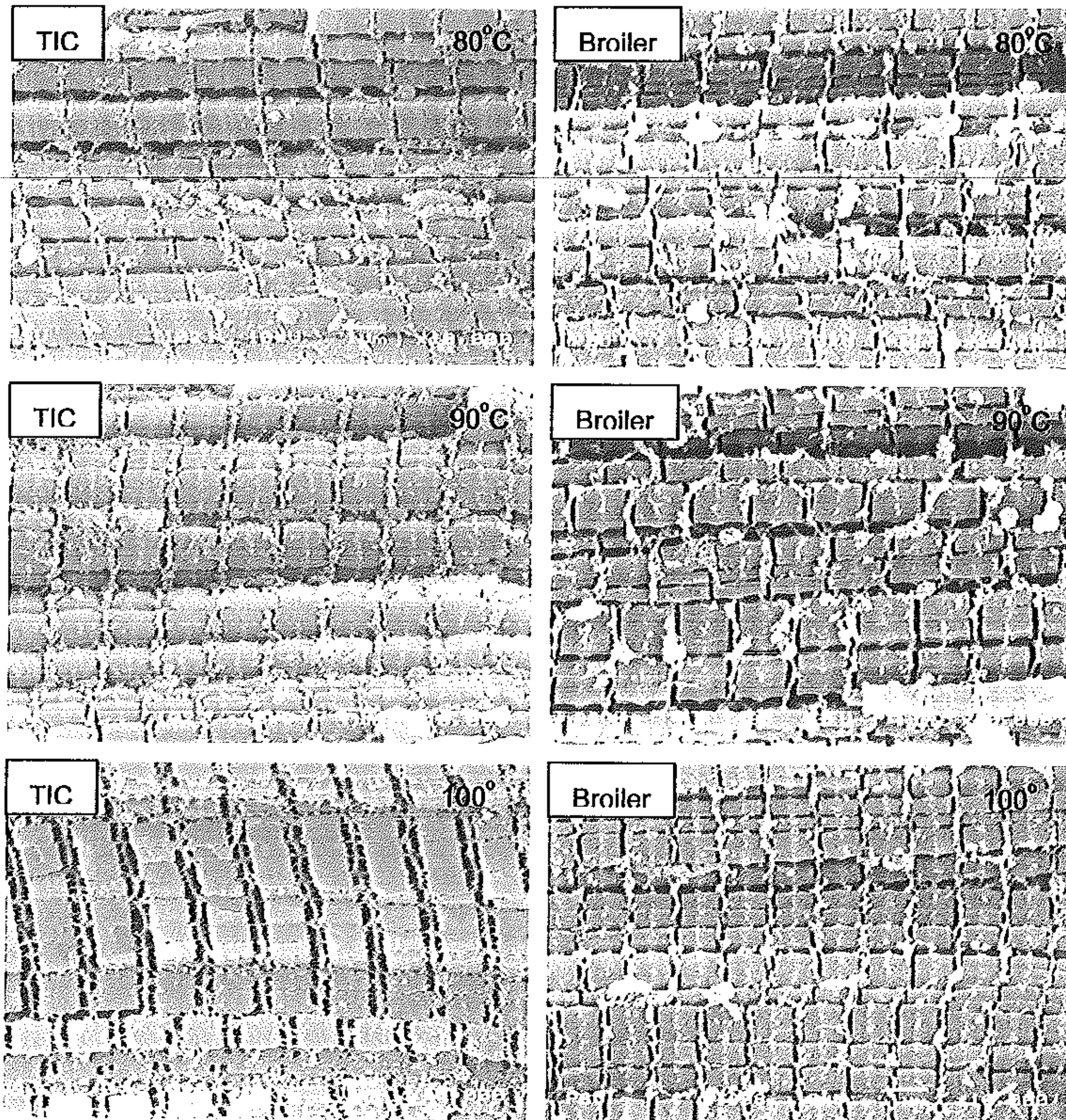

Fig 19. (Cont.) SEM micrographs of longitudinal sections of raw and cooked broiler and indigenous *Pectoralis* muscle

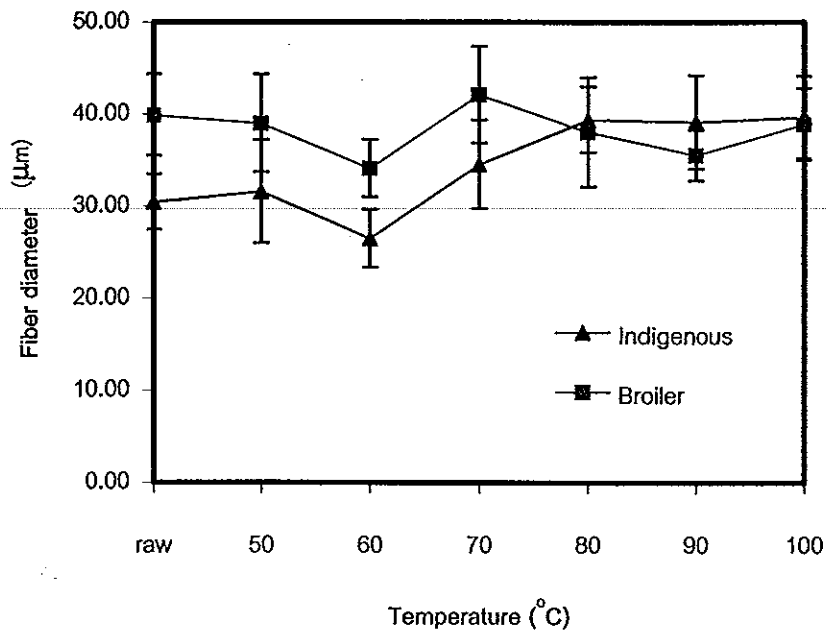

Fig 20. Effect of heating temperature on fiber diameter of broiler and indigenous *Pectoralis* muscle. Bars indicate standard deviation from forty determinations.

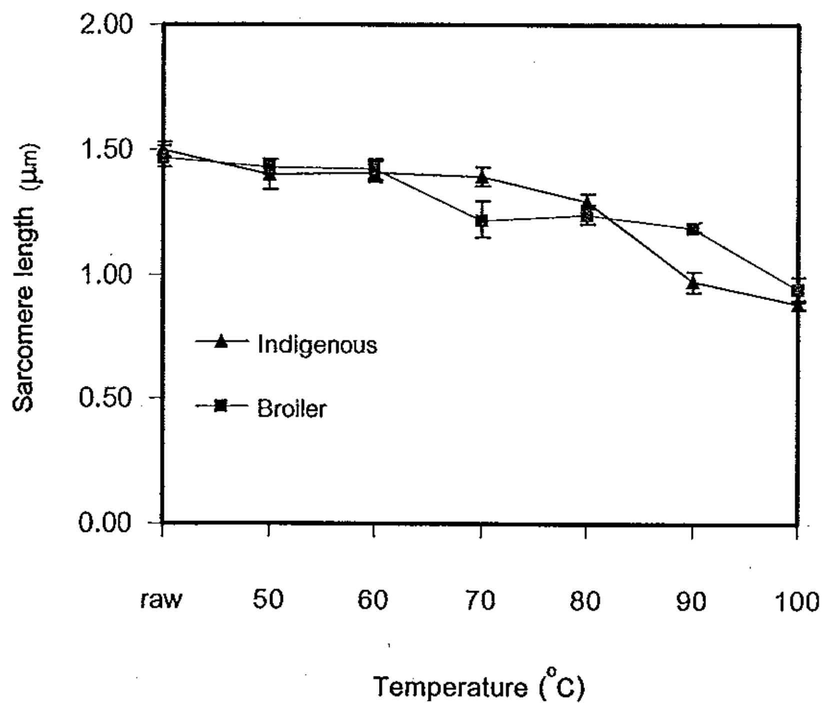

Fig 21. Effect of heating temperature on sarcomere length of broiler and indigenous *Pectoralis* muscle. Bars indicate standard deviation from thirty determinations.

decrease in fiber diameter was observed in samples heated to the endpoint temperature of 60°C. This shrinkage might be due to the thermal denaturation of intramuscular collagen, which was found at 60.7°C and 61.7°C for indigenous chicken and broiler *Pectoralis* muscle, respectively (Table 15). The fiber diameter of both chicken muscles was expanded when heated to higher endpoint temperature and tended to stabilise in the temperature ranges of 80-100°C ($P>$ 0.05). The change in fiber diameter was related to the change in sarcomere length as shown in Fig 21. Sarcomere length decreased with increasing temperature. The sarcomere length decreased non-significantly when heated to temperature of 50-60°C for broiler muscle sample and 50-70°C for the indigenous chicken muscle ($P>0.05$). However, the greatest shrinkage of sarcomere was observed in samples heated to endpoint temperatures of 70-100°C for the broiler muscle and 80-100°C for the indigenous chicken muscle ($P<0.05$). The result indicated that the shrinkage of chicken meat during cooking in the range of 50-100°C occurred in two phases. At a temperature of about 50-60°C, the shrinkage was primarily transverse and was primarily parallel to the fiber axis at 70-100°C. Changes in muscle fibers observed in this study are in agreement with Offer *et al.* (1984) and Palka and Daun (1999) who studied the process in cooked bovine muscles.

2. Changes in physical properties of muscle

The amount of water bound by the tissue system decreased with increasing temperature (Palka and Daun, 1999). The cooking losses of broiler and indigenous chicken muscles are presented in Fig 22. The cooking losses of broiler and indigenous chicken muscles were not significantly different in the temperature range 50-70°C. The greatest increase in cooking losses for indigenous chicken muscles were observed in the temperature range 80-100°C. Significantly higher cooking losses were found in indigenous chicken muscle compared with those of the broiler ($P<0.001$). The difference might be related to the difference in content of crosslinked collagen between chicken breeds (Wattanachant *et al.*, 2004). For the older indigenous birds, the more highly

crosslinked collagen remained insoluble and shrank during heat treatment and effectively squeezed the heat denatured myofibrillar gel. This led to the loss of moisture and a tougher texture.

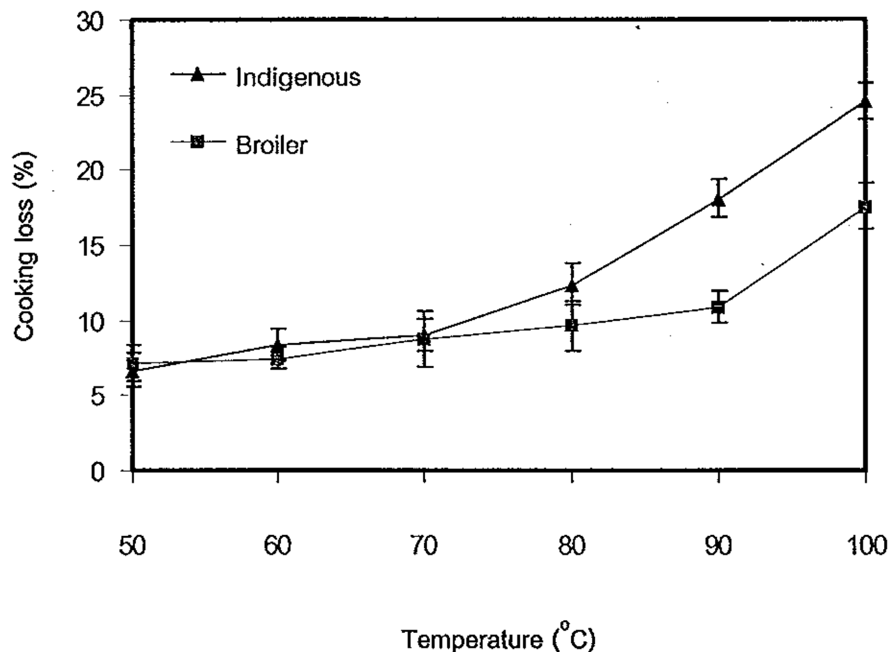

Fig 22. Effect of heating temperature on cooking losses of broiler and indigenous *Pectoralis* muscle

Note: Bars indicate standard deviation from seven determinations.

Changes in color of broiler and indigenous chicken muscles during heating are shown in Fig 23. The CIE system values of lightness (L*) and yellowness (b*) of both chicken muscles increased significantly with increasing temperature in the range 50-70°C ($P<0.05$) and no changes were observed when heated to higher temperature ($P>0.05$). The redness (a*) of indigenous chicken muscle increased significantly when heated to endpoint temperature of 70°C and

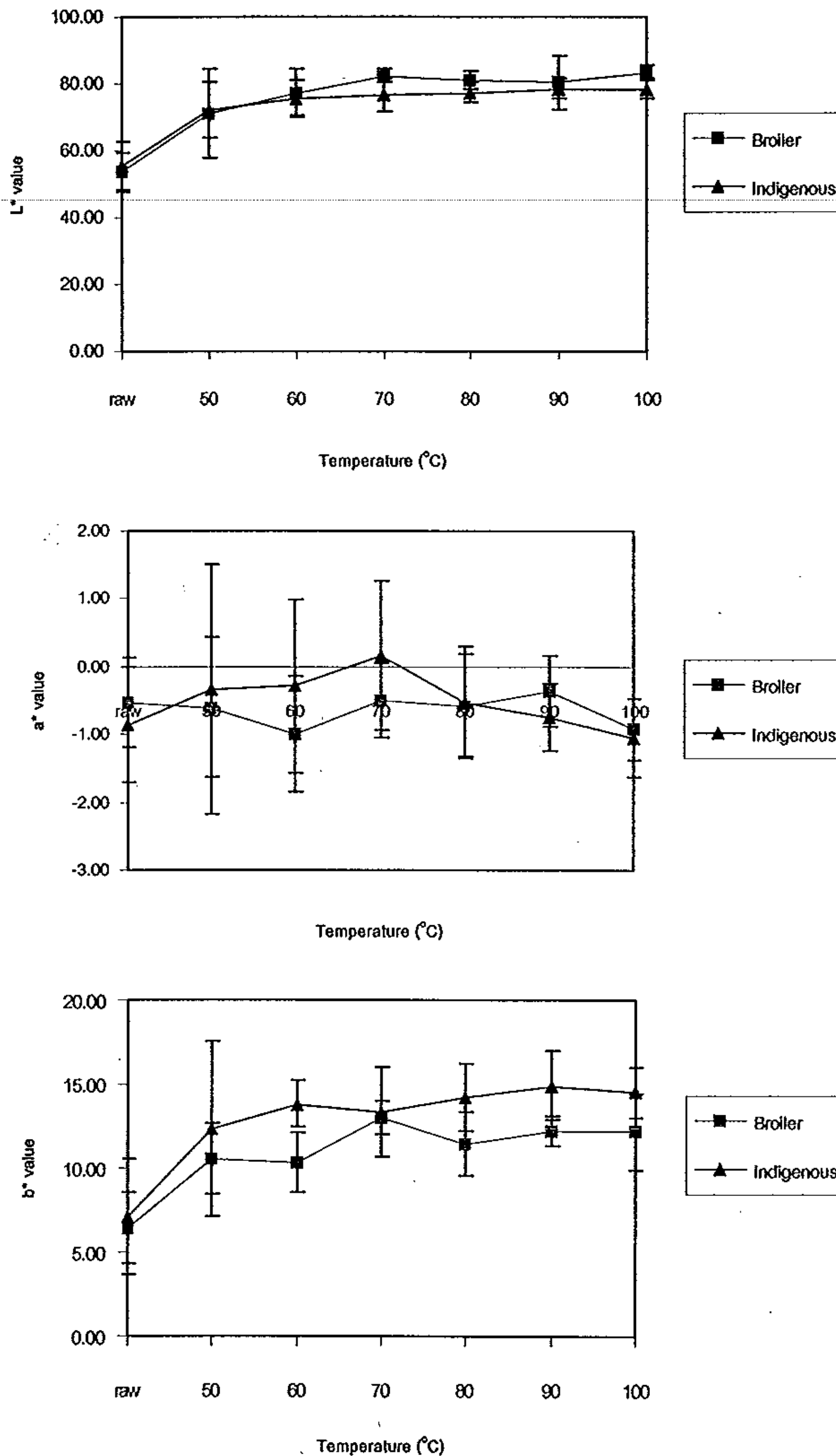

Fig 23. Effect of heating temperature on color of broiler and indigenous *Pectoralis* muscle. Bars indicate standard deviation from fourteen determinations.

decreased when heated to higher temperatures ($P<0.05$). On the other hand, the a* value of broiler muscle decreased with heat treatment up to 60°C, followed by continuous increase up to 90°C. A sharp decrease was noticeable at 100°C. The increase in L* and b* value and decrease in a* value of broiler muscle after cooking at 95°C and 98°C have been reported (Fletcher *et al.*, 2000; Qiao *et al.*, 2002). With increasing heating temperature, meat tended to be lighter and also turned to a brown-grey hue. The lightening is due to an increased reflection of light arising from light scattering by denatured proteins (Young and West, 2001). The loss of chroma and change in hue resulted from the changes in myoglobin. Myoglobin is one of the more heat-stable of the sarcoplasmic proteins, which is almost completely denatured between 80-85°C (Lawrie, 1991). According to Lawrie (1991) and Young and West (2001), the compound involved in increasing redness of muscles should be globin hemochrome, in which the iron is in Fe^{2+} state. Its color is typically dull red. Globin hemichrome with the iron in the Fe^{3+} state, is largely responsible for the brown-grey hue. The balance between hemochromes and hemichromes is affected by the state of the meat before cooking and other factors including species, animal maturity and muscle type (Young and West, 2001).

3. Changes in solubility of proteins

Changes in solubility of muscle proteins in S1-S5 are shown in Fig 24. The contents of soluble protein in both S1 and S2 markedly decreased with increasing temperature ($P<0.05$). The decrease in total soluble proteins of chicken breast patties with increasing temperatures has been reported by Marphy and Marks (2000). This result indicated denaturation or other structural changes in the proteins during heating. The indigenous chicken muscle had lower content of protein solubilized in S1 and S2 compared with that of broiler muscle. This might be attributed to the lower myofibrillar protein in the former chicken muscle (Table 9). Gradual increases in protein solubility in the presence of denaturating agents, especially SDS and urea (S3 and S4) were observed in the

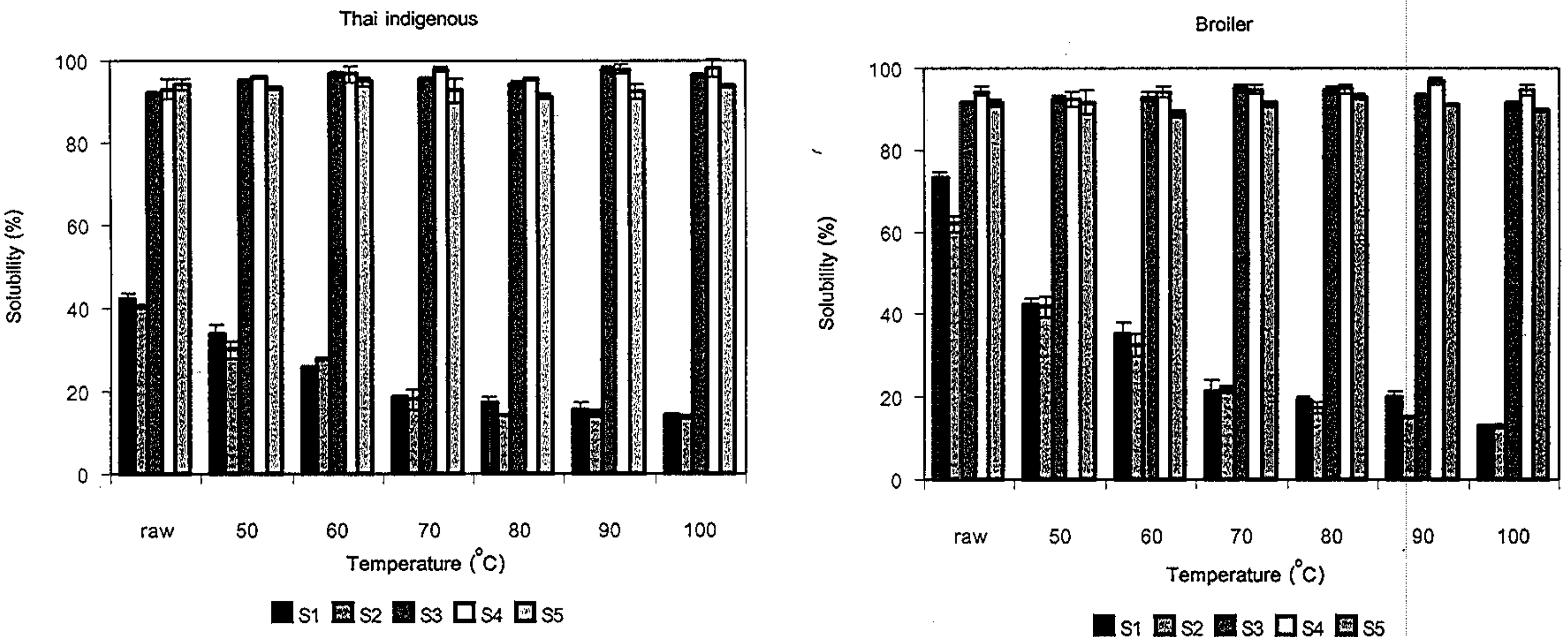

Fig 24. Effect of heating temperatures on solubility of broiler and indigenous *Pectoralis* muscle

Note: S1 = 0.6 M KCl; S2 = 20 mM Tris, pH 8.0; S3 = 20 mM Tris, pH 8.0 containing 1% (w/v) SDS; S4 = 20 mM Tris, pH 8.0 containing 1% (w/v) SDS, 8 M urea; S5 = 20 mM Tris, pH 8.0 containing 1% (w/v) SDS, 8 M urea, and 2% (v/v) β-mercaptoethanol. Bars indicate standard deviation from triplicate determinations.

muscle of indigenous chickens up to 70°C. The higher content of proteins solubilized in S3, S4 and S5 in indigenous chicken muscle suggested that there were more contribution of hydrogen bonds, hydrophobic interactions and disulfide bonds compared with that of broiler muscle. The result also indicated that the hydrophobic interaction and hydrogen bonds were important interactions in stabilising the structure of chicken muscle.

SDS-PAGE patterns of muscle and exudate of both chickens during heating are shown in Fig 25 and 26. The decrease in myosin heavy chains was observed in the indigenous muscle with increasing temperature. However, the changes in SDS-PAGE patterns for broiler muscle were not noticeable. Murphy and Marks (2000) reported that the protein subunits with a molecular weight greater than 40 kDa decreased with increasing temperature. As shown in Fig 26, the low molecular weight proteins were observed in the exudate of cooked muscle at lower heating temperature and decreased with increased temperatures. In contrast, solubilised collagen was observed with increasing temperature in the exudate from the cooked muscle of both breeds. The results also indicated that the myofibrillar protein of indigenous muscle was less heat stable since a lower temperature caused the disappearance of those protein bands in this muscle compared with that of the broiler.

Changes in solubility of collagen during heating of both chicken muscles are shown in Table 20. The soluble collagen content of both chicken muscles increased gradually ($P<0.05$) with increasing temperature from 50 to 100°C. Larick and Turner (1992) stated that collagen began to shrink at 60 to 70°C and was converted to gelatin at 80°C and that these changes weakened the connective tissue. Therefore, the reduction in shear value above 80°C (Fig 17) could also be due to the increase in collagen solubility. The indigenous chicken muscle contained less soluble collagen than those of the broiler muscles at all heating temperatures tested except at 100°C. This resulted in higher shear value in the indigenous muscle, compared with the broiler. The difference in the amount of

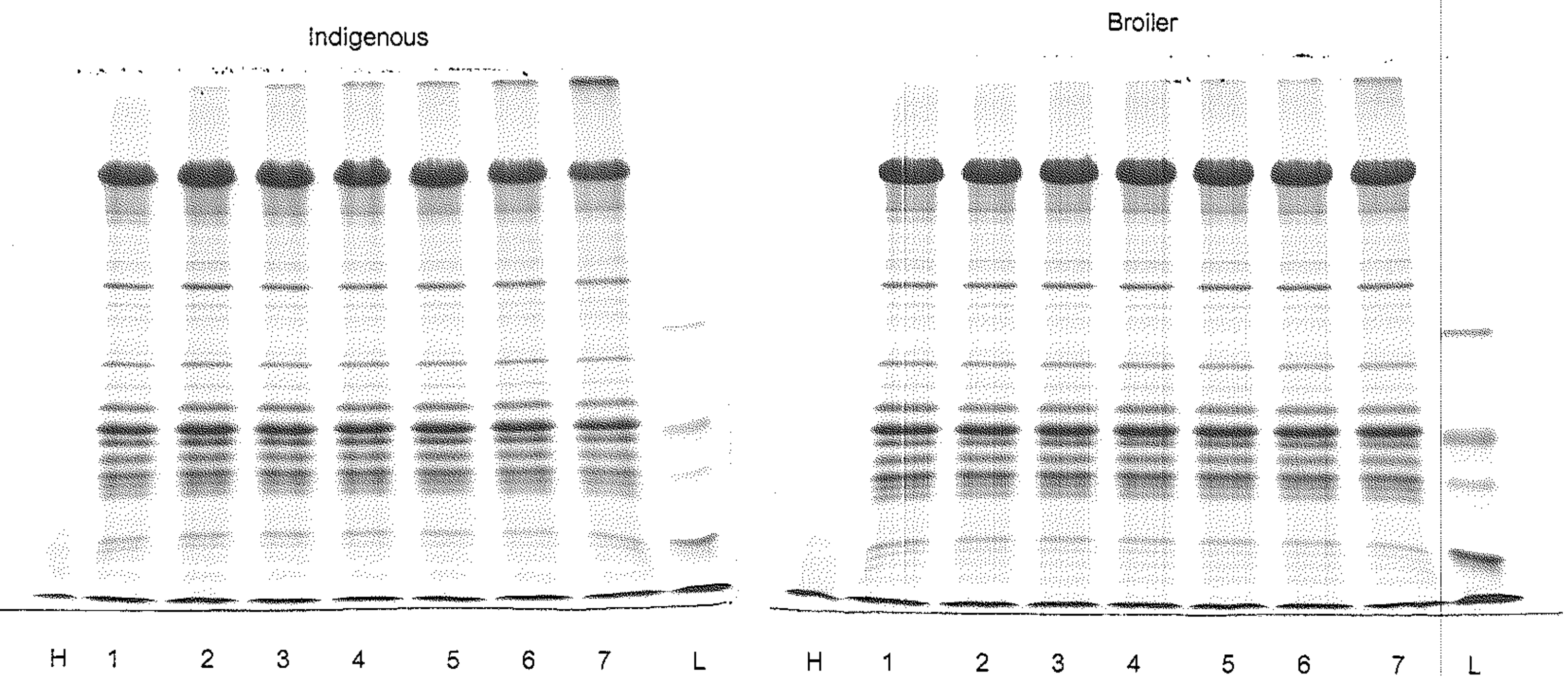

Fig 25. SDS-PAGE patterns of raw and cooked broiler and indigenous *Pectoralis* muscles (H) and (L) high and low molecular weight protein standards, (1) raw, (2-7) muscles cooked to 50, 60, 70, 80, 90, and 100°C, respectively.

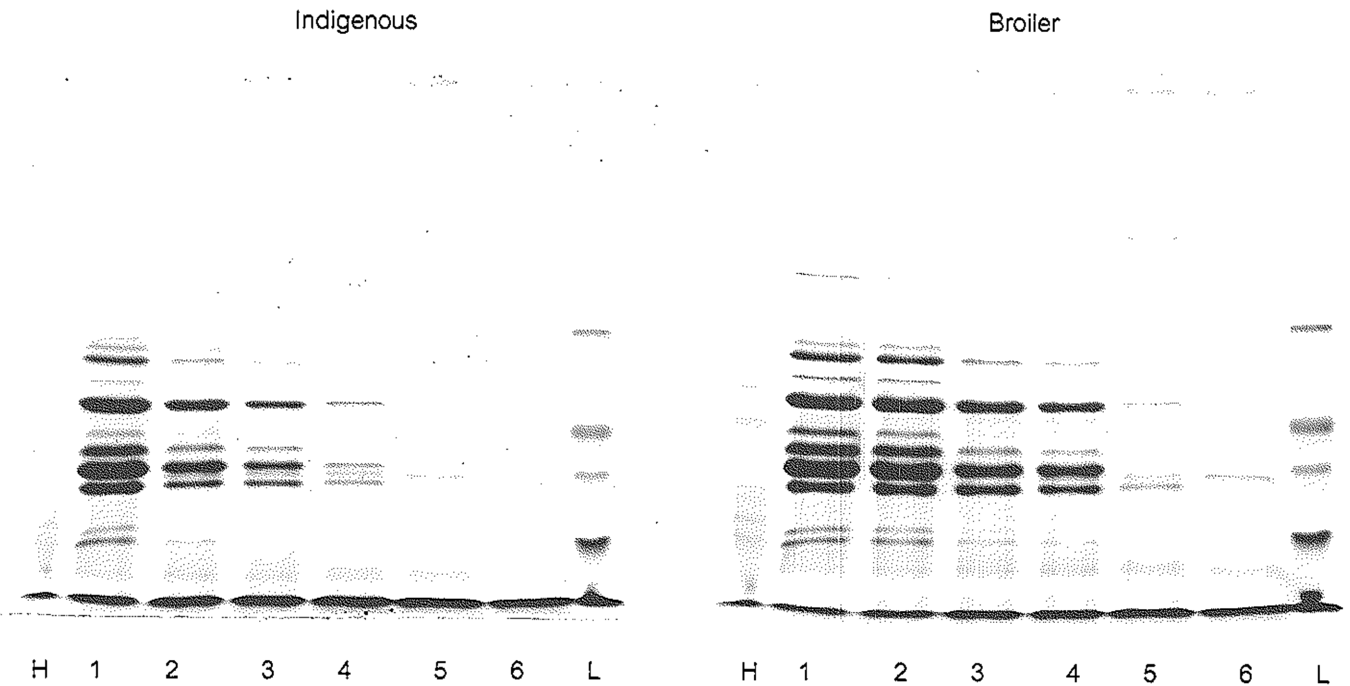

Fig 26. SDS-PAGE patterns of exudates from cooked broiler and indigenous *Pectoralis* muscles (H) and (L) high and low molecular weight protein standards, (1-6) exudates from cooked muscle to 50, 60, 70, 80, 90, and 100°C, respectively.

heat soluble collagen between broiler and indigenous chicken might be due to the differences in amount of crosslinked collagen, which related to the age of bird. The heat solubility of collagen decreases with increased collagen crosslinking and crosslinking increases with animal age (Pearson and Young, 1989; Foegeding and Lanier, 1996).

Table 20. Effect of heating on soluble collagen of broiler and indigenous *Pectoralis* muscles.

Temperature (°C)	Soluble collagen (%)		Significance
	Broiler	Indigenous	Between breeds
raw	3.59 ± 0.85^{a}	2.34 ± 0.63^{a}	ns
50	6.09 ± 0.64^{b}	3.60 ± 1.18^{ab}	*
60	6.54 ± 1.33^{bc}	5.12 ± 0.92^{bc}	ns
70	7.87 ± 1.11^{bcd}	5.17 ± 0.75^{bc}	*
80	7.66 ± 0.91^{bcd}	5.93 ± 1.00^{cd}	ns
90	8.13 ± 1.40^{cd}	7.48 ± 0.92^{d}	ns
100	9.41 ± 0.34^{d}	10.69 ± 1.15^{e}	ns

Data are presented as mean ± standard deviation. n = 3
$^{a-d}$Means within a column with differing superscripts are significantly different (P<0.05).
Significant differences between breeds were determined by t-test: * P<0.05; ** P<0.01; *** P<0.001; ns = no significant difference.

4. Relationship evaluation

Relationships between changes in sarcomere length, fiber diameter, shear value, cooking loss and solubility of protein and collagen were evaluated using linear regression as shown in Table 21. A strong linear correlation was obtained between sarcomere length and cooking loss of indigenous (R^2=0.92) and broiler muscle (R^2=0.83), solubility of protein and shear value of broiler muscle (R^2=0.95), soluble collagen and shear value of indigenous (R^2=0.88) and broiler

muscle (R^2=0.86), and shear value and cooking loss of indigenous muscle (R^2=0.80). The solubility of muscle protein showed the highest correlation with the shear value of broiler muscle but did not for indigenous chicken muscle. Lopez-Bote *et al.* (1989) found that total soluble protein was a good potential predictor of pig lean meat quality. However, the solubility was not significantly related to shear values or sensory tenderness ratings in young beef (Seideman *et al.*, 1987). Therefore, the correlation tended to depend on the species studied. For indigenous chicken, the result indicated that sarcomere length and collagen solubility were very important factors influencing cooking loss and texture of cooked indigenous chicken muscle, and cooking loss directly affected the shear value. The high correlation between sarcomere length and cooking loss was in agreement with Laakkonen *et al* (1970) and Bouton *et al* (1975) who stated that the change in water content contributes to changes in sarcomere length with temperature. The results suggested that changes in muscle structure during heating might influence the texture and cooking loss of cooked chicken meat, especially for indigenous chicken muscle.

Table 21. Linear correlation for changes in structure, texture, cook loss and solubility of chicken *Pectolaris* muscle

Variable	Indigenous	Broiler
Sarcomere length : cooking loss	0.92	0.83
Cooking loss : shear value	0.80	0.67
Sarcomere length : shear value	0.77	0.60
Fiber diameter : shear value	0.79	0.10
Cooking loss : fiber diameter	0.57	0.02
Protein solubility : shear value	0.77	0.95
Collagen solubility : shear value	0.88	0.86

Changes in Intramuscular Connective Tissue (IMCT) of Thai Indigenous and Broiler Chicken Muscles during Thermal Processing

1. Hydroxyproline and collagen content in chicken IMCT

The hydroxyproline and collagen content in IMCT from broiler and indigenous chicken muscles are shown in Table 22. The IMCT extracted from broiler *Biceps femoris* muscle had significantly higher hydroxyproline and collagen content ($P<0.05$) than those extracted from broiler *Pectoralis* muscle and indigenous *Pectoralis* and *Biceps femoris* muscles. The differences in total collagen content in the perimysium from various bovine muscles has been reported and correlated with the differences in meat texture (Lawrie, 1991). From our preliminary study, *Biceps femoris* muscle was tougher than *Pectoralis* muscle from both breeds (Wattanachant *et al.*, 2004). However, for the same muscle tested, Thai indigenous chicken muscle was tougher than broiler muscle, particularly after cooking. The results indicated that the collagen content in their IMCT was not correlated well with the toughness of the muscle. Lawrie (1991) concluded that elastin contributed to the toughness of cooked meat to about the same extent as denatured collagen. Therefore, the significance of elastin should not be overlooked (Bailey and Light, 1989).

Table 22. Hydroxyproline and collagen content of chicken IMCT

Breed	Muscle	Hydroxyproline (%)	Collagen (mg/g dry basis)
Broiler	*Pectoralis m.*	3.51 ± 0.47^a	254.7 ± 34.0^a
	Biceps femoris m.	5.52 ± 0.43^b	400.0 ± 31.4^b
Indigenous	*Pectoralis m.*	3.49 ± 0.43^a	252.8 ± 31.0^a
	Biceps femoris m.	3.52 ± 0.53^a	254.9 ± 38.7^a

N=12, Means with differing superscripts in the same column are significantly different ($p<0.05$).

2. Thermal transition of native IMCT

Typical thermograms of IMCT from both muscles of Thai indigenous and broiler chickens are presented in Fig 27. One endothermic peak was observed for all samples. In both breeds, the larger peak area was observed for IMCT of *Biceps femoris* muscles than that of *Pectoralis* muscles. DSC allows the study of phase transition and chemical reactions occurring within a muscle and can be successfully applied to measuring thermal transitions or denaturation temperatures of proteins. These transition temperatures are most commonly specified as temperature at maximum peaks, enthalpy changes (ΔH) or the amount of specific heat involved in protein denaturation (Kijowski and Mast, 1988). However, Akta (2003) stated that T_{onset} measures the thermal stability of less stable components better than T_{peak}, which is considered a measure of average thermal stability. The effect of breed, muscle and their interactions on T_{onset}, T_{peak} and the denaturation enthalpy (ΔH) were tested using analysis of variance (Table 23-25). The probability values indicated that breed had significant effects on T_{onset} and ΔH as calculated based on the weight of IMCT ($P < 0.05$). This was caused by the higher content of collagen in broiler IMCT compared with indigenous IMCT as a non significant difference in ΔH was observed when calculated on the weight of collagen (Table 23). Muscle type showed highly significant effect only on the denaturation enthalpy (Table 24). A higher ΔH was observed for collagen in IMCT from *Biceps femoris* muscle, compared with that from *Pectoralis* muscle. This was probably due to the difference in the ratio of heat stable (oxo-imino) to heat labile (aldimine) cross links in these collagens and in their histological distribution in the different muscles (Lawrie, 1991). Significant interactions between breed and muscle type were observed only on ΔH (J/g IMCT), which was influenced by the difference in collagen content among the IMCT samples. T_{onset}, T_{peak} and ΔH (J/ g collagen) of all samples were not significantly different ($P > 0.05$) being in the range of 64.2

– 64.7°C, 68.4 – 68.8°C and 35.3 – 44.0 J/g collagen, respectively. Previously, Kijowski and Mast (1988) found one major peak at 65.3°C for IMCT isolated from breast broiler muscle. Some thermal curves of connective tissue showed minor thermal transitions at temperatures lower than this study. This may be due to the fact that connective tissue in muscle is not homogeneous and consists of endomysium, perimysium and epimysium, which have different denaturation temperatures (Kijowski and Mast, 1988; Lawire, 1991).

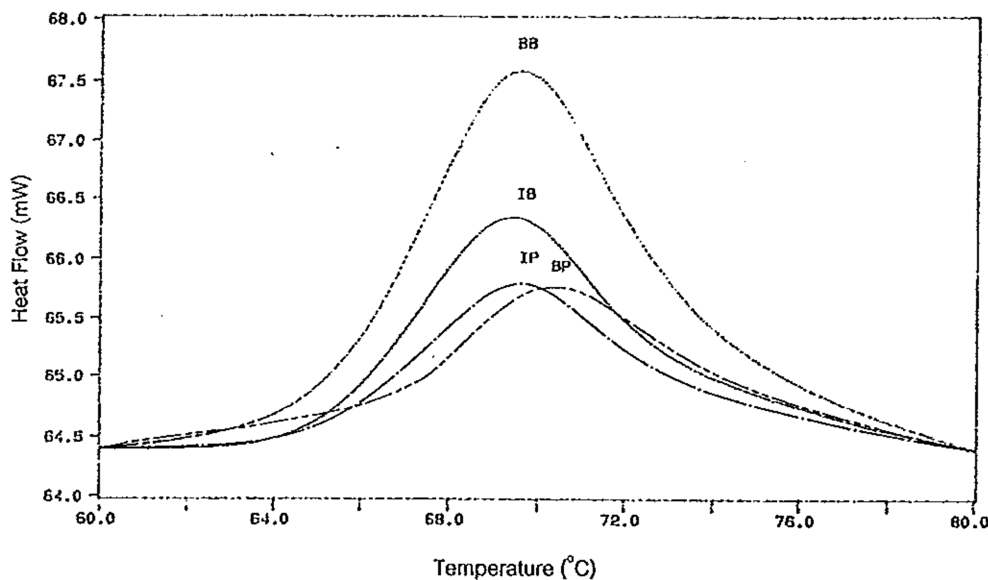

Fig 27. Thermal transition of IMCT of Thai indigenous and broiler chicken muscles (BB = Broiler *Biceps femoris* muscle, IB = indigenous *Biceps femoris* muscle, BP = Broiler *Pectoralis* muscle, IP = Indigenous *Pectoralis* muscle).

Table 23. Effect of breeds on DSC parameters of IMCT

Chicken Breed	T_{onset} (°C)	T_{peak} (°C)	ΔH (J/g IMCT[1])	ΔH (J/g collagen[1])
Broiler	64.68 ± 0.26	68.63 ± 0.23	2.24 ± 0.75	40.20 ± 4.50
Indigenous	64.41 ± 0.22	68.57 ± 0.38	1.62 ± 0.21	38.27 ± 4.86
Significant (p<0.05)	0.027	0.685	0.001	0.247

N=8, [1]=wet basis

Table 24. Effect of muscles on DSC parameters of IMCT

Chicken Muscle	T_{onset} (°C)	T_{peak} (°C)	ΔH (J/g IMCT[1])	ΔH (J/g collagen[1])
Pectoralis m.	64.43 ± 0.30	68.56 ± 0.38	1.52 ± 0.14	35.85 ± 3.32
Biceps femoris m.	64.66 ± 0.20	68.64 ± 0.21	2.34 ± 0.65	42.62 ± 2.96
Significant (p<0.05)	0.057	0.588	<0.001	0.001

N=8, [1]=wet basis

Table 25. Effect of breed x muscle on DSC parameters of IMCT

Breed	Muscle	T_{onset}(°C)	T_{peak}(°C)	ΔH(J/g IMCT[1])	ΔH(J/g collagen[1])
Broiler	*Pectoralis m.*	64.63 ± 0.28	68.75 ± 0.21	1.55 ± 0.07^{ab}	36.43 ± 1.73
	Biceps femoris m.	64.74 ± 0.27	68.52 ± 0.20	2.93 ± 0.17^{c}	43.97 ± 2.50
Indigenous	*Pectoralis m.*	64.24 ± 0.16	68.38 ± 0.46	1.49 ± 0.20^{a}	35.28 ± 4.68
	Biceps femoris m.	64.58 ± 0.06	68.77 ± 0.16	1.75 ± 0.13^{b}	41.25 ± 3.05
Significant	(p<0.05)	0.290	0.050	<0.001	0.630

N=4, [1]= wet basis, Means with differing superscripts in the same column are significantly different (p<0.05).

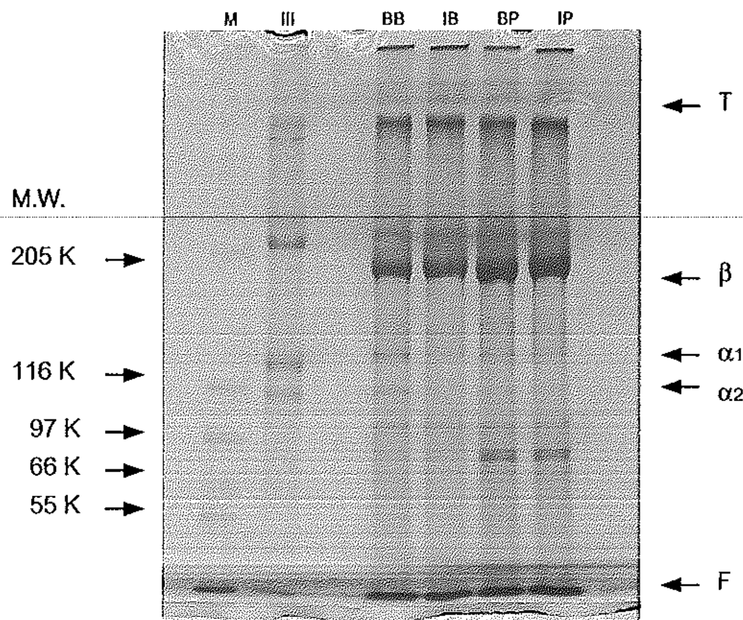

Fig 28. SDS-PAGE patterns (5% gel) of IMCT obtained from broiler *Biceps femoris m.* (BB), indigenous *Biceps femoris m.* (IB), broiler *Pectoralis m. (BP)*, and indigenous *Pectoralis m.* (IP), III: bovine Type III collagen; M: the molecular weight standards (Sigma M3788). Letters T and F show the top and buffer front of gel, respectively.

3. SDS-PAGE patterns of chicken IMCT

SDS-PAGE pattern of IMCT from broiler *Biceps femoris* and *Pectoralis* muscles and Thai indigenous *Biceps femoris* and *Pectoralis* muscles are shown in Fig 28. Pearson and Young (1989) stated that the molecular weights of α, β, and γ components of collagen were in the range of 80,000 – 125,000 D, 160,000 – 250,000 D and 240,000 – 375,000 D, respectively. All IMCT samples had two major bands corresponding to γ and β components and some lower molecular weight components (α_1(I-III) and α_2(I)). The collagen pattern in all IMCT seemed to be type III and I compared with previous research (Cliché *et al.*, 2003). However, IMCT from Thai indigenous chicken muscles exhibited lower densities of α_1 and α_2 bands compared to broiler IMCT. While IMCT from

Pectoralis muscle showed higher density of β band as compared to IMCT from *Biceps femoris* muscle. These Thai indigenous chicken muscle and *Pectoralis* muscle might have a higher crosslinked collagen content than broiler muscles and *Biceps femoris* muscle, respectively. Lower soluble collagen contents were observed in Thai indigenous chicken muscles and *Pectoralis* muscle, compared with those of broiler and *Biceps femoris* muscle, respectively (Wattanachant *et al.*, 2004) which agrees with the above conclusion.

4. Effect of heating on thermal transition of IMCT

Typical thermograms of unheated and heated IMCT from *Pectoralis* muscle of Thai indigenous and broiler chicken after heating at different temperatures are shown in Fig 29. It is seen that the peak shifted to higher temperatures after the IMCT was cooked at temperatures up to 60°C. When the temperature was increased to 70 and 80°C, small peaks with lower T_{peak} were observed in IMCT of Thai indigenous muscle. The peak of IMCT samples disappeared when samples were heated to 80°C for broiler IMCT and 90°C for indigenous IMCT. Collagen is main component in IMCT (Lawrie, 1991). The fibrillar collagen molecules are composed of long triple helices comprising three intertwined primary chains. The three chains in the triple helix molecule, as well as neighboring helices in collagen fibrils, are held together through hydrogen bonds. There may also be one or more interstrand (intramolecular) covalent crosslinks in individual molecules, as well as helix-to-helix (intermolecular) crosslinks in fibrils (Chang *et al.*, 2000). Under denaturing conditions, the hydrogen bonds between the helices or between the three chains of the triple helix break down and dissociate into individual units, which are constrained by the various covalent intra- and intermolecular crosslinks (Chang *et al.*, 2000). The effects of heating temperature on the thermal transitions of the IMCT samples are shown in Table 26. The heat treatment first causes breaking of hydrogen bonds which stabilise the native helical structure of collagen in intramuscular connective tissue (Rochdi *et al.*, 2000). The cleavage of hydrogen

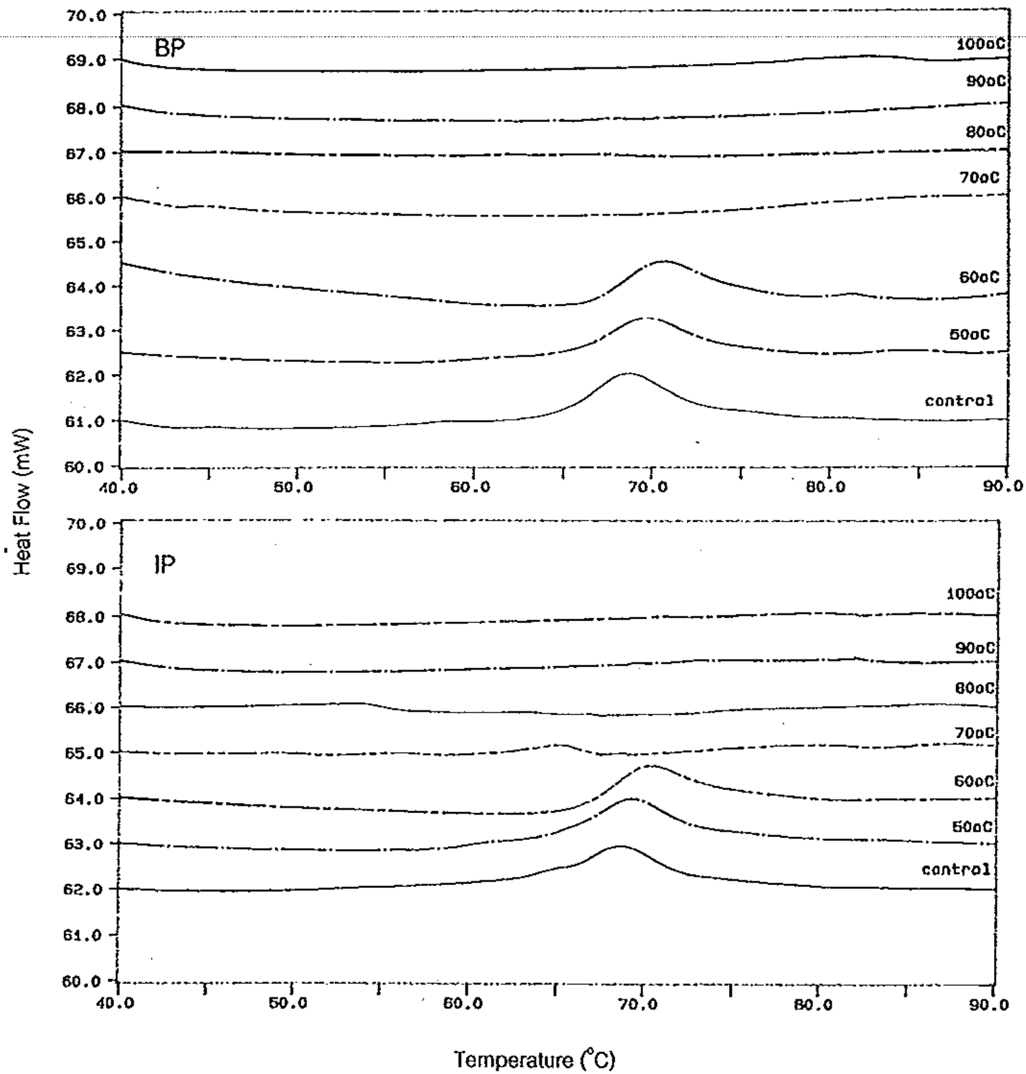

Fig 29. Thermal transition of IMCT obtained from *Pectoralis* muscle of broiler (BP) and Thai indigenous (IP) chickens after heating for 20 min at 50, 60, 70, 80, 90, and 100°C.

Table 26. Effect of heating on thermal transition of IMCT from *Pectoralis m.*

Temperature	T_{onset} (°C)		T_{peak}(°C)		ΔH (J/g IMCT)	
(°C)	Broiler	Indigenous	Broiler	Indigenous	Broiler	Indigenous
Control	64.63 ± 0.10^{b}	64.48 ± 0.36^{c}	68.64 ± 0.15^{b}	68.67 ± 0.37^{b}	1.377 ± 0.128^{c}	1.205 ± 0.148^{c}
50	66.07 ± 0.61^{b}	65.61 ± 1.46^{c}	70.19 ± 0.53^{b}	69.87 ± 1.44^{b}	1.382 ± 0.126^{cA}	1.750 ± 0.183^{dB}
60	66.42 ± 0.39^{b}	66.40 ± 0.18^{c}	70.07 ± 0.43^{b}	70.19 ± 0.10^{b}	1.123 ± 0.147^{b}	1.181 ± 0.259^{c}
70	45.38 ± 3.27^{aA}	59.59 ± 3.12^{b}	46.99 ± 2.71^{aA}	65.96 ± 4.04^{bB}	0.029 ± 0.018^{aA}	0.516 ± 0.271^{bB}
80	Not seen	52.64 ± 0.80^{aB}	Not seen	54.57 ± 1.35^{a}	Not seen	0.015 ± 0.016^{a}
90	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen
100	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen

Data are presented as mean ± standard deviation. n = 3

[a-c]Means within a column with differing superscripts are significantly different ($P<0.05$).

[A-B]Means under the same parameter within a row with differing superscripts are significantly different between breeds by t-test ($P<0.05$).

bonds (an endothermic process) in native collagens lowered both the onset melting temperature and enthalpy of denaturation (Finch *et al.*, 1974). This was supported by the results for the control samples, which exhibited lower T_{onset}, T_{peak} and enthalpy compared to samples heated at 50°C. T_{onset} and T_{peak} of IMCT samples increased with increasing heating temperature to 60°C. The increase in thermal stability of samples after heating at 50 and 60°C might be due to both changes in bound water structure and the greater freedom of movement of the polypeptide chains (Finch and Ledward, 1972). After cooling, the orientation of the hydrophilic and hydrophobic residues at the interface, further unfolding of the proteins may take place, and a more stable system with many protein-water interactions may occur. In this situation, the enthalpy of denaturation would be expected to increase (Akta and Kaya, 2001). However, if many hydrophobic groups are exposed to the aqueous phase, decreased denaturation enthalpy would be observed (Akta and Kaya, 2001). This evidence was found after samples were heated at 60°C. Denaturation of collagen is accomplished by unfolding of the triple helix but the decrease in temperature during cooling allows the refolding of the collagen triple helix if the three chains are kept in register (Pearson and Young, 1989; Rochdi *et al.*, 2000). After heating at 70°C for 20 min, the collagen in IMCT samples was fully denatured and partially refolded during cooling accompanied by significant decrease in T_{onset}, T_{peak} and enthalpy. Increasing temperature of heating at 80°C and 90°C, could prevent the renaturation of collagen from broiler IMCT and indigenous IMCT, respectively. This result indicated that collagen from IMCT of Thai indigenous chicken muscle was more heat stable than that from broiler IMCT. A higher denaturation enthalpy for heated indigenous IMCT was also observed compared with that of heated broiler IMCT (Table 26). This was probably due to the cleavage of more exothermic hydrophobic bonds in indigenous IMCT (Rochdi *et al.*, 2000). Hydrophobic interactions are known to stabilise the cross-linked collagen fibers (Finch and Ledward, 1972).

5. Effect of heating on soluble collagen of IMCT

Higher cross-linking can be displayed by the lower solubility in Thai indigenous IMCT than in broiler IMCT (Table 27). With increasing heating temperature, the soluble collagen of both IMCTs increased significantly (*P* <0.05). The greatest increment in soluble collagen was observed when the sample was heated from 60 to 70°C, temperatures in the range of the thermal transition temperature of IMCT (Table 26). This result also agrees with those of Murphy and Marks (2000) and Palka (1999) who found that the percentage of soluble collagen almost doubled in chicken breast patties and bovine muscle when heated at 60-70°C, compared to raw samples.

Table 27. Effect of heating on soluble collagen of IMCT from *Pectoralis m.*

Temperature (°C)	Soluble collagen (% of total collagen)		Significance
	Broiler	Indigenous	Between breeds
Control	18.71 ± 3.09^{a}	14.99 ± 4.38^{a}	ns
50	24.89 ± 4.18^{b}	16.05 ± 3.90^{a}	**
60	26.06 ± 3.52^{b}	21.08 ± 1.85^{b}	*
70	38.63 ± 1.84^{c}	23.57 ± 3.91^{bc}	***
80	39.13 ± 1.09^{c}	26.09 ± 4.09^{c}	***
90	41.76 ± 1.00^{cd}	30.26 ± 1.84^{d}	***
100	44.38 ± 3.20^{d}	33.75 ± 1.31^{d}	***

Data are presented as mean ± standard deviation. n = 6
$^{a-d}$Means within a column with differing superscripts are significantly different (*P*<0.05).
Significant differences between breeds were determined by t-test: * *P*<0.05; ** *P*<0.01; *** *P*<0.001; ns = no significant difference.

6. Effect of heating on SDS-PAGE pattern of IMCT

Protein pattern of the soluble fractions from IMCT heated at 50 to 100°C were examined by SDS-PAGE (Fig 30). SDS-PAGE patterns of the heat soluble fractions from both chicken IMCT were similar to that of Type III collagen from calf skin (Fig 28).For both broiler and Thai indigenous IMCT, no notable

difference was observed in protein patterns among the temperatures used. However, more thermal degradation components appeared at the higher temperature. The soluble fractions from unheated IMCT and IMCT heated at 50°C of both chicken breeds showed no bands although soluble collagen was detected (Table 27). This was probably because at low heating temperature, renaturation of unfolding triple helix to high molecular weight components occurred during cooling of the samples, resulting in the formation of insoluble fraction. Transformation of soluble collagen into an insoluble form during cooling of sample was also postulated by Palka (1999).

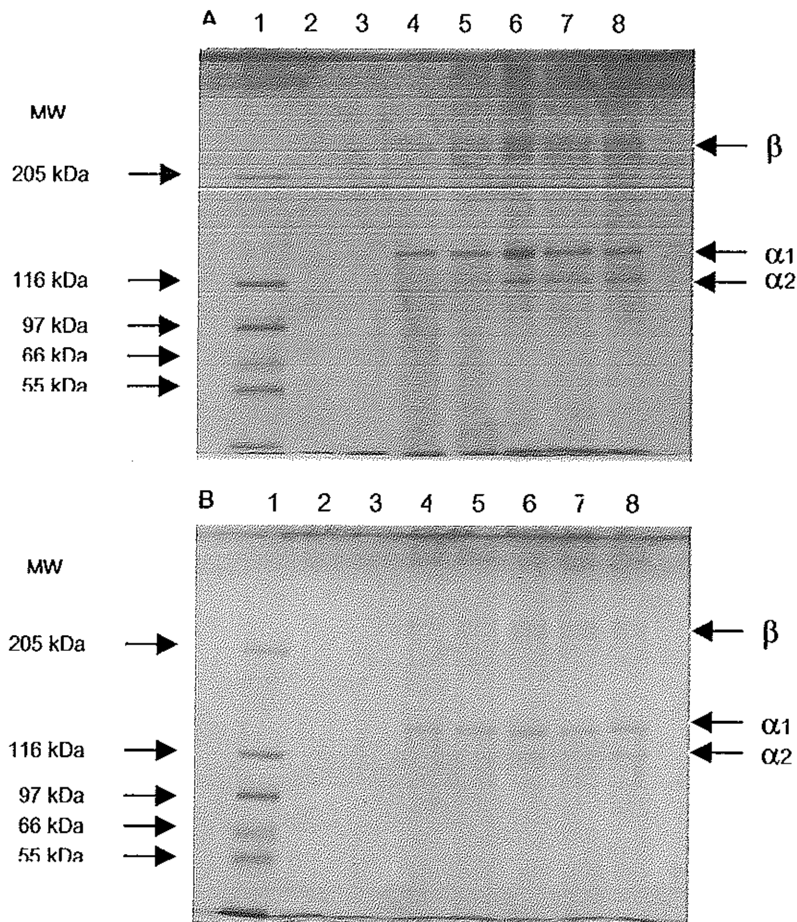

Fig 30. SDS-PAGE patterns (5% gel) of soluble fractions in IMCT from broiler *Pectoralis m.* (A), and indigenous *Pectoralis m.* (B), treated at different heating temperatures Lane 1: high molecular weight standards, lane 2: unheated IMCT, lanes 3-8: IMCT heated at 50, 60, 70, 80, 90 and 100°C, respectively.

Chapter 4

CONCLUSIONS

The post-mortem metabolic rate of Thai indigenous chicken carcasses was slower than that of the broilers. The ultimate pH of Thai indigenous muscle was lower than that of the broiler muscle. Improvement in tenderness of muscle caused by proteolysis required at least 4 h and 6 h post-mortem ageing at 4°C for broiler and Thai indigenous chicken carcasses, respectively.

Thai indigenous chicken muscle contained higher total protein contents but with lower myofibrillar protein and higher stromal protein contents compared with broiler muscle. The total collagen contents of indigenous chicken muscles were higher whereas the heat soluble collagen content was lower than found in broiler muscles. Higher glutamic amino acid contents and differences in fatty acid composition might contribute to the better taste of Thai indigenous chicken muscles, compared with broiler. The shear values of indigenous chicken muscles either raw or cooked were higher than those of broiler muscles. After cooking, the shear values decreased for broiler muscles, but increased for indigenous chicken *Pectoralis* muscle.

The thermal characteristics and microstructural results indicated that the thickness of the perimysium directly contributed to the texture of raw chicken muscles, whereas the crosslinked collagen content primarily influenced the texture of cooked muscles. The broiler muscle fibers shrank more in parallel than transverse to the fiber axis and expanded in transverse dimensions after cooking, resulting in increased tenderness of the muscles. The indigenous chicken muscle fibers slightly shrank in both dimensions and the toughening of muscle fibers was caused by heat coagulation and shrinkage of myofibrils and intramuscular

connective tissue after cooking. As a consequence, muscles from both breeds were different in textural characteristics.

The indigenous chicken muscles were tougher than the broiler muscle at all heating temperatures from 50 to 100°C. The change in shear value of both chicken muscles occurred in two steps: 50-70°C and 80-100°C, respectively. A significant decrease in fiber diameter was observed in samples heated to an endpoint temperature of 60°C, while the greatest shrinkage of the sarcomeres was observed in samples heated to the endpoint temperatures in the range 70-100°C for the broiler and 80-100°C for the indigenous chicken muscles. The shrinkage of chicken meat during cooking in the temperature range 50-100°C occurred in two phases. At a temperature of about 50-60°C, the shrinkage was primarily transverse and at 70-100°C primarily parallel to the fiber axis. With increasing heating temperature, the cooked chicken muscle became lighter and yellower. Cooking loss and collagen solubility increased with increasing internal temperature of samples. In contrast, the solubility of muscle protein decreased with increasing temperatures and had a very high correlation with the texture of broiler muscle. During heating, sarcomere length changes and collagen solubility were very important factors influencing the cooking loss and texture of cooked indigenous chicken muscle, and the cooking loss directly affected the shear value. The changes in muscle structure during heating directly affected the texture and cooking loss of cooked chicken meat, especially for the indigenous chicken muscles.

Broiler IMCT had a higher content of collagen than Thai indigenous IMCT. The high content of collagen in IMCT was associated with high beginning denaturation temperature and enthalpy. No difference in thermal stability of native IMCT between broiler and Thai indigenous chicken was observed. However, after thermal processing the differences between IMCT from both breeds were detected. Heating at temperatures lower than 70°C resulted in increased thermal stability of IMCT. This result suggested that the toughness of

muscle would be increased if the meat was precooked at temperatures lower than 70°C. Higher temperatures were required for Thai indigenous IMCT to achieve complete denaturation and prevent renaturation of collagen, compared with broiler IMCT. This result indicated that intermolecular crosslinks were the main factor in stabilising the structure of Thai indigenous chicken IMCT. Thai indigenous IMCT was more heat stable due to its high content of crosslinked collagen, leading to less change in the intramuscular connective tissue after cooking. Therefore, the crosslinked collagen primarily influences the texture of cooked muscle.

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APPENDIX

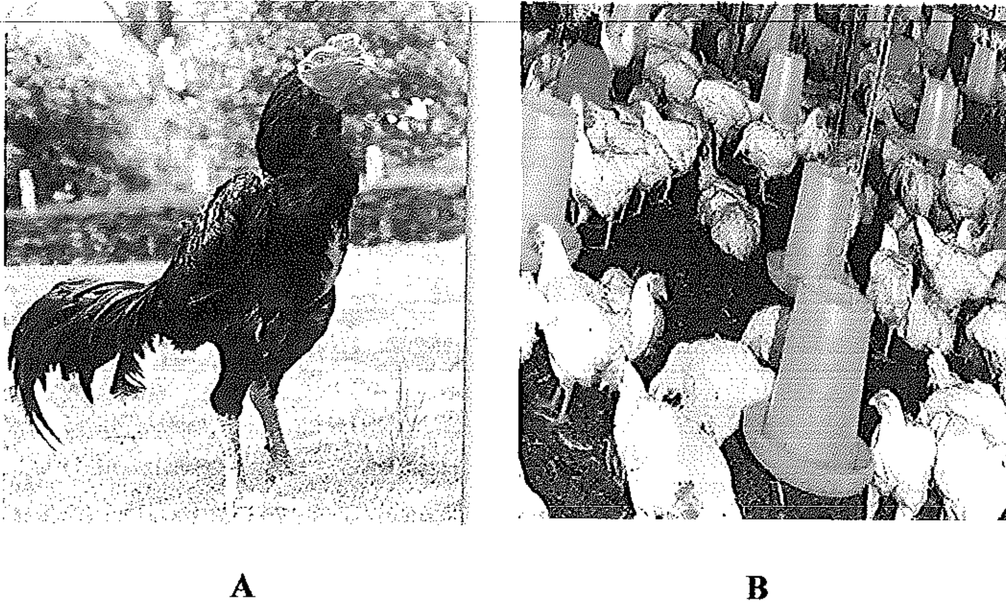

Fig 31. Characteristics of Thai indigenous chicken (A) and broiler (B)

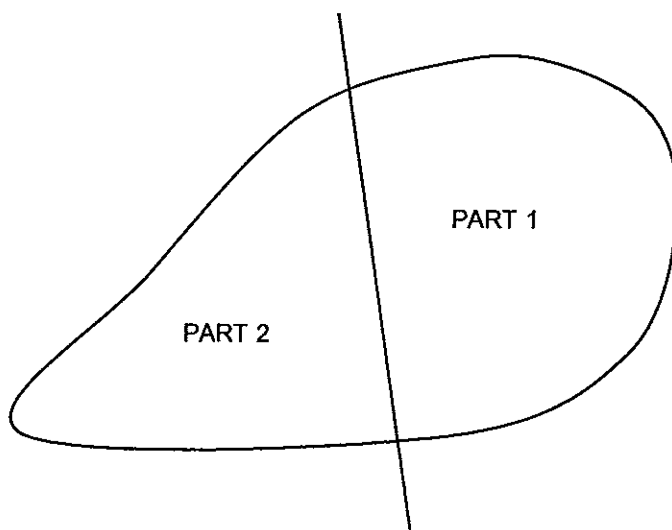

Fig 32. Parts of *Pectoralis* muscle for study of post-mortem ageing

PUBLICATION

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Wattanachant, S., Benjakul, S. and Ledward, D. A. 2004. Effect of heat treatment on changes in texture, structure and properties of Thai indigenous chicken muscle. Food Chem.: Submitted.

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