Quality Changes of Fresh and Processed Spent Hen *Pectoralis major* Muscles during Chilled and Frozen Storage

Atchariya Chueachuaychoo

A Thesis Submitted in Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Food Technology

Prince of Songkla University

2010

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Thesis Title	Quality Changes of Fresh and Processed Spent Hen *Pectoralis major* Muscles during Chilled and Frozen Storage
Author	MissAtchariya Chuaechuaychoo
Major Program	Food Technology

Major Advisor:

.....

(Asst. Prof. Dr.Saowakon Wattanachant)

Co-advisor:

.....

(Prof. Dr.Soottawat Benjakul)

Examining Committee:

.....Chairperson

(Dr.Pitaya Adulyatham)

.....

(Asst. Prof. Dr.Saowakon Wattanachant)

.....

(Prof. Dr.Soottawat Benjakul)

.....

(Asst. Prof. Dr.Kongkarn Kijroongrojana)

.....

(Assoc. Prof. Dr.Kanok-Orn Intarapichet)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Degree of Doctor Philosophy in Food Technology.

.....

(Prof. Dr.Amornrat Phongdara)

Dean of Graduate School

ชื่อวิทยานิพนธ์ การเปลี่ยนแปลงคุณภาพของกล้ามเนื้อไก่ไข่ปลดระวางส่วนอกแบบสดและแบบที่ผ่านการแปรรูปขณะเก็บรักษาแบบแช่เย็นและแช่แข็ง
ผู้เขียน นางสาวอัจฉริยา เชื้อช่วยชู
สาขาวิชา เทคโนโลยีอาหาร
ปีการศึกษา 2553

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีและคุณลักษณะทางกายภาพของกล้ามเนื้อไก่ไข่ปลดระวางส่วนอก และศึกษาการเปลี่ยนแปลงคุณภาพระหว่างการเก็บรักษาแบบแช่เย็นและแช่แข็งของกล้ามเนื้อสดและกล้ามเนื้อต้มสุกที่ผ่านการแช่สารละลายผสมของเกลือ (ร้อยละ 1 โดยน้ำหนักต่อปริมาตร) และสารละลายโซเดียมไตรโพลีฟอสเฟต (ร้อยละ 10 โดยน้ำหนักต่อปริมาตร) พบว่า กล้ามเนื้อสดของไก่ไข่ปลดระวางมีคุณค่าทางโภชนาการเช่นเดียวกับกล้ามเนื้อไก่กระทง สำหรับการแช่กล้ามเนื้อสดด้วยสารละลายผสมของเกลือและสารละลายโซเดียมไตร-โพลีฟอสเฟต ส่งผลให้น้ำหนักที่สูญเสียภายหลังการเก็บรักษาแบบแช่เย็น ($P<0.05$) และน้ำหนักที่สูญเสียภายหลังการให้ความร้อน ($P<0.05$) มีค่าสูญเสียน้อยกว่าชุดทดลองที่ไม่ผ่านการแช่สารละลายหลังผ่านการเก็บรักษาเป็นระยะเวลา 6 และ 9 วัน อย่างไรก็ตามน้ำหนักที่สูญเสียภายหลังการเก็บรักษาแบบแช่เย็นของกล้ามเนื้อต้มสุกชุดที่ไม่ผ่านการแช่สารละลายมีค่าสูญเสียน้อยกว่าชุดที่ผ่านการแช่สารละลายเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น สารละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟตมีประสิทธิภาพต่อการชะลอการเพิ่มขึ้นของค่า TBARS ของกล้ามเนื้อต้มสุกได้ดีกว่ากล้ามเนื้อสดตลอดระยะเวลาการเก็บรักษาแบบแช่เย็น สารละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟต มีประสิทธิภาพต่อการลดลงของน้ำหนักที่สูญเสียภายหลังการละลายน้ำแข็ง ($P<0.05$) ทั้งกล้ามเนื้อสดและกล้ามเนื้อต้มสุกตลอดระยะเวลาการเก็บรักษาแบบแช่แข็ง ตลอดระยะเวลาการเก็บรักษาแบบแช่เย็นและแช่แข็งไม่พบค่าความแตกต่างทางสถิติระหว่างตัวอย่างที่แช่และไม่แช่สารละลายต่อคุณภาพทางเคมี และกายภาพอื่น ๆ ทั้งในตัวอย่างกล้ามเนื้อสดและกล้ามเนื้อต้มสุก ซึ่งแสดงให้เห็นว่าละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟตไม่มีผลต่อคุณภาพดังกล่าว

การศึกษาผลของคุณภาพที่แตกต่างกันของวัตถุดิบ (วัตถุดิบสด วัตถุดิบที่ผ่านการแช่ละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟต และวัตถุดิบที่ผ่านการแช่แข็งและการละลายน้ำแข็ง) และกรรมวิธีการให้ความร้อนที่แตกต่างกัน (การอบด้วยลมร้อน การอบด้วยไมโครเวฟ การต้มในน้ำเดือด และการนึ่งภายใต้ความดันบรรยากาศ) ต่อคุณภาพของกล้ามเนื้อสุกพบว่า การนำวัตถุดิบสดแช่ละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟต ก่อนการให้ความร้อนทุกชนิดแสดงค่าการละลายของโปรตีนสูงสุด และน้ำหนักที่

สูญเสียภายหลังการให้ความร้อนต่ำที่สุด รวมทั้งสามารถปรับปรุงคุณลักษณะความชอบทางเนื้อสัมผัสได้ดีกว่าการใช้วัตถุดิบชนิดอื่น สำหรับการใช้วัตถุดิบทุกชนิด การให้ความร้อนด้วยวิธีการอบด้วยลมร้อนส่งผลให้เกิดการสูญเสียน้ำหนักภายหลังการให้ความร้อนสูงที่สุด ให้ค่าความยาวซาร์โคเมียร์ของเส้นใยกล้ามเนื้อสูงที่สุด แสดงค่าความเหนียวสูงสุด ให้คุณลักษณะด้านกลิ่นรสดีที่สุด และมีผลกระทบต่อด้านสีของกล้ามเนื้อสุกมากกว่าการให้ความร้อนด้วยวิธีการอื่น ๆ วัตถุดิบทุกชนิดเมื่อผ่านการต้มในน้ำเดือด และนึ่งภายใต้ความดันบรรยากาศ มีผลให้ค่าความเหนียวลดลง และมีคุณลักษณะทางเนื้อสัมผัสดีกว่าการให้ความร้อนแบบอบด้วยลมร้อน จากลักษณะโครงสร้างทางจุลภาคของกล้ามเนื้อสุกในวัตถุดิบทุกชนิด หลังผ่านการให้ความร้อนทุกวิธีแสดงให้เห็นการลดลงของซาร์โคเมียร์ การละลายของแอนโดไมเซียม และการสูญเสียสภาพทางธรรมชาติของไมโอไฟบริลลาร์โปรตีน ซึ่งทำให้สามารถสังเกตการพองตัวของกล้ามเนื้อได้อย่างชัดเจน

การศึกษาผลการนำกล้ามเนื้อไก่ไข่ปลดระวางสดแช่ในสารละลายขิงผงร้อยละ 2.5 น้ำหนักโดยปริมาตร ที่ระยะเวลาแตกต่างกัน (ไม่แช่สารละลาย และแช่สารละลายเป็นระยะเวลา 1 ชั่วโมง 2 ชั่วโมง และ 24 ชั่วโมง) ก่อนการให้ความร้อนแบบนึ่ง ต่อคุณภาพของกล้ามเนื้อไก่ไข่ปลดระวางสดและกล้ามเนื้อสุกระหว่างการเก็บรักษาแบบแช่เย็นพบว่า การนำกล้ามเนื้อสดแช่ในสารละลายขิงผงช่วยเสริมประสิทธิภาพการย่อยสลายโปรตีนและการละลายของคอลลาเจน ส่งผลให้ค่าแรงเฉือนของตัวอย่างที่ผ่านการแช่ในสารละลายขิงผงมีค่าต่ำกว่าตัวอย่างที่ไม่ได้ผ่านการแช่สารละลาย ตลอดระยะเวลาการเก็บรักษาแบบแช่เย็น ซึ่งแสดงให้เห็นว่าเอนไซม์ย่อยโปรตีนในขิงผงสามารถปรับปรุงคุณลักษณะทางเนื้อสัมผัสของกล้ามเนื้อไก่ไข่ปลดระวางได้

การศึกษาผลการใช้สารแอนติออกซิแดนซ์จากชาเขียว (ทีแคเทชิน) ที่ระดับความเข้มข้นแตกต่างกัน (0 100 150 และ 200 มิลลิกรัมต่อกิโลกรัมตัวอย่างโดยน้ำหนักต่อน้ำหนัก) ต่อความคงตัวของการเกิดออกซิเดชันของกล้ามเนื้อไก่ไข่ปลดระวางสดและกล้ามเนื้อสุกระหว่างการเก็บรักษาแบบแช่เย็น พบว่าการเติมทีแคเทชินมีผลต่อการชะลอการเกิดออกซิเดชันในกล้ามเนื้อทั้ง 2 ชนิด เมื่อเก็บรักษาแบบแช่เย็น โดยไม่มีผลกระทบต่อค่าความนุ่มเหนียว และการเปลี่ยนแปลงของน้ำหนักเนื้อตลอดระยะเวลาการเก็บรักษา อย่างไรก็ตามพบว่าการเติมทีแคเทชินทำให้เกิดการเปลี่ยนแปลงสีในกล้ามเนื้อสุกมากกว่ากล้ามเนื้อสด

การศึกษาผลกระทบจากการใช้สารเติมแต่งอาหารร่วม (สารละลายขิงผงร้อยละ 2.5 โดยน้ำหนักต่อปริมาตร สารละลายผสมของเกลือและสารละลายโซเดียมไตรโพลีฟอสเฟตร้อยละ 1 และร้อยละ 10 โดยน้ำหนักต่อปริมาตรตามลำดับ และทีแคเทชิน150 มิลลิกรัมต่อกิโลกรัมตัวอย่าง โดยน้ำหนักต่อน้ำหนัก) ต่อคุณภาพของกล้ามเนื้อไก่ไข่ปลดระวางสดและกล้ามเนื้อสุกระหว่างการเก็บรักษาแบบแช่เย็น พบว่าการใช้สารเติมแต่งอาหารร่วมในกล้ามเนื้อไก่ไข่ปลดระวางสดและกล้ามเนื้อสุก ส่งผลทำให้ค่าการละลายของโปรตีนและค่าการละลายของ

คอลลาเจนสูงกว่าตัวอย่างที่ไม่ใช้สารเติมแต่งอาหาร ในขณะเดียวกันสามารถชะลอการเพิ่มขึ้นของค่า TBARS และการลดลงของค่าแรงเฉือนได้ดีกว่าตลอดระยะเวลาการเก็บรักษาแบบแช่เย็น การเติมสารเติมแต่งอาหารร่วมสามารถปรับปรุงค่าความอุ้มน้ำในตัวอย่างกล้ามเนื้อสุก แต่ให้ค่าน้ำหนักที่สูญเสียภายหลังการให้ความร้อนของกล้ามเนื้อสดสูงกว่าตัวอย่างที่ไม่ใช้วัตถุเจือปนอาหาร นอกจากนี้การเติมสารเติมแต่งอาหารร่วมทำให้เกิดการเปลี่ยนแปลงสีในกล้ามเนื้อสุกมากกว่ากล้ามเนื้อสด ดังนั้นจึงส่งผลกระทบต่อค่าความชอบด้านสีโดยผู้ทดสอบทางประสาทสัมผัสของตัวอย่างที่เติมสารเติมแต่งอาหารร่วมมีค่าต่ำกว่าตัวอย่างที่ไม่ใช้สารเติมแต่งอาหาร

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Author	MissAtchariya Chueachuaychoo
Major Program	Food Technology
Academic Year	2010

ABSTRACT

Quality characteristics of spent hen *Pectoralis major* muscles and effect of 1.0% NaCl and 10% sodium tripolyphosphate (STPP) on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled and frozen storage were investigated. Meat from spent hen had the similar nutritional values, compared to commercial broiler meat. Raw samples treated with NaCl and STPP showed lower drip loss during storage ($P<0.05$) and had the lower cooking loss ($P<0.05$) at day 6 and day 9 of storage, compared with untreated samples. However, weight loss of cooked-untreated samples was lower ($P<0.05$) than that of cooked-treated samples as storage time increased. NaCl and STPP treatments had more effectiveness to reduce TBARS values of cooked samples than those found in raw samples during chilled storage. NaCl and STPP were effective in decreasing the thawing loss of both raw and cooked muscle samples during frozen storage. There were no differences between treated and untreated samples for other chemical and physical properties between raw and cooked spent hen during chilled and frozen storage, demonstrating the ineffectiveness of NaCl and STPP.

The effects of raw material pretreatment (untreated, NaCl/STPP treated, and freeze-thawed) and heating methods (oven heating, microwave heating, steaming and boiling) on quality of cooked spent hen meat were investigated. For all heating methods, spent hen meat treated with NaCl/STPP showed the highest protein solubility, the lowest cooking loss, and improved texture sensory scores than those found in other raw materials ($P<0.05$). Cooked meat by oven heating had the highest cooking loss, the highest sarcomere length, the most toughness, the highest flavor scores ($P<0.05$). Oven heating had more influence on surface color than other heating methods for all kinds of raw material used. Different raw materials cooked by boiling and steaming exhibited lower shear force and had higher texture scores than those

cooked by oven heating ($P<0.05$). After cooking, the shrinkage of sarcomeres, melting of the endomysium together with the denatured myofibrils resulted in swelling of the fibers. Those changes were observed clearly for all spent hen meat samples cooked by different heating methods.

The effects of ginger powder solution at different soaking times (0, 1, 2 and 24 h) on tenderization of raw and cooked spent hen *Pectoralis major* muscles during chilled storage were investigated. Soaking spent hen meat in ginger powder solution could enhance proteolysis and collagen solubility, resulting in lower shear force of cooked sample with ginger powder treatment, compared to untreated samples throughout chilled storage ($P<0.05$). This clearly indicated an increase tenderness of spent hen meat by using protease from ginger powder.

The effects of different concentrations (0, 100, 150, and 200 mg/kg samples) of antioxidant from green tea (tea catechins; TC) on oxidative stability of raw and cooked spent hen *Pectoralis major* muscles during chilled storage were studied. TC could delay the onset of oxidation in raw and cooked meat without any effect on shear force and yield throughout chilled storage. However, discoloration of meat caused by adding TC was more pronounced in cooked samples than that found in raw meat.

The influence of combination additives (2.5% GP for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled storage were investigated. Both raw and cooked treated samples exhibited higher ($P<0.05$) protein solubility and collagen solubility content, but lower TBARS values and shear force values compared to untreated samples throughout storage ($P<0.05$). Adding additives could improve WHC of cooked samples but increased cooking loss of raw samples. Discoloration of treated samples was more pronounced in cooked meat than that found in raw meat. This could result in the less color preference scores evaluated by panelists of cooked-treated, compared to cooked-untreated samples.

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CHAPTER 1

INTRODUCTION

At present, chicken is one of the most important commercial animals in Thailand. According to statistic report of poultry industry in 2008, there were 235,599,566 heads of total chicken in Thailand consisting of 17.34% laying-hens, 58.46% broilers, and 24.20% native chickens (Department of Livestock Development, 2008a). Of total chicken output, 50-60% is for domestic sales and 40-50% has been sent to poultry processing plant to process chicken meat for export. Thailand exports broilers in form of frozen and chilled, cooked and semi-cooked chicken meat to Japan, Germany, Singapore, United Kingdom, Netherlands, and Iceland (Department of Livestock Development, 2008b). The statistic report for exporting chicken meat and chicken meat products between 2000 and 2008 are shown in Table 1. Before 2003, the chicken meat exported mainly in form of frozen and chilled had steadily expended in terms of quantity and value. However, from year 2003 to 2005, the exported raw chicken meat in these forms have decreased dramatically because consumers feel fear on avian influenza H5N1 (bird flu) which affects on chicken consumption around the world. Inversely, the export of semi-cooked and cooked chicken meat has been increased since 2000 until present due to confidence in killing this virus by heating. According to the USDA guideline, chicken meat should be heated to 68.3-72^{o}C to ensure that it is free from pathogen and viruses, especially avian influenza H5N1 (Apenten, 2002; Larsen, 2006). Moreover, The Department of Livestock Development of Thailand has pronounced hygiene standard of chicken farm management guidelines for safety to protect avian influenza H5N1 and promoted the practical information among consumers both in domestic and oversea. This is also helps to increase opportunity of Thai chicken market. The other sources of poultry meat in Thailand are spent hen. The toughness prevents spent hen use in whole meat food and reduces the market value in many countries such as Korea, Taiwan, Japan, and United States (Sams, 1990; Nowsad *et al*., 2000a, b; Lee *et al*., 2003; Li, 2006). However, meat from spent hen is a good protein source (Rhee *et al*., 1999; Lee *et al*.,

2003); high omega-3 fatty acids and low in cholesterol content particular in breast muscles (Ajuyah *et al*., 1992) which have been shown to have health promoting benefits (Illingworth and Ullmann, 1990). Therefore, decreasing toughness need to be developed to increase the value of spent hen meat.

Table 1. Statistics of export for chicken meat and chicken meat product in Thailand between 2000 and 2008.

Year	Chicken meat (frozen and chilled chicken meat)		Chicken product (cooked and semi-cooked chicken meat)		Total	
	Quantity (tons)	Value (million baht)	Quantity (tons)	Value (million baht)	Quantity (tons)	Value (million baht)
2000[a]	240,924	15,690	69,329	8,750	310,253	24,440
2001[a]	309,543	23,931	89,159	11,546	398,702	35,477
2002[a]	303,966	22,591	127,974	15,725	431,940	38,316
2003[a]	370,781	24,767	154,464	18,631	525,245	43,398
2004[a]	22,568	1738	193,767	23,910	216,335	25,648
2005[a]	64	2	263,419	31,551	263,483	31,553
2006[a]	2,286	96	270,345	32,075	272,631	32,171
2007[a]	23,841	1,308	290,345	33,136	314,186	34,444
2008[b]	26,506	2,346	347,691	48,916	374,197	51,262

Source: [a]Dept. of Livestock Development (2007) and [b]Dept. of Livestock Development (2008b).

Chicken meat usually stored in form of chilled and frozen to extend shelf-life before heating process is carried out. The storage condition may affect on meat quality. Although refrigeration can protect poultry against spoilage (Bender, 1992), however, during refrigerated storage and handling, raw or precooked poultry undergo several changes such as microbiology growth, chemical and physical changes leading to deterioration that can affect their quality attributes resulting in reduced consumer acceptance (Kim and Marshall, 1999; Woods and Church, 1999; Bustabad, 1999). In addition, thermal processing of poultry results in chemical and physical changes that will strongly influence cooking loss, yield, and other important quality factors, such as texture, juiciness, color, and flavor, which are associated with

palatability and consumer acceptance of the final products (Murphy and Marks, 2000; Wattanachant *et al*., 2005a, b). Therefore, the effect of processing such as chilling, freezing, marinating, and heating on the quality of chicken meat should be concerned and informed to meat processors. However, there is less published report about quality of spent hen meat producing in Thailand either raw or cooked during refrigerated storage and quality of spent hen meat after heat processing.

Many additives have been used to improve meat quality during processing and storage, however, among them sodium chloride (NaCl) and sodium tripolyphosphate (STPP) were commonly used. The combined additives providing greater positive effects on quality of meat have been reported by many investigators (Nakhost and Karel, 1985; King *et al*., 1986; Young and Lyon, 1986; Ahn and Maurer 1989). More recently, tenderizing tough meat by application of ginger proteases (GP), a new source of plant proteolytic enzyme, have been reported in beef (Lee *et al*., 1986); buffalo (Naveena *et al*., 2004) and spent hen (Bhaskar *et al*., 2006) because it is not only active on actomyosin but also highly active against collagen and other connective tissue proteins (Thomson *et al*., 1973). This may be of benefit to improve tenderness of cooked spent hen meat for export. Due to highly polyunsaturated fatty acids in spent hen meat (Ajuyah *et al*., 1992), cooked meat products may promote lipid oxidation during refrigerated storage (Pikul *et al*., 1984; Ang and Huang, 1993; Rhee *et al*., 1996; Dawson *et al*., 1990). Antioxidant from tea (tea catechins, TC) has been reported to be more powerful than the synthetic antioxidants (He and Shahidi, 1997; Chen and Chan, 1996; McCarthy *et al*., 2001a; Tang *et al*., 2000; Tang *et al*., 2001a, b; Bozkurt., 2006). Thus, application of TC may be another way to prevent lipid oxidation in cooked spent hen meat during refrigerated storage.

Therefore, the objective of this study was to obtain the information on chemical and physical properties of spent hen meat and the effect of NaCl, STPP, GP, and TC on quality of spent hen meat either raw or cooked during refrigerated storage. The information gained from this research would be beneficial to food processors for using food additives in quality improvement of raw or cooked spent hen *Pectoralis major* muscles during chilled and frozen storage. Moreover, the knowledge regarding the appropriated heating methods for cooking spent hen *Pectoralis major* muscles could be obtained.

Literature Reviews

1. Poultry meat quality

Quality is the most important criterion from the point of view of the consumer (Levie, 1970). There are two major aspects of meat quality, "nutrition quality" which is objective and "eating quality" as perceived by the consumer which is highly subjective (Bender, 1992).

1.1 Nutritional quality

Meat from poultry contains several important classes of nutrients and is low in calories. The fat contains essential fatty acids; the proteins are a good source of essential amino acids (Mountney and Parkhurst, 1995; Van Heerden *et al*., 2002; Wattanachant *et al*., 2004) and also excellent sources of the water-soluble vitamins and minerals such as iron and zinc (Van Heerden *et al*., 2002; Boccia *et al*., 2005). For this reason, poultry is a good foodstuff for weight control diets, convalescents, and elderly people who are not physically active.

Chicken contains about 16.44-23.31 % protein, 0.37-7.20 % fat, 0.19-6.52 % ash, and 72.8-80.82 % moisture content (Xiong *et al*., 1993; Smith *et al*., 1993; Abeni and Bergoglio, 2001; Van Heerden *et al*., 2002; Al-Najdawi and Abdullah, 2002; Wattanachant *et al*., 2004; Chuaynukool *et al*., 2007). Chemical composition of poultry meat has been shown to be related to species, breed, muscle type, sex, age, and method of carcasses processing (Ding *et al*., 1999; Ngoka *et al*., 1982; Smith *et al*., 1993; Abeni and Bergoglio, 2001; Van Heerden *et al*., 2002; Al-Najdawi and Abdullah, 2002; Wattanachant *et al*., 2004; Boccia *et al*., 2005; Chuaynukool *et al*., 2007). Ding *et al*. (1999) showed significant differences in fat contents between broiler and local chicken. Abeni and Bergoglio (2001) reported the significant difference ($p<0.01$) for ash content of breast muscle from 3 stains chicken broiler. Several researchers have reported that carcasses in young birds have a higher proportion of moisture than the old one, while fat content increase with age (Mountney and Parkhurst, 1995; Moran, 1995; Ngoka *et al*., 1982; Twining, 1978). Wattanachant *et al*. (2004) found that Indigenous chicken muscles contained higher protein contents but lower fat and ash contents compared to broiler muscles ($P<0.001$). Ang and Hamm (1983) found that hand deboned meat (HDM) and

mechanically deboned meat (MDM) have similar protein contents. However, Al-Najdawi and Abdullah (2002) reported that HDM spent leg horn chicken without skin has a higher protein content but lower fat and ash content than MDM. Muscle types of different chicken breeds also different in chemical composition has been reported by Chuaynukool *et al*. (2007) as shown in Table 2.

Table 2. Chemical composition of *Pectoralis m.* and *Biceps femoris m.* from different breeds of Thai chicken.

Chemical composition	Broiler		Thai Indigenous chicken		Spent hen	
	Pectoralis muscle	*Biceps femoris muscle*	*Pectoralis muscle*	*Biceps femoris muscle*	*Pectoralis muscle*	*Biceps femoris muscle*
Protein (%)	21.02	16.98	23.05	17.43	20.34	16.44
Fat (%)	1.33	0.51	2.88	1.14	1.64	1.28
Ash (%)	1.44	1.43	0.60	0.75	0.19	1.29
Moisture (%)	76.62	77.30	74.39	80.82	74.83	79.42

Source: Chuaynukool *et al*. (2007).

Much of the value of a protein food is based on its amino acid contents which high nutritional value is related to a complete component of “essential” amino acids (Bender, 1992). Amino acids represent over 90% of the crude protein in the body of poultry (Hunton, 1995). Proportions of the amino acids are similar among the species of poultry which contain all of essential amino acids as shown in Table 3.

Table 3. Amino acids represented in the crude protein of some poultry.

Amino acid	Thai broiler (38 days)[a]		Thai Indigenous chicken (16 weeks)[a]		Turkey (8 weeks)[b]	Goose (7 weeks)[c]
	Biceps femoris m.	*Pectoralis m.*	*Biceps femoris m.*	*Pectoralis m.*		
Histidine	2.49	2.90	2.75	2.85	2.0	2.2
Arginine	4.75	4.39	4.48	4.58	6.5	6.7
Threonine	2.96	3.02	2.87	3.12	3.9	4.2
Cystine	0.31	0.31	0.30	0.34	1.7	2.4
Tyrosine	2.90	3.03	2.85	3.10	2.8	3.6
Valine	2.08	2.16	2.04	2.20	5.0	4.6
Methionine	1.83	1.88	1.81	1.93	1.8	1.5
Lysine	3.16	3.41	3.15	3.35	5.6	5.5
Isoleucine	2.29	2.41	2.26	2.45	4.0	3.5
Leucine	4.19	4.29	4.11	4.39	7.0	7.0
Phenylalanine	2.94	3.01	2.89	3.07	3.5	4.0
Aspartic acid	3.48	3.64	3.34	3.68	6.6	8.5
Serine	2.44	2.38	2.36	2.46	4.7	3.6
Glutamic acid	6.33	6.35	6.63	6.54	12.6	12.3
Glycine	2.95	2.70	2.29	2.83	7.1	8.1
Alanine	2.66	2.80	2.60	2.80	6.0	6.1
Proline	2.03	1.93	2.05	1.98	5.7	5.6

Source: [a]Wattanachant *et al*. (2004), [b]Fisher and Scougall (1982), and [c]Nitsan *et al*. (1981).

Poultry, like mammals, store excess energy as fat in adipose tissues (Rose, 1997). Adipose tissue contains 60-80% lipid of which 99% is triacylglycerol, whereas white muscle may contain 1% lipid, only half of which is triacylglycerol (Katz *et al*., 1966). The fatty acid composition of triacylglycerols tends to consist mainly of oleic, palmitic, linoleic, stearic and palmitoleic acids in decreasing amounts with very little C_{20} and C_{22} PUFA (Enser, 1999). Recent study by Wattanachant (2003) reported the fatty acid composition of Thai indigenous and broiler chicken muscle as shown in Table 4. Huton (1995) noted that high unsaturated fat intakes may be preferable for human; however, unsaturated fatty acids are more prone to oxidation.

Table 4. Fatty acid composition of chicken muscles from Thai indigenous and broiler.

Fatty acid	**Broiler(commercial breed, CP707)**		**Thai Indigenous *(Gallus domesticus)***	
(% of total fatty acids)	***Biceps femoris m.***	***Pectoralis m.***	***Biceps femoris m.***	***Pectoralis m.***
C8:0	0.18	0.21	0.21	0.74
C10:0	0.00	0.00	0.11	0.16
C12:0	0.37	0.36	2.74	1.85
C14:0	0.95	0.87	3.06	2.31
C16:0	32.65	31.82	32.04	33.01
C17:0	0.29	0.21	0.32	0.33
C18:0	14.97	14.56	25.61	22.72
C20:0	0.21	0.23	0.42	0.38
C22:0	0.26	0.31	0.63	0.57
C24:0	0.13	0.17	0.42	0.56
Saturated	**50.01**	**48.76**	**65.55**	**62.64**
C16:1	3.44	3.33	1.47	1.24
C18:1	35.13	37.76	26.35	31.25
C20:1	0.68	0.74	0.74	0.41
C24:1	0.00	0.00	0.21	0.20
Monounsaturated	**39.25**	**41.82**	**28.77**	**33.09**
C18:2 n-6	8.86	7.63	4.74	3.36
C18:3 n-3	0.13	0.16	0.00	0.00
C20:2 n-6	0.27	0.32	0.00	0.00
C20:3 n-3 n-6	0.14	0.16	0.00	0.00
C20:4 n-6 n-3	0.56	0.45	0.21	0.27
C20:5 n-3	0.40	0.34	0.21	0.16
C22:6 n-3	0.38	0.37	0.53	0.47
Polyunsaturated	**10.74**	**9.42**	**5.69**	**4.26**

Source: Wattanachant (2003).

1.2. Eating Quality

Eating quality of meat include color (appearance), flavor, texture, and juiciness. These 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 (Xiong *et al*., 1999; Fletcher, 1999a).

1.2.1 Color

Color is an important attribute for customer satisfaction (Sams and Alvarado, 2004). It is important for both the consumer's initial selection of raw meat and for the consumer's final evaluation of the cooked product (Fletcher, 1999b). The characteristic color of meat is a function of two factors: the meat pigments and the light-scattering properties (Varnam and Sutherland, 1995).

1.2.1.1 Meat pigments

The basic pigment of fresh meat is myoglobin (Figure 1). The myoglobin structure consists of a protein, the globin, and a heme group, which comprises the flat protoporphyrin IX ring system with a central iron atom (Young and West, 2001). The porphyrin ring structure hold in the confines of the myoglobin protein accounts for four of the six coordination sites available on the iron atom. 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. The basic structure of myoglobin from all animals is similar, but small differences exist which may account for observed differences in visual appearance and color stability of different meats (Varnam and Sutherland, 1995).

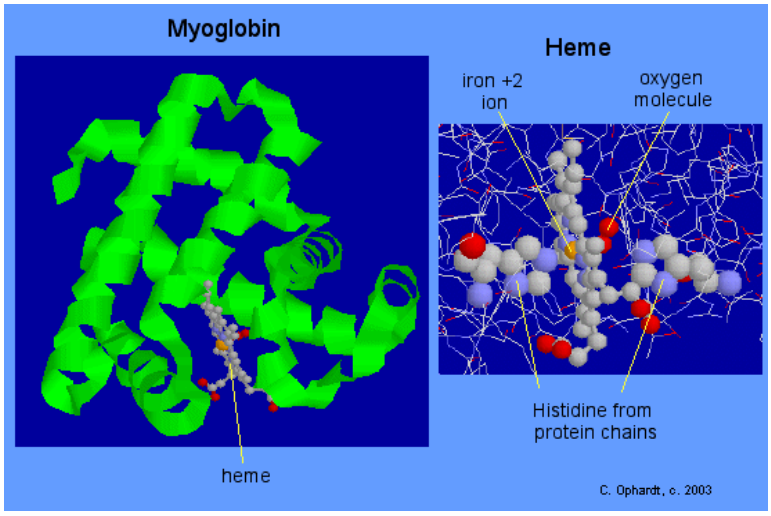


Figure 1. Myoglobin structure and heme.

Source: Ophardt (2003).

Myoglobin content has been shown to be primarily related to species, muscle type, sex, and age of the animal (Levie, 1970; Varnam and Sutherland, 1995; Miller, 1994; Fletcher, 1999a; 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 meat), 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), and 3-year-old beef (16-20 mg/g).

Myoglobin in fresh meat usually exists in three forms, the colors of which differ (Varnam and Sutherland, 1995) as shown in Figure 2. All three forms are present in fresh meat and are in equilibrium with each other. Reduced myoglobin has reduced iron (Fe^{2+}) and H_2O at the sixth co-ordination point. The pigment is purple-red in color and found in the centre of a piece of meat, where O_2 is absent, and also in vacuum packs. Oxymyoglobin is the oxygenated form of myoglobin and while the iron is in reduce (Fe^{2+}) form; O_2 occupies the sixth co-ordination link. This pigment is bright red in color. Metmyoglobin has iron in the oxidized state (Fe^{3+}) and H_2O at the sixth co-ordination point. The pigment, which is brown in color, is unable to form an

oxygen adduct. In red meats a bright red color associated with a high content of oxymyoglobin is a positive determinant of quality, while metmyoglobin content is a negative determinant. Other myoblobin-derived pigments can be presented in fresh meat under specific, and usually uncommon, circumstances. These include the green-pigmented sulphmyoglobin, formed by combination of myoglobin with H_2S of bacterial origin. This is a common problem with dressed (unevicerated) poultry due to production of H_2S in the intestine and its diffusion into the meat.

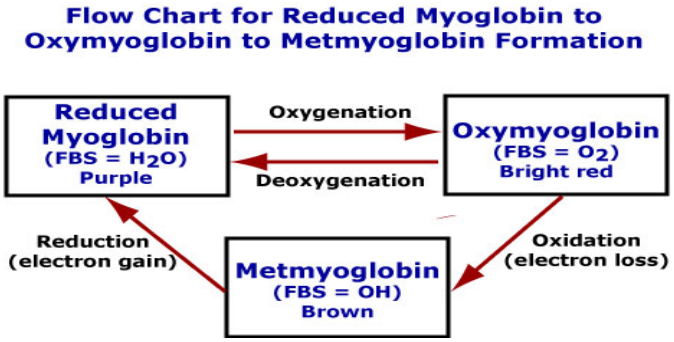


Figure 2. Interrelationship between pigments of raw meat.
Source: Taxas A&M (2007).

1.2.1.2 Light-scattering

The L^* value of the meat depends upon the amount of light scattered (Sams and Alvarado, 2004). The extent of light-scattering is related to the structure of the muscle and appears to depend on myofibrillar volume. The pale, soft, exudative meat (PSE) and dark, firm, dry meat (DFD) conditions represent extremes and variation in the extent of light scattering will occur in "normal" meat, modifying the visual appearance to a greater or lesser extent. PSE, which is of low myofibrillar volume, has a high light-scattering ability. An increased scattering of light due to denaturation of sarcoplasmic proteins and an increase in the amount of extra cellular water was responsible for pale meat (Swatland, 1993). Light is unable to penetrate a significant distance into the meat without being scattered. This means that there is relatively little absorption by myoglobin and the meat appears pale (Varnam and Sutherland, 1995). DFD has only very limited light-scattering ability, permitting incident light to penetrate for a considerable distance (Varnam and Sutherland, 1995). Considerable absorption by myoglobin occurs and meat appears dark.

1.2.1 Flavor

Flavor is one of the main eating quality attributes which, together with appearance, and texture (Farmer, 1999). It plays an important sensory aspect of the overall acceptability of meat product (Shahidi, 1994a). Factor which might affect flavor include the production factors of age, sex, genotype, diet and stocking density, the method of slaughter, the post slaughter factors of time of evisceration, time and temperature of chilling and storage, and finally, cooking method (Farmer, 1999). Fresh meat is characterized by two distinct types of flavors: 'species flavors' and 'meat flavor' (Xiong *et al*., 1999). Species flavors are due generally to fat-soluble substances, including aromatic compounds and short chain fatty acids and their derivatives from high-energy nucleotides such as inosine monophosphate (IMP). Substances contributing to meat flavor can be divided into taste compounds and aroma compounds (Farmer, 1999). Taste compounds are non-volatile or water soluble substances with taste or tactile properties. These can cause the sensations of salt, sour or acid, bitter, sweet, umami, etc. It has been suggested that amino acids, peptides, proteins and nucleotides are most important for the taste of red meats (Farmer, 1999). Interaction of these components and/or their breakdown products produces a large number of intermediates and/or volatiles which contribute to meat flavor development and aroma generation during heat processing (Shahidi, 1994a).

Studies on red meats have shown that cooking meat may affect the concentrations of taste substances (Macy *et al*., 1964; Macy *et al*., 1970; Cambero *et al*., 1992). In contrast to the taste compounds, aroma compounds are largely formed during the cooking process (Farmer, 1999). Nearly 1000 compounds have so far been identified in the volatile constituents of meat from beef, chicken, pork, and sheep (Shahidi *et al*., 1986; Shahidi, 1989). These volatiles were representative of most classes of organic compounds such as hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, furans, pyridines, pyrazines, pyrroles, oxazoles and oxazolines, thaizoles and thaizolines, thiophenes and other sulphur- and halogen-containing substances. It is believed that the predominant contribution to aroma is made by sulphurous- and carbonyl-containing volatiles (Shahidi, 1994a).

Lipid also plays an important role in the overall flavor of meat which is distinct and species dependent (Mottram and Edwards, 1983). Lipid oxidation is well known as the cause of rancidity development, but it can also contribute to desirable food flavor (Farmer, 1999). When catalysed by heat, the thermal oxidation of lipid plays an important role in the generation of the desirable flavor of meat (Mottram, 1991 cited in Farmer, 1999). The reactivity of lipids in oxidation reactions is related to their degree of unsatuation; polyunsaturated lipids will be the most reactive both in the generation of desirable flavor and in the formation of off-flavor (Gasser and Grosch, 1988 cited in Farmer, 1999), including the unpleasant flavor of reheated cooked meat, known as "warmed-over-flavor" (Tims and Watts, 1958; Asghar *et al*., 1988). Susceptibility of meat to lipid oxidation depends on several factors such as: (1) the unsaturated fatty acid content (Pikul *et al*., 1984; Ang, 1988; Mistry and Min, 1992; Porter *et al*., 1995; Jensen *et al*., 1997a, b; Woods and Church, 1999); (2) the fatty acid composition of lipid fractions (Wilson *et al*., 1976; Pikul *et al*., 1984; Ang, 1988; Morrissey *et al*., 1998; Dowson *et al*., 1990; Nawar, 2000; Jensen *et al*., 1997a, b); (3) the balance between antioxidant/prooxidant factors (Ang, 1988; Cava *et al*., 2000; Gray *et al*., 1996; Jensen *et al*., 1997a, b; Rhee and Ziprin, 1987; Rhee *et al*., 1996; Botsoglou *et al*., 2003); and (4) processing methods (Pikul *et al*., 1984; Dawson *et al*., 1990; Ang and Huang, 1993).

Fats in poultry are more susceptible to rancidity since they are richer in unsaturated fatty acids than other meats (Jensen *et al*., 1997a, b; Pikul *et al*., 1984; Rhee *et al*., 1996). Wilson *et al*. (1976) reported that oxidative changes, as measured by the thiobarbituric acid (TBA) numbers, occurred most readily in turkey meat, followed by chicken, pork, beef, and mutton. Within the same species of poultry, dark meat was found to generate higher TBA numbers than white meat (Wilson *et al*., 1976; Pikul *et al*., 1984; Ang, 1988; Rhee *et al*., 1996; Jensen *et al*., 1997a; Botsoglou *et al*., 2003). Both triacylglyceride (TG) and phospholipid (PL) will undergo oxidation; however, several studies have shown that the PL fraction contributes more lipid oxidation in meat than the TG (Younathan and Watts, 1960; Wilson *et al*., 1976; Igene and Pearson, 1979; Igene *et al*., 1980; Pikul *et al*., 1984; Dawson *et al*., 1990; Alasnier *et al*., 2000), which may be due to a higher degree of polyunsaturation (Pikul *et al*., 1984; Jensen *et al*., 1997a, b; Alasnier *et al*., 2000).

Pikul *et al*. (1984) pointed out that fat from chicken breast meat contained two times more malondialdehyde than fat from leg meat, due to a larger PL fraction in fat from breast meat. However, the TBA numbers were higher in leg meat because the breast meat contained much less total fat. Ang (1988) similarly reported that when tissues were freshly cooked, the initial TBA numbers were highest in leg meat, followed by liver, breast, and skin. After 5-days storage at 4^{o}C, however, liver had the highest TBA numbers, followed by leg meat, breast meat, and skin due to the fact that skin PL contains only about one half of polyunsaturated fatty acids with 20- and 22-carbon atoms compared to leg and breast tissues (Pikul *et al*., 1985).

1.2.3 Texture

The most important quality attribute of chicken meat is texture (Levie, 1970; Varnam and Sutherland, 1995; Smith and Fletcher, 1988; Chrystall, 1994). Tenderness is a quality attribute uniquely important to meat texture. Tenderness of meat may be simply defined as the ease of teeth to cut meat fibers during mastication. The word tenderness is often used in reciprocation of its antonym, toughness. In general terms the degree of tenderness is directly related to quality, rated very high on the palatability scale (Levie, 1970). For intact or noncomminuted meat, tenderness or toughness is determined by two groups of components: the connective tissues and the muscle fibers (Xiong *et al*., 1999), and other factors such as post-mortem carcass aging temperature and time (Dunn *et al*., 1993; Varnam and Sutherland, 1995; Dunn *et al*., 2000; Wattanachant, 2003).

1.2.3.1 Connective tissues

The structure of muscles is largely defined by sheaths of connective tissues (Lluch *et al*., 2001). There are three levels of organization (Figure 3). Individual muscle fibres are surrounded by a fine network of the connective tissue, the endomysium. Bundles of fibres are surrounded by the perimysium, and the whole muscle is contained within the epimysium. The main component of the connective tissue is collagen, together with the protein elastin (Levie, 1970; Warriss, 2000). Collagen fibres are straight, inextensible and non-branching, while elastin fibres are branched and elastic (Warriss, 2000).

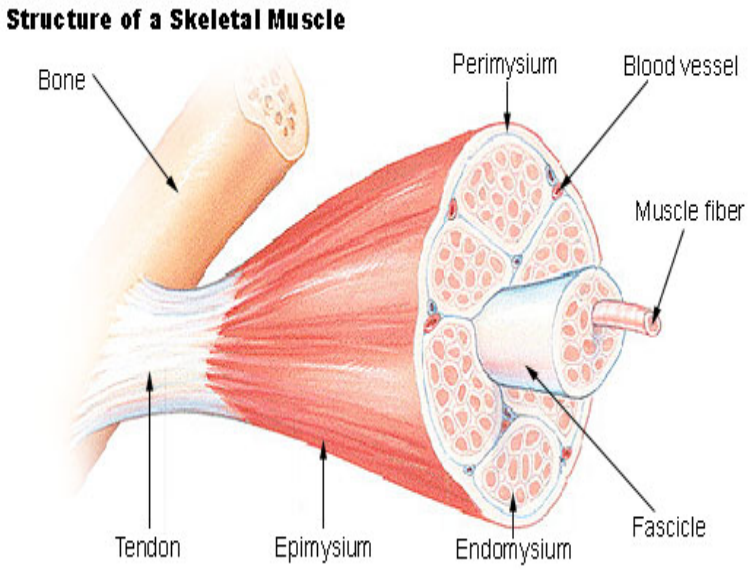


Figure 3. A transverse sections of the muscle bundles and the connective tissues: epimysium, perimysium, and endomysium.

Source: Musclehelp (2010).

So far the role of connective tissues on meat tenderness has been elucidated (Nishimura *et al*., 1995; Liu *et al*., 1995, 1996; Nakamura *et al*., 2003; Brooks and Savell, 2004). Since epimysium is normally removed when cooked meat is consumed, only the intramuscular connective tissue (IMCT), perimysium and endomysium, present a realistic toughness problem to meat and meat product (Xiong *et al*., 1999). The amount, composition, and arrangements of IMCT directly affect the texture of meat (Bailey and Light, 1989; Lawrie, 1991; Liu *et al*., 1996; Wattanachant *et al*., 2004). Total amount of connective tissue from different muscles of the same animal are different (Miller, 1994; Liu *et al*., 1996; Wattanachant *et al*., 2004). The specific influence of intramuscular connective tissues depends on their thickness, the amount of collagen present, as well as the density and type of cross linkages between collagen fibrils (Xiong *et al*., 1999; Wattanachant *et al*., 2004, 2005a). A high correlation between the thickness of perimysium and shear values have been reported by Liu *et al*. (1996) and Wattanachant *et al*. (2004, 2005a). Wattanachant *et al*. (2005a) reported that perimysium thicknesses were 14.2 μm for *Biceps femoris* muscle and 7.10 μm for *Pectoralis* muscle of indigenous chicken muscles, thicker

than those of broiler muscles, which were 9.93 μm for *Biceps femoris* muscle and 3.87 μm for *Pectoralis* muscle. Perimysium thickness of the muscles correlated well with the magnitude of the shear values of the muscles as previously reported by Wattanachant *et al*. (2004), Thai indigenous *Biceps femoris* (5.20 kg), broiler *Biceps femoris* (2.89 kg), Thai indigenous *Pectoralis* (1.78 kg), and broiler *Pectoralis* (1.20 kg).

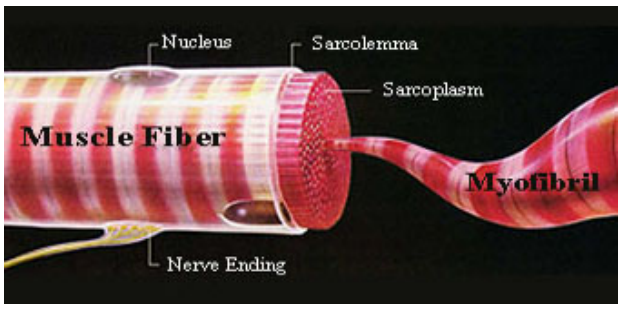
The total collagen contents from different muscles of the same animal are different and its property is important because it contributes significantly to toughness in muscle (Liu *et al*., 1996; Ding *et al*., 1999; Wattanachant *et al*., 2004). Wattanachant *et al*. (2004) reported that the total collagen contents of Thai indigenous *Pectoralis* and *Biceps femoris* muscles were 5.09 and 12.85 mg/g respectively, which were higher than those found in broiler *Pectoralis* (3.86 mg/g) and *Biceps femoris* muscles (8.70 mg/g). The authors also found that the shear value of the indigenous chicken muscles, either raw or cooked, were significantly higher than for the broiler muscles. In addition, raw meat from the *Biceps femoris* muscle had higher shear values than the *Pectoralis* muscle for both breeds, although no differences in shear values were observed between the two muscles after cooking.

The properties of collagen are dependent on its structure and composition which change as animal age increase and can contribute to the difference in tenderness among the muscles (Levie, 1970; Dawson *et al*., 1991; El, 1995; Liu *et al*., 1996; Wattanachant *et al*., 2004). 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. As animal age increase, collagen cross-links 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 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; Wattanachant *et al*., 2004). This fact also suggests that collagen molecules of

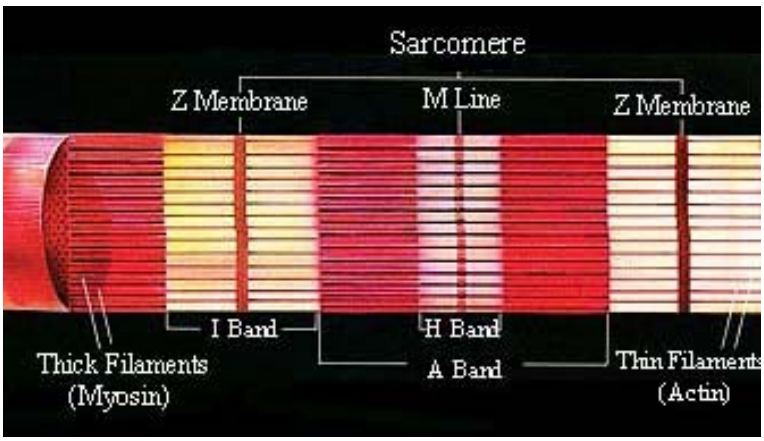
intramuscular connective tissue change to a structure of higher heat resistant through the formation of cross-linkages (Nakamura *et al*., 1975). As cross-linking of collagen increase it becomes less soluble in a variety of solvents, such as salt and acid solutions (Wattanachant *et al*., 2004).

1.2.3.2 Muscle fibers

Muscle fibers or muscle cells (Figure 4a) have a threadlike appearance and surrounded by sarcolemma, which is a cell membrane overlayed with endomysial connective tissue (Lluch *et al*., 2001). Muscle fiber is composed about one thousand finer units called myofibrils, themselves containing a number of smaller long thin filaments called myofilaments (Wilson *et al*., 1981). Myofibrils are cross-striated, and the light and dark bands of adjacent myofibrils lie in register (Lluch *et al*., 2001). The dark band is called the A-band, the light band is called I-band, and the dark line bisecting the light band is called Z-disk, the contractile unit of the myofibril is called a sarcomere (Figure 4b), which is the distance from one Z-disk to the next. The sarcomere has two sets of interdigitating filaments, thin and thick; the dark band in the light microscope is that area of the myofibril that contains thick filaments and the light band is that area contains no thick filaments. As shown in Figure 4c, thin filaments are composed predominantly of the actin molecules wrapped around one another, tropomyosin and troponin, and are connected to the Z-disk; thick filaments, composed of strongly packed protein myosin, run from the center of the sarcomere toward the Z-disk (Lluch *et al*., 2001). The region of I-band contains only thin filaments, and the A-band contains both. The H-zone is the region between the ends of the thin filaments that contains only thick filament interconnected by protein links which give rise to the denser M-line (Lewis, 1979 cited in Lluch *et al*., 2001).



(a)



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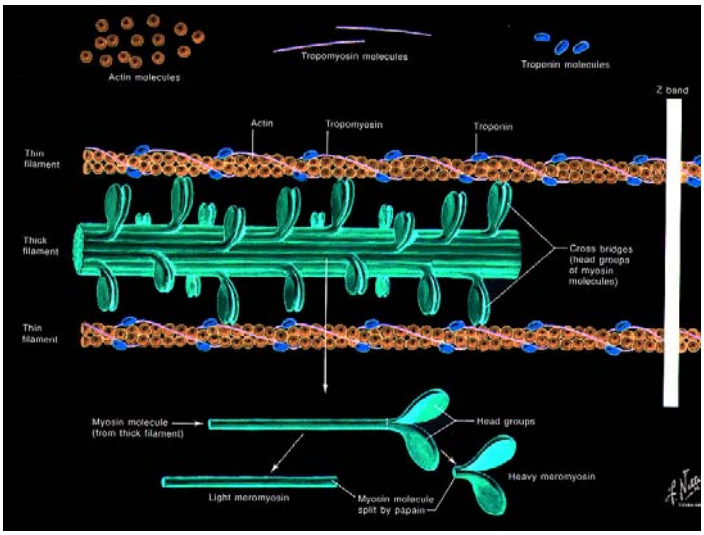


(c)

Figure 4. Muscle fiber, sarcomere structure, and different types of protein in filaments; (a) Longitudinal muscle fiber; myofibrils and sarcolemma, (b) a longitudinal section of sarcomere, (c) different types of protein in thin and thick filament.

Source: Rima Kavade (2007).

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. Wide ranges of diameters among chicken white fibers have been reported as 68.2 μm (Kiessling, 1977), 38 to 46 μm (Smith and Fletcher, 1988), 32.6 μm (Smith *et al*., 1993), 26.6-28.9 μm (Wattanachant *et al*., 2005a) and 16.1-18.7 μm (Chuaynukool *et al*., 2007). The size of the muscle fiber bundles can determine the texture of the muscle (Lawrie, 1991). However, there is an indirect correlation between muscle fiber diameter and tenderness. The report by Ozawa *et al*. (2000) found that the white muscle fiber content (larger diameter and narrow Z-lines) was negatively correlated ($p<0.05$) with shear value, while at the same time red muscle fiber content (wide Z-lines) was positively correlated ($p<0.05$) to this value. The average of sarcomeres length of raw muscles from Thai indigenous, spent hen, and broiler were significant difference ($p<0.05$) in ranges of 1.55-1.62 μm for *Pectoralis* muscle and 1.53-1.64 μm for *Biceps femoris* muscle (Chuaynukool *et al*., 2007). Sarcomere length is used as a measure of muscle contraction and is highly correlated with tenderness of pre-rigor 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 is 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).

1.2.3.3 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 it is weakening during post-mortem ageing of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animal and fowl. To obtain meat of high quality, post-mortem ageing of meat at around 4^{o}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 is mainly due to the structural weakening of myofribrils 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 is 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. Two groups of endogenous protease in skeletal muscle, the calpain and the cathepsin, have been implicated in the degradation of the myofibrillar protein matrix (Ouali, 1990; Walker *et al*., 1995; Koohmaraie, 1992). However, post-mortem ageing induced tenderness is associated with decreases in calpain but not cathepsin enzyme level (Lyon and Buhr, 1999). In most mammalian and avian tissues, the calpain proteolytic system consists of two isoforms (μ and m) of a neutral (pH) calcium-dependent protease (Northcutt *et al*., 1998). Veeramuthu and Sams (1999) reported that μ-calpain is an unstable enzyme and is inactivated by either proteolysis or intermolecular rearrangement during post-mortem storage of *Pectoralis* muscle of broiler. In contrast, m-calpain activity slightly decline during 24 h of ageing. 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 tenderization 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.

2. Spent hen meat

Chicken raised primarily for egg production are referred to as laying hens (The Humane Society of The United States, 2006). Laying hens are considered "spent hen" at the end of their laying cycle, typically after 52 weeks in production (White, 2005). Excessive expansion of egg industry resulted in abundant availability of spent hen (Sangtherapitikul *et al*., 2004) and become available for use in further processed products (Ajuyah *et al*., 1992; Nowsad *et al*., 2000a, b). Age makes its muscle objectionably tough (Nurmahmudi *et al*., 1997; Nurmahmudi and Sams, 1997a, b; Woods *et al*., 1997; Rhee *et al*., 1999; Nowsad *et al*., 2000a, b; Lee *et al*., 2003; Sangtherapitikul *et al*., 2004; Bhaskar *et al*., 2006) and poor in functional properties (Singh *et al*., 2001; Sangtherapitikul *et al*., 2004), due to formation of a high amount of heat stable collagen (Nakamura *et al*., 1975) or the number and maturity state of the collagen cross-links (Sams, 1990). The toughness prevents its use in whole meat food and reduces the market value in many countries such as Korea, Taiwan, Japan, and United States (Hollender *et al*., 1987; Sams, 1990; Nowsad *et al*., 2000a, b; Kim, 2002; Lee *et al*., 2003; Li, 2006). Therefore, it has been utilized primarily for chicken soups and emulsified chicken products such as frankfurter, restructured steaks, patties, and bologna (Roland *et al*., 1981; Seideman *et al*., 1982a, b, c; Lee *et al*., 2003). However, meat from spent hen is a good protein source (Hollender *et al*., 1987; Rhee *et al*., 1999; Lee *et al*., 2003; Chuaynukool *et al*., 2007) and highly enriched with omega-3 fatty acids; in particular α-linolenic acid (C18:3ω3), eicosapentaenoic acid (C20:5ω3), and docosahexaenoic acid (C22:6ω3), when oil seeds were added to layer diets (Ajuyah *et al*., 1992). As the result of their modulating effects on blood platelet aggregation, systolic blood pressure and the control of diabetes in humans, omega-3 fatty acids have been shown to have health promoting benefits (Illingworth and Ullmann, 1990). In addition, Ajuyah *et al*. (1992) also found that the white meat from spent hen was lower in cholesterol content when compared with dark meat and whole carcass. Therefore, additional usages and decreasing toughness need to be developed to increase the value of the spent hen meat. In recent years, many investigators have developed new products from spent hen meat such as smoke chicken sausage (Singh *et al*., 2001), surimi (Xiong and Brekke, 1990, 1991; Nowsad *et al*., 2000a, b), an ingredient for pork ham (Li, 2006),

snack foods (Rhee *et al*., 1999), and spent hen meat enzymic hydrolysate (Sangtherapitikul *et al*., 2004). Decreasing toughness of spent hen has been reported by many investigators. The study in poultry feeds, reported by Sams (1990) suggested that β-aminopropionitrile (BAPN) was effective in preventing the formation of collagen cross-links by inhibiting lysyl oxidase but no improvement in the shear value for cooked meat was observed. Tenderizing effects of ionic strength from the calcium chloride ($CaCl_2$) solution on spent hen breast fillets have been reported by many researchers (Nurmahmudi and Sams, 1997a, b; Nurmahmudi *et al*., 1997; Woods, 1997). Although injecting meat with $CaCl_2$ tenderizes meat, detrimental effects include reduced palatability and loss of moisture binding capacity (Nurmahmudi and Sams, 1997a). High temperature processing such as retorting and aseptic heat processing have been used to solubilize the cross-links found in the structure of collagen in spent hen in order to process canned products as solid particles in liquid products (Dawson *et al*., 1991; Dawson and Sheldon, 1993; Voller-Reasonnover *et al.,* 1997). Other investigators have been focused on enzymatic improvement (Tsenga *et al*., 2002; Naveena and Mendiratta, 2001; Bhaskar *et al*., 2006). Tsenga *et al*. (2002) found that transglutaminase (TGase) from pig plasma could improve functional properties of myofibrillar proteins from spent hens by catalyzed the formations of glutamyl-lysine bonds in myosin and actin. Naveena and Mendiratta (2001) marinated spent hen chunks with different concentrations of ginger extract (GE) and found that GE treatments increased the pH, moisture content, cooking yield, water holding capacity, collagen solubility, protein extractability, muscle fiber diameter and decreased the shear force values. They also reported that 3% of GE was optimum for tenderization. Bhaskar *et al*. (2006) reported that 2.5% (w/w) ginger powder obtained by ethanol treatment was very effective in tenderizing the tough meat and improving the sensory-flavor quality of spent hen traditional meat product.

3. Processing methods of poultry meat

Poultry meat products are prepared in many forms, mainly available fresh, chilled or frozen, broil, roast, canned, sometimes in combination with ingredient and other food. Processing methods of poultry meat is a very important part of the poultry meat business. There are two major methods of processing poultry meat: refrigerating and thermal processing.

3.1 Refrigeration

Most processed meat products also need to be kept refrigeration from the time of processing until their consumption. Poultry meat can be refrigerated in either chilled or frozen storage. It may be kept chilled if there is only short period of time for distribution, whereas for longer storage periods meat has to be frozen (Bender, 1992). Crushed ice, mechanical refrigeration, or a combination of two are used as the source of refrigeration for chilled storage and liquefied gases and mechanical refrigeration for frozen storage (Mountney and Parkhurst, 1995). The temperature at which poultry is held during chilled storage determines to a large extent its shelf-life (Mountney and Parkhurst, 1995). Chilled poultry stored at 2-4^{o}C have its shelf-life for one or two days at maximum before dispatch to retail outlets and may be kept a day or so longer if stored at –1^{o}C (Silverside, 1992). Poultry kept in frozen storage at –20^{o}C may be kept for up to 6 months (Mountney and Parkhurst, 1995). Sawyer *et al*. (1960 cited in Mountney and Parkhurst, 1995) reported that well-packaged turkeys and cut-up chicken stored at –18^{o}C will remain in good condition for one year, when stored at –12.2^{o}C they will remain in good condition only six months, and when held at –6.6^{o}C only three months.

Silverside (1992) noted that poultry may be frozen by one of several ways. The usual system is to place the wrapped carcasses or portions into metal trays and place them in a blast freezer for 2-3 h at –40^{o}C. The process is followed by storage in a freezer store at –20^{o}C or below for a period up to six months. There are alternative methods of freezing. Regular packages lend themselves to plate freezing. In this system, “bricks” of meat packages are sandwiched between two plates. There are several plates and each will sandwich several packs of poultry. The plates are brought down into direct contact with the pack and refrigerant is run through so that

the packs reach –18°C in about 1 1/2 h. The packs are then removed, placed in boxes and stored at –20°C. The other freezing methods use adding refrigerant directly to the pack or cryogenic freezing. The two best known examples are lipid nitrogen and solid carbon dioxide. Varnam and Sutherland (1995) reported that retail cuts and poultry portions may also freeze using blast freezer. Where blast freezing is used, it is usual to use plastic film wraps to prevent dehydration and protect from contamination during handling. Plate freezers may be used for deboned. Mince can be frozen in final packaging using plate or blast freezer. Brine immersion freezing has been used, in conjunction with blast freezing, for freezing whole poultry. Cryogenic freezing, however, leads to improved quality with small pieces.

After storage, frozen food needs thawing. Thawing is the reverse process of freezing (Varnam and Sutherland, 1995). Frozen meat can be thawed by the following ways: overnight in the refrigerator, in a microwave oven, and under cold water with the meat kept within a water tight plastic bag (AVA, 2004). However, thawing over night at 4°C have shown to be greater positive effects on quality and the most flavor used by many investigators (Allen *et al*., 1998; Johnston *et al*., 2005; Smith, 1987; Zhang *et al*., 2005).

3.2 Thermal processing

Meat is usually cooked before being eaten (Levie, 1970; Combes *et al*., 2003). Darey and Gilbert (1974) defined cooking as the heating of meat to sufficiently high temperature to denature proteins. Woods and Church (1999) noted that there are at least two reasons why heat processing is carried out. The first is to cook the product. This process involves aspects of the palatability and acceptability of the food. The second reason for heat processing is to preserve the product and extend its shelf-life. Heat destroys certainly microorganisms and can therefore be used to prevent or delay spoilage and to reduce the risk of food poisoning. According to the USDA guideline, chicken should be heated to 68.3-72°C to ensure that it is free from pathogen and viruses (Apenten, 2002). However, poultry meat may be cooked to a higher end point temperature, such as 80-82°C, for flavor preference (Ang and Huang, 1993). There are two basic methods of cooking meat: moist heat, and dry heat (Sharrara, 2007). Moist heat involves cooking with water or some variation on water (stock, wine, beer, etc.) such as; poaching, steaming, boiling, braising, and

microwaving. Dry heat involves non-aquatic heat transfer methods such as; roasting, broiling, pan-broiling, pan-frying, and grilling. Less tender cuts of meat require moist heat cooking methods to help break down the tough connective tissues, whereas tender cuts of meat usually cooked with a dry heat method (Sharrara, 2007).

3.2.1 Steaming

Steaming refers to cooking food over a small amount of simmering or boiling liquid in a covered pot and the trapped steam does the cooking (Cookcheap, 2007). Steaming is an ideal method for cooking boneless chicken breasts and small whole birds such as Cornish hens (Hormel, 2006). It retains the flavor, tenderness and moisture through the use of steam. It is a healthy method of cooking because no additional fat used. Chicken can be steamed with a traditional steamer that fits on top of a saucepan, by using a rack that sits in the bottom of a tightly covered pan, suspending the chicken above the water, or by the use of an electric steamer.

3.2.2 Boiling

Boiling refers to cooking foods fully immersed in boiling water, where bubbles are constantly breaking the surface of the water (Cookcheap, 2007).

3.2.3 Microwave cooking

Microwave cooking is extremely easy, but it does differ from conventional methods. Unlike a conventional oven, which uses currents of hot, dry air to cook food, microwave oven uses high-frequency radio waves. These waves cause the moisture inside the food to vibrate and generate heat. Rosen (1972) noted that the difference between the supply of heat *via* microwave or conventional methods is the initial distribution of heat. Microwave deposit heat throughout a three-dimensional space. This distribution is complicated not only by the gradual decrease of intensity as the energy is absorbed by successive layers of material, but also, by differing dielectric properties of the various materials; thus it is possible that “hot spots” may develop in the material being heated. In addition, the total time of heat exposure for a microwave oven is shorter than that for a conventional oven (Lyon and Ang, 1990). Microwave cooking is a quick and convenient method for cooking chicken that will provide a tender, juicy meat but the flavor will not be enhanced as it is when comparing with other cooking methods (Hormel, 2006).

4. Effect of processing on quality of poultry meat

4.1 Refrigerated poultry meat

The primary aim of poultry refrigeration is protection against spoilage by microorganism and also gives a measure of protection against other forms of meat deterioration (Silverside, 1992). However, during refrigerated storage and handling, raw or cooked poultry undergo several changes such as microbiology growth, chemical and physical changes that can affect their quality attributes and results in reduced consumer acceptance (Kim and Marshall, 1999; Woods and Church, 1999; Bustabad, 1999).

4.1.1 Physical changes

Physical changes in the food can result in negative effects on the shelf-life (Woods and Church, 1999). During freezing and thawing of meat, ice crystal growth causes physical changes resulting in the disruption of cellular organelles and release of their contents into the meat drip juice (Downey and Beauchene, 1997). The term “drip” has been used to describe exudate both from frozen thawed meat (drip) and from chilled or fresh meat (weep) (Ngapo *et al*., 1999). In the case of frozen foods sublimation of water from exposed parts can cause localized dehydration (freezer burn) (Woods and Church, 1999). Changes in drip loss, water-holding capacity, cooking yields and microstructure during refrigerated storage have been reported in chicken meat (Dhillon and Maurer, 1975; Smith, 1987; Barbut and Mittal, 1990; Galobart and Moran, 2004; Johnston *et al*., 2005), pork (Ngapo *et al*., 1999; Bustabad, 1999; Mortensen *et al*., 2006) and beef (Carroll *et al*., 1981; Martino and Zaritzky, 1998; Bustabad, 1999; Zhang *et al*., 2005). Several of the published data reported higher drip loss and lower cooking yields from frozen meat and meat product compared to fresh meat (Sebranek, 1982; Smith, 1987; Nusbaum *et al*., 1983; Ngapo *et al*., 1999; Galobart and Moran, 2004; Johnston *et al*., 2005). During long-term frozen storage, freezing out of water can lead to insolubility of protein which results in alteration of water holding capacity (Sikorski, 1978; Patel *et al*., 1988). Johnston *et al*. (2005) reported that drip loss of chicken breast frozen increased during storage at -18^{o}C for 150 days. They also found that samples frozen in a conventional home freezer (slow rate, -0.03^{o}C/min) had a greater drip loss than the mini blast/plate

freezer (fast rate, -0.13^{o}C/min) and the samples thawed at room temperature (22^{o} C for 3 h) showed a higher drip loss than those thawed at refrigerated temperature (4^{o}C for 24 h). Galobart and Moran (2004) reported that frozen broiler fillets at -20^{o}C and allowed to thaw for 3 days at 4^{o}C in a walk-in refrigerator had 10% decreased thaw loss from the frozen state and 26% decreased cooking loss from thawed state. Smith (1987) reported that cook yield of mechanical deboned turkey meat (MDT) and hand deboned turkey meat (HDT) decreased by 6-11% during frozen storage at -20^{o}C for 26 weeks and MDT had lower cook yields than those of HDT throughout storage. Changes in microstructure during freezing, frozen storage and subsequent thawing can cause mechanical damage in beef and poultry (Carroll *et al*., 1981; Smith, 1987; Ngapo *et al*., 1999). Smith (1987) found that myofibril gel microstructure of the turkey meat changed from a continuous filamentous matrix to a globular matrix and large spaces occurred in the gels during 26 weeks of frozen storage. As observed by Carroll *et al*. (1981), slices beef (*Semitendinosus muscle*) frozen in liquid nitrogen at -18^{o}C showed distortion, compaction and pitting of fibers and this deterioration increased with frozen time from 1 to 26 weeks.

4.1.2 Chemical changes

During refrigerated storage lower temperatures prevent or minimize many undesirable changes in meat, however, some chemical reactions still occur which can affect product quality (Smith, 1987). Changes in proteins during long-term frozen storage have been reported in some studies (Sikorski, 1978; Smith, 1987) that can alter physical, chemical, functional or sensory properties of meat. Freezing out of water can lead to insolubility of protein which results in alteration of textural properties and hardening of the fresh meat (Sikorski, 1978). Smith (1987) reported that frozen storage (at -20^{o}C, 26 weeks) caused protein insolubilization in both mechanical and handed deboned turkey meat as indicated by decreasing in protein solubility test, however, there were no changes in the electrophoretic patterns of the myofibrillar proteins during frozen storage as observed on SDS polyacrylamide gels. Protein denaturation during frozen storage of meat may be caused by one or more of the following factors: (1) ice crystal damage to cells and membranes; (2) dehydration of protein molecule; (3) increase in solute concentration in the unfrozen water phase; (4) enzymatic activity; and (5) reaction of proteins with free fatty acids and other

intact lipids, and reaction of proteins with oxidizing lipids (Matsumoto, 1980; Shenouda, 1980; Smith, 1987).

Lipid oxidation is one of the most important degradation processes during meat refrigeration. Lipid oxidation leads to rancidity in meat, which is associated with the development of warmed-over-flavor in refrigerated cooked meat (Byrne *et al*., 2001; Poste *et al*., 1986; St. Angelo *et al*., 1987; 1990). After refrigerated storage poultry meat showed increase rate of lipid oxidation (Igene *et al*., 1979b; Pikul *et al*., 1984; Ang, 1988; Ang and Huang, 1993; Rhee *et al*., 1996; Jensen *et al*., 1997a; Dawson *et al*., 1990; Alasnier *et al*., 2000; Botsoglou *et al*., 2003). As reported by Botsoglou *et al*. (2003) found that lipid oxidation in thigh and breast muscles at day 7 of refrigerated storage following 1, 3, 6 or 9 months of frozen storage were significantly ($P<0.05$) higher compared with that on day 0 of refrigerated storage and then more gradually increased as storage time increased. Processing methods have been also reported to promote lipid oxidation. According to Dawson *et al*. (1990), reported that both raw and cooked washed mechanically deboned chicken meat (MDCM) oxidized during the washing treatment and had TBA values higher than the raw and cooked unwashed MDCM after 4 days of refrigerated storage. Pikul *et al*. (1984) reported that TBA numbers of convection cooked chicken meat increased 98% in breast and 170% in leg compared to TBA numbers before cooking after six months of frozen storage and samples cooked by microwave were lower TBA numbers compared to samples cooked by convection oven. When studied in raw chilled meat, Rhee and Ziprin (1987) and Rhee *et al*. (1996) found that chicken and pork muscles had lower TBARS values compared to beef muscles during storage at $4^{o}C$ for 6 days. However, when studied in cooked samples either chilled or frozen storage, Rhee *et al*. (1996) found higher lipid oxidation potential for cooked chicken muscles, compared to cooked beef and pork during storage. They concluded that catalase activity and heme pigment have more affected on lipid oxidation than unsaturated fatty acids in raw meat, whereas, the amount of reactants (PUFA), rather than catalysts, would become a more important determinant of inter-species differences in lipid oxidation rate in cooked meat. Ang and Huang (1993) concluded that TBARS values of chicken leg patties increased with time and patties heat to higher end point temperature (EPT) oxidized at the faster rate than cooked to lower

EPT. Ang and Su (1989 cited in Ang and Huang, 1993) similarly showed higher TBARS values of broiler breast patties cooked to EPT of 83°C compared with EPT of 73°C after 3 days storage at 4°C.

4.2 Cooked poultry meat

Thermal processing of poultry results in chemical and physical changes that will strongly influences chemical composition, cooking loss, yield, and other important quality factors, such as texture, juiciness, color, and flavor, which are associated with palatability and consumer acceptance of the final product (Califano *et al*., 1997; Murphy and Marks, 2000; Wattanachant *et al*., 2005a, b).

With increasing temperature, the denaturation of myosin and actin caused shrinkage of the muscle fibers and expelled the sarcoplasmic fluid from the muscle fibers, resulting in water losses from meat tissues (Bertola *et al*., 1994 cited in Murphy and Marks, 2000) that can affect moisture content, yield and cooking loss of chicken, beef, bull, bovine, cattle, and rabbit meat (Dawson *et al*., 1991; Lyon and Wilson, 1986; Lyon and Lyon, 1993; Voller-Reasonover *et al*., 1997; Palka and Daun, 1999; Murphy and Marks, 2000; Combes *et al*., 2003; Wattanachant *et al*., 2005b; Silva *et al*., 1993; Wu *et al*., 1995; Swan and Boles, 2002). Dawson *et al*. (1991) reported a moisture loss of 0.8 to 2.9 % for broiler meat and 2.9 to 7.4 % for hen meat during aseptic processing. Voller-Reasonover *et al*. (1997) found that spent fowl meat gel pressure cooking at 115.6°C resulting in the better yield and moisture retention compared to heat at 121.1 and 126.7°C. Palka and Daun (1999) pointed out that cooking loss and sarcomere length seemed to be a good indicator of changes in meat during cooking. They observed a reverse proportionality of changes in the sarcomere length of bovine ST muscle and cooking losses during heating in the range of 50-121°C. Wattanachant *et al*. (2005b) found that the sarcomere length decreased non-significantly when heated to temperature of 50-60°C for broiler muscle and 50-70°C for indigenous chicken muscle ($P>0.05$) and the cooking losses of broiler and indigenous chicken muscles were not significantly different in the range 50-70°C. However, the authors observed the greatest shrinkage of the sarcomeres in samples heated to end-point temperatures of 70-100°C for broiler and 80-100°C for indigenous chicken and the greatest increases in cooking loss for indigenous chicken muscles were observed in the range 80-100°C.

The color of the product is also influenced by cooking. The increase in L^* and b^* value and decrease in a^* value of broiler muscle after cooking have been reported by Fletcher *et al*. (2000), Qiao *et al*. (2002a), and Wattanachant *et al*. (2005b). Young and West (2001) pointed out that with increasing heating temperature, meat tends to be lighter and also turn to a brown-grey hue. The lightening is due to an increased reflection of light, arising from light scattering by denatured proteins. The loss of chroma and change in hue resulted from the changes in myoglobin which is one of the more heat-stable of the sarcoplasmic proteins. Lawrie (1991) noted that denaturation of red myoglobin and conversion to brown myohaemochromogen starts at 40^{o}C and is almost completely denatured between 80-85^{o}C. 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 the 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).

Cooking brings out the best flavor attributes (Levie, 1970). Cooked flavor resulted from a number of reactions including changes in lipids, carbohydrate and protein, with heat breakdown of peptides and amino acids, and reaction between proteins and carbohydrates (Bender, 1992). A few studies have examined aspects of cooking method and their effect on flavor of poultry (Joseph *et al*., 1996; Salama, 1993; Farmer, 1999). Joseph *et al*. (1996) examined the effect of final internal cooking temperature on the flavor of various species of poultry found in Nigeria. In all cased they found that the flavor improved from 65^{o}C to 80^{o}C and then decline slightly when cooked to 85^{o}C. Farmer (unpublished data cited in Farmer, 1999) found that chicken portions cooked at different oven temperatures gave some flavor differences; the higher oven temperature tended to give more chicken flavor. Salama (1993) showed that conventional heating gave a preferable flavor for both breast and leg meat than microwave heating and premarinading in sodium chloride or sodium tripolyphosphate tended to give increased scores for flavor for both cooking methods. Bender (1992) pointed out that meat from older animals richer in connective tissue

requires longer cooking at 50-60^{o}C, a temperature at which collagen can be hydrolysed, but if heated for long periods at temperature above 80^{o}C amino acids begin to decompose with the production of unpleasant flavor. Cooked-meat sometimes cause flavor deterioration during storage. Off-flavor normally increased along with storage time (Penner and Bowers, 1973; Lyon, 1988). Flavor deterioration due to storage is usually related to lipid oxidation. Lipid oxidation has been considered the primary cause of flavor deterioration and the development of oxidized flavors generally called ‘‘warmed-over flavor’’ in cooked–stored meat (Tims and Watts, 1958; Shahidi, 1994b; Rhee *et al*., 2005). Rhee *et al*. (2005) found difference flavor changes during 4^{o}C storage of beef, pork, and chicken cooked patties and cooked meats from all the species can oxidize and loose species-specific natural flavor during storage. Lyon *et al*. (1988) reported the TBA values were higher in microwave (MW) reheated chicken patties after 2 to 3 days of storage; however, the sensory data indicated no effect from the reheating method. Lyon and Ang (1990) found that the TBA values of precooked chicken patties showed significant ($P<0.05$) storage time (stored at 4^{o}C for 4, 24 or 48 h) effects but no effects from the reheating methods by using MW or conventional oven (CO). They also noted that sensory changes between MW and CO reheating were not supported by chemical analyses at storage time less than 48 h.

The texture of cooked meat is generally considered to be affected by heat-induced changes in connective tissue, soluble proteins, and myofibrillar proteins (Dawson *et al*., 1991; Califano *et al*., 1997; Murphy and Marks, 2000; Wattanachant *et al*. 2004; Wattanachant *et al*., 2005a, b). Cooking generally results in increased tenderness of poultry muscles, but the temperature of cooking can affect tenderness. Voller-Reasonover *et al*. (1997) found that spent fowl meat gels pressure cooking at 115.6^{o}C increased the soluble collagen and decreased in hardness in the samples compared to heat at 126.7^{o}C. As suggested by many investigators (Bouton and Harris, 1972; Harris and Shorthose, 1988; Lewis and Purslow, 1989; Mutungi *et al*., 1996; Christensen *et al*., 2000), meat toughness is found to increase in two distinct phases. The initial rise in meat toughness was probably due to thermal shrinkage of the intramuscular connective tissue at the temperature below 60^{o}C, whereas the second rise in meat toughness between 65 and 80^{o}C could be due to heat denaturation of

myofibrillar proteins. Wattanachant *et al*. (2004) reported that the indigenous chicken muscles, both *Pectoralis muscle* and *Biceps femoris muscle* possessed firmer textures after cooking than broilers due to the difference in total and soluble collagen contents between the muscles of the two chicken breeds. A series of research reported by Wattanachant *et al*. (2005a, b) found that the perimysium and endomysium of broiler muscles melted after cooking at 80^{o}C, however, only slight disintegration was observed in this tissue in the indigenous chicken muscle. They also found that the protein solubility of muscle was very highly correlated with the texture of cooked broiler muscle while collagen solubility was important factors influencing texture of cooked indigenous chicken muscle.

5. Additives used in meat and poultry

5.1 Sodium chloride (NaCl)

Sodium chloride (NaCl) or salt is the most common additive in processed meat products because of its important contribution to produce flavoring, solubilizing proteins for binding, improve water holding capacity (WHC), decrease cooking losses, and increase total yield (Huffman *et al*., 1981; Schwartz and Mandigo, 1976; Sams and Janky, 1986; Ingram and Kitchell, 1967; Cerveny, 1980; Hand *et al*., 1982; Kastuer and Kropf, 1986; Chow *et al*., 1986; Lamkey *et al*., 1986; Shackelford *et al*., 1989; Stoick *et al*., 1991).

The use of salt in meat product to enhance flavor is an old practice (Miller, 1998). The increased yields associated with added salt are mainly due to the decreased in cooking losses affected by the greater water-holding capacity of processed meats (Schwartz and Mandigo, 1976; Pepper and Schmidt, 1975; Chow *et al*., 1986; Lamkey *et al*., 1986; Shackelford *et al*., 1989). According to Shackelford *et al*. (1989), reported that as salt level increased from 1.0 % to 1.25%, water-holding capacity of beef roast increased and higher salt level resulted in decreased cooking losses and increased total yields. Sodium chloride acts to improve water-holding capacity by lowering the isoelectric point of meat proteins and contributes to the swelling of the proteins (Miller, 1998). The swelling provides a higher number of protein side chains that can bind water and therefore, water-holding capacity increased. Combinations of salts with extracted protein from muscle fibers result in an

improved binding capacity for the meat that involve in the development of texture and juiciness (Acton, 1983; Maurer, 1983). Addition of NaCl to the chill solution during conventionally processed aged broiler carcasses increased tenderness of breast meat (Janky *et al*., 1982, 1983; Dukes and Janky, 1984). NaCl at the level of 1.0 - 2.0 % has been used to extract protein from several types of poultry muscle (Gillett *et al*., 1977; Hand *et al*., 1982; Prusa and Bowers, 1984). For example, Prusa and Bowers (1984) reported that protein extraction from chicken breast muscle increased as the NaCl concentration was raised from 1 % to 2 %. In contrast, using NaCl at the level higher than 2% has been shown to promote lipid oxidation in several meat products (Huffman *et al*., 1981; Chen *et al*., 1984; Patel *et al*., 1988; King and Earl, 1988; King and Bosch, 1990; Stoick *et al*., 1991). Although NaCl can be used to extract proteins from poultry meat, a combination of NaCl and sodium tripolyphosphate seems to be more effective than either salt alone (Gillet *et al*., 1977; Siegel *et al*., 1978; Theno *et al*., 1978; Prusa and Bowers, 1984; Nakhost and Karel, 1985; Young and Lyon, 1986; Lamkey *et al*., 1986; Kijorski and Mast, 1988; Yoon, 2002).

5.2 Sodium tripolyphosphate (STPP)

Phosphates have a marked effect on the characteristics of meat and poultry products. They serve other functions in meat and poultry products, such as, stabilize color and flavor (Tims and Watt, 1958; Steinhauer, 1983); improved texture (Miller, 1998; Yoon, 2002); enhance water-holding capacity (Hamm, 1970; Pepper and Schmidt, 1975; Moore *et al*., 1976; Knipe *et al*., 1985; Chow *et al*., 1986; Lamkey *et al*., 1986; Barbut *et al*., 1988); improve yield by reducing cooking loss and drip loss (Mann *et al*., 1989; Young and Lyon, 1986, 1997; Yoon, 2002); promote water binding (Kijorski and Mast, 1988); retard oxidation and minimize the development of warmed-over flavor in precooked and cooked meat (Tims and Watts, 1958; Steinhauer, 1983; Shahidi *et al*., 1987; Mann *et al*., 1989; Stoick *et al*., 1991). In addition, phosphate can be used as antimicrobial surface treatments to decrease populations of pathogens, prevent growth of spoilage microorganisms, and extend shelf-life of fresh poultry (Nelson *et al*., 1983; Yoon, 2002; Kim *et al*., 1994; Kim and Slavik, 1994; Kim and Marshall, 1999). Moreover, phosphates are especially important in reduced salt products where there is insufficient salt to extract salt-soluble protein (Whiting, 1984). There are different phosphates, such as, sodium

tripolyphosphate (STPP or STP, $Na_5P_3O_{10}$), sodium acid pyrophosphate (SAPP, $Na_2H_2P_2O_7$), sodium hexametaphosphate (HMP, $(NaPO_3)_{13-15}$), potassium tripolyphosphate (KTPP, $K_5P_3O_{10}$) and trisodium phosphate (TSP, Na_3PO_4) etc., which have been approved for use in meat. Each type of phosphate is difference in its functional properties. Among the phosphates commonly used in the meat industry, sodium tripolyphosphate is the most popular (Barbut *et al*., 1988; Miller, 1998), and accounts for about 80% of the phosphates in corporated either as a single phosphate or in blends (Everson, 1984 cited in Miller, 1998).

Sodium tripolyphosphate has an alkaline pH (Miller, 1998). Although meat is a very good buffer (Keeton *et al*., 1983; Miller, 1998; Hinton and Corry, 1999), the addition of STPP to meat systems increases the meat pH (Smith *et al*., 1984; Jone *et al*., 1987; Sutton *et al*., 1997; Detienne and Wicker, 1999) that moving the meat pH further from the meat protein isoelectric point (about 5.5 in chicken) (Miller, 1998). As the pH moves further from the isoelectric point, water-holding capacity increases due to an increase in the amount of negative charges on the meat proteins that can bind water (Miller, 1998). The net result is an increase in the amount of water that can be bound to the meat proteins. The addition of STPP to pork loin, ground pork and chicken breasts have been shown to improve meat yields by decreased cooking loss and drip loss (Keeton *et al*., 1983; Smith *et al*., 1984; Young and Lyon, 1986, 1997; Jone *et al*., 1987; Sutton *et al*., 1997; Detienne and Wicker, 1999; Yoon, 2002). As reported by Young and Lyon (1986), the yields of all STPP-treated chicken breast pieces were greater than those which were not treated with STPP. Young and Lyon (1997) found that treating chicken breast with STPP after previous postchill storage decreased cooking loss. So far, a synergistic effect of salt and phosphates in increasing water holding capacity has been reported by many researchers (Young and Lyon 1986; King *et al*., 1990; Patel *et al*., 1988). The challenge is to balance the amount of NaCl addition with the amount of STPP that is added to maximize water-holding capacity without getting too high of a salt flavor or altering texture (Miller, 1998). According to Young and Lyon (1986), both NaCl and STPP increased the water holding capacity of the ground chicken breast and also appeared to have a synergistic effect between NaCl and STPP, in contrast, $CaCl_2$ reducing these effect.

With increased water-holding capacity, meat with STPP added has been shown to be juicier and there are some changes in meat flavor. As reported by Smith *et al*. (1984), when STPP level increased from 0 to 0.2 to 0.4%, pork roasts were juicier but the addition of 0.4% STPP resulted in lower flavor intensity scores. The addition of STPP alone associated with increasing the off-flavors such as soapy and sour have been reported in pork and beef (Sutton *et al*., 1997; Vote *et al*., 2000).

Sodium tripolyphosphate addition has been associated with changes in meat texture. Meat containing STPP have been characterized as more tender, also shown to be softer and more rubbery (Lyon, 1983; King *et al*., 1986; Sheard *et al*., 1999; Yoon, 2002). The interaction of the postchill storage time by STPP treatment on chicken breast meat was investigated by Young and Lyon (1997) who showed that both the control and STPP treated meat, Warner-Bratzler shear values decreased as aging time increased. However, in no postchill aging, the shear values of STPP-treated meat (9.14 kg shear value) was more than 60% greater than for the control (5.69 kg shear value), which mean that the meat may have toughness problem by consumer when using benchmarks established by Lyon and Lyon (1991) who noted that Warner-Bratzler shear values below 3.62 kg corresponded to "very tender"; 3.63 to 6.61 kg "moderately to slightly tender"; 6.62 to 9.60 kg "slightly tender to slightly tough"; 9.61 to 12.60 kg "slightly to moderately tough" and greater than 12.60 kg "very tough". They concluded that the toughness problem of plant-marinated chicken breast can be avoided by aging the meat at least 2 h postchill before treating with polyphosphate.

As the pH of meat increases with the addition of STPP, changes in meat color would be expected (Ahn and Maurer, 1989; Young and Lyon, 1994, 1997; Young *et al*., 1996; Femandez-Lopez *et al*., 2003). According to Ahn and Maurer (1989), found that both NaCl (1, 1.5%) and STPP (0.5%) treated increased a* value and decreased L* and b* values of ground turkey breast meat. As mentioned by Fletcher (1999a, b), higher muscle pH is associated with darker meat, whereas lower muscle pH values are associated with lighter meat. Additionally, pH affects the ability of myoglobin to oxidize to metmyoglobin. It has been shown that as pH decreases, myoglobin is more easily oxidized to metmyoglobin (Femandez-Lopez *et al*., 2003). Young *et al*. (1996) found that the STPP treatment had no effect on C.I.E. color value

of broiler (*Pectoralis major* muscles) prior to cooking, but resulted in a slightly elevated L* and decreased a* value in the cooked product. Miller (1998) stated that in general during cooking, myoglobin degrades and as a result, meat goes from red-based color to gray, however, meat pH can effect on color due to at higher pH, myoglobin does not degrade as rapidly with heat.

The addition of STPP to meat has been shown to have antioxidant properties in cooked meat products (Stoick *et al*., 1991; Shahidi *et al*., 1987) by chelating prooxidant metals such as ferrous iron in cooked meats system which can catalyze the oxidation reaction (Tims and Walts, 1958). Moreover, addition of STPP would also increase pH of the meat system, possibly creating a more oxidative stable environment (Judge and Aberle, 1980). Several investigators have shown that STPP combined with NaCl retarded lipid oxidation and deterioration (Nakhost and Karel, 1985; Ahn and Maurer 1989).

Meat with a higher pH has been shown to be more conducive to microbial growth, however, successfully on limited microbial growth have been reported by Miller (1998) and Yoon (2002). Miller (1998) reported that the addition of STPP decreased total plate counts in pork loin chops. Yoon (2002) reported that washing fresh chicken carcasses with 10% salt and various phosphates solutions, including TSP, STPP and tetrapotassium pyrophosphate has been practiced to control microbial contamination and growth but only TSP and STPP improved textural and sensory properties of frozen chicken breasts. Of the various phosphates available TSP, alkaline phosphates, is the most effective antimicrobial agent for raw, unchilled carcasses and giblets in poultry industry (Crozier-Dodson *et al*., 2003). The allowable use is 8-12% solution used as a dip or spray, with 30 seconds allowed for giblets and 15 seconds for carcasses (21 CFR 182.1778 cited in Crozier-Dodson *et al*., 2003).

5.3 Ginger proteases

Proteolytic enzymes used in meat have been shown to increase the tenderness of meat as a result of proteolysis of the various meat protein fractions (Kang and Rice, 1970). Among the proteolytic enzymes of plant origin, papain, ficin, actinidin, and bromelain have been most extensively studied and are most commonly used for tenderization of meat (Choi and Laursen, 2000; Lee *et al*., 1986). More recently, ginger rhizome was investigated as a new source of plant proteolytic enzyme

by many researchers (Ichikawa *et al*., 1973; Thompson *et al*., 1973; Lee *et al*., 1986; Ohtsuki *et al*., 1995; Choi *et al*., 1999; Choi and Laursen, 2000; Adulyatham, 2001; Adulyatham and Apenten, 2005). The meat tenderizing component is ginger proteases or zingibain (Thompson *et al*., 1973) as shown in Figure 5. The ginger proteases are inactivated by sulfhydryl reagents (Ichikawa *et al*., 1973) and thus are grouped with other plant cysteine proteinases or thiol proteinases, such as papain, actinidin, and bromelain, and with similar enzymes from nonplant sources, such as cathepsin B (Choi *et al*., 1999; Choi and Laursen, 2000). The molecular weight of ginger proteases is 34.8 kDa (Adulyatham and Apenten, 2005) with an optimum temperature of 60^{o}C (Thompson *et al*., 1973; Adulyatham, 2001) and rapid denaturation of the enzyme occurs at 70^{o}C (Thompson *et al*., 1973). The optimum pH of GP has been found in the range of 5.0-5.6 (Thompson *et al*., 1973), 6.5-7.5 (Ichikawa *et al*., 1973), and 6.0-8.0 (Adulyatham and Apenten, 2005).

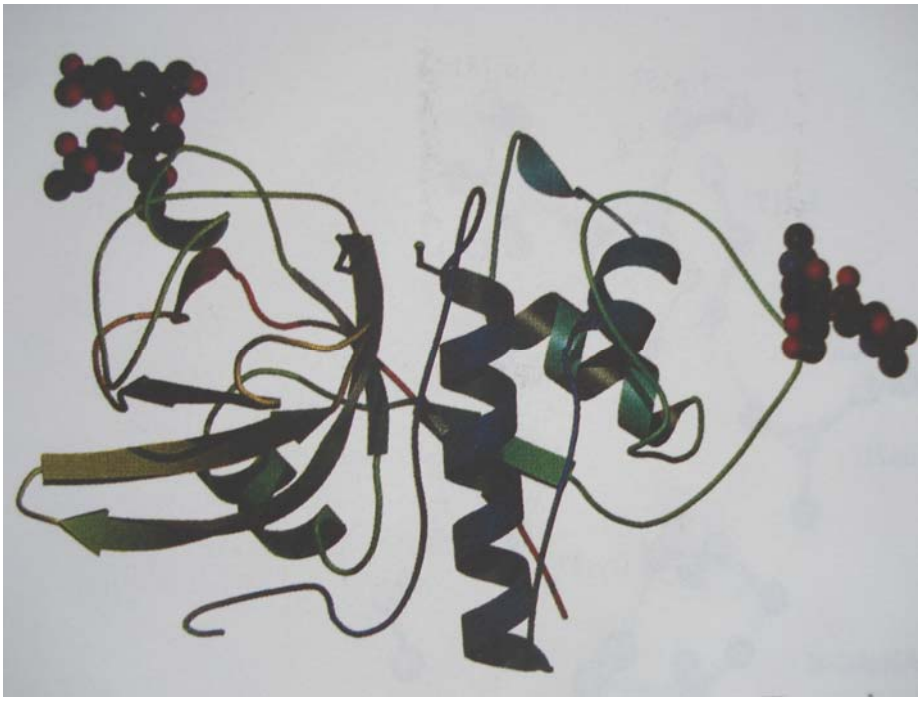


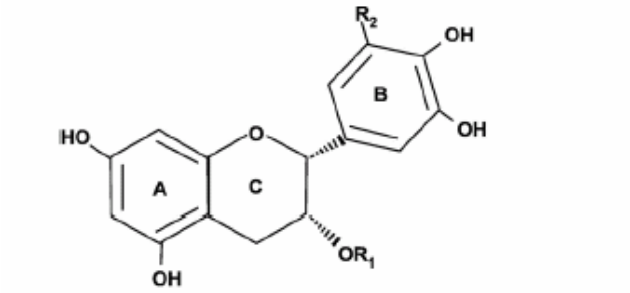
Figure 5. Three dimensional monomer structure of ginger protease.
Source: Choi *et al*. (1999).

Ginger protease is highly active against collagen and other connective tissue proteins (Thompson *et al*., 1973; Choi *et al*., 1999). Moreover, its proteolytic activity on collagen appeared to be many fold greater than that on actomyosin (Thompson *et al*., 1973). The combined proteolysis of these two muscle proteins resulted in significantly more tender meat (Thompson *et al*., 1973). Tenderizing by direct application of an aqueous ginger extract has been reported in beef (Lee *et al*., 1986) and buffalo muscles (Naveena *et al*., 2004). Lee *et al*. (1986) reported that crude ginger extracted at low levels was an effective meat tenderizer in beef steaks and sliced-marinated beef. The tenderization was achieved through the fragmentation of myofibrils by preferential degradation of thin filaments in the I-bands (Lee *et al*., 1986). In buffalo meat, Naveena *et al*. (2004) found that ginger extract improved the cooking yield, reduced the shear forces value, and showed higher sensory score in tenderness, flavor, and overall palatability than control due to the degradation of muscle fibers and connective tissue layers surrounding muscle fibers in ginger extract treated. Although crude ginger extract has been used successfully for tenderization of meat, it is unstable. Adulyatham and Apenten (2005) found that crude ginger extracted from fresh ginger (*Zingiber offinale* Roscoe) had a half-life ($t_{1/2}$) of 2 days at 5^{o}C decreasing to 20 min at 30^{o}C, however, addition of ascorbate (0.2% w/v) increased the $t_{1/2}$ for crude GE from 2 to 20 days at 5^{o}C. Thus, crude GE is undesirable in a commercial enzyme. Therefore, ginger powder, obtained by treating fresh ginger rhizome with polar solvent has become more useful than ginger extract in recent year. Thompson *et al*. (1973) mentioned that the treatment of ginger rhizome with polar solvents enhance the specific activity of ginger protease by the removal of various constituents of ginger and also eliminate the problem of sprouting or molding of the ginger rhizome. Adulyatham and Apenten (2005) prepared ginger powder by extracting with acetone and showed that the stability of GE greatly improved with $t_{1/2}$ of 18 months at 5^{o}C. Bhaskar *et al*. (2006) reported that ginger powder obtained by treating fresh ginger rhizome with ethanol had a higher proteolytic activity compared to acetone and isopropanol treatments. Moreover, ethanol, being a generally recognized as safe (GRAS) chemical, is an added advantage. The authors also reported effectiveness in tenderizing breast and leg muscles of spent hen as indicated by lower shear values and higher sensory texture scores than control.

5.4 Tea catechins (TC)

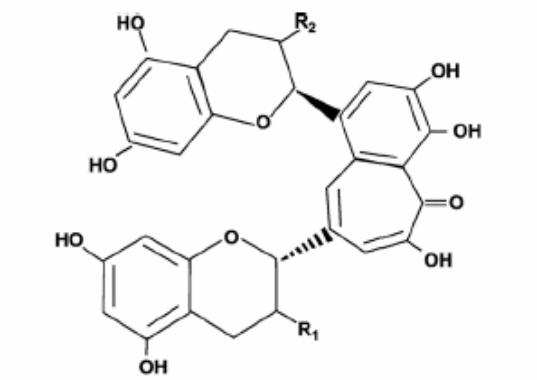
Lipid oxidation in muscle foods is one of the major degradative process responsible for loss in quality (Rhee *et al*., 1996; O'Grady *et al*., 2006), which may be delayed by the presence of antioxidants (Mason *et al*., 2005). Antioxidants are classified as compounds capable of delaying, retarding, or preventing autoxidation processes (Shahidi and Wanasundara, 1992). Synthetic antioxidants such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) are commonly used in the food industry (Wanasundara and Shahidi, 1998; Bozkurt, 2006). However, the use of synthetic antioxidants has been associated with potential health risks resulting in their strict regulation in food applications (Wanasundara and Shahidi, 1998). Antioxidant properties of natural plant and other food extracts are apparently related to their phenolic contents, thus, their antioxidant action is similar to synthetic phenolic antioxidants (McCarthy *et al*., 2001a). Recently, natural antioxidants have become a part of the diet in human nutrition with the aim of decreasing the risk of disease such as coronary heart disease, cancer (Higdon and Frei, 2003; Zandi and Gondon, 1999), and also diabetes (McCune and Johns, 2002). Moreover, natural antioxidants are reported to be more powerful than the synthetic antioxidants, especially, antioxidant from tea (Chen and Chan, 1996; McCarthy *et al*., 2001a; Tang *et al*., 2000; Tang *et al*., 2001a, b; Zandi and Gondon, 1999; Yanishlieva and Marinova, 2001; Bozkurt., 2006).

Tea is one of the most popular nonalcoholic beverages worldwide (Chen and Chan, 1996; Lee *et al*., 2000; Gamberucci *et al*., 2006; Bozkurt., 2006) and manufactured in three basic forms. Green tea is prepared by dehydration the young shoots of *Camellia sinensis* leaves, whereas black tea is the "fermented" product (Luczaj and Skrzydlewska, 2005). The third form of tea is oolong tea, which is less fermented than the black tea (Koo and Cho, 2004). The beneficial effects of tea are generally attributed to the antioxidant activity of their polyphenolic compounds or tea flavonoids (Majchrzak *et al*., 2004; Lambert and Yang, 2003) as shown in Figure 6.



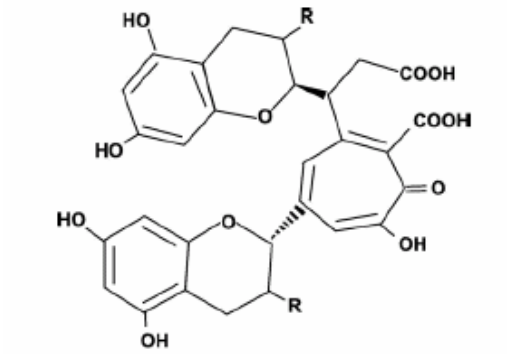
Epicatechin [EC]: $R_1 = R_2 = H$
Epigallocatechin [EGC]: $R_1 = H$; $R_2 = OH$
Epicatechin-3-gallate [ECG]: R_1 = Galloyl; $R_2 = H$
Epigallocatechin-3-gallate [EGCG]: R_1 Galloyl; $R_2 = OH$

(a)



Theaflavin [TF_1]: $R_1 = R_2 = OH$
Theaflavin-3-gallate [TF_2A]: R_1 = Galloyl; $R_2 = OH$
Theaflavin-3'-gallate [TF_2B]: $R_1 = OH$; R_2 = Galloyl
Theaflavin-3,3'-digallate [TF_3]: $R_1 = R_2$ = Galloyl

(b)



(R=Galloyl or other groups)

(c)

Figure 6. Structure of tea flavonoids: (a) catechins, (b) theaflavins, and (c) thearubigins.
Source: Lambert and Yang (2003).

Green tea is an excellent source of phenolic compounds, known as green tea catechins (GTCs or TC) (Pelillo *et al*., 2002; Majchrzak *et al*., 2004; Chen and Chan, 1996; Sang *et al*., 2003), while black tea is rich in thearubigins and theaflavins, which are dimers of catechins, formed by enzymatic oxidation during the manufacture of black tea (Majchrzak *et al*., 2004). Chen and Chan (1996) suggested that the beneficial effect of green tea over black tea was attributed to the content of TC. In general, green tea contains about 30% catechins (dry mass base), while black tea contains about 2-10% catechins, 2-6 % theaflavins and 10–20% thearubigins (Harbowy and Balentine, 1997 cited in Lee *et al*., 2000). Other compounds obtainable in green tea are the flavonols (quercetin, kaempferol and rutin), caffeine, phenolic acids (such as gallic acid, cumaric acid or caffeic acid), theanine, and flavor compounds (Graham, 1992; Pelillo *et al*., 2002). The major tea catechins are a mixture of epicatechin isomers, including (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) (Pelillo *et al*., 2002; Sang *et al*., 2003; Bonoli *et al*., 2003; Azam *et al*., 2004). Although the total green tea catechins extracted vary with variety of teas, methods used, and different laboratories, the composition of epicatechin derivatives is relatively consistent with EGCG being most abundant (>50%) followed by EGC (> 18%), EC (> 12%) and ECG (> 11%) (Graham, 1992; Ho *et al*., 1992; Wang *et al*., 1992; Chen and Chan, 1996). Many researchers reported that these substances showed a strong antioxidative (Ho *et al*., 1992; Wiseman, 1997; Wanasundara and Shahidi, 1998), antimicrobial properties (Diker *et al*., 1991; Hu *et al*., 2001; Sugita *et al*., 1999, anti-viral (Mukoyama *et al*., 1991), anti-carcinogenic and antimutagenic activity (Gao *et al*., 1994; Dashwood *et al*., 1999; Fujiki, 1999; Hamilton-Miller, 2001; Imai *et al*., 1997; Jankun *et al*., 1997; Kuroda and Hara, 1999; Lambert and Yang, 2003; Oguni *et al*., 1992) and anti-diabetic (Zeyuan *et al*., 1998).

Tang *et al*. (2002) stated that the powerful free radical-scavenging activity and some metal chelating effects of TC may be a mechanism for TC as potential antioxidants for meat and fatty foods. Thus, TC has received considerable attention in food products in recent years. Green tea extracts or green tea epicatechin derivatives have been used in vegetable oil (Chen and Chan, 1996; Zandi and Gondon, 1999; Yanishlieva and Marinova, 2001), marine oil (Wanasundara and

Shahidi, 1998), animal diets (Tang *et al*., 1998; Tang *et al*., 2000; Tang *et al*., 2001a, b; Tang *et al*., 2002; O'Grady *et al*., 2006), fish muscle model systems (He and Shahidi, 1997), cooked beef and chicken meat (Tang *et al*., 2000; Tang *et al*., 2001a), raw red meat, poultry, and fish muscle (Tang *et al*., 2001b), patties formed from fresh and previously frozen pork (MaCarthy *et al*., 2001b), minced raw beef (Maher *et al*., 2002; Tang *et al*., 2006) and found to be more effective to reduce the lipid oxidation than vitamin C, ∞-tocopherol, BHT, BHA or TBHQ.

The antioxidant potential of green tea extracts in meat systems was first reported by Shahidi *et al*. (1992 cited in Tang *et al*., 2006) who applied different types of tea extracts in a pig meat model system. However, the concentration and the form of TC used are different among meat and meat products. He and Shahidi (1997) reported that addition of TC at a concentration equivalent to 0.68 mmol/kg samples decreasing the formation of TBARS in a mackerel fish muscle and the potency of TC to inhibit the formation of TBARS was found to be stronger than that of BHT, BHA, TBHQ, and a-tocopherol. McCarthy *et al*. (2001b) reported that optimum concentrations of TC ranged from 0.25% to 1% (2500–10,000 mg/kg) in fresh and previously frozen pork patties. Jo *et al*. (2003) reported that raw pork patties with added 0.1% (1000 mg/kg meat) green tea extract powder had a higher Hunter a value, and sensory color of cooked pork patties was scored better with green tea extract powder than control during chilled storage. Maher *et al*. (2002) reported that addition of TC (1000 mg/kg muscle) to minced raw beef greatly improved color and lipid stability under aerobic and modified atmosphere conditions. In contrast, some investigators reported that TC at high level concentration cause discolorations in beef and chicken. Although TC at the level of 400 mg/kg meat inhibited lipid oxidation in beef and chicken meat greater than vitamin C, also found discoloration in beef and chicken meat caused by the same level of TC addition (Mitsumoto *et al*., 2005). Tang *et al*. (2006) found that TC added at a level of 200, 400, 600, 800 and 1000 mg/kg meat were effective in reducing lipid oxidation of fresh minced beef patties held in both aerobic and MAP conditions during 7 days of refrigerated storage. In addition, TC at a level of 200 mg/kg could improve color stability by delaying the formation of metmyoglobin and the reduction of oxymyoglobin, however, TC at levels of over 200 mg/kg did not give rise to such effects. Thus, several studies have focused on the use

of TC at the level lower than 400 mg/kg. Dietary TC, at the level of 200 mgTC/kg feed, possessed antioxidative effects equivalent to dietary α-tocopherol acetate at the same concentration for fresh and 1-year frozen-stored chicken meat (Tang *et al*., 2000; 2001c; 2002). When studied in cooked beef and chicken meat (Tang *et al*., 2001a) and in raw minced red meat, poultry and fish muscle (Tang *et al*., 2001b), TC at a level of over 300 mgTC/kg meat was reported to be more effective compared to vitamin E (300 mg α-tocopherol/kg meat) for inhibition of lipid oxidation.

Objectives

1. To determine quality characteristics of raw spent hen *Pectoralis major* muscles.
2. To study effect of NaCl and STPP on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled and frozen storage.
3. To study effects of different raw material pretreatments and heating methods on chemical and physical properties of cooked spent hen *Pectoralis major* muscles.
4. To improve quality of raw and cooked spent hen *Pectoralis major* muscles by using ginger powder and tea catechins.

CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Spent hen muscle

Spent hens (*H* and *M Brown Nick*) used throughout the study were obtained from Department of Animal Science, Faculty of Natural Resource, Prince of Songkla University. Spent hens raised under commercial farming system with the aged between 62-68 weeks (lived weights approximately 1.2-1.3 kg) used for study in section 1-4, 6-7 and spent hens aged between 74-80 weeks (lived weights approximately 1.2-1.3 kg) obtained from the same source were used for study in section 5.

1.2 Ginger rhizome

Five kg of the ginger rhizome (*Zingiber officinale* Roscoe) with the age between 10-12 months were obtained from Klong Reain Market, Hat Yai, Songkhla, Thailand.

1.3 Tea catechins

Tea catechins (Teanova^{TM} EGCg 70, purity of 84.44%) were obtained from Kinglong Natural Plant Industry Ltd., Changsha, Hunan, China.

1.4 Chemicals

Sodium tripolyphosphate (STPP) was purchased from High Science, Ltd. Partnership (Songkhla, Thailand) as Food Grade types. Other chemicals were analytical grade. Ammonium persulphate, copper (II) sulphate and trisodium citrate were obtained from Ajax Finechem (Wellington, Auckland, New Zealand). Glutaraldehyde, dithiothreitol, glycine, malondialdehyde tetrabutylammonium salt, L-hydroxyproline, L-tyrosine, and albumin from bovine serum were obtained from Fluka Chemical Corp. (Milwaukee, WI, USA). Trichloroacetic acid and *p*-dimethylamino-benzaldehyde were obtained from Carlo Erba Reagenti (Strada Rivoltana, Rodano, Italy). Sodium chloride, potassium chloride, calcium chloride, chloramine T and folin-ciocalteu's phenol were obtained from Merck Ltd. (Darmstadt, Germany). β-mercaptoethanol (β-ME) and 2-thiobarbituric acid were

obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS) and N,N,N',N'-tetramethyl ethylene diamine (TEMED) were obtained from Bio-Rad Laboratories (Hercules, CA, USA).

2. Equipments

Equipments used in this experiment were listed in Table 5.

Table 5. Equipments used in the experiment.

Equipments	Model	Company/City/Country
- pH meter	SevenGo SG2	Mettler Toledo, Schwerzenbach, Switzerland
- Homogenizer	D-500	Wiggen Hauser, Germany
- Water bath	W350	Memmert, Schwabach, Germany
- Refrigerated centrifuge	RC-5B plus	Sorvall, Norwalk CT, USA
- Colorimeter	ColorFlex	HunterLab Reston, VA, USA
- Texture analyzer	TA-XT2i	Stable Micro Systems, Surrey, UK
- Scanning electron microscope	JSW-5200	JEOL, Akishima, Japan
- Differential scanning calorimeter	DSC7	Perkin Elmer, California, USA
- Spectrophotometer	UV-16001	SHIMADZU, Kyoto, Japan

3. Methods

3.1 Study on the quality of raw spent hen *Pectoralis major* muscles

3.1.1 Sample preparation

Five spent hens were slaughtered by conventional neck cut, bled for 2 min, scalded at 60^{o}C for 2 min, plucked in a rotary-drum picker for 30 s and eviscerated. *Pectoralis major* muscles (breast muscles) were dissected from the carcasses and trimmed of obvious fat and connective tissue after chilling at 4^{o}C for 24 h. Ten fillets were taken randomly and subjected to chemical and physical analysis.

3.1.2 Analyses

3.1.2.1 pH measurement

Two grams of ground samples were used for pH determination. Samples were homogenized with distilled water at a ratio of 1:5 (w/v) at a speed of 11,000 rpm for 60 s using Wiggen Hauser D-500, Germany. The homogenate was subjected to pH measurement using a combined glass electrode pH meter (SevenGo SG2, Mettler Toledo, Switzerland).

3.1.2.2 Proximate analysis

Moisture was determined by oven method (AOAC, 1999), protein was determined by Kjeldahl method (AOAC, 1999), fat was analyzed by the Soxhlet apparatus method (AOAC, 1999), and ash was determined with a furnace 600^{o}C (AOAC, 1999).

3.1.2.3 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 homogenized with 10 volumes of solution A (15.6 mM Na_2HPO_4 and 3.5 mM KH_2PO_4, pH 7.5) at a speed of 11,000 rpm for 60 s. The homogenate was centrifuged at 7,500xg for 15 min with the Sorvall centrifuge (Sorvall, Norwalk CT, USA). 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 7,500xg for 15 min (the sarcoplasmic protein fraction). The filtrate is the non-protein nitrogen (NPN) fraction. The pellet was extraction with 10 volumes of solution B (0.45 M KCl, 15.6 mM Na_2HPO_4, and 3.5 mM KH_2PO_4, pH 7.5) using homogenizer at 11,000 rpm for 60 s and centrifuged at 7,500xg 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 0.1 M NaOH with continuous stirring overnight. The mixture was centrifuged and supernatant is the alkali-soluble protein fraction. The final residual is used as stroma protein fraction. The nitrogen content of all protein and non-protein fractions was determined by the Kjeldahl method (AOAC, 1999).

3.1.2.4 Determination of total collagen and soluble collagen content

Total collagen content was determined after acid hydrolysis according to the method of Palka and Daun (1999) as modified by Wattanachant *et al*. (2004). Finely ground muscle (1 g) was hydrolyzed with 25 ml of 6 M HCl at 110^{o}C for 24 h. After clarification with active carbon and neutralization with 10 N NaOH, the amount of hydroxyproline (Hyp) was determined as described by 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.

Soluble collagen of samples was determined according to the method of Liu *et al*. (1996) as modified by Wattanachant *et al*. (2004). Minced breast muscle (2 g) was homogenized for 1 min at 11,000 rpm in a 4-fold volume of ¼ strength Ringer's solutions. The homogenates were heated for 70 min at 77^{o}C and centrifuged for 30 min at 2,400xg at 4^{o}C. The supernatant was decanted, and then the pellet was suspended with the same solution and re-centrifuged. The supernatant solutions were combined and hydrolyzed in 6 N HCl for 24 h at 110°C. 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 total collagen (collagen content in sediment plus that in the supernatant).

3.1.2.5 Determination of myoglobin content

Myoglobin was measured by spectrophotometric method according to the method of Lee *et al*. (1998). Muscle samples (5g) were homogenized at 11,000 rpm for 2 min to fine slurry in 25 ml of cold 40 mM phosphate buffer, pH 6.8. The homogenized sample was allowed to stand for 1 h at 4^{o}C, and then centrifuged at 5,000 rpm for 30 min at 4^{o}C. The supernatant was filtered with Whatman number 1 filter paper into a 25 ml volumetric flask and made up to volume of 25 ml with 40 mM phosphate buffer. The myoglobin in the extract was determined by measuring absorbance at 525 and 700 nm using a spectrophotometer (SHIMADZU UV-16001, Japan). The myoglobin was calculated according to the equation mentioned by Krzywicki (1982):

total myoglobin (mg/ml) = (A_{525} - A_{700}) x 2.303 x dilution factor.

3.1.2.6 Fatty acid determination

The fatty acid composition of the muscle samples was determined after extraction 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).

3.1.2.7 Determination of cholesterol content

Cholesterol determination was carried out based on AOAC 994.10 (AOAC, 2005).

3.1.2.8 Cooking loss

Cooking loss of raw muscle samples was determined according to the method of Wattanachant *et al*. (2004). To prepare cooked muscles, small pieces (1.5x3.0x0.5 cm^3) of raw samples were cut, weighed, put in a tightly sealed plastic bag, and cooked in water bath at 80^oC for 10 min. After being cooked, the samples were cooled by immersing in an ice water bath for 2 min and then removed from the container, blotted with a paper towel, and reweigh to determine the cooking loss as a percentage of initial weight (w/w, wet basis).

3.1.2.9 Warner-Bratzler shear force measurement

Samples were cut (1.0x3.0x0.5 cm^3) paralleled with the muscle fibre at the middle portion of the fillets fiber for shear analysis using the Texture Analyzer equipped with a Warner-Bratzler shear apparatus (Stable Micro System, TA-XT 2i, UK). The operating parameters consisted of a cross head speed of 2 mm/s and a 25 kg load cell (Wattanachant *et al*., 2005b). The shear force perpendicular to the axis of muscle fibers was measured in eight replicates for each treatment. The peak of the shear force profile was regarded as the shear force value (kg).

3.1.2.10 Surface color measurement

Raw muscle slices were measured in eight replicates at the inner surface of breast muscle using a Hunterlab colorimeter (ColorFlex, Hunter Lab Reston, USA) and reported as the complete International Commission on Illumination (CIE) system color profiles of lightness (L*), redness (a*), and yellowness (b*).

3.1.2.11 Determination of thermal transition

Samples were ground and determined for thermal transition according to the method of Wattanachant *et al*. (2005a). Differential scanning calorimeter (DSC) was performed on a Perkin Elmer DSC7, California, USA. Minced muscle (10-15 mg) was weighed in aluminum pan which were then sealed with a crimper. In DSC studies, empty aluminum pans were used as a reference and for baseline corrections. Gallium (T=29.8°C, ΔH 80.22 J/g) and Indium (T=156.6°C, ΔH 28.46 J/g) were used as the standards for temperature and enthalpy calibration. The heating conditions were programmed and fully controlled by microcomputer over the range of 20-90°C. A heating rate was set at 10°C/min. The peak transition temperature (Tp) and enthalpy of transition (ΔH) were calculated using a Perkin Elmer Thermal Analysis Data Station. The determination was done in duplicate.

3.1.2.12 Determination of fiber diameter and sarcomere length

Fiber diameter and sarcomere length of spent hen muscle were determined from transverse section and longitudinal section of electron micrographs according to the method of Palka and Daun (1999) and Wattanachant *et al*. (2004).

3.2 Study on the effect of NaCl and STPP on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

3.2.1 Sample treatments preparation and storage condition

Thirty-two spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then randomly divided into 4 groups (16 fillets per groups = 64 fillets) of raw-untreated, cooked-untreated, raw-treated and cooked-treated samples. To prepare cooked-untreated samples, breast muscles were cooked with steam at atmospheric pressure until an internal temperature reached at 80^{o}C and then allowed to cool and gently blotted with a paper towel to remove surface moisture. The internal temperature was monitored by thermocouple inserted into the thickest part of the samples. Raw-treated samples were prepared by dipping each muscle for 15 seconds in solution containing 1% NaCl and 10 % STPP with muscle/solution ratio of 1:2 (w/v) and draining for 15 min on aluminum tray. To prepare cooked-treated samples, breast muscles were dipped into the same solution before cooking with steam using the same method as described previously.

All samples of each group were weighed, placed individually on the supporting mesh and put in the labelled polyethylene bags before sealing. Samples were kept in chilled room at 4^{o}C for 9 days. At 0, 3, 6, and 9 days of storage, four packs of each group were randomly taken for analysis.

3.2.2 Analyses

3.2.2.1 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 at speed of 11,000 rpm for 60 s with a homogenizer. The mixture was incubated at 4^{o}C for 15 min and centrifuged at 12,000xg for 5 min. TCA-soluble peptides in the supernatant were measured according to the methods of Lowry *et al*. (1951) and expressed as micromole of tyrosine per gram muscle.

3.2.2.2 Determination of metmyoglobin (MetMb)

MetMb was determined according to the method of Lee *et al*. (1998). Muscle samples (5g) were extracted with cold 40 mM phosphate buffer, pH 6.8 and then centrifuged using the same equipment and methods as described previously section 3.1.2.6. The supernatant was filtered, made up volume of 25 ml and subjected to absorbance measurement at 700, 572, and 525 nm by scanning the visible spectrum. The MetMb was calculated using the following equation (Krzywicki, 1982): MetMb (%) = {1.395-[(A572-A700) / (A525-A700)] x100

3.2.2.3 Determination of Thiobarbituric acid-reactive substances (TBARS)

Lipid oxidation of samples was quantified using thiobarbituric acid reactive substances (TBARS) determined spectophotometrically by method of Buege and Aust (1978). Butylated hydroxytoluene (0.08% in hexane) was added to 5 g samples (0.03 % by wt) to protect oxidation prior to homogenize with 25 ml of TBARS solutions (0.375% TBA, 15% TCA, and 0.25 N HCl) at speed of 11,000 rpm for 60 s. The mixture was heated for 10 min in boiling water (95-100^{o}C) to develop a pink color, cooled in an ice water bath and then centrifuged at 5,500 rpm for 25 min. The absorbance of the supernatant was measured at 532 nm using a

spectrophotometer. TBARS value was calculated from the standard curve of malondialdehyde (MDA) and expressed as μg MDA/g sample.

3.2.2.4 Determination of drip loss and weight loss

Drip loss of raw and weight loss of cooked samples were determined in four replicates by calculating the weight loss during chilling as a percentage of the initial weight as described by Woelfel *et al*. (2002) and Honikel (1998) with a slight modification. Each sample was weighed at the time before chilling (initial weight). After a storage period, samples were taken immediately from the containers, gently blotted dry and reweighed.

3.2.2.5 Determination of cooking loss as described previously in section 3.1.2.8.

3.2.2.6 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.2.2.7 Surface color measurement as described previously in section 3.1.2.10.

3.3 Study on the effect of NaCl and STPP on quality changes of raw and cooked spent hen *Pectoralis major* muscles during frozen storage

3.3.1 Sample treatments preparation and storage condition

Thirty-two spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then randomly divided into 4 groups (16 fillets per groups = 64 fillets) of raw-untreated, cooked-untreated, raw-treated and cooked-treated samples. Cooked, raw-treated, and cooked-treated samples were prepared using the same method as described previously in section 3.2.1.

All samples of each group were weighed, placed individually on the supporting mesh and inserted in the labelled polyethylene plastic bags before sealing. Samples were frozen at -18^{o}C for 24 h and then put in polystyrene bock before keeping in storage room at -18^{o}C for 6 months. At 0, 2, 4, and 6 months of storage, four packs of each group were thawed approximately 24 h at 4^{o}C prior to analysis**.**

3.3.2 Analyses

3.3.2.1 Determination of protein solubility

Protein solubility of muscle was determined by the procedure of Roussel and Cheftel (1990) with slight modification. Twenty ml of 0.6 M KCl (for myofibrillar protein) or 0.5 M NaOH (for total protein solubility) was added to 2 g of sample. The mixture was homogenized for 1 min at speed of 11,000 rpm and then agitated with a magnetic stirrer for 4 h at room temperature. Samples were centrifuged at 5000xg for 30 min at 4°C. The supernatant was defined as salt-soluble fraction, which is considered to be native proteins. Protein content was estimated by Biuret method (Robinson and Hodgen, 1940) using bovine serum albumin as standard. The solubility of protein was expressed as the percentage of the soluble protein, compared with total protein in the sample.

3.3.2.2 Determination of MetMb as described previously in section 3.2.2.2.

3.3.2.3 Determination of TBARS as described previously in section 3.2.2.3.

3.3.2.4 Determination of thawing loss

Thawing loss of samples was determined in four replicates by calculating the weight loss during frozen as a percentage of the initial weight as described by Woelfel *et al*. (2002) and Honikel (1998) with a slight modification. Each sample was weighed at the time before freezing (initial weight). After a storage period, samples were thaw approximately 24 h at 4^{o}C and taken from the containers, gently blotted with a paper towel prior to reweigh.

3.3.2.5 Determination of cooking loss as described previously in section 3.1.2.8.

3.3.2.6 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.3.2.7 Surface color measurement as described previously in section 3.1.2.10.

3.3.2.8 Determination of microstructure

Muscle structures of samples were determined using scanning electron microscopy (SEM) according to the procedure of Palka and Daun (1999). Pieces (1.0x1.0x0.5 cm^3) were excised from muscle samples and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 2 h at room temperature. The specimens were then rinsed with distilled water and dehydrated in graded ethanol solutions of 25%, 50%, 70%, 95%, and absolute ethanol (twice), with dehydration conducted for 1 h in each solution. The samples were cut in liquid nitrogen. Fragments of dried specimens were mounted on aluminum stubs and coated with gold. The specimens were examined and photographed in a SEM (JEOL, JSW-5200, Japan), using an accelerating voltage of 5 or 10 kV. The micrographs and videoprints were taken at a magnification of 500x (10 kV) for transverse sections and 10,000x (10 kV) for longitudinal sections. The areas of muscle fibers and the length of sarcomeres were measured on videoprints, using a special morphometric facility. Three video prints from each cooking treatments samples were taken for transverse sections and ten measurements of fibre area on each were made (n=30). The fiber diameter was calculated from the fiber area. Three videoprints from each muscle sample were taken for longitudinal sections and ten measurements of sarcomere length on each were made (n=30).

3.4 Study on the effects of different raw material pretreatments and heating methods on quality of cooked spent hen *Pectoralis major* muscles

3.4.1 Sample treatments preparation

Ninety-six spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then cut with the same size (4.0x6.0x1.0 cm^3). Samples were randomly divided into 3 groups (64 pieces per groups) of untreated, NaCl/STPP treated, and freeze-thawed samples. To prepare NaCl/STPP treated samples, pieces of muscles were weighed and immersed separately for 15 seconds in solution containing 1.0% NaCl and 10% STPP with muscles/solution ratio of 1:2 (w/v) and drained for 15 min on aluminum tray prior to process. Freeze-thawed samples were prepared by dipping pieces of muscles for 15 seconds in same solution. After drained, the samples were frozen in air blast freezer at -18^oC for 24 h and stored in freezer room at -18^oC. After 7 days of storage, the

samples were thawed by being kept in chilled room at 4°C for 24 h prior to freeze again. This freeze-thaw cycle were repeated twice (3 freeze-thaw cycles). Each group of sample was processed with dry and moist heat including; oven heating, steaming, boiling, and microwave heating (16 pieces per cooking methods).

(a) Oven heating

Samples were weighed, placed in baking pans on aluminum racks (one piece of muscle per pan) and then cooked in convection oven which were preheated to 200°C before cooking. The samples were heated until reaching an internal temperature of 80°C. Temperature was monitored by thermocouple inserted into the thickest part of the samples and the cooking time was recorded.

(b) Steaming

Samples were weighed and cooked with steam without pressure by putting the muscles on aluminum racks and placed in the stovetop steamer until an internal temperature of 80°C was obtained. Temperature was monitored and the cooking time was recorded as previous described.

(c) Boiling

Samples were weighed and cooked in boiling distilled water with muscles/distilled water ratio of 1:4 until an internal temperature of 80°C was obtained. Temperature was monitored and the cooking time was recorded as previous described.

(d) Microwave heating

Samples were weighed, placed in a Pyrex dishes (one piece of muscle per dish) and heated for 30 second in electric microwave oven on the high power setting. After cooking, a thermocouple was inserted into the thickest part of the samples and temperature was noted. If the temperature is less than 80°C, additional 5 second increments were applied to reach 80°C.

After heating all samples were allowed to cool and gently blotted with a paper towel to removed surface moisture prior to reweigh. Samples were randomly designated for physical/chemical analysis and sensory evaluation.

3.4.2 Analyses

3.4.2.1 Determination of protein solubility as described previously in section 3.3.2.1.

3.4.2.2 Determination of soluble collagen content as described previously in section 3.1.2.4.

3.4.2.3 Determination of cooking loss

The percentage cooking loss was determined by difference in weight before and after cooking following the method of Bhaskar *et al*. (2006).

3.4.2.4 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.4.2.5 Surface color measurement as described previously in section 3.1.2.10.

3.4.2.6 Determination of microstructure as described previously in section 3.3.2.8.

3.4.2.7 Sensory evaluation

Tenderness, flavor, and color of samples were evaluated by 30 panelists from Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, using a nine-point hedonic scale according to the method of Capita *et al*. (2000). The samples prepared from each cooking method were reheated for 30 s in electric microwave oven on the high power setting before serving to the panelists. For each panelist, two sample pieces of approximately equal size (1.0x1.0x1.5 cm^3) were cut randomly from muscle samples and placed in sample cups with three-digit coded numbers. Panelists were asked to assign scores for texture, color, flavor, and overall characteristics by a nine-point hedonic scale (9=like extremely; 8=like very much; 7=like moderately; 6=like slightly; 5=neither like nor dislike; 4=dislike slightly; 3=dislike moderately; 2=dislike very much; 1=dislike extremely). The scores from all panelists were averaged and the mean was be used as the score for each treated muscle sample.

3.5 Study on the effect of ginger powder (GP) on tenderization of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

3.5.1 Sample preparation

3.5.1.1 Ginger powder (GP)

Ginger powder was prepared according to the method of Adulyatham and Apenten (2005) and Bhaskar *et al*. (2006) with a slight modification. The ginger rhizome were cleaned off soil and extraneous matter by being soaked in water, peeled, and diced. Diced ginger were mixed with 0.2 % sodium ascorbate (w/w) and frozen at -18^{o}C until used. Samples were homogenized with chilled ethanol with solvent to material ratio of 4:1 (v/w) for 5 min using household blender and then filtered through 2 layers of muslin cloth. The residue was homogenized again with the same solution at speed of 11,000 rpm for 2 min using Wiggen Hauser D-500, Germany. The obtained residue was collected and followed by air drying for 5 h. The dried residues were powdered and sieved (mesh No. 25 and 35) prior to use. The yield (as percentage of fresh cleaned and peeled ginger rhizome) of GP was determined. Moisture content was determined by the oven method (AOAC, 1999). The proteolytic activity of GP was determined following the method of Thompson *et al*. (1973) as modified by Bhaskar *et al*. (2006).

3.5.1.2 Muscle samples and storage condition

Sixty-four spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then each muscle was cut into 2 pieces with the same size (4.0x6.0x1.0 cm^3). Muscle samples were randomly divided into 4 groups of untreated sample and treated samples with different holding time in dissolved GP. The samples were prepared by immersing muscles separately in water (1:1 w/v) containing 2.5% GP solution (the unit activity of GP was recorded) and held in solution for 1 and 2 h at room temperature (RT) and for 24 h at 4^{o}C. The samples were drained for 15 min on aluminum tray and reweighed**.** Each group of sample was subdivided into 2 groups of raw and cooked samples. Cooked samples were prepared using the same method as described previously in section 3.3.1.

All samples of each group were weighed, placed individually on the supporting mesh and inserted in the labelled polyethylene bags before sealing. Samples were kept in chilled room at 4^{o}C for 9 days. At 0, 3, 6, and 9 days of storage, four packs of each group were randomized for analysis.

3.5.2 Analyses

3.5.2.1 Estimation of proteolytic activity of GP

The proteolytic activity of GP was determined following the method of Thompson *et al*. (1973) with minor modification by Bhaskar *et al*. (2006). The GP was homogenized at a speed of 11,000 rpm for 5 min in 20 parts (w/v) of 0.2 M phosphate buffer of pH 6.0 containing 0.3 mM dithiothreitol (DTT). The extract was filtered into a resulting filtrate of 25 ml with 0.2 M phosphate buffer containing 0.3 mM DTT. Aliquots of this enzyme extract were used for the estimation of protein content by the Biuret method (Robinson and Hodgen, 1940) and proteolytic activity. Two ml of 1% bovine serum albumin (BSA) solution prepared in 0.2 M phosphate buffer (pH 6.0) were mixed with 500 µl enzyme extract and allowed to stand at 30 ± 2^{o}C for 15 min. Reaction was terminated by the addition of 3 ml of 5% (w/v) trichloroacetic acid (TCA) solution, cooled in a 10^{o}C water bath for 5 min, filtered though a Whatman filter paper No. 1 and the TCA-solution peptides quantified by measuring optical density (OD) at 280 nm. A blank was prepared by the addition of TCA solution before the enzyme extract. Specific activity of the enzyme extract was expressed as units per mg of protein in GP (1 unit = 0.01 OD increase/min).

3.5.2.2 Determination of protein solubility as described previously in section 3.3.2.1.

3.5.3.3 Determination of soluble collagen content as described previously in section 3.1.2.4.

3.5.3.4 Determination of cooking loss and weight loss

Cooking loss of raw samples was determined according to the method of Wattanachant *et al*. (2004) as described previously in section 3.1.2.8, whereas weight loss of cooked samples was determined following the method of Woelfel *et al*. (2002) as described previously in section 3.2.2.4.

3.5.3.5 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.5.3.6 Surface color measurement as described previously in section 3.1.2.10.

3.5.3.7 Sensory evaluation

Tenderness, flavor, and color of cooked samples were evaluated by 30 panelists using a nine-point hedonic scale according to the method of Capita *et al*. (2000).

3.6 Study on the effect of tea catechins (TC) on oxidative stability of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

3.6.1 Sample treatments preparation

Sixty-four spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then cut with the same size (4.0x6.0x1.0 cm^3). Samples were randomly assigned to one of the following 4 groups; control, TC100 (meat plus 100 mg TC/kg muscle), TC150 (meat plus 150 mg TC/kg muscle) and TC200 (meat plus 200 mg TC/kg muscle). To ensure even distribution of the additive throughout the pieces of meat, TC was freshly dissolved in boiled water (100, 150 or 200 mg antioxidant/10 ml water for 1 kg muscle) and sprayed as a fine aerosol on the both side of the pieces. Each group of sample was subdivided into 2 groups of raw and cooked samples. Cooked samples were prepared using the same method as described previously in section 3.2.1.

All samples of each group were weighed, placed individually on the supporting mesh and inserted in the labelled polyethylene plastic bags before sealing. Samples were kept in chilled room at 4^{o}C for 9 days. At 0, 3, 6, and 9 days of storage, four packs of each group were randomized for analysis.

3.6.2 Analyses

3.6.2.1 Determination of TBARS as described previously in section 3.2.2.3.

3.6.2.2 Determination of cooking loss and weight loss as described previously in section 3.5.3.5.

3.6.2.3 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.6.2.4 Surface color measurement as described previously in section 3.1.2.10.

3.6.2.5 Sensory evaluation

Oxidized odor was evaluated by 30 experienced panelists using a six-point category scale (1= very strong, 2= strong, 3= moderately strong, 4=moderate, 5= just detectable, 6= not detectable) according to the intensity scale for evaluation off flavors in meat by Tarladgis *et al*. (1959 cited in Melton *et al.,* 1987). The panelists were explained for standard scores of oxidized odor. To prepare standard odor scores, subcutaneous fat kept at 4^{o}C for 7 days was blended with distilled water at the ratio of 1:1 (w/v), kept in screw caps which corresponding to very strong oxidized odor sample, while distilled water was used as not detectable sample. The strong, moderately strong, moderate, and just detectable oxidized odor samples were prepared by dilution the suspension of very strong oxidized odor sample with distilled water at a ratio of 1:1, 1:2, 1:3, and 1:4 (v/v), respectively.

3.7 Study on the combination effect of NaCl, STPP, GP, and TC on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

3.7.1 Sample treatments preparation

Thirty-two spent hens were prepared for *Pectoralis major* muscles using the same methods as described previously in section 3.1.1 and then cut with the same size (4.0x6.0x1.0 cm^3). Samples were randomly and assigned to one of the following 2 groups; untreated and treated with additives (GP, NaCl/STPP and TC). The best treatment of GP and TC was chosen from section 5 and 6. Samples were weighed before treating with additives. To prepare meat samples with additives, pieces of muscles were treated with GP using the same methods in section 3.5.1.2 before treating with NaCl and STPP as described previously in section 3.2.1 and followed by TC treated as described previously in section 3.6.1. Each group of sample was subdivided into 2 groups of raw and cooked samples. Cooked samples were prepared using the same method as described previously in section 3.2.1.

All samples of each group were weighed, packed and sealed as described previously in section 3.2.1 before keeping in chilled room at 4^{o}C for 9 days. At 0, 3, 6, and 9 days of storage, four packs of each group were randomized for analysis.

3.7.2 Analyses

3.7.2.1 Determination of protein solubility as described previously in section 3.3.2.1.

3.7.2.2 Determination of soluble collagen content as described previously in section 3.1.2.4.

3.7.2.3 Determination of TBARS as described previously in section 3.2.2.3.

3.7.2.4 Determination of cooking loss and weight loss as described previously in section 3.5.3.5.

3.7.2.5 Warner-Bratzler shear force measurement as described previously in section 3.1.2.9.

3.7.2.6 Surface color measurement as described previously in section 3.1.2.10.

3.7.2.7 Sensory evaluation

Texture, color, and flavor of cooked samples were evaluated by 30 panelists using a nine-point hedonic scale according to the method of Capita *et al*. (2000).

4. Statistics Analysis

The completely randomized design (CRD) was applied for all experiments. The samples of each treatment and replication were randomly taken for physical analysis in eight replicate determinations and for chemical evaluation in triplicate determinations. Data were analyzed statistically using analysis software computer as one way ANOVA. Significant difference between treatments was analyzed by Duncan's multiple range tests (Stell and Torrie, 1980). Significance was accepted at the 5% probability level.

CHAPTER 3

RESULTS AND DISCUSSION

1. Proximate composition, chemical and physical properties of raw spent hen *Pectoralis major* muscles

Proximate composition of raw spent hen *Pectoralis major* muscles are shown in Table 6. Moisture, protein, and ash contents were in the range of chicken *Pectoralis* muscle as reported previously by many investigators (Xiong *et al*., 1993; Smith *et al*., 1993; Van Heerden *et al*., 2002; Al-Najdawi and Abdullah, 2002; Qiao *et al*., 2002b; Wattanachant *et al*., 2004) and were consistent with the early reported of Chuaynukool *et al*. (2007) and Liwa (2009) who showed that spent hen breast meat consisted of moisture, protein, and ash content between 74.64-74.83 %, 20.34-20.50 % and 0.19-1.19 %, respectively. The fat content was higher than those reported in broiler at 0.68 - 2.78 % (Wattanachant *et al*., 2004; Qiao *et al*., 2002b; Aberni and Bergoglio, 2001), and in spent hen breast meat at 0.89 - 1.64 % (Chuaynukool *et al*., 2007; Liwa, 2009) probably due to difference in breeds and the old age of spent hens used in this study. This finding seemed reasonable because it is generally recognized that fat content increase with age (Mountney and Parkhurst, 1995; Ngoka *et al*., 1982). The results performed that spent hen *Pectoralis major* muscle had similar nutritional value to commercial broiler meat except higher fat content.

The low content of cholesterol from current result (Table 6) was consistent with earlier observations in breast muscle of spent hen at 34.29 mg/100 g by Al-Najdawi and Abdullah (2002), 43 mg/100 g by Jantawat and Dawson (1980), 52.98 mg/100g by Liwa (2009), and between 50.07-62.64 mg/100 g by Ajuyah *et al*. (1992). The cholesterol content found in spent hen meat were lower than early report in breast muscle of broiler at 81 mg/100 g by Ang and Hamm (1982). This might confirm health promoting benefits from spent hen meat.

Protein compositions from raw spent hen *Pectoralis major* muscles are shown in Table 6. The spent hen muscle had lower sarcoplasmic protein but higher myofibrillar and stroma protein as compared with broiler muscle reported previously

by Wattanachant *et al*. (2004) who found that broiler breast muscle comprised of 43.45 % myofibrillar and 48.83 % sarcoplasmic protein, respectively. This was probably due to the older age of spent hen. Higher fraction of stroma protein in spent hen meat at 8.01 % compared with broiler between 1.50-1.81% (Lan *et al*., 1995; Wattanachant *et al*., 2004) might result in low functional properties of spent hen meat.

Myoglobin content of raw spent hen *Pectoralis major* muscle (Table 6) was observed a higher when compared with broiler *Pectoralis* as reported previously (between 0.1-0.17 mg/g muscle) (Nishida and Nishida, 1985; Boulianne and King, 1995) and in good agreement with those found in spent hen breast muscle at 3.99 % by Liwa (2009). It was probably due to the higher age of spent hen and difference in breed and feed of animals caused difference in Mb content of muscle (Miller, 1994). However, it was not shown any differences in color value L*, a*, and b* of spent hen *Pectoralis major* muscle (Table 6) compared to those of broiler *Pectoralis* muscle, 52.51, -1.18, and 6.96, respectively (Wattanachant *et al*., 2004).

The collagen content of spent hen *Pectoralis major* muscle in this study (Table 6) was observed a higher than broiler *Pectoralis* as reported previously between 1.27-3.86 mg/g (Smith *et al*., 1993; Ruantrakool and Chen, 1986; Wattanachant *et al*., 2004) and at 5.09 mg/g for Thai indigenous chicken muscle aged 16 weeks by Wattanachant *et al*., (2004). Differences in collagen content could be attributed to differences in the age of birds at the time of slaughter (Dawson *et al*., 1991). Trindade *et al*. (2004) stated that high contents of collagen in any meat can negatively influence its technological and nutritional characteristics, since collagen is a protein with inferior functionality and low nutrition value because of its poor balance of amino acids. Moreover, it has also been shown that the solubility of collagen decreases with increased collagen cross-linking and cross-linking increases as the animal ages (Pearson and Young, 1989; Foegeding and Lanier, 1996). From the result, therefore spent hen had more highly cross-linked collagen as indicated by the much lower soluble collagen content, (18.76 % of total collagen) compared to that at 31.38 % of total collagen content for younger broiler muscle (aged 38 days) in previous study by Wattanachant *et al*. (2004). The lower soluble collagen content of spent hen breast muscle at 15.23 % of total collagen was recently reported by

Wongwiwat (2009).The highly cross-linked collagen might result in low functional properties of spent hen meat.

The fiber diameters of raw spent hen *Pectoralis major* muscles (Table 6) was higher with those found in spent hen breast muscle at 18.74 μm by Chuaynukool *et al*. (2007). The average diameters of chicken white fibers were variously reported as 38 to 46 μm (Smith and Fletcher, 1988), 32.6 μm (Smith *et al*., 1993) and 26.6 μm (Wattanachant *et al*., 2004) for broiler and 28.9 μm for Thai indigenous chicken muscle aged 16 weeks by Wattanachant *et al*., (2004). These differences in muscle fiber diameter may be due to the differences in age, rate of rigor onset, and degree of sarcomere shortening (Smith and Fletcher, 1988). As shown in Table 6, the sarcomere length of raw spent hen *Pectoralis major* muscles was in consistent with previous report of Chuaynukool *et al*. (2007) at 1.62 μm. The sarcomere lengths of chicken white muscles were reported at 1.64 μm for broiler and at 1.61 μm for Thai indigenous chicken muscle by Wattanachant *et al*. (2004).

The ultimate pH of raw spent hen *Pectoralis major* muscle (Table 6) was in the range of an average ultimate pH of poultry breast muscle, 5.76-6.10 (Kahn and Nakamura, 1970; Rasmussen and Mast, 1989; Xiong *et al*., 1993; Qiao *et al*., 2002a; and Wattanachant *et al*., 2004; Wongwiwat, 2009; Liwa, 2009).

As shown in Table 6, shear force value obtained from spent hen muscle (2.6 kg) showed double higher than that of broiler *Pectoralis* (1.20 kg) in previous report of Wattanachant *et al*. (2004). This was supported by more stroma protein content and highly cross-linking collagen found in older age of spent hen as discussed earlier. Wattanachant *et al*. (2004) stated that 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 structure elements. This may partially explain why meat from older animals is tougher than that from younger animals, even though muscle from younger animals generally contain more collagen (Pearson and Young, 1989; Foegeding and Lanier, 1996).

Cooking loss of raw spent hen *Pectoralis major* muscle (Table 6) found in current study was in good agreement with those reported in broiler *Pectoralis major* muscles at 19.93 % as reported by Wattanachant (2003).

Table 6. Chemical composition, pH, C.I.E. values, and shear force value of raw spent hen *Pectoralis major* muscles.

Quality characteristics	
Moisture (%)	74.16 ± 0.21
Protein (%)	22.34 ± 0.25
Fat (%)	3.11 ± 0.59
Ash (%)	1.19 ± 0.07
Cholesterol content (mg/100g)	34.60 ± 0.01
Protein composition	
- *Myofibrillar protein (%)*	55.53 ± 0.33
- *Sarcoplasmic protein (%)*	34.11 ± 0.50
- *Stroma protein (%)*	8.01 ± 0.84
- *Alkali soluble (%)*	2.35 ± 0.03
Myoglobin content (mg/ g sample)	3.47 ± 0.06
Total Collagen (mg/g muscle)	6.47 ± 0.20
Soluble collagen (% of total collagen)	18.76 ± 0.81
Fibre diameter (μm)	32.78 ± 2.80
Sarcomere length (μm)	1.66 ± 0.21
pH	5.78 ± 0.11
L*	52.26 ± 1.29
a*	-2.86 ± 0.46
b*	7.19 ± 2.60
Shear force value (kg)	2.60 ± 0.81
Cooking loss	19.43 ± 2.32

Data are presented as mean ± standard deviation.

N=3 for chemical properties, n=8 for physical properties.

The fatty acid composition of raw spent hen *Pectoralis major* muscle (Table 7) was generally similar to those of fatty acid composition in poultry muscle reported by Smith *et al*. (1993), Qiao *et al*. (2002b), and Wattanachant *et al*. (2004). The results was also in good agreement with Liwa (2009) who reported that spent hen breast meat contained 35.67% saturated fatty acids (SFA), 44.57% monounsaturated fatty acids (MUFA), and 19.79% polyunsaturated fatty acids (PUFA), respectively. In addition, the high contents in palmitic acid, stearic acid, oleic acid, and linoleic acid found in current study were similar with those found in spent hen breast meat reported by Liwa (2009). Moreover, the content of linoleic acid (essential fatty acid) obtained in this study was higher than those reported in *Pectoralis muscle* of broiler at 7.63 (% of total fatty acid) by Wattanachant *et al*. (2004) and between 15.62-15.99 (%) by Qiao *et al*. (2002b) elucidated to the high nutritional value of spent hen meat. However, meat from spent hen contained a higher percentage of total MUFA and total PUFA and a lower percentage of SFA as compared with broiler in previous report of Wattanachant *et al*. (2004). Huton (1995) noted that high unsaturated fat intakes may be preferable for human; however, unsaturated fatty acids are more prone to oxidation. This result indicated that meat from spent hen may promote faster oxidation than broiler during processing and storage.

The onset temperature of transition (T_o), maximum thermal transition (T_{peak}) temperatures, and denaturation enthalpy (ΔH) were determined for raw spent hen *Pectoralis major* muscle. Five endothermic peaks were obtained at 55.92^{o}C, 62.58^{o}C, 65.08^{o}C, 72.75^{o}C, and 77.83^{o}C. These results were consistent with earlier observations in broiler *Pectoralis* muscle at 54.88^{o}C, 61.66^{o}C, 65.37^{o}C, 70.63^{o}C, and 76.14^{o}C by Wattanachant *et al*. (2004). Water-washed myofibrillar proteins from broiler *Pectoralis minor* muscle have been reported to produce two major thermal transitions at 55.2^{o}C and 77.7^{o}C and corresponded with pure myosin and actin at 57.9^{o}C and 80.8^{o}C, respectively (Kijowski and Mast, 1988). Therefore, the first and the fifth peaks from these results were most likely belonging to myofibrillar proteins. For sarcoplasmic proteins, only one peak (second peak) in current study was in agreement with Kijowski and Mast (1988) who found 2 main heat transitions at 62.3^{o}C and 68.3^{o}C. Difference results perhaps caused by the authors collected sarcoplasmic proteins as the drip from thawed muscle, which had been frozen

at -20^{o}C for 24 h. However, sarcoplasmic proteins of chicken breast muscle have also been reported at 72^{o}C by Wang and Smith (1994) and 71.9^{o}C by Murphy *et al*. (1998). Conversely, Kijowski and Mast (1988) stated that a minor heat transitions at 72.8^{o}C in broiler *Pectoralis minor* muscle could be contributed to thin filaments, such as tropomyosin, troponin, actinin, as well as the other intracellular proteins (connectin). These can be attributed with peak four of the present study. Stroma proteins isolated from breast muscle have been reported at 65.3^{o}C by Kijowski and Mast (1988), and 65.37^{o}C by Wattanachant *et al*. (2004) which was probably associated with the third peak in current result.

Table 7. Fatty acid composition (% of total fatty acids) of raw spent hen *Pectoralis major* muscles.

Fatty acid	% of total fatty acids
Lauric acid (C12:0)	0.60 ± 0.00
Myristic acid (C14:0)	0.90 ± 0.10
Palmitic acid (C16:0)	24.60 ± 0.20
Palmitoleic acid (C16:1)	1.40 ± 0.01
Stearic acid (C18:0)	8.00 ± 0.10
Oleic acid (C18:1)	31.40 ± 0.20
Linoleic acid (C18:2)	17.50 ± 0.10
Linolenic acid (C18:3)	0.40 ± 0.01
Eicosanoic acid (C20:0)	0.10 ± 0.01
Eicosenoic acid (C20:1)	0.20 ± 0.10
[1]SFA	**34.27±0.41**
[2]MUFA	**50.95±0.31**
[3]PUFA	**17.90±0.11**

Data are presented as mean ± standard deviation from duplicate determinations.

[1]SFA = Total saturated fatty acids (C12:0, C14:0, C16:0, C18:0, and C20:0).

[2]MUFA = Total monounsaturated fatty acids (C16:1, C18:1, and C20:1).

[3]PUFA = Total polyunsaturated fatty acids (C18:2 and C18:3).

2. Effect of NaCl and STPP on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

2.1 Changes in chemical properties

2.1.1 TCA-soluble peptides

Proteolysis of raw spent hen *Pectoralis muscle* treated with NaCl and STPP (raw-treated) and untreated samples (raw-untreated) during chilled storage was evaluated by TCA-soluble peptides (Table 8). Significant differences between both samples in TCA-soluble peptides were not found over storage time ($P \geq 0.05$) although raw-treated samples exhibited slightly higher values than did raw-untreated samples. At the beginning of chilled storage, no change on TCA-soluble peptides of both samples was observed ($P \geq 0.05$), while after day 6 of storage TCA-soluble peptides of both samples increased gradually ($P < 0.05$). It was probably due to the proteolysis in meat during the initial stages caused by endogenous proteases in skeletal muscles (Ouali, 1990; Koohmaraie, 1992; Walker *et al*., 1995), whereas proteases of microorganism might be involved with the extended storage time (Allen *et al*., 1998).

2.1.2 MetMb content

The MetMb (oxidized state=Fe^{3+}) is an oxidized form of Myoglobin (reduced state=Fe^{2+}) that can be generated during refrigerated storage (Chaijan *et al*., 2005). The formation of MetMb in spent hen *Pectoralis major* muscles treated with NaCl and STPP (raw-treated) and untreated samples (raw-untreated) during chilled storage are shown in Figure 7. No change in MetMb content was observed in both samples up to day 3 of chilled storage ($P \geq 0.05$). A gradual increase in MetMb content of raw-treated muscle samples was found when the storage time increased from 3 to 9 days, whereas MetMb content of raw-untreated increased sharply on day 6 ($P < 0.05$) and then approached a plateau thereafter. The sharp increase in MetMb formation of raw-untreated with extended storage time suggested that myoglobin underwent more oxidation. The constant of MetMb formation in raw-untreated at the end of storage time was probably caused by the lower myoglobin content remaining or bound in the muscle which was in concomitant with the increasing of drip loss (Figure 9) resulted in more water loss from muscle leading to more Mb release and less Mb to continue the formation of MetMb. The addition of STPP to meat has been shown to have antioxidation properties by chelating the heavy metal ions and keeping a part of the

iron in the reduced state (Stoick *et al*., 1991; Shahidi *et al*., 1987), resulting in a gradual increase of MetMb content in raw-treated samples throughout storage time.

Table 8. TCA soluble peptides (mmol tyrosine/g muscle) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time(days)	TCA soluble peptides (mmol tyrosine/g muscle)	
	Raw-untreated	Raw-treated
0	0.0965±0.0038 [a, x]	0.1150±0.0052 [a, x]
3	0.1009±0.0018 [a, x]	0.1160±0.0185 [a, x]
6	0.1242±0.0101 [b, x]	0.1420±0.0096 [b, x]
9	0.1782±0.0004 [c, x]	0.1571±0.0097 [b, x]

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

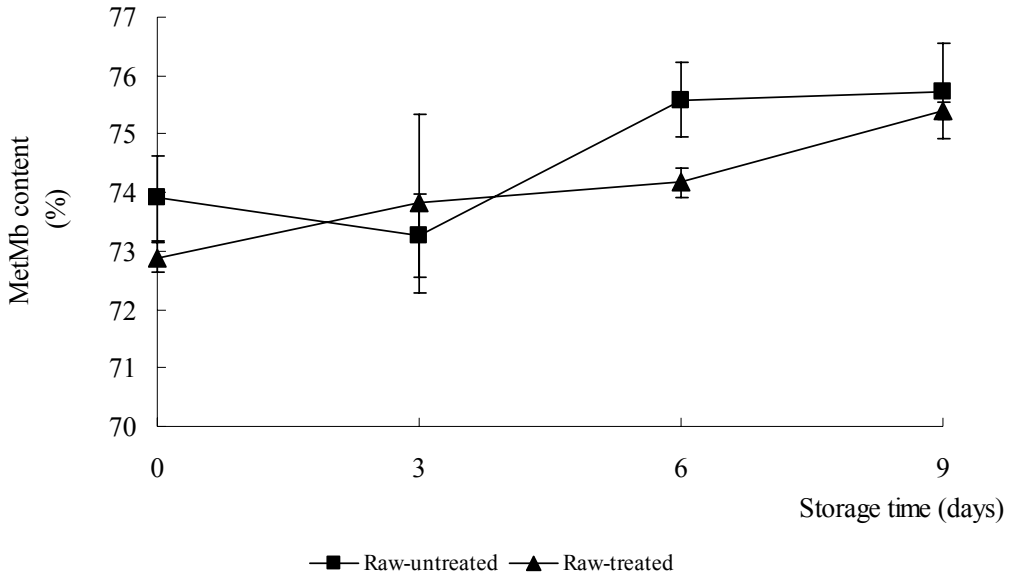


Figure 7. Changes in MetMb content (%) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for triplicate determinations.

2.1.3 TBARS

The 2-thiobarbituric acid reactive substances (TBARS) assay is the most widely used to measure lipid oxidation in meat which determines malondialdehyde (MDA) content (Broncano *et al*., 2009). TBARS values of spent hen *Pectoralis major* muscles treated with NaCl and STPP (raw-treated/cooked-treated) and untreated samples (raw-untreated/cooked-untreated) are presented in Figure 8. Storage time had influence on lipid oxidation of raw spent hen meat as elucidated by an increase in TBARS values of both samples during chilled storage. As storage time increased from day 0 to day 6, a dramatic increase in TBARS values of both raw-untreated and raw-treated muscle samples were found ($P<0.05$). Thereafter, no change in TBARS values of both samples was observed ($P\geq0.05$). An increase in TBARS values during chilled storage have been reported in chicken, beef, and turkey by Ang (1988), Ahn *et al*. (1993), and Rhee and Ziprin (2001). Lipid oxidation in meat is initiated when polyunsaturated fatty acids (PUFA) react with molecular oxygen, via a free radical chain mechanism, forming peroxides (Gray, 1978). The primary auto-oxidation is followed by a series of secondary reactions which lead to degradation of the peroxidized fatty acid. The susceptibility of meat to oxidative process depends on several factors and one of the most important being the level of highly oxidizable substrates, such as PUFA in the phospholipids fraction of cell membranes (Luciano *et al*., 2009); where lipid oxidation is initiating (Buckley *et al*., 1995). The rapid increase in TBARS values in current study probably due to high content of polyunsaturated fatty acids in spent hen meat that containing more double bonds in molecules leading to sensitive to become free radicals when activated by prooxidant compounds. In addition, high phospholipids fraction in spent hen meat could enhance lipid oxidation during chilled storage. Higher content of phospholipids fraction in spent hen breast meat (21.03%) compared with (5.71%) in Thai indigenous chicken have been reported by Liwa (2009). Moreover, some chemical composition in meat such as heme pigment could effect on lipid oxidation during chilled storage. The primary catalysts of lipid oxidation in skeletal muscle have been suggested to be hemoprotein and iron (Igene *et al*., 1979a). Recently studied in different breeds of chicken meat by Liwa (2009) found that the increase of MDA content during chilled storage when extended storage time from 0 to 6 days in each meat sample associated with the

increase in non-heme iron content and metmyoglobin formation, and relating to the decrease in heme iron and myoglobin content of chicken meat. The high content of myoglobin in spent hen meat compared to other chicken meat as mentioned earlier might result in high prooxidant compounds in meat system that could directly stimulated lipid oxidation during chilled storage. Adding NaCl and STPP had little effect on retarding lipid oxidation of raw spent hen meat during chilled storage as indicated by raw-untreated samples exhibited higher TBARS values than did in raw-treated muscle samples on day 6 of chilled storage ($P<0.05$), while no significant differences between both samples was found on other days of storage ($P\geq0.05$).

As expected, cooked samples exhibited higher TBARS values than raw samples because when meat is cooked and exposed to atmospheric conditions, it becomes rapidly oxidized. Cooking not only disrupts the membrane structure but also facilitates release of iron from the iron carrier proteins or iron storage conditions (Ahn *et al*., 1993). Under such conditions oxygen can directly react with the ferrous iron in the meat and activate the ground state oxygen to a highly reactive form (Halliwell and Gutteridge, 1990) resulted in rapid oxidation leading to high TBARS value in cooked meat.

For cooked samples, at the beginning of storage (day 0-3), no significant difference in TBARS values of both treated and untreated samples was found ($P\geq0.05$), thereafter muscle samples treated with NaCl and STPP showed lower in this values than did found in untreated samples ($P<0.05$) indicated that NaCl and STPP treated could retard lipid oxidation in cooked spent hen meat during long time of storage. This could be caused by STPP can act like antioxidant as described previously. Storage time also had influence on lipid oxidation of cooked spent hen meat as elucidated by a dramatic increase in TBARS values of both samples were found from day 0 to day 6 ($P<0.05$) and then approached a plateau thereafter. No change in TBARS value of both raw and cooked samples when extended storage time perhaps caused by during prolonged storage removal or losses of MDA that might have occurred due to microbial growth/metabolism (Rhee and Ziprin, 2001) and the reaction of MDA with proteins or to MDA polymerization reactions (Whang *et al*., 1986; Wen *et al*., 1996).

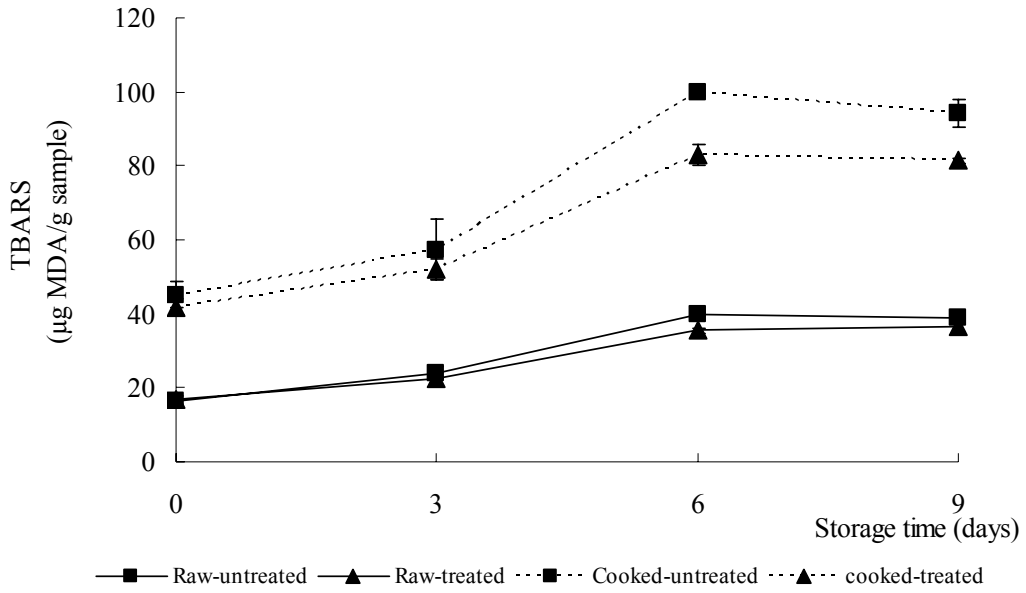


Figure 8. Changes in TBARS (µg MDA/g sample) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for triplicate determinations.

2.2 Changes in physical properties

2.2.1 Drip loss, weight loss, and cooking loss

Drip loss was measured to obtain an overall assessment of the water binding properties of muscle. As expected, increasing storage time caused increasing in drip loss of both raw-untreated and raw-treated samples ($P<0.05$) (Figure 9). Similar trend of increasing drip loss during chilled storage of raw breast broiler muscles was also reported by Allen *et al*. (1998). NaCl and STPP treated had influence on drip loss of spent hen meat as indicated by raw-untreated samples exhibited higher drip loss than did in raw-treated samples ($P<0.05$). This probably caused by treated samples with NaCl and STPP performed more binding water during storage than did in untreated samples resulted in more water retention in muscle samples leading to lower drip loss. Combinations of NaCl and STPP have been reported to synergistically improve moisture absorption and WHC of poultry meat (Young *et al*., 1996). The addition of STPP to meat systems increased the meat pH (Jone *et al*., 1987; Sutton *et al*., 1997) that moving the meat pH further from the meat protein isoelectric point (about 5.5 in chickens) (Miller, 1998) and an increase in

negative charge of protein molecule resulting in higher WHC (Zayas, 1987). In addition, NaCl acts to improve WHC by increasing ionic strength of meat proteins (Miller, 1998; Rhee and Ziprin, 2001; Zayas, 1987) and contributes to the swelling of the proteins (Miller, 1998; Zayas, 1987). The swelling provides a higher number of protein side chains that can bind water and therefore WHC increased.

For cooked samples, the same trend was observed by increasing in weight loss as storage time increased (Figure 10). However, cooked-treated samples showed higher weight loss than cooked-untreated samples ($P<0.05$). This was probably caused by more water retention in treated muscle samples was carried out during cooking than did in untreated samples. In addition, the treated samples might be more structure changes and expelled more fluid from the muscle fibers due to heat denaturation. Larik and Turner (1992) stated that WHC of muscle tissue was related to the extent of heat denaturation of myofibrillar proteins during thermal processing that leading to structure changes and expelled the sarcoplasmic fluid from the muscle fibers, resulting in water loss from meat tissue (Murphy and Mast, 2000).

Cooking loss is another parameter to evaluate WHC and an increase in cooking loss was related to low WHC of proteins due to their denaturation (Zayas, 1987). Because of muscle protein changes with heating, water content within the muscle in the narrow channels between the filaments changes as meat shrinks (Bertola *et al*., 1994), resulting in cooking loss with heating. The data from Figure 10 showed that cooking loss of raw-untreated samples increased gradually throughout chilled storage, whereas that of raw-treated samples slightly increased on day 3 and then decreased gradually until 9 days of storage. It was probably due to degradation of myofibrillar and sarcoplasmic proteins via proteolysis process during chilled storage as indicated by the increase in TCA-soluble peptides (Table 8). Protein degradation resulted in low property of proteins to hold water in muscles and exhibited increase in cooking loss during storage in untreated samples. As described earlier, the STPP treatment increased meat WHC in raw-treated samples than did in raw-untreated samples leading to slightly decline in cooking loss for longer time of storage.

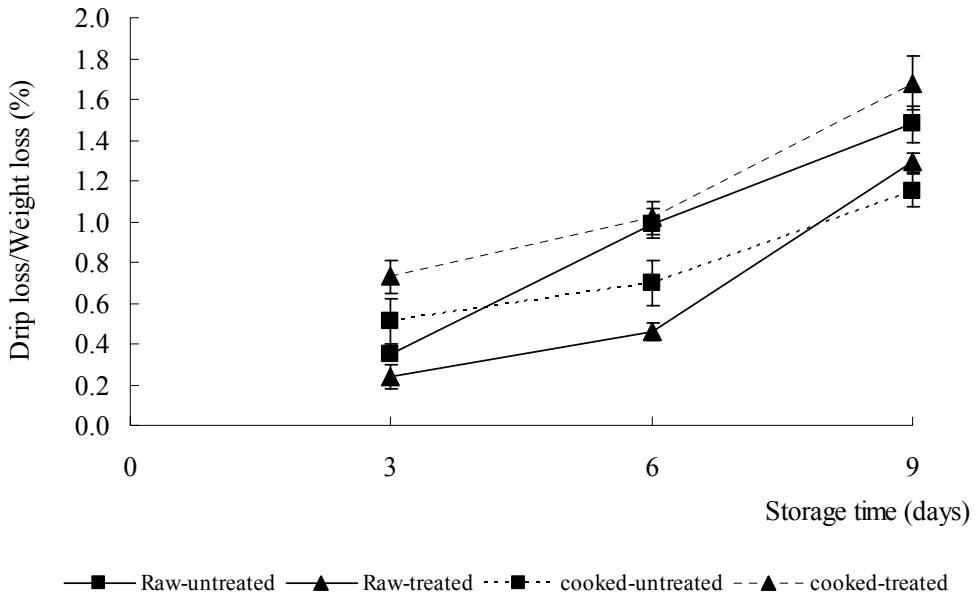


Figure 9. Changes in drip loss (%) of raw-untreated and raw-treated and weight loss (%) of cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP during chilled storage at 4°C. Bars represent the standard deviations for four determinations.

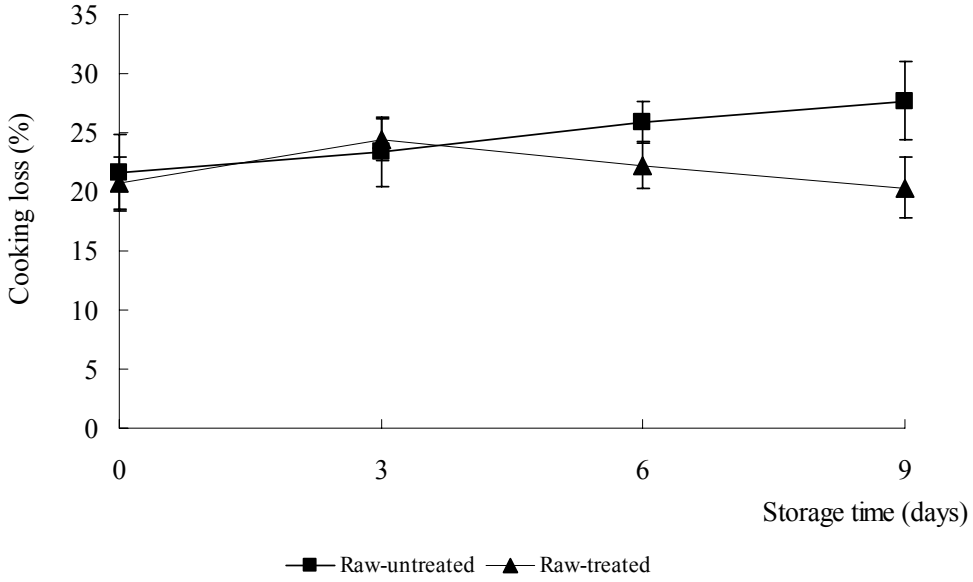


Figure 10. Changes in cooking loss (%) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for eight determinations.

2.2.2 Warner-Bratzler shear force values

Changes in texture during chilled storage of raw and cooked spent hen *Pectoralis major* muscle samples were determined by Warner-Bratzler shear force values as presented in Figure 11. NaCl and STPP treatments showed no impact on shear force values of raw and cooked muscle samples throughout storage time ($P \geq 0.05$). However, shear force values of samples were affected by storage time. The highest shear force value was observed on day 6 of storage ($P < 0.05$). The highest value on day 6 was probably due to water loss from muscle as indicated by higher drip loss than the early of storage time as described previously. The decrease in this values after prolong storage probably caused by increase in the degree of proteolysis resulted in degradation of protein.

The results showed no improvement on texture of muscle after cooking at 80^{o}C neither NaCl/STPP treated samples ($P \geq 0.05$). Wattanachant *et al*. (2005b) reported a slightly increased in shear value when heated broiler from 60 to 80^{o}C and tended to decrease at higher temperature from 80 to 100^{o}C. The increase in shear value with heating up to 80^{o}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 degradation of actomyosin (Bailey and Light, 1989). Therefore, the results could be demonstrating the ineffectiveness of NaCl and STPP on decreasing toughness of both raw and cooked spent hen muscles during chilled storage. This was due to the spent hen muscle had high content of stroma protein (Table 6) resulted in more difficulty for both additives to penetrate into the muscle. In addition, high collagen cross-linking found in current study leading to less collagen was melt to become gelatin which tenderness of muscle is carried out.

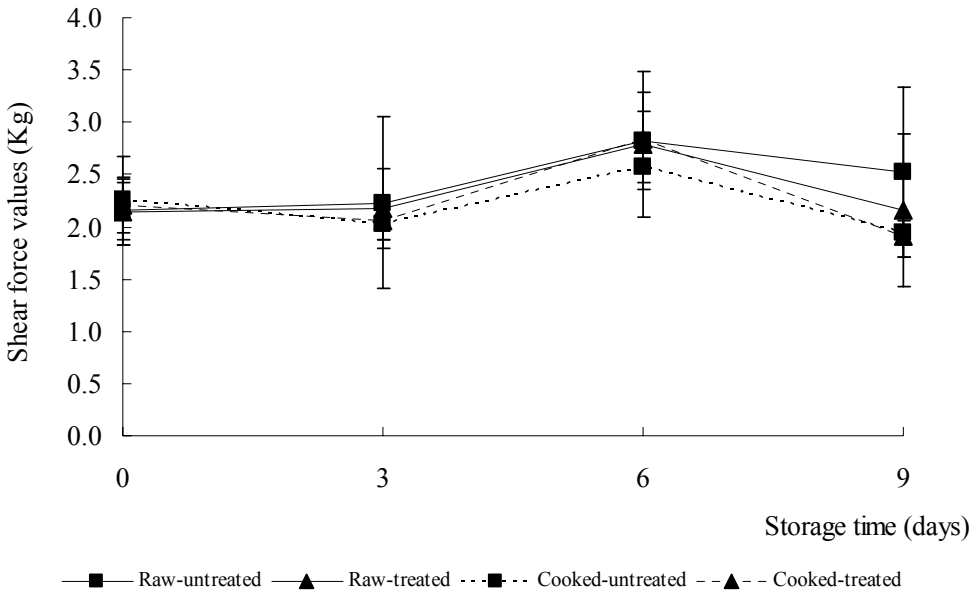


Figure 11. Changes in shear force values (kg) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated, and cook-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for eight determinations.

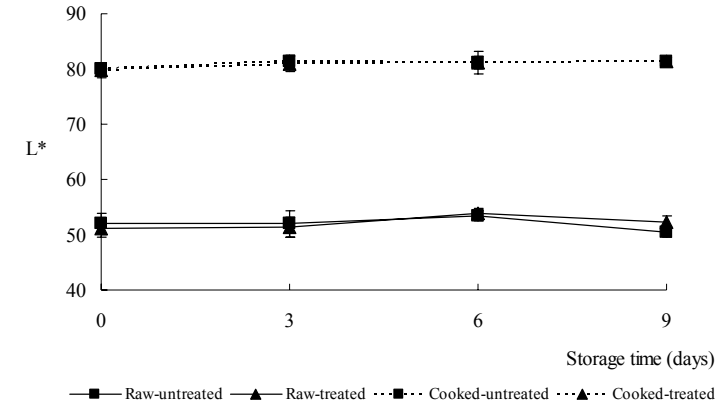
2.2.3 Surface color

Surface color (L*, a* and b* values) of raw spent hen *Pectoralis major* muscles during storage are shown in Figure 12. At day 0, the surface color values of spent hen found in current study were consistent with those of spent hen *Pectoralis* muscles reported in previous researches for L* value (47.79 to 58.38), a* value (-1.94 to -0.73), and b* value (7.74 to 8.76) (Chuaynukool, 2007; Wongwiwat, 2009; Liwa, 2009). Negative a* value (greenness) of spent hen meat and other chicken compared to pig and beef muscles which has positive a* value (redness) might be contributed from low in myoglobin content (Miller, 1994) and difference in breeds, feed diet, age and fat content (Fletcher, 1999a, b). After heating spent hen muscle changed surface color from dark yellowish to paler color as shown in Figure 44 (Appendix). This could explain why cooked meat has increased in L* and b* value (yellowness) and a* value increase close to zero after heating. The L* value of the meat depends upon the amount of light scattered (Sams and Alvarado, 2004). Young and West (2001) pointed out that with increasing heating temperature, meat tends to be lighter and also turn to a brown-grey hue and the lightening is due to an increased reflection of light, arising

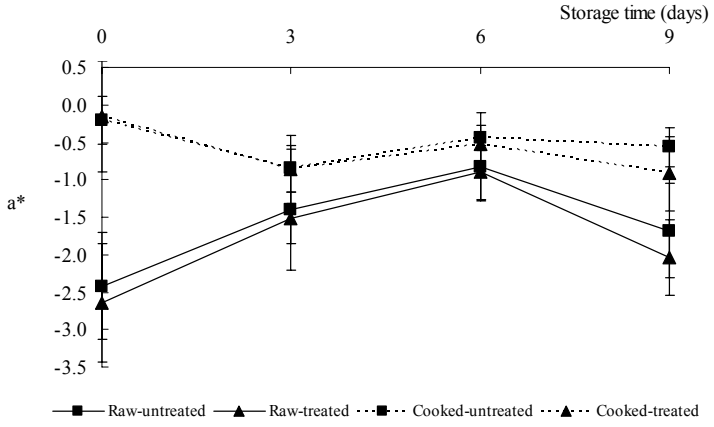
from light scattering by denatured proteins. Lawrie (1991) noted that denaturation of red myoglobin and conversion to brown myohaemochromogen starts at 40^{o}C and is almost completely denatured between 80-85^{o}C.

Changes in surface color during chilled storage of raw and cooked spent hen *Pectoralis major* muscles were presented in Figure 12. There was no significant difference in C.I.E. color values between untreated and NaCl and STPP treated samples for both raw and cooked muscle samples throughout chilled storage ($P \geq 0.05$). However, L* and a* values of samples were affected by storage time ($P < 0.05$), while b* value was unchanged with storage time ($P \geq 0.05$). In addition, the degree of color variation during chilled storage in cooked meat was not pronounced as in raw meat. L* value of both raw-untreated and raw-treated samples exhibited highest value on day 6 and a slight change was observed in raw-untreated, while no change in L* value of raw-treated was found on the other days of storage (Figure 12a). It was indicated that the decrease in pigment concentrations increased meat lightness (Boulianne and King, 1995; Fletcher *et al*., 2000). In general, as drip loss increased, L* value increased with little effect of pH (Woelfel *et al*., 2002). The highest L* value on day 6 was in concomitant with the increasing drip loss (Figure 9), and the increased pH of meat due to rapid growth of spoilage microorganism.

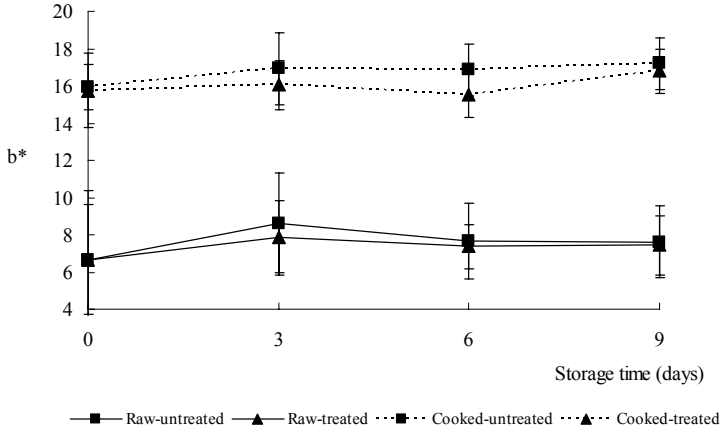
Storage time had no effect on L* value of cooked-untreated ($P \geq 0.05$), while it showed significant increase in this value of cooked-treated samples when extended storage time ($P < 0.05$). The color a* value of raw meat samples either treated and untreated increased gradually from 0 to 6 days and then it slightly decreased thereafter (Figure 12b). An increase in a* value of spent hen muscle might be contribute to water loss (Figure 9) and MetMb formation (Figure 7) during chilled storage. Boulianne and King (1995) stated that a* value was positively correlated with total pigment, myoglobin, and iron concentrations and change in a* value was correlated well with Mb content that might undergo oxidation during chilled storage to form MetMb resulting in appear more brownish in color. For a* value of cooked meat either treated or untreated samples was slightly changed throughout storage ($P < 0.05$). No significant difference in b* value was observed for raw and cooked muscle samples during chilled storage ($P \geq 0.05$) (Figure 12c).



(a)



(b)



(c)

Figure 12. Changes in L* values (a), a* values (b), and b* values (c) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for eight determinations.

3. Effect of NaCl and STPP on quality changes of raw and cooked spent hen *Pectoralis major* muscles during frozen storage

3.1 Changes in chemical properties

3.1.1 Protein solubility

Protein solubility is a physicochemical property that is related to other functional properties which reflects the processing condition (Zayas, 1997). Protein solubility of raw and cooked spent hen meat in current study was slightly higher than those found in *Pectoralis* muscle of broiler in previous report. Wattanachant (2003) reported the solubility in 0.6 M KCl of raw *Pectoralis* broiler muscle at approximately 70% and this content decreased to approximately 20% when the muscle was heated at 80^{o}C. This might be attributed to the higher myofibrillar protein of spent hen meat compared to that of broiler as described previously. The decrease in protein solubility of cooked meat indicated denaturation or structural changes in the meat protein. Alteration in protein solubility during heating is evidence of conformational changes in the structure of protein result in diverse and complicated precipitation reactions. At temperature of 40-50^{o}C or greater, the increase in thermal kinetic energy causes protein unfolding (denaturation), expose of non polar groups, the formation of new electrostatic and hydrogen cross-linkages leading to aggregation and precipitation result to decrease solubility (Zayas, 1997; Marphy and Marks, 2000; Wattanachant, 2003). Myofibrillar proteins start to coagulate at 30-40^{o}C, they are losing solubility and the coagulation is nearly completed at 55^{o}C (Zayas, 1997).

Changes in protein solubility, a measure of protein denaturation, during frozen storage of raw and cooked spent hen *Pectoralis major* muscles treated with NaCl/STPP and untreated samples are presented in Figure 13. As storage time increased, raw-untreated and raw-treated samples showed slight decrease in protein solubility throughout storage ($P<0.05$), whereas both cooked samples had the slight decrease at the beginning of storage ($P<0.05$). Thereafter no change in this value was found ($P\geq0.05$). This result indicated protein denaturation during frozen storage. A decrease in solubility in a 0.6 M KCl solution of frozen beef muscle stored at -20^{o}C, -10^{o}C, and -5^{o}C for 40-45 weeks has been reported by Wagner and Anon (1986). Zayas (1997) demonstrated that protein denaturation, in particular myofibrillar

proteins, is the main factor of meat quality deterioration during frozen storage. Freeze-denatuartion of actomyosin was mainly caused by formation of disulfide, hydrogen, and hydrophobic bonds (Jiang *et al*., 1988). Matsumoto (1980) reported formation of aggregates during frozen storage of fish muscle. The formation of aggregates was caused by the progressive formation of hydrogen bonds, ionic bonds, hydrophobic interactions, and disulfide bridges. In addition, Sikorski (1978) stated that freezing out of water can lead to insoluble of protein which results in lower protein solubility. Moreover, it is known that during frozen storage the oxidative processes occurring in both lipid and protein fractions of meat are one of the major causes for changes in its quality parameters (Teodora, 2009). Several proteins contain amino acids that are very susceptible to oxidation such as cysteine, histidine, methionine, lysine, and tryptophan (Xiong, 2000). Oxidative reactions involving the side chains of amino acids can lead to the formation of carbonyl groups as this conversion may ultimately result in a loss of catalytic activity and increased susceptibility to protein degradation (Stadtman, 1990) or protein aggregation and loss of solubility.

Treatment of spent hen meat with NaCl and STPP had no influence on protein solubility of both raw and cooked samples at the beginning of storage, but it was more influence on this value during long time of storage. Raw-treated samples showed higher protein solubility than those found in raw-untreated samples on month 4 of frozen storage ($P<0.05$), while for cooked muscle samples, this phenomenal was observed on month 6 ($P<0.05$). As mentioned earlier, NaCl and STPP increased functionality of muscle protein mainly by increasing ionic strength and pH (Trout and Schmidt, 1984) resulting in swelling of protein that can function by the dissociation of the actomyosin complex binding with water leading to less protein aggregation during long time of storage when compared to untreated samples.

3.1.2 MetMb content

Metmyoglobin contents of raw samples during 6 months of frozen storage are presented in Figure 14. Significant difference in MetMb content was not observed between untreated and treated muscle samples throughout storage ($P\geq0.05$). However, MetMb content of samples was affected by storage time. Those in raw-treated and untreated samples increased gradually from 0 to 4 months of storage and

slightly decreased thereafter. The decrease in MetMb after 4 months of storage was probably caused by the lower pigment content remaining or bound in the muscle. The decrease in this content during prolong storage at 6 months was in concomitant with the increasing of thawing loss (Figure 16) resulted in more water loss from muscle leading to more Mb release and exhibited lower MetMb than initial storage time.

3.1.3 TBARS

TBARS values of both raw and cooked spent hen muscle samples during frozen storage are shown in Figure 15. Treatment of spent hen muscle with NaCl and STPP in this study showed no significant effect on TBARS value during frozen storage ($P \geq 0.05$). From 0 to 4 months of frozen storage, no change in TBARS value of both raw and cooked samples was observed ($P \geq 0.05$). Thereafter a dramatic increase in this value was found ($P < 0.05$). This finding was in accordance with that of previously reported by Al-Najdawi and Abdulla (2002) and the researchers suggested that freezing delayed chicken meat from oxidation and lipid rancidity within shorter storage time.

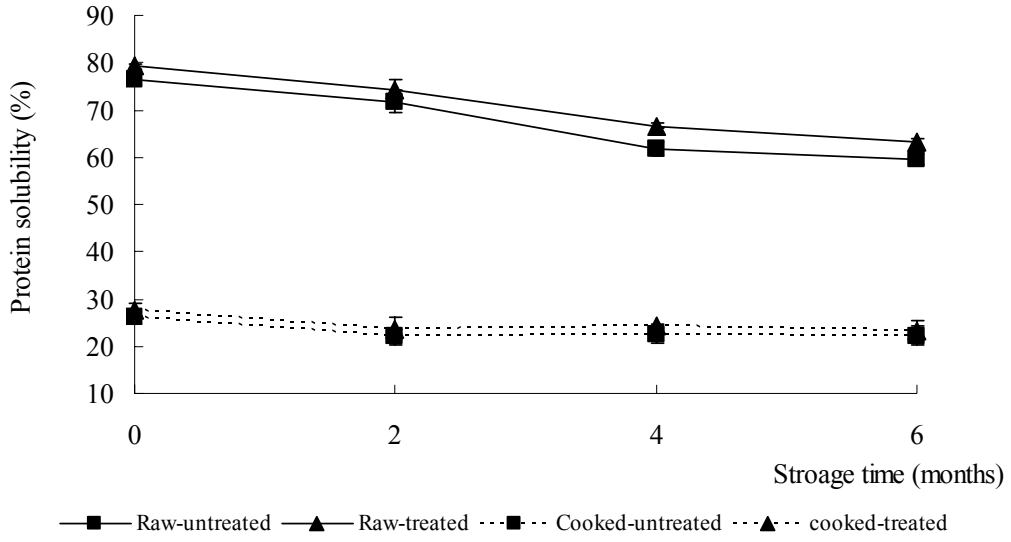


Figure 13. Changes in protein solubility (%) during frozen storage at -18°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for triplicate determinations.

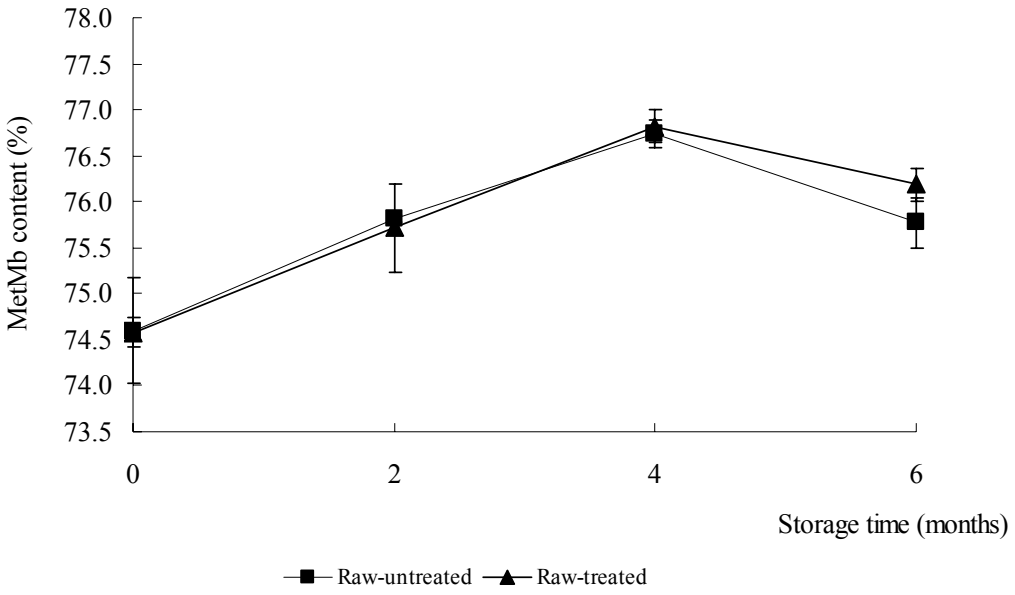


Figure 14. Changes in MetMb content (%) during frozen storage at -18°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for triplicate determinations.

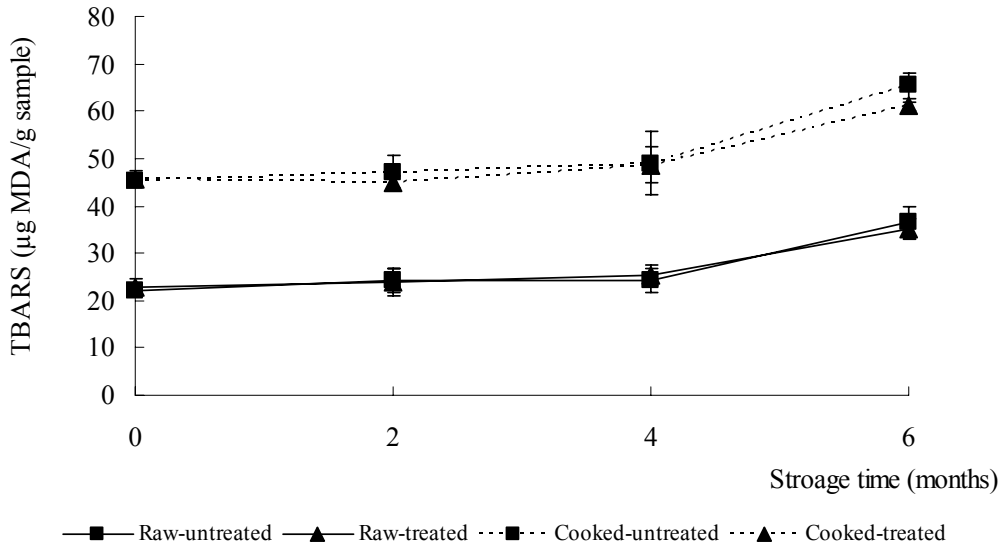


Figure 15. Changes in TBARS (μg MDA/g sample) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations from triplicate determinations.

3.2 Changes in physical properties

3.2.1 Thawing loss and cooking loss

The increasing thawing loss with frozen storage time of both raw and cooked muscle samples which reflects the degree of protein denaturation is shown in Figure 16. Miller *et al*. (1980) demonstrated a significant decline in the capacity of meat proteins to remain water during extended frozen storage. Significantly higher thawing loss was found in raw-untreated samples compared with those found in raw-treated samples ($P<0.05$). Heating reduced thawing loss of cooked meat, as indicated by lower thawing loss than that of raw meat, was due to the lower amount of water in cooked muscle. Apparently, adding NaCl and STPP was effective in decreasing thawing loss of both raw and cooked muscle samples.

No significant difference in cooking loss was found between raw untreated and treated samples throughout frozen storage ($P\geq0.05$) (Figure 17), demonstrating that NaCl and STPP had no affect on cooking loss. However, cooking loss of untreated and treated frozen samples were affected by storage time ($P<0.05$). An increase in this value of both samples were found on month 4 of storage and tended to constant thereafter. An increase in cooking loss was related to low WHC of proteins due to their denaturation during frozen storage (Zayas, 1987). Freezing out of water can lead to insoluble of protein which results in alteration WHC during long time of frozen storage (Sikorski, 1978) and this was in concomitant with the increasing of thaw loss (Figure 16) and decreasing of protein solubility (Figure 13).

3.2.2 Warner-Bratzler shear force values

Changes in Warner-Bratzler shear force values of raw and cooked samples during frozen storage are presented in Figure 18. Significant difference was not observed in shear force values between untreated and treated with NaCl and STPP on both raw and cooked muscle samples throughout frozen storage ($P\geq0.05$). However, shear force values of samples were affected by storage time ($P<0.05$). The shear force of raw spent hen muscle samples did not change at the beginning of frozen storage and exhibited the highest value on month 4, whereas cooked samples was gradual increase in this value from 0 to 4 months of frozen storage. Both raw and cooked muscle samples showed slightly decrease in shear force when extended storage time for 6 months ($P<0.05$). The highest value on month 4 could be due to the

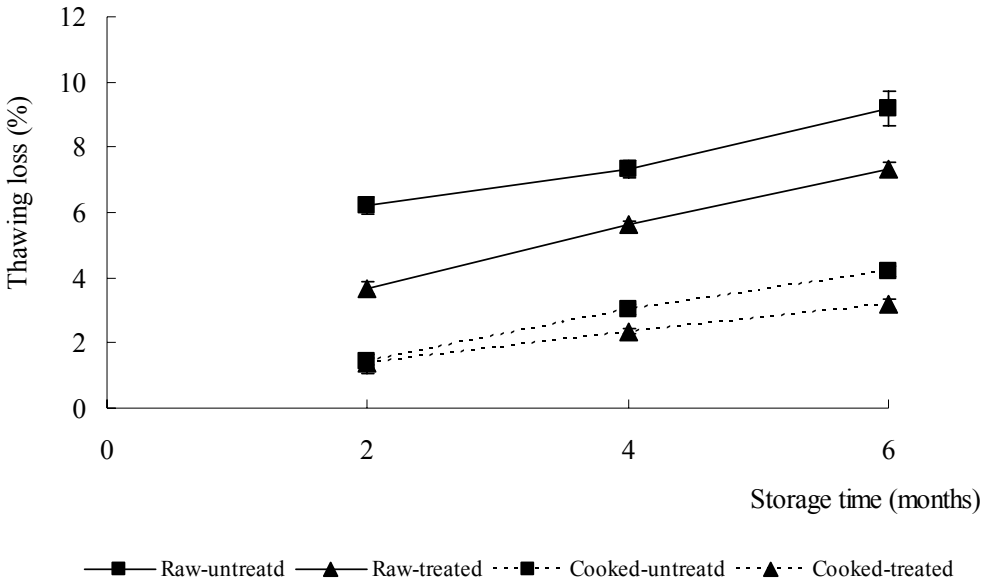


Figure 16. Changes in thawing loss (%) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations from four determinations.

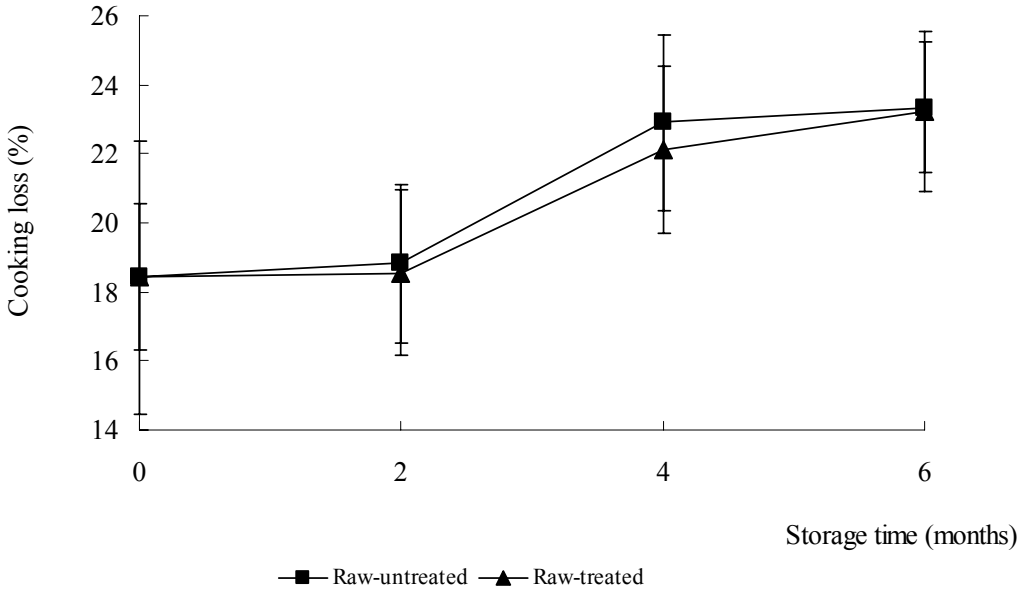


Figure 17. Changes in cooking loss (%) during frozen storage at -18°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations from eight determinations.

denaturation of protein leading to more water loss from muscle resulted in firmer texture. Freezing out of water can lead to insoluble of protein which results in alteration WHC and texture during long time of frozen storage (Sikorski, 1978) and this was in concomitant with the decreasing of protein solubility (Figure 13), increasing of thaw loss (Figure 16) and the more compact of muscle fiber (Figure 20) A decrease in shear force during long time of storage perhaps caused by recrystallization phenomenon resulted in the disruption of cellular organelles (Figure 20) As mentioned by Marino and Zaritzky (1988), recrystallization is probably the most important change of purely physical nature; it consists of an enlargement of the larger crystals at the expense of the smaller ones; as a consequence the smaller crystals tend to disappear leading to a reduction in the total number of crystals and to an increase of the mean crystal diameter. This phenomenon could cause physical damage of muscle fiber and connective tissue as shown by wider gap between muscle fiber leading to lower shear force during long time of storage. Cooked samples exhibited slightly higher in shear force values when compared with raw samples. Sikorski (1978) stated that hardening of the fresh meat may occur if there is marked denaturation of protein. Cooked meat had more protein denaturation as elucidated by lower protein solubility (Figure 13) leading to toughness of cooked spent hen meat.

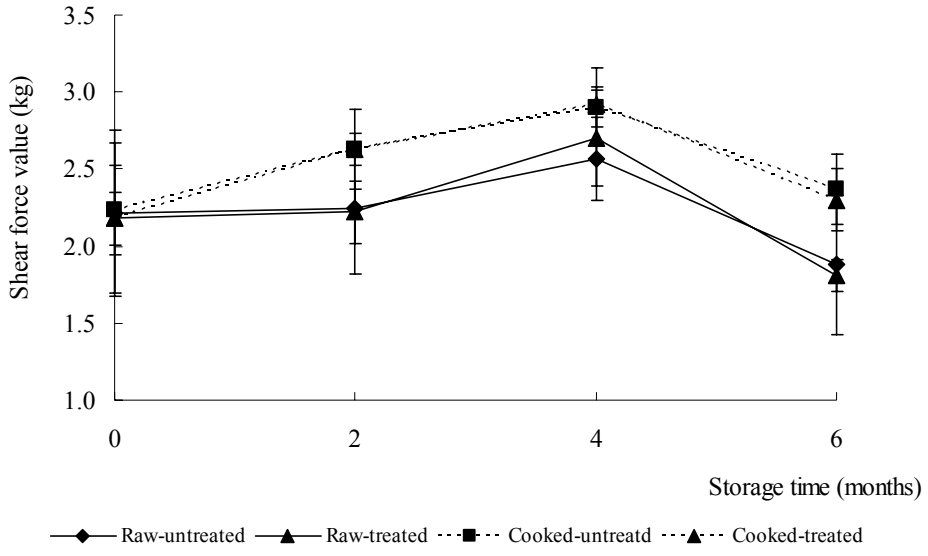


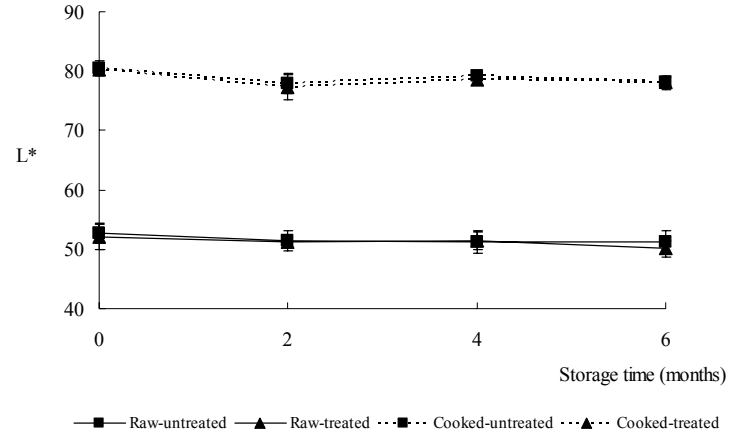
Figure 18. Changes in Warner-Bratzler shear force values (kg) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations; n=8.

3.2.3 Surface color

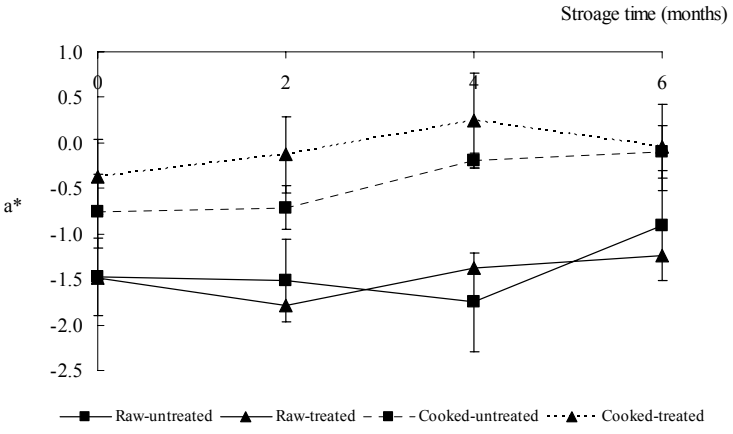
The difference in surface color between raw and cooked muscle samples showed the same results in section 2. Changes in surface color during frozen storage of raw and cooked spent hen *Pectoralis major* muscles are presented in Figure 19. Significant differences in L* and b* values were not observed between untreated and treated with NaCl and STPP muscle samples either raw or cooked muscle samples throughout storage ($P \geq 0.05$). L* value of raw-treated samples was slightly decreased by storage time ($P < 0.05$), while no change in L* value of raw-untreated sample was observed throughout storage ($P \geq 0.05$). For a* value of raw-treated (Figure 19b), there had a little change from 0 to 4 months ($p < 0.05$) and then slightly decreased thereafter, whereas no change in a* value of raw-untreated was observed from 0 to 4 months and then slightly decreased on month 6 of storage. No significant difference was found on b* value of both raw meat samples throughout storage ($P \geq 0.05$). L* value of cooked meat showed the highest value on the beginning of storage time and then slightly decreased in cooked-untreated ($P < 0.05$) and cooked-treated samples ($P \geq 0.05$) from 2 to 6 months of storage. Cooked-treated samples showed lower negative a* value than cooked-untreated did on month 2 and 4 of storage ($P < 0.05$), whereas no significant difference was observed on other months of storage ($P \geq 0.05$). As shown in Figure 19c, b* value of cooked-untreated trended to increase slightly as storage time increased ($P < 0.05$), while no change in this value was observed in cooked-treated throughout storage ($P \geq 0.05$).

3.2.4 Microstructure changes

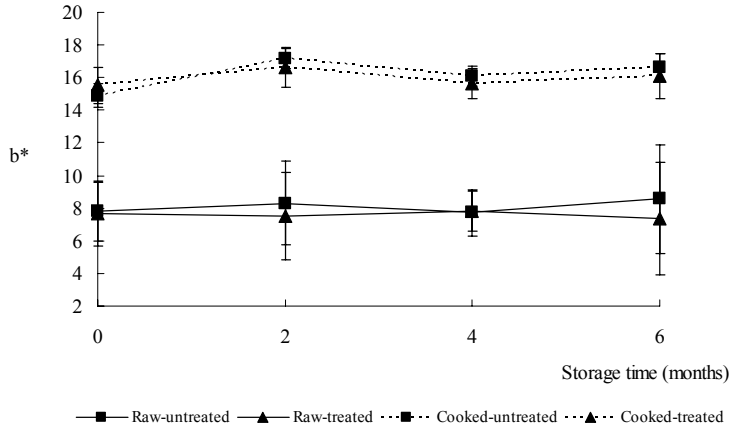
Changes in fiber diameter during frozen storage of raw and cooked spent hen *Pectoralis major* muscles with NaCl and STPP are shown in Table 9. The fiber diameter of cooked samples were greater than raw samples, indicated the expanded in transverse of protein to the fiber direction after heating. As reported by Wattanachart (2003), the fiber diameter of broiler *Pectoralis* muscle increased by 12.4% compared to their raw counterpart after heating. Treatment of spent hen meat with NaCl and STPP had more effect on fiber diameter of cooked meat than raw meat as indicated by cooked-treated samples showed higher fiber diameter than those found in cooked-untreated samples throughout storage ($P < 0.05$). This could be combination effect of NaCl and STPP on structural changes of protein leading to increase in protein



(a)



(b)



(c)

Figure 19. Changes in L* values (a), a* values (b), and b* values (c) during frozen storage at -18^{o}C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP. Bars represent the standard deviations for eight determinations.

swelling as described previously. The decrease in fiber diameter were observed in both raw and cooked meat either treated or untreated samples when storage time increased from 0 to 4 months ($P<0.05$). This was in concomitant with the increasing of shear force value on 4 months of storage (Figure 19).

Table 9. Fibre diameter (μm) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Months	Fibre diameter (μm)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	33.32±2.43$^{a, y}$	35.98±2.00$^{a, x}$	37.13±2.63$^{a, y}$	43.56±1.98$^{a, x}$
4	23.82±2.18$^{b, x}$	24.85±2.21$^{b, x}$	35.22±3.25$^{b, y}$	38.29±2.47$^{b, x}$
6	24.17±1.97$^{b, y}$	25.55±1.78$^{b, x}$	34.97±3.57$^{b, y}$	37.68±3.59$^{b, x}$

Data are presented as mean ± standard deviation; n=30.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Changes in transverse sections during frozen storage of raw and cooked spent hen *Pectoralis major* muscles are shown in Figure 20 and 21. For both raw and cooked muscle samples, the more compact muscle fibers were found at month 4 of frozen storage either treated or untreated samples. Denaturation of protein led to more water loss from muscle resulted in firm structure and the highest shear force value (Figure 18). It was also in concomitant with the increasing thawing loss when storage time increased (Figure 16). Thereafter, the spaces between muscles were clearly observed in both samples resulted in lower shear force value. This phenomenal might be affected from ice crystal growth during freezing and storage, which causes biochemical and physical changes as well as the disruption of cellular organelles (Downey and Beauchene, 1997).

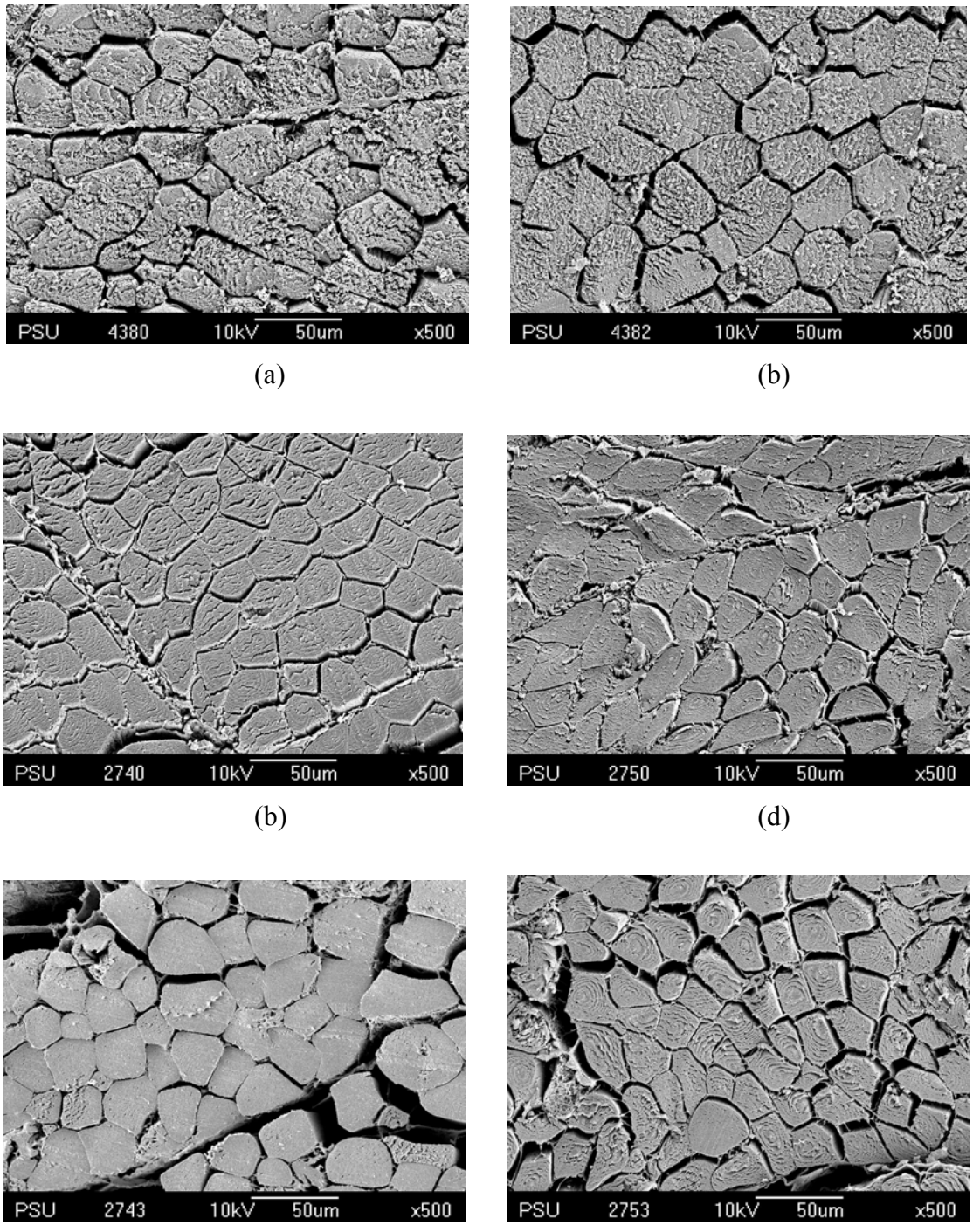


Figure 20. Scanning electron micrographs of transverse sections of raw spent hen *Pectoralis major* muscles during frozen storage at -18^{o}C; raw-untreated month 0 (a), raw-treated/month 0 (b), raw-untreated/month 4 (c), raw-treated/month 4 (d), raw-untreated/month 6 (e), and raw-treated/month 6 (f). Scale bar indicates 50 μm, magnification=500x.

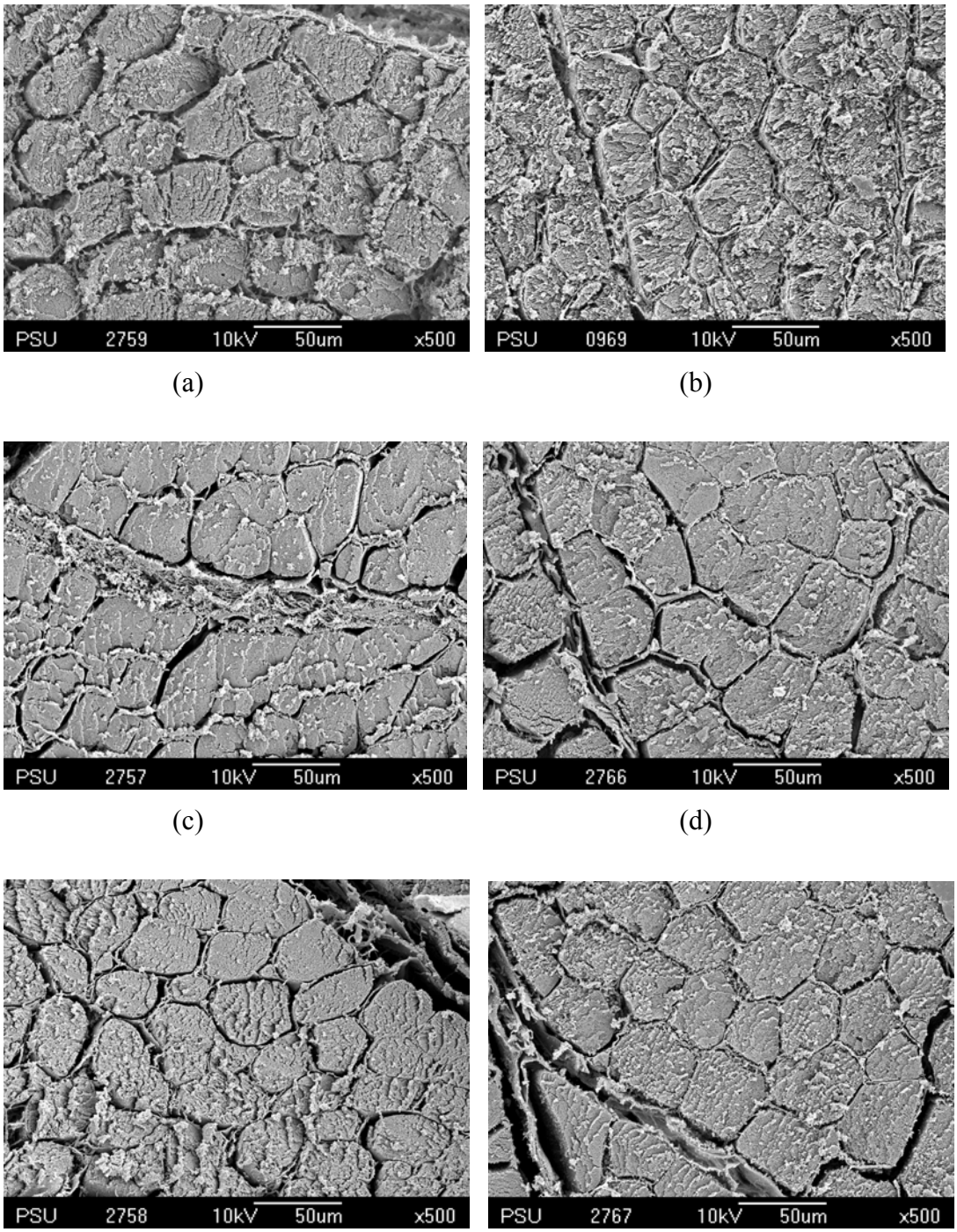


Figure 21. Scanning electron micrographs of transverse sections of cooked spent hen *Pectoralis major* muscles during frozen storage at -18°C; cooked-untreated month 0 (a), cooked-treated/month 0 (b), cooked-untreated/month 4 (c), cooked-treated/month 4 (d), cooked-untreated/month 6 (e), and cooked-treated/month 6 (f). Scale bar indicates 50 μm, magnification=500x.

4. Effect of different raw material pretreatments and heating methods on quality of cooked spent hen *Pectoralis major* muscles

The chemical, physical properties and sensory characteristics were evaluated to study the effect of different raw material pretreatments (untreated, NaCl/STPP treated, and freeze-thawed) and heating methods (oven heating, microwave heating, steaming and boiling) on quality of cooked spent hen meat.

4.1 Chemical properties

4.1.1 Protein solubility

The influence of different raw material pretreatments and heating methods on protein solubility of cooked spent hen *Pectoralis major* muscles is shown in Table 10. For all heating methods, spent hen meat treated with NaCl and STPP showed the highest protein solubility than those found in other raw materials ($P<0.05$). On the other hand, freeze-thawed samples exhibited the lowest values ($P<0.05$), indicating the most protein denaturation. The highest protein solubility of samples treated with NaCl and STPP was likely due to combine effect of physical changes to structural protein. It was due to phosphates increased functionality of muscle protein mainly by increasing ionic strength and pH (Trout and Schmidt, 1984) that moving the meat pH further from the meat protein isoelectric point (about 5.5 in chickens) resulted in increasing in solubility of protein. Wongwiwat (2009) found that spent hen breast muscles treated with STPP, DSP (disodium orthophosphate) and TSPP (tetrasodium pyrophosphate) had greater myofibrillar protein solubility than without phosphate treatment. The lowest protein solubility was found in freeze-thawed samples probably due to protein denaturation and oxidation. Xia *et al*. (2009) stated that freeze-thaw process may cause protein and lipid oxidation and one of the consequences of protein oxidation is the formation of protein aggregates through both non-covalent and covalent intermolecular bonds leading to decrease in protein solubility. Non-significant on protein solubility of freeze-thawed samples was observed for all heating methods, elucidated that this sample could decrease the effect of heating methods on protein solubility.

4.1.2 Soluble collagen content

The texture of cooked meat is generally considered to be affected by heat-induced changes in connective tissues. The cross-linkage between the collagen molecules within the connective tissue is associated with collagen solubility and changes in collagen solubility during heating could significantly influence the texture of poultry meat (Zayas and Naewbanij, 1986). As shown in Table 10, both raw materials and heating methods had influence on soluble collagen content of cooked spent hen *Pectoralis major* muscles. Freeze-thawed spent hen meat before heating exhibited the highest soluble collagen content than those found in other raw materials for all heating methods ($P<0.05$). Moreover, it could reduce the effect of heating methods on soluble collagen content as indicated by non-significant ($P\geq0.05$) between all heating methods were found when used freeze-thawed meat as raw material. This probably caused by during freezing and thawing, ice crystal growth causes biochemical and physical changes resulted in the disruption of cellular organelles (Figure 22, 23) (Downey and Beauchene, 1997) leading to more collagen expose to heat resulted in higher collagen was melt than other raw materials. Oven method showed higher soluble collagen content than other heating methods when used fresh raw meat or NaCl and STPP treated samples as raw material. These probably was because this method taking a longer time of heating to reach end point temperature leading to more solubilization of cross-linked collagen.

Table 10. Protein solubility (%) and soluble collagen content (% of total collagen) of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating method.

Heating methods	Protein solubility (%)			Soluble collagen content (% of total collagen)		
	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed
Oven heating	$9.77\pm0.27^{ab, y}$	$15.72\pm0.11^{a, x}$	$6.66\pm0.81^{a, z}$	$17.62\pm0.08^{a, z}$	$27.93\pm2.02^{a, y}$	$32.71\pm1.17^{a, x}$
Steaming	$8.60\pm0.70^{b, xy}$	$10.25\pm0.24^{c, x}$	$7.86\pm1.24^{a, y}$	$12.67\pm0.62^{c, z}$	$17.68\pm0.95^{c, y}$	$29.82\pm2.02^{a, x}$
Boiling	$8.64\pm1.25^{b, xy}$	$10.17\pm0.45^{c, x}$	$7.36\pm0.29^{a, y}$	$15.41\pm0.10^{b, z}$	$21.61\pm0.97^{b, y}$	$30.95\pm1.69^{a, x}$
Microwave heating	$10.93\pm1.14^{a, y}$	$13.82\pm0.99^{b, x}$	$7.88\pm0.68^{a, z}$	$11.33\pm0.28^{d, z}$	$17.58\pm0.34^{c, y}$	$29.01\pm1.17^{a, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

4.2 Physical properties

4.2.1 Cooking loss

Because of muscle protein changes with heating, water content within the muscle in the narrow channels between the filaments changes as meat shrinks (Bertola *et al*., 1994), resulting in cooking loss with heating. Cooking loss of spent hen *Pectoralis major* muscles cooked by different raw material pretreatments and heating methods are shown in Table 11. Raw meat treated with NaCl and STPP before cooking showed lower cooking loss than those found in other raw materials indicating more water retention after heating. This probably due to combination effect of NaCl and STPP caused protein swelling. Swelling capacity of meat is defined as the spontaneous uptake of water from any surrounding fluid, resulting in an increase of weight and volume of muscle fibers (Hamm, 1986). The swelling provides a higher number of protein side chains that can bind water and therefore WHC increased. Cooked meat by oven heating had the highest cooking loss ($P<0.05$) than those found in other heating methods for all kinds of raw material used. This results probably caused by protein in meat cooked by oven heating had long time expose to dry heat at high temperature leading to more denaturation of protein resulted in loosing ability to hold water. WHC of muscle tissue has been related to the extent of heat denaturation of myofibrillar proteins during thermal processing (Larick and Turner, 1992). With increasing temperature, the denaturation of myosin and actin caused structure changes and expelled the sarcoplasmic fluid from the muscle fibers, resulting in water losses from meat tissues (Bertola *et al*., 1994). For moist heat methods, boiling had less effect on WHC of fresh raw and NaCl/STPP treated samples than steaming as elucidated by the lower cooking loss ($P<0.05$). However, it did not show any difference when used freeze-thawed samples as raw material ($P\geq0.05$).

4.2.2 Warner-Bratzer shear force value

Warner-Bratzer shear force value is commonly used for determining meat texture. As shown in Table 11, heating methods had more influence on Warner-Bratzer shear force value of cooked spent hen *Pectoralis major* muscles than fresh raw materials. Cooked meat by oven heating resulted to the most toughness as indicated by the highest shear force ($P<0.05$) than those found in other heating methods for all kinds of raw materials used. However, using freeze-thawed samples before cooking by oven heating could reduce toughness in spent hen meat as elucidated by the lowest shear value than other raw materials ($P<0.05$). This probably caused by more disruption of cellular organelles (Figure 22, 23) than other raw materials as mentioned earlier. Different raw material pretreatments cooked by moist heat did not effect on texture as indicated by non significance on Warner-Bratzer shear force among all samples were found ($P\geq0.05$). However, using boiling could obtain slight decrease in toughness of fresh raw and NaCl/STPP treated samples. These result probably caused by heat transfer via liquid medium of this method had more efficiency to solubilize collagen within short time leading to high soluble collagen content (Table 10) after heating resulted in more tender meat. Bouton and Harris (1972) postulated that heating produced a softening of connective tissue caused by conversion of collagen to gelatin and a toughening of meat fibers caused by heat coagulation of myofibrillar proteins. Larick and Turner (1992) stated that collagen began to shrink at 60 to 70^{o}C and was converted to gelatin at 80^{o}C and that these changes weakened the connective tissue. On the other hand, myofibrillar protein shortening during heating could also result in increasing toughness in chicken (Dawson *et al*., 1991). Although spent hen meat cooked by oven heating had high content of soluble collagen, it had lower water retention in muscle than did by moist heat methods due to lower heat transfer of dry heat taking for a long time of cooking resulted in more tough meat.

Table 11. Cooking loss (%) and Warner-Bratzler shear force value (kg) of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods.

Heating methods	Cooking loss (%)			Warner-Bratzler shear force value (kg)		
	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed
Oven heating	26.13±1.25$^{a, y}$	27.15±1.57$^{a, y}$	32.00±1.19$^{a, x}$	2.64±0.12$^{a, x}$	2.40±0.37$^{a, xy}$	2.30±0.18$^{a, y}$
Steaming	19.91±0.76$^{c, y}$	17.39±2.15$^{c, y}$	18.36±1.34$^{b, xy}$	2.09±0.15$^{b, x}$	2.13±0.16$^{ab, x}$	2.15±0.10$^{ab, x}$
Boiling	17.46±2.32$^{d, y}$	15.75±0.91$^{d, y}$	19.82±2.72$^{b, x}$	2.10±0.16$^{b, x}$	2.11±0.28$^{b, x}$	1.96±0.23$^{b, x}$
Microwave heating	22.29±1.55$^{b, x}$	19.63±0.96$^{b, y}$	19.42±1.99$^{b, y}$	2.18±0.22$^{b, x}$	2.19±0.12$^{ab, x}$	2.11±0.30$^{ab, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

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4.2.3 Surface color

The effect of different raw material pretreatments and heating methods on surface color of cooked spent hen *Pectoralis major* muscles was evaluated by L*, a* and b* color values as shown in Table 12. Heating all raw materials by oven heating had more influence on surface color of cooked spent hen meat than all other heating methods. It was elucidated by oven heating exhibited the lowest L* and the highest a* and b* values than other heating methods ($P<0.05$), whereas no significant differences among microwave heating and moist heat methods on L* and b* values of all raw materials were found ($P\geq0.05$). The highest b* values of meat cooked by oven heating might be affected from non enzymatic browning process via Maillard reaction. The Maillard reaction is a chemical reaction between an amino acid and a reducing sugar, usually requiring the addition of heat above 140^{o}C (Harrison and Dake, 2005). The heat in an oven is provided by convection, i.e., circulating air currents, which is mostly higher than 140^{o}C to produce a browning reaction (Vogel, 2004). In contrast, moist heat methods require water for heat transfer. Since water cannot be heated above 140^{o}C, boiled, poached and steamed foods will never brown (Vogel, 2004). In addition, the Maillard reactions occur only at the surface of the meat, because the moisture in the meat keeps the interior from getting above 100^{o}C. For all moist heat methods, freeze-thawed samples had the highest L* value and the lowest a* values than at that of other samples. This was probably caused by more denaturation of protein. The L* value of the meat depends upon the amount of light scattered (Sams and Alvarado, 2004). Young and West (2001) pointed out that with increasing heating temperature, meat tends to be lighter and the lightening is due to an increased reflection of light, arising from light scattering by denatured proteins. For color a* values is associated with meat pigment, and change in a* value was correlated well with Mb content (Boulianne and King, 1995). The lowest a* values of freeze-thawed samples perhaps due to more meat pigments loss during freeze-thaw cycle process.

Table 12. Surface Color (L*, a*, and b* values) of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods.

Heating methods	L*			a*			b*		
	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	treated freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed
Oven heating	77.62±0.93$^{b, x}$	75.33±1.76$^{b, y}$	76.57±1.17$^{b, xy}$	0.61±0.30$^{a, x}$	0.35±0.68$^{a, x}$	0.11±0.44$^{a, x}$	18.12±1.92$^{a, x}$	16.83±2.72$^{a, x}$	17.54±1.81$^{a, x}$
Steaming	80.73±0.70$^{a, y}$	80.46±0.25$^{a, y}$	82.56±0.52$^{a, x}$	-0.24±0.28$^{b, x}$	-0.10±0.71$^{a, x}$	-0.78±0.21$^{b, y}$	15.49±0.61$^{b, x}$	12.97±1.16$^{b, y}$	12.48±0.70$^{b, y}$
Boiling	80.47±1.07$^{a, y}$	80.70±0.78$^{a, y}$	82.22±1.27$^{a, x}$	-0.75±0.46$^{c, y}$	-0.13±0.64$^{a, x}$	-0.93±0.15$^{b, y}$	14.96±0.90$^{b, x}$	12.79±1.29$^{b, y}$	12.21±0.63$^{b, y}$
Microwave heating	80.79±1.08$^{a, xy}$	80.38±0.54$^{a, y}$	81.53±0.88$^{a, x}$	-0.85±0.23$^{c, x}$	-0.82±0.25$^{b, x}$	-1.28±0.28$^{c, y}$	14.89±1.35$^{b, x}$	12.53±1.03$^{b, y}$	12.13±0.78$^{b, y}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

4.2.4 Microstructure

Microstructure of cooked spent hen *Pectoralis major* muscles were evaluated fibre diameter and sarcomere length as presented in Table 13. Palka and Daun (1999) stated that springiness of meat is probably related to the degree of the fiber swelling which in turn should be reflected in the fiber diameter. For all heating methods, fresh raw and NaCl/STPP treated samples showed variation in fiber diameters ($P<0.05$), whereas no different on this value was observed in freeze-thawed samples ($P\geq0.05$). This could be indicated that meat cooked from fresh raw and NaCl/STPP treated samples with any heating methods might affect on texture of meat than did find from freeze-thawed samples. The lowest fibre diameter after heating with all methods was found in cooked samples prepared from fresh raw material compared to other raw materials. The sarcomere length of raw and NaCl/STPP treated samples cooked by oven heating exhibited the highest values compared to other heating methods. This result probably caused by differences in heat transfer rate among methods used. The result found in this study was in good agreement with Silva *et al*. (1993) who reported that rapid heating beef muscles produced greater percentage shorter sarcomeres than did slow heating.

Table 13. Fiber diameter (μm) and sarcomere length (μm) of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods.

Heating methods	Physical properties					
	Fibre diameter (μm)			Sarcomere length (μm)		
	unterated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed
Oven heating	**35.34±3.48$^{bc,\,y}$**	**40.12±2.25$^{c,\,x}$**	**39.84±5.33$^{a,\,x}$**	**1.20±0.06$^{a,\,x}$**	**1.15±0.05$^{a,\,y}$**	**0.90±0.05$^{b,\,z}$**
Steaming	**37.17±3.17$^{b,\,z}$**	**43.49±3.19$^{b,\,x}$**	**39.99±5.02$^{a,\,y}$**	**0.90±0.06$^{c,\,y}$**	**0.91±0.14$^{bc,\,y}$**	**0.97±0.04$^{a,\,x}$**
Boiling	**39.20±4.44$^{a,\,xy}$**	**38.30±3.28$^{c,\,y}$**	**41.25±5.24$^{a,\,x}$**	**1.02±0.07$^{b,\,x}$**	**0.91±0.18$^{b,\,y}$**	**0.90±0.06$^{b,\,z}$**
Microwave heating	**33.64±3.41$^{c,\,z}$**	**46.10±5.29$^{a,\,x}$**	**40.31±4.22$^{a,\,y}$**	**0.89±0.06$^{c,\,x}$**	**0.89±0.06$^{c,\,x}$**	**0.90±0.05$^{b,\,x}$**

Data are presented as mean ± standard deviation; n=30.

$^{a\text{-}c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x\text{-}y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Scanning electron micrographs of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods are shown in Figure 22. and 23. After cooking, denaturation and melting of the endomysium together with the denatured myofibrils resulted in swelling of the fibers were observed clearly on the transverse sections (Figure 22) of all spent hen meat samples cooked by different heating methods. Wattanachant *et al*. (2005a) reported that the denaturation and disintegrating of perimysial and endomysial collagen, together with the denaturation of myofibrils, were observed in the range 80-100^{o}C for Thai indigenous and broiler. The authors explained that the increase in fibre diameter when heating above 60^{o}C might be due to the denaturation and progressive loss of crimp structure, and shrinkage of collagen fibres in intramuscular connective tissue, leading to swelling of muscle fibres. In Addition, the gaps between fibres and endomysial tubes were very clear, especically spent hen meat cooked by moist heat methods. However, for meat cooked by oven heating this phenomenal only found in NaCl/STPP treated samples. Moreover, the granulations between muscles of all cooked meat were visible, especially in cooked spent hen meat prepared by using freeze-thawed samples. This could be indicated more denaturation of protein than other raw materials. On the longitunidual sections, the shrinkage of sarcomeres and gradual disintegration of myofibrillar structure were observed for all samples. However, the shrinkage sarcomeres of fresh raw and NaCl/STPP treated samples cooked by oven heating were more compact and with little loss of the Z-disks compared with samples cooked by moist heat. In addition, the thick filaments of meat cooked by oven heating was slightly degraded, and M-lines did not appear different from those of cooked meat prepared by moist heat methods. This could elucidate more toughness meat cooked by oven heating probably due to different in rate of heating. As studied in prerigor beef muscles, Silva *et al*. (1993) reported that rapid heating caused more extensive degradation of A and I bands, greater loss of the trimensional pattern of myofibrils, more fragmentation and melting of myofibrils, widened intermyofibrillar spaces, and maximum separation of fiber bundles as compared to slow heating. For all heating methods, treated freeze-thawed samples caused more coagulation of thick filaments, loss of z-disks structure, and more inter-

and intramyofibrillar spaces resulted probably resulted from escape of fluid as indicated by greater cooking loss after heating compared to other raw materials.

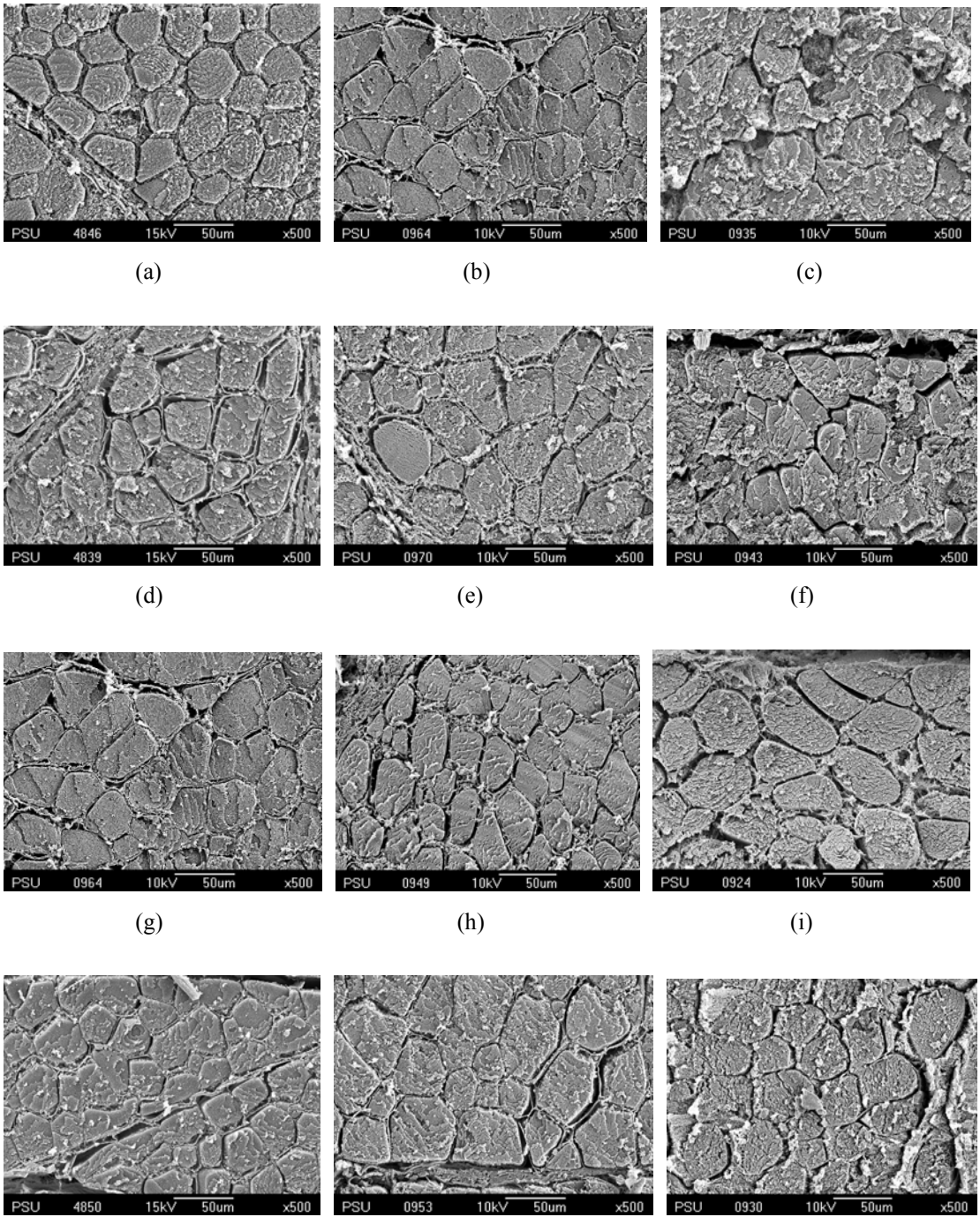


Figure 22. Scanning electron micrographs of transverse sections of cooked spent hen *Pectoralis major* muscles; untreated/oven heating (a), NaCl+STPP treated/oven heating (b), freeze-thawed/oven heating (c), untreated/steaming (d), NaCl+STPP treated/steaming (e), freeze-thawed/streaming (f), untreated/boiling (g), NaCl+STPP treated/boiling (h), freeze-thawed/boiling (i), untreated/microwave heating (j), NaCl+STPP treated/microwave heating (k) and freeze-thawed/microwave heating (l). Scale bar indicates 50 μm, magnification=500x.

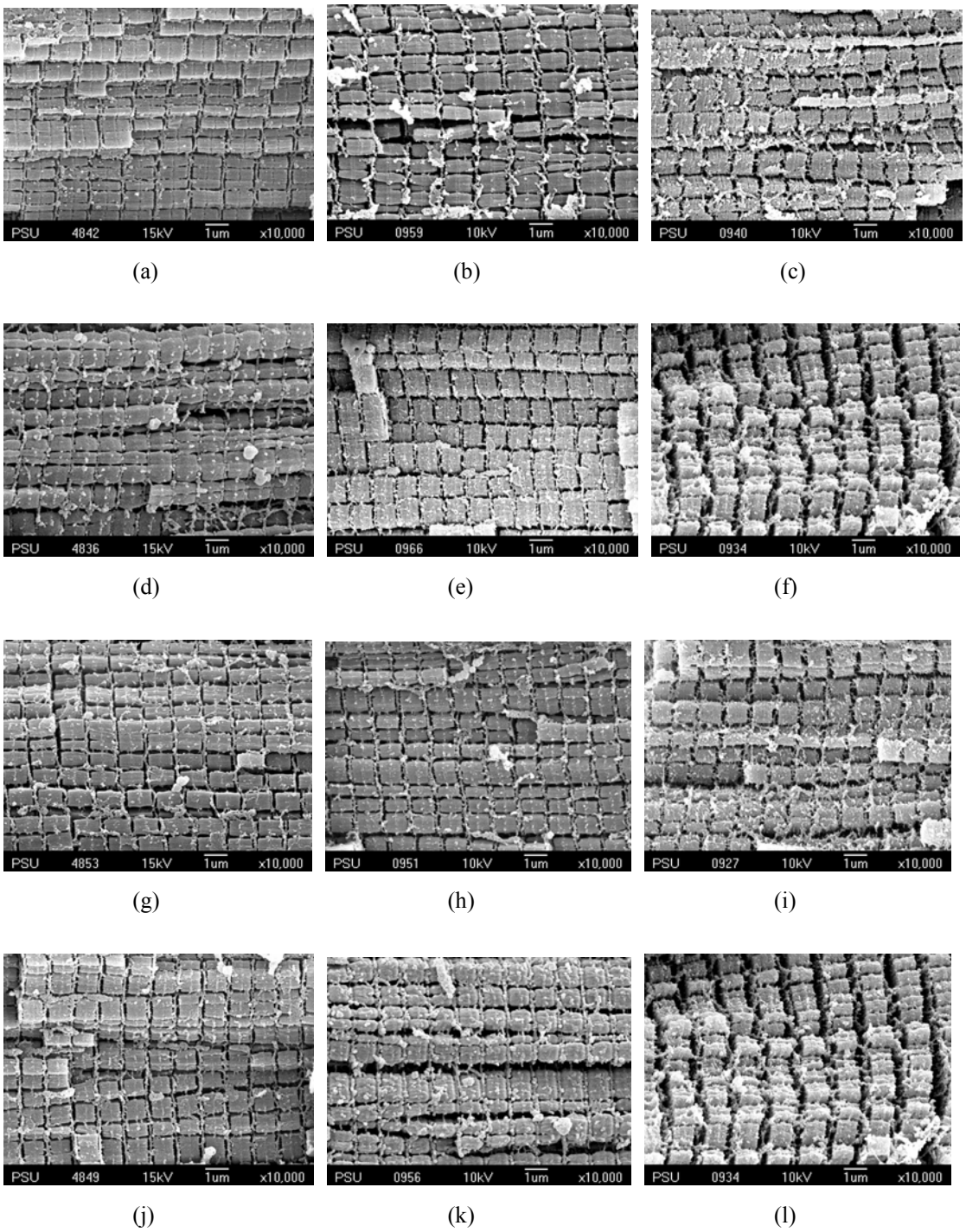


Figure 23. Scanning electron micrographs of longitunidual sections of cooked spent hen *Pectoralis major* muscles; untreated/oven heating (a), NaCl+STPP treated/oven heating (b), freeze-thawed/oven heating (c), untreated/steaming (d), NaCl+STPP treated/steaming (e), freeze-thawed/streaming (f), untreated-boiling (g), NaCl+STPP treated/boiling (h), freeze-thawed/boiling (i), untreated/microwave heating (j), NaCl+STPP treated/microwave heating (k), and freeze-thawed/microwave heating (l). Scale bar indicates 1 μm, magnification=10,000x.

4.3 Sensory evaluation

Flavor, texture, color, and overall preference of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods are presented in Table 14**.** For all heating methods, cooking meat by fresh raw samples exhibited the highest flavor scores than other raw materials ($P<0.05$). In addition, significant differences on flavor scores of cooked fresh raw meat samples were found ($p<0.05$), whereas these phenomenal did not observed when using samples treated with NaCl/STPP or freeze-thawed samples ($P\geq0.05$). Cooked fresh raw samples by using oven heating showed the highest flavor scores than all other heat methods. Treated samples with NaCl/STPP and freeze-thawed meat before heating with all methods could improve texture as indicated by both sample showed higher texture scores than those found in fresh raw samples ($P<0.05$). Moist heat methods exhibited higher texture scores than oven heating ($P<0.05$). The result was probably due to higher water retention in muscle samples by using moist heat as described previously. The same trends were found on color scores, moist heat methods exhibited higher color scores than oven heating ($P<0.05$) elucidated panelists expected more white in color for cooked spent hen meat. Boiling meat showed significant difference in color scores among all materials used ($P<0.05$), while other heating methods did not find these phenomenal. Using different heating methods did not affect on overall preference scores ($P\geq0.05$), while fresh raw samples showed higher overall preference scores than those found in samples treated with NaCl and STPP or freeze-thawed samples.

Table 14. Sensory characteristics of cooked spent hen *Pectoralis major* muscles prepared by different raw material pretreatments and heating methods.

Heating methods	Flavor			Texture			Color			Over all		
	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed	untreated	NaCl/STPP treated	freeze-thawed
Oven heating	6.90±0.80$^{a, x}$	5.40±0.72$^{a, y}$	5.60±0.67$^{a, y}$	4.70±0.88$^{b, y}$	5.33±0.84$^{c, x}$	5.53±0.82$^{b, x}$	4.97±0.81$^{c, x}$	5.20±1.16$^{b, x}$	5.27±1.23$^{b, x}$	6.20±1.00$^{a, x}$	5.50±0.82$^{a, y}$	5.63±1.19$^{a, y}$
Steaming	6.17±0.83$^{b, x}$	5.23±0.90$^{a, y}$	5.13±0.73$^{a, y}$	6.00±1.11$^{a, y}$	6.43±0.77$^{a, xy}$	6.67±0.84$^{a, x}$	6.87±0.94$^{a, x}$	6.33±1.06$^{a, x}$	6.57±0.97$^{a, x}$	6.47±0.97$^{a, x}$	5.87±1.04$^{a, x}$	5.97±1.03$^{a, x}$
Boiling	5.87±0.97$^{b, x}$	5.07±0.83$^{a, y}$	5.33±0.96$^{a, y}$	6.13±1.11$^{a, x}$	6.17±0.91$^{ab, x}$	6.33±0.76$^{a, x}$	6.83±0.84$^{a, x}$	6.30±1.06$^{a, y}$	6.27±1.08$^{a, y}$	6.40±0.93$^{a, x}$	5.67±1.06$^{a, y}$	5.97±1.16$^{a, xy}$
Microwave heating	5.73±0.98$^{b, x}$	5.17±0.79$^{a, y}$	5.43±0.86$^{a, xy}$	5.17±0.83$^{b, y}$	5.97±0.72$^{b, x}$	5.80±0.85$^{b, x}$	6.00±1.05$^{b, x}$	6.07±1.05$^{a, x}$	6.43±1.04$^{a, x}$	5.90±0.96$^{a, x}$	5.77±0.90$^{a, x}$	5.83±0.91$^{a, x}$

Data are presented as mean ± standard deviation; n=30. (9=like extremely, 1=dislike extremely).

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-z}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

5. Effect of ginger powder (GP) on tenderization of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

5.1 Quality characteristics of ginger powder

The ginger powder (GP) was creamy-white in color as shown in Figure 24 and its characteristics are presented in Table 15.

Figure 24. Ginger powder obtained by ethanol extraction.

The yield of GP obtained in this study (Table 15) was lower than those reported in Bangalore ginger (3.80%) and Coorg ginger (7.60%) by Bhaskar *et al*. (2006) who prepared ginger powder by using ethanol extraction. This probably caused by different variety of ginger used and the quantity of pulp removed during the extraction process. Ginger powder prepared by using acetone precipitation also shown large variation in yield. Thomson *et al*. (1973) reported a yield of 2.26 %, whereas the yield obtained by Adulyatham (2001) showed higher content between 12-15%. Duke

(2003) noted that ginger contains a proteolytic enzyme in present at level of 2.26% of fresh rhizome, such that 50 kg ginger can yield 1 kg of the enzyme; by contrast, it takes 8000 kg papaya to produce 1 kg papain that showed an advantage of ginger protease as an alternative to papain in commercial application. In addition, low moisture content of GP (Table 15) obtained after dehydrated by ethanol leading to low water activity in system that could be advantage for high stability because enzyme 3-D structure is more rigid within low water activity systems (Lui *et al*., 1991; Apenten, 1998). Adulyatham and Apenten (2005) found that crude ginger extracted from fresh ginger (*Zingiber offinale* Roscoe) had a half-life ($t_{1/2}$) of 2 days at 5^{o}C decreasing to 20 min at 30^{o}C, whereas ginger powder prepared by acetone precipitation greatly improved the stability of GE with $t_{1/2}$ of 18 months at 5^{o}C. Moreover, the treatment of ginger rhizome with polar solvents also significantly enhanced the specific activity of ginger protease by the removal of various constituents of ginger (Thomson *et al*., 1973), especially hydrophobic plant polyphenols which causing enzyme deactivation via enzymatic browning reaction (Adulyatham and Apenten, 2005). Bhaskar *et al*. (2006) reported an increase in the specific activity of proteases obtained by ethanol treatment were 6.9- and 8.3-fold higher for Bangalore and Coorg ginger powder, respectively when compared to fresh ginger. The specific activity of GP in current study (Table 15) was higher than those obtained from Bangalore (20.60 units/mg protein) and Coorg (16.60 units/mg protein) ginger as reported by Bhaskar *et al*. (2006). This could be different variety of ginger rhizome used and different process in adding sodium ascorbate to protect the active size of enzyme (Adulyatham, 2001) resulted in higher specific activity. Adulyatham and Apenten (2005) found that at temperature lower than 40^{o}C, ginger extract and ginger powder obtained by acetone precipitation could stabilize by sodium ascorbate. Adding sodium ascorbate during process might reduce deactivation of enzyme due to inhibitors effect of ascorbate via enzymatic browning reaction. Adulyatham and Apenten (2005) explained that the process involved the oxidation of polyphenols to from quinines catalyzed by an endogenous polyphenol oxidase (PPO). The quinines could then react with amine, sulhydryl, and other nucleophilic residues from ginger rhizome, thereby causing enzyme deactivation.

Table 15. Some quality characteristics of ginger powder obtained by ethanol extraction.

Quality characteristics	
Yield (% as peeled)	2.67±0.21
Moisture content (%)	12.25±0.12
Specific activity of protease (units/mg protein)*	33.59±0.59

Data are presented as mean ± standard deviation from two lots of ginger rhizome. (n=3×2, triplicate determination × lots).

*Protein in ginger powder=4.02±0.12 mg/g sample.

5.2 Changes in chemical properties

5.2.1 TCA-soluble peptides

The effects of different soaking times in 2.5% GP solution of spent hen *Pectoralis major* muscles on TCA soluble peptides are presented in Figure 25. Significantly higher TCA soluble peptides were observed in all GP treated samples compared to untreated samples ($P<0.05$) throughout chilled storage. This could be elucidated that soaking spent hen meat in GP solution could enhance proteolysis. Among all treated samples, 2 h treated had slightly higher efficiency for proteolysis spent hen meat than other GP treated samples. This result perhaps caused by the longer soaking time at room temperature (RT) which was closer to optimum temperature of ginger protease enzyme at 60^{o}C (Thompson, *et al*., 1973; Adulyatham, 2001). Storage time also influence on proteolysis for all samples either treated or untreated samples. At the beginning of storage, slightly increased in TCA soluble peptides of all samples were observed, thereafter a dramatic increase in these values were found ($P<0.05$). It was probably due to the proteolysis in meat during the initial stages caused by endogenous proteases in skeletal muscles (Ouali, 1990; Koohmaraie, 1992; Walker *et al*., 1995), whereas for longer time of storage might be resulted from proteases of microorganism (Allen *et al*., 1998).

5.2.2 Protein solubility

An effect of GP on solubilizing protein of cooked spent hen *Pectoralis major* muscles was found as indicated by increasing in protein solubility (Figure 26). Significantly higher protein solubility values were observed in all GP solution treated

samples compared to untreated samples ($P<0.05$). An increase in protein solubility with ginger extract treatment has been reported on spent hen meat by Naveena and Mendiratta (2001) and on buffalo meat chunks by Naveena *et al*. (2004). Increase in solubility of enzyme treated samples might be due to increase in permeability of myofibrils, which will disintegrate easily (Naveena *et al*., 2004). Lee *et al*. (1986) stated that the mechanism of the tenderization of muscles by proteolytic enzymes from ginger is attributed to the fragmentation of myofibrils by preferential degradation of these filaments in the I-bands. In untreated samples, regularly aligned filaments of myofibrils prevent buffer penetration, thus making action seeming resistant to extraction. The higher protein solubility of sample at RT treated than that of 24 h treated at 4°C samples indicated more pronounced proteolysis. Treated muscle samples held at 4°C for 24 h had less activity than treated at RT ($P<0.05$) perhaps caused by the enzyme has an optimum temperature of 60°C (Thompson, *et al*., 1973; Adulyatham, 2001) so such longer periods of time were needed due to the enzyme activity is lower at refrigerated temperature.

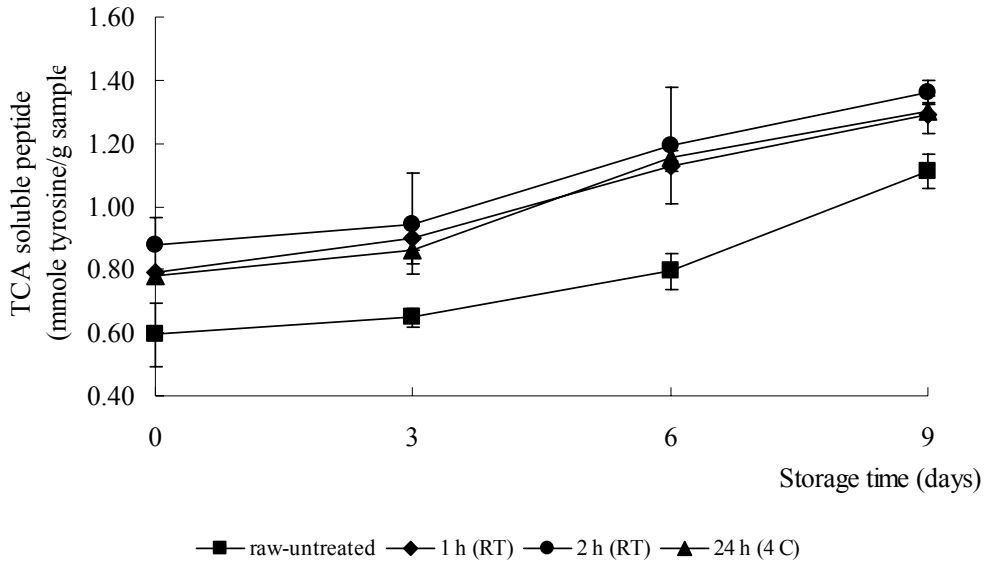


Figure 25. Changes in TCA-soluble peptides (mmole tyrosine/g sample) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

5.2.3 Soluble collagen content

The collagenase activity of GP on spent hen *Pectoralis major* muscles was found as indicated by an increasing in soluble collagen content (Figure 27). An increase ($P<0.05$) in collagen solubility were observed in all GP treated samples compared to untreated samples throughout storage time and 1 h treated samples showed the highest values. Similar observations in increasing soluble collagen of spent hen meat treated with 5% (v/w) of ginger extract for 24 h at 4^{o}C on day 0 had been reported by Naveena and Mendiratta (2001). High proteolytic activity against collagen and other connective tissue proteins of ginger protease have been reported (Thompson *et al*., 1973, Choi *et al*., 1999; Kim *et al*., 2007). Previous report of Choi *et al*. (1999) showed that a cysteine protease from ginger rhizome (GPII) cleaves peptides and proteins with proline at the P_2 position. The preference of this enzyme on proline at the P_2 position has been confirmed by Kim *et al*. (2007) who found that ginger protease (GP2) showed at least 10-fold higher efficiency of hydrolysis than that of papain. As discussed earlier, spent hen muscles had high content of collagen (Table 6) at 6.47±0.20 mg/g sample which 20% of the amino acids in the collagen structure are proline and hydroxyproline (Zhao, 2010). Therefore, ginger protease with interested in proline, could degrade collagen of spent hen muscles resulted in higher soluble collagen of GP treated samples than untreated sample. Treated muscle samples held at 4^{o}C for 24 h had less activity than treated at RT ($P<0.05$) perhaps caused by the enzyme has an optimum temperature of 60^{o}C (Thompson *et al*., 1973; Adulyatham, 2001) so such longer periods of time were needed due to the enzyme activity is lower at refrigerated temperature. Surprisingly, 1 h treated samples at RT exhibited more activity than 2 h. This was probably due to longer time of soaking might result in some soluble collagen release into GP solution, thus resulted in less soluble collagen content remained in muscles. Storage time had no effect on soluble collagen content of spent hen meat treated with GP solution as elucidated by non significant differences of these values for all treated samples were found throughout chilled storage ($P\geq0.05$). The results indicated that GP could be used as an effective protease enzyme for tenderizing spent hen *Pectoralis major* muscles due to it could hydrolyze high collagen content of spent hen meat as the target substrate.

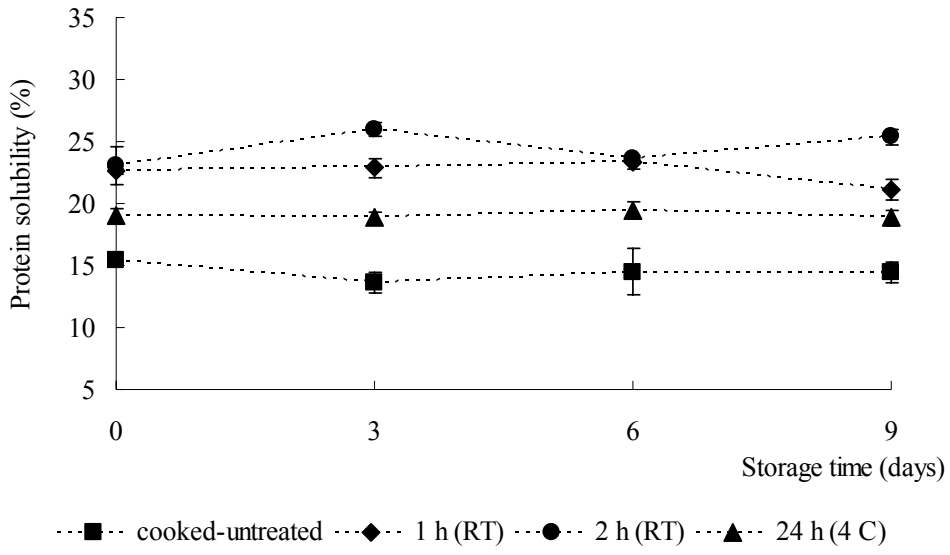


Figure 26. Changes in protein solubility (%) during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

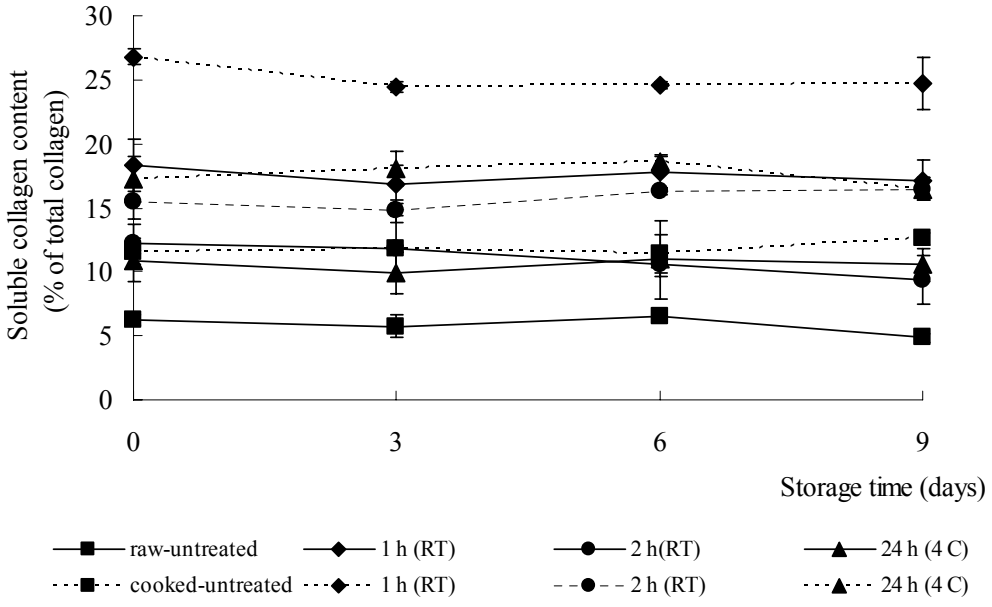


Figure 27. Changes in soluble collagen content (% of total collagen) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

5.3 Change in physical properties

5.3.1 Cooking loss and weight loss

The effect of GP on WHC of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated by cooking loss of raw samples and weight loss of cooked samples as presented in Figure 28 and 29. All GP treated samples showed higher cooking loss compared to untreated samples throughout storage time ($P<0.05$). In addition, soaking time with GP had marked effect on cooking loss as indicated by an increase in cooking loss of samples in the order of soaking time were observed for all storage time. Naveena *et al*. (2004) reported that the cooking yield of 5% ginger extract-treated buffalo muscle had shown to be marginally higher compared to control which soaking muscle in distilled water. Breast muscles from layer-chicken treated with ginger powder (25.5%) had lower cooking loss compared to control (36.2%) (Bhaskar *et al*., 2006). This was indicated that GP treated had an effect on WHC of raw samples during chilled storage. It was probably caused by degradation of myofibrillar and sarcoplasmic proteins in spent hen meat via proteolysis process which could be enhanced by adding GP as indicated by the higher TCA-soluble peptide of treated than untreated samples (Figure 25). As mentioned earlier, protein degradation resulted in low property of proteins to hold water in muscles and exhibited increase in cooking loss for all samples during chilled storage. The evidences of increase in cooking loss when increasing soaking time of muscles in GP solution at RT were expected because enzymes could work well near optimum temperature leading more protein degradation for 2 h than 1 h and 0 h. The optimum temperature of ginger protease at 60^{o}C has been reported (Thompson *et al*., 1973; Adulyatham, 2001). The highest cooking loss of meat samples soaking at 4^{o}C was associated with higher TCA soluble peptide (Figure 25) probably involved with protease enzyme from psychrophillic bacteria resulted in protein degradation still occurred during chilled storage.

As shown in Figure 29, no changes on weight loss of 1 h and 24 h cooked treated and untreated samples up to day 6 of storage were observed ($P\geq0.05$), thereafter an increase in weight loss of all these samples were found ($P<0.05$), whereas 2 h treated samples exhibited a dramatic increase throughout storage ($P<0.05$). This could be demonstrated that storage time had an effect on weight loss of

samples for longer storage time than at the beginning of storage. Soaking meat in GP solution showed good efficiency to reduce weight loss of cooked spent hen meat as elucidated by lower weight loss of treated samples than untreated samples and 1 h treated samples showed the lowest values throughout storage ($P<0.05$). The greatest effect of 1 h treated samples on weight loss might be caused by this samples had more collagen were melt (Figure 27) and exhibited gel matrix when heating had carried out which could prevent water molecule in muscles leading to lower weight loss than other samples. The results could be summarized that although soaking spent hen meat in GP solution reduced WHC of raw meat, it could prevent weight loss of cooked spent hen meat, especially at 1 h treatment before cooking. Thus, 1 h soaking time in GP solution at RT is suitable for tenderizing spent hen meat and obtains a great benefit in yield during chilled storage.

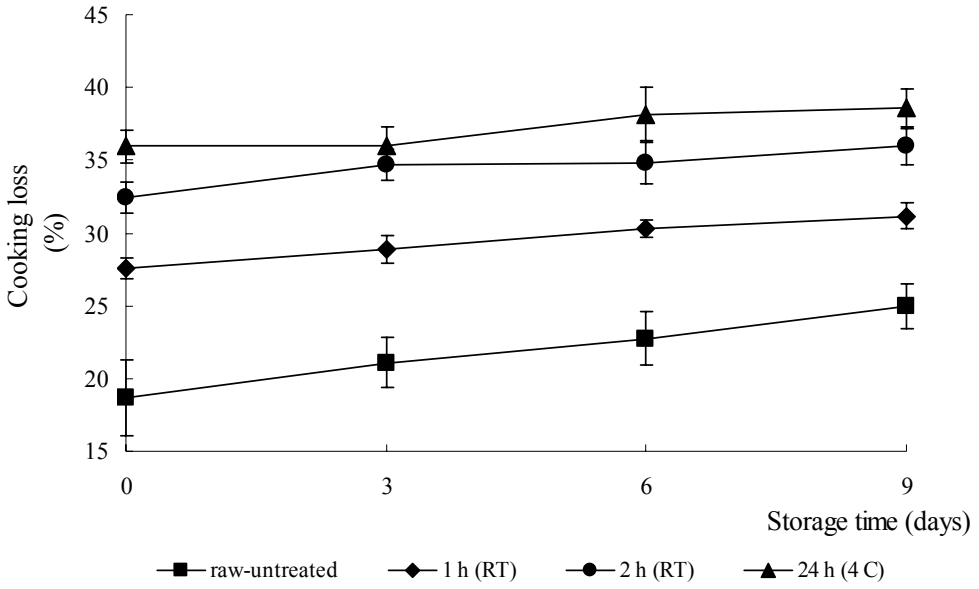


Figure 28. Changes in cooking loss (%) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for eight determinations.

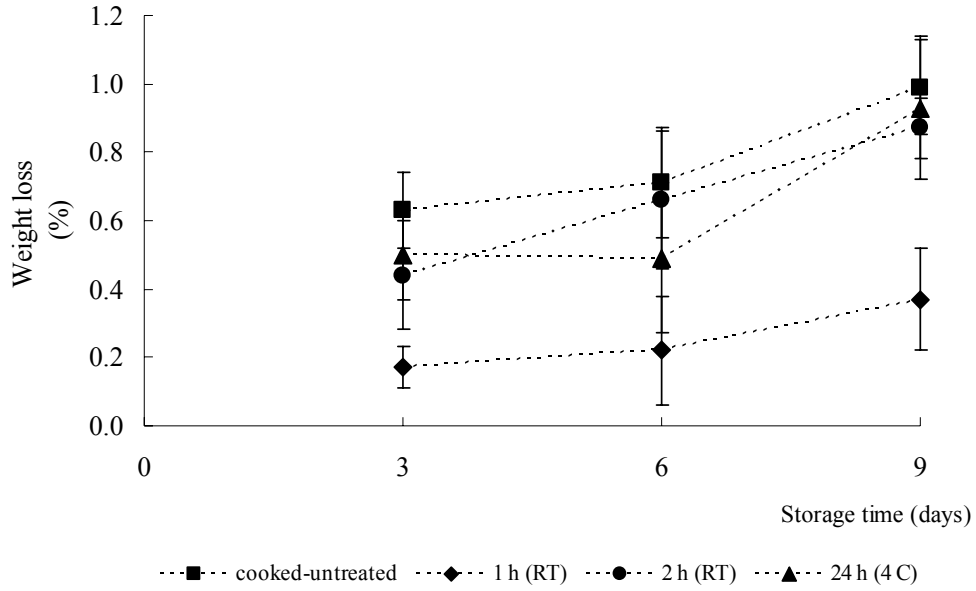


Figure 29. Changes in weight loss (%) during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for 4 determinations.

5.3.2 Warner-Bratzler shear force values

Change in texture during chilled storage of raw and cooked spent hen *Pectoralis major* muscles treated with GP solution at different soaking time are shown in Figure 30. All samples treated with GP showed lower shear force values at day 0 and day 3 of storage than that of untreated sample ($P<0.05$), thereafter significant differences were not found ($P\geq0.05$). This was indicated that GP treated had little effect on shear force values of raw samples during chilled storage. It was probably due to protease from GP could induce more proteolysis at the beginning of storage than for longer time of storage. A decrease in shear force values was in good agreement with the report of Bhaskar *et al*. (2006) stated that raw breast and leg muscles of spent hen treated with ginger powder were found to be significantly more tender as indicated by lower shear values compared to the shear values of control samples. Tenderizing by direct application of an aqueous ginger extract as elucidated by lower shear force values has been reported in beef muscles (Kim and Lee, 1995), spent hen meat (Naveena and Mendiratta, 2001), and buffalo meat chunks (Naveena *et al*., 2004). Treated muscle samples with GP at RT for 1 h has marked effect on decrease in toughness than other time treatments as indicated by lower shear force

values. The tenderizing spent hen meat with ginger powder had been hold at 28 ± 2^{o}C for 3 h (Bhaskar *et al*., 2006); hence, the results from current study required shorter period of time for tenderizing spent hen meat. Treated muscle samples held at 4^{o}C for 24 h had less activity than treated at RT ($P<0.05$) perhaps caused by the enzyme has an optimum temperature of 60^{o}C (Thompson *et al*., 1973; Adulyatham, 2001) so such longer periods of time were needed due to the enzyme activity was lower at refrigerated temperature. Holding ginger extract-treated muscles at 4^{o}C for periods of 48 h (Naveena *et al*., 2004) or 5 days (Syed Ziauddin *et al*., 1995) have been reported in buffalo meat. Although GP treated had little effect on texture of raw samples during chilled storage, it had more improvement on texture of cooked samples. It was postulated by significantly lower shear force values of cooked muscle with GP treated compared to untreated samples throughout storage ($P<0.05$). This clearly indicated an increase proteolysis of muscle proteins by protease enzyme from GP. The mechanism of the tenderization of muscles by proteolytic enzymes from ginger is attributed to the fragmentation of myofibrils by preferential degradation of these filaments in the I-bands (Lee *et al*., 1986). In addition, highly active against collagen and other connective tissue proteins of ginger protease (Thompson *et al*., 1973, Ichikawa *et al*., 1973; Choi *et al*., 1999; Kim *et al*., 2007) could degrade collagen of spent hen muscles leading to more collagen was melt (Figure 27) to become gelatin during cooking which tenderness of muscle is carried out. The same trends for the great effective to reduce toughness of raw muscles were found in cooked samples treated with GP at RT for 1 h. Therefore, spent hen meat treated with GP at RT for 1 h may be an advantage method for tenderizing. The results could be demonstrating the effective of GP on decreasing toughness of both raw and cooked spent hen muscles during chilled storage and support a role of ginger protease as meat tenderizer in commercial application, where collagen is the target substrate. Significant differences in shear force values were not observed in both raw and cooked samples throughout storage ($P\geq0.05$).

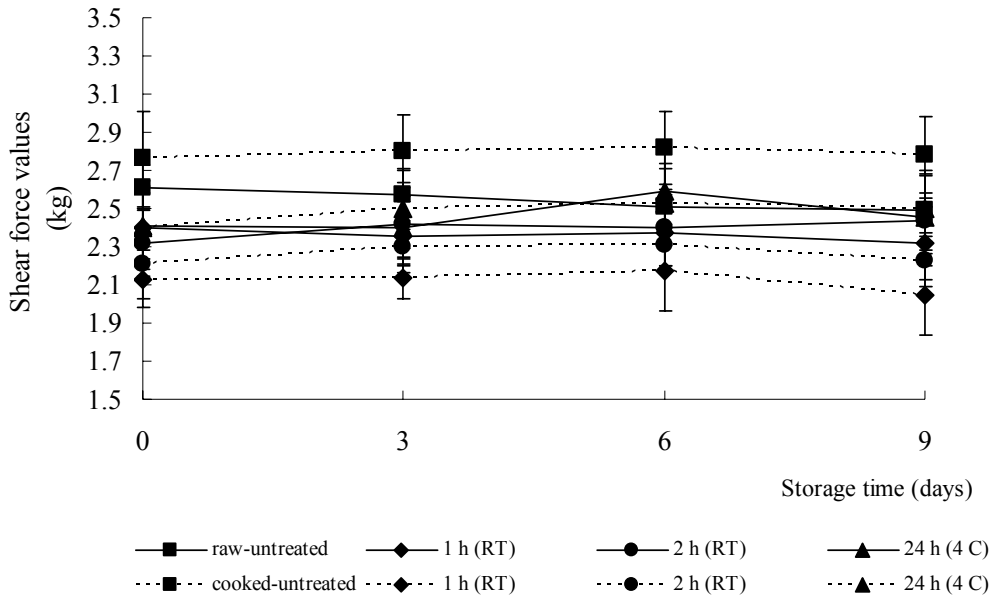


Figure 30. Changes in shear force values (kg) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples. Bars represent the standard deviations for eight determinations.

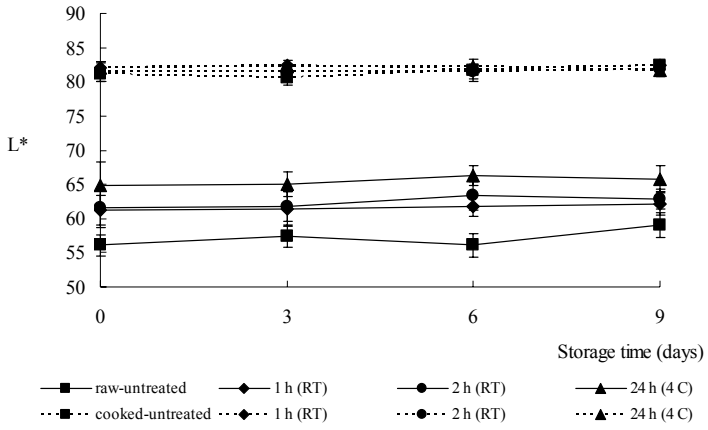
5.3.3 Surface color

Surface color of raw spent hen muscle samples in this section exhibited higher L*, a* and b* compared to previous section perhaps due to difference source of raw materials supplied. Color of meat could be influenced by breeds, feed diets and age of animals (Miller, 1994; Fletcher, 1999a, b).

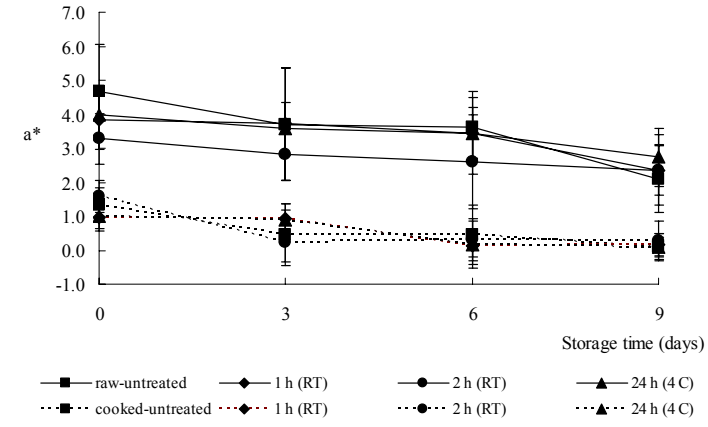
Changes in surface color during 0, 3, 6, and 9 days of storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples are presented in Figure 31. Treated raw spent hen meat with GP had influence on L* values as indicated by significantly higher L* values of treated samples than that of untreated samples throughout storage ($P<0.05$) (Figure 31a). In addition, increasing soaking time of meat in GP solution at RT had non-significance on L* values between 1 and 2 h treated ($P\geq0.05$), while 24 h treated at 4°C showed slightly increase in this values ($P<0.05$). However, heat processing could reduce this effects as elucidated by non significant differences in L* values among all samples ($P\geq0.05$). L* values of raw untreated samples increased with storage time ($P<0.05$), whereas no changes on L*

values of all raw and cooked treated samples was found throughout storage ($P \geq 0.05$). Thus, treatment with GP solution could stabilize L* value of spent hen meat during chilled storage. As shown in Figure 31b, soaking spent hen meat in GP solution had little effect on a* values of both raw and cooked samples during chilled storage as elucidated by significant difference between all treated and untreated samples were found only at the beginning of storage ($P<0.05$). A slightly decrease in a* values of all treated and untreated samples were found when storage time increased ($P<0.05$). This could be due to reducing in myoglobin content via drip loss during chilled storage as described previously. The same trends of decrease in a* values ($P<0.05$) for all cooked treated and untreated samples were observed as storage time increase. The effect of GP on b* values of both raw and cooked spent hen meat were observed from day 0 to day 6 of chilled storage (Figure 31c). All raw and cooked treated samples exhibited lower b* values compared to untreated samples ($P<0.05$), although it pronounced this phenomenal at different time of storage. Slightly effect of storage time on b* values were found in raw untreated and 1 and 2 h raw treated samples but did not observed this effect on 24 h treated samples. Storage time had no effect on b* values of cooked untreated samples ($P \geq 0.05$), whereas a slight increase in all treated samples during storage were found ($P<0.05$). The different between a* and b* value of spent hen muscle in this section compare to other sections probably due to different raw material source of spent hen used in this section, which was difference in age and feed diets. The high variations on color of chicken meat also reported by many investigators (Miller, 1994;Young and Lyon, 1994, 1997; Fletcher, 1999a, b; Kim and Marshall, 1999; Fletcher *et al*., 2000; Young and West, 2001) which were related to species, breed, muscle type, sex, feed diets, age and method of carcasses processing.

Therefore, adding GP had effect on color values of raw spent hen meat, however, heat processing could reduce this effect as summarized by it showed reducing in different L* and a* color values among spent hen cooked meat.



(a)



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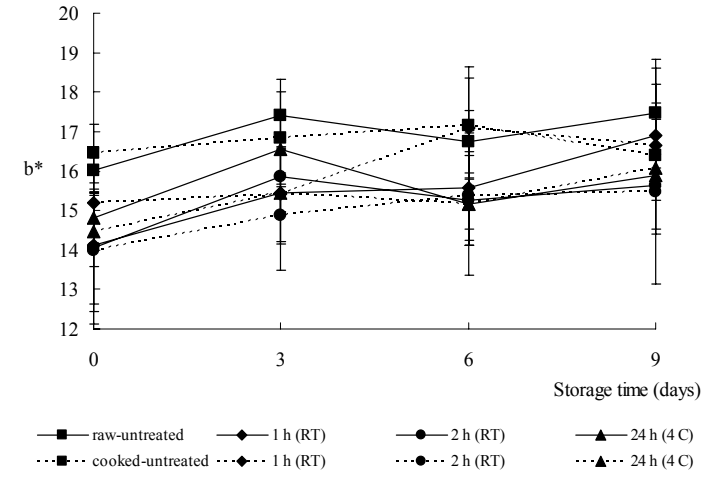


(c)

Figure 31. Change in L* values (a), a* values (b), and b* values (c) during chilled storage of raw and cooked spent hen *Pectoralis major* muscles treated with ginger powder solution at RT for 1, 2 h and treated at 4^{o}C for 24 h compared to untreated samples. Bars represent the standard deviations; n= 8.

6. Effect of tea catechins (TC) on oxidative stability of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

6.1 Changes in chemical properties

6.1.1 Changes in TBARS

Effect of TC on lipid oxidation of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated changes in TBARS values as presented in Figure 32. Storage time had effect on lipid oxidation of both raw and cooked spent hen meat either treated or untreated muscle samples. As storage time increased from day 0 to day 6, a dramatic increase in TBARS values of raw untreated samples were found ($P<0.05$) and then approached a plateau thereafter ($P\geq0.05$). Whereas a slight increase in all TC treated samples were observed throughout storage ($P<0.05$). Treated raw muscle samples with TC could reduce lipid oxidation of both raw and cooked muscle samples during chilled storage as evidenced by lower TBARS values of all treated samples compared to untreated samples throughout storage ($P<0.05$). In addition, TC treated at higher level exhibited greater efficient to retard lipid oxidation in both raw and cooked spent hen meat. In raw muscle samples, adding TC at the level of 150 and 200 mg/kg muscle showed significantly lower TBARS values than at the level of 100 mg/kg muscle throughout storage ($P<0.05$), whereas in cooked samples the lowest values were found when treated with TC at the level of 200 mg/kg muscle ($P<0.05$). The results indicated that TC could delay the accumulation of oxidation products in spent hen meat during chilled storage. The results obtained were in agreement with previous researchers reported effective of TC to reduce lipid oxidation in cooked beef, beef patties, and chicken meat (Tang *et al*., 2001a; Mitsumoto *et al*., 2005), in raw red meat, poultry and fish muscle (Tang *et al*., 2001b). The tea catechins and other flavonoids in green tea have been recognized as efficient antioxidants for scavenging oxygen radicals and chelating metal ions (Wanasundara and Shahidi, 1998; Bozkurt *et al*., 2006). Tea catechins have been studied to have a wide concentrations range of 200-10,000 mg/kg meat to reduce lipid oxidation in raw and cooked meat (Mitsumoto *et al*., 2005). This study indicated that lower TC concentration of 150-200 mg/kg meat had ability to retard lipid oxidation in spent hen meat probably due to more purify of catechins (84.84%) consisting of high contents of

(3)-epigallocatechin gallate (EGCG) (70%) used in current study. The relationships between the free radical scavenging activities and the chemical structures of tea catechins and their corresponding epimers have been reported by Tang *et al*. (2002). Their results showed that the presence of the gallate group at the 3 position plays the most important role in free radical-scavenging ability of catechins resulted in the effects of galloyated catechins on free radicals were stronger than those non galloyated catechins. In addition, EGCG could permeate with the lipid bilayer membrane structure due to their high affinity for lipid bilayers (Hashimoto *et al*., 1979) that could be more efficiency to use with spent hen meat which containing high fat content. Moreover, cooked muscle samples had faster oxidation than raw muscle samples as elucidated by all treated samples and control of cooked sample showed dramatic increase in TBARS values compared with gradual increased in these values of raw samples during chilled storage ($p<0.05$). Cooking not only disrupts the membrane structure but also facilitates release of iron from the iron carrier proteins or iron storage conditions (Ahn *et al*., 1993). Under such conditions oxygen can directly react with the ferrous iron in the meat and activate the ground state oxygen to a highly reactive form (Halliwell and Gutteridge, 1990) resulted in rapid oxidation leading to high TBARS value. However, the chelating effect of TC could reduce this phenomenon by binding with the iron components, stabilizing them in an inactive or insoluble form (Allen and Cornforth, 2009) resulted in more efficiency of TC to reduce lipid oxidation in cooked meat.

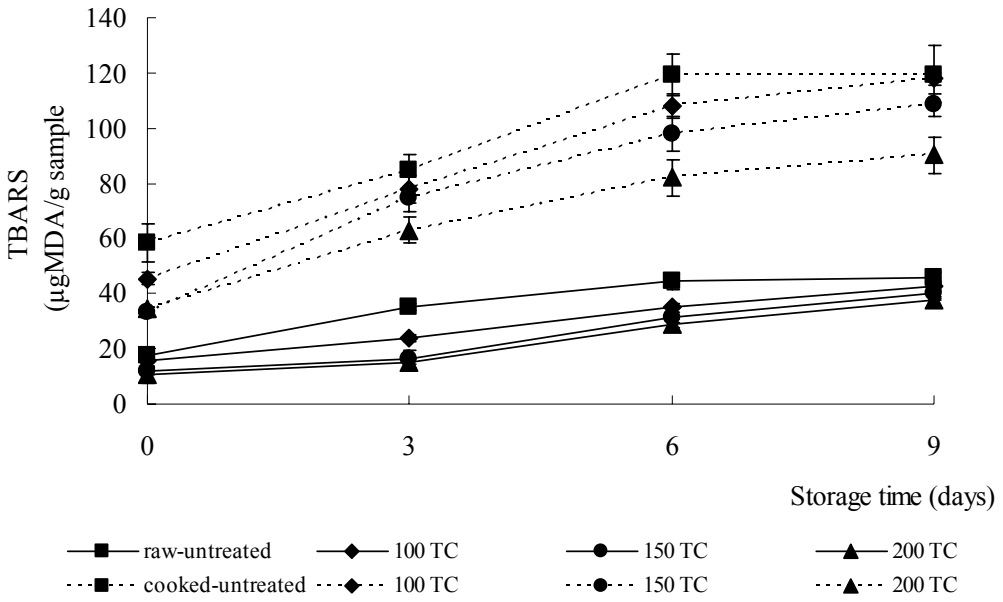


Figure 32. Changes in TBARS values (µg MDA/g sample) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle. Bars represent the standard deviations for triplicates determinations.

6.2 Changes in physical properties

6.2.1 Cooking loss and Weight loss

The effect of TC on WHC of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated by determining cooking loss of raw and weight loss of cooked samples as presented in Figure 33 and 34. Cooking loss of raw and weight loss of cooked samples was not affected by adding TC as elucidated by significant differences between untreated and treated samples were not found throughout storage ($P \geq 0.05$). This was indicated that TC treated had no effect on WHC of both raw and cooked muscle samples. However, both parameters were affected by storage time. An increase in cooking loss of raw samples were found in all TC treated and control untreated samples ($P < 0.05$). The same trends were found in weight loss of cooked samples. These results were expected. As mentioned earlier, during chilled storage caused protein degradation resulted in low property of proteins to hold water in muscles and exhibited increase in both values.

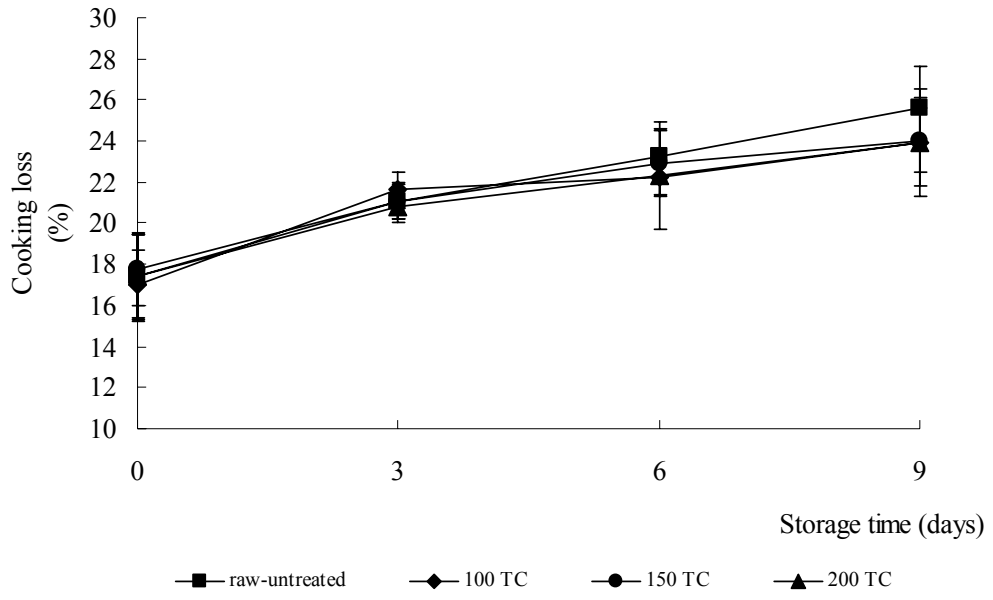


Figure 33. Changes in cooking loss (%) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle. Bars represent the standard deviations for eight determinations.

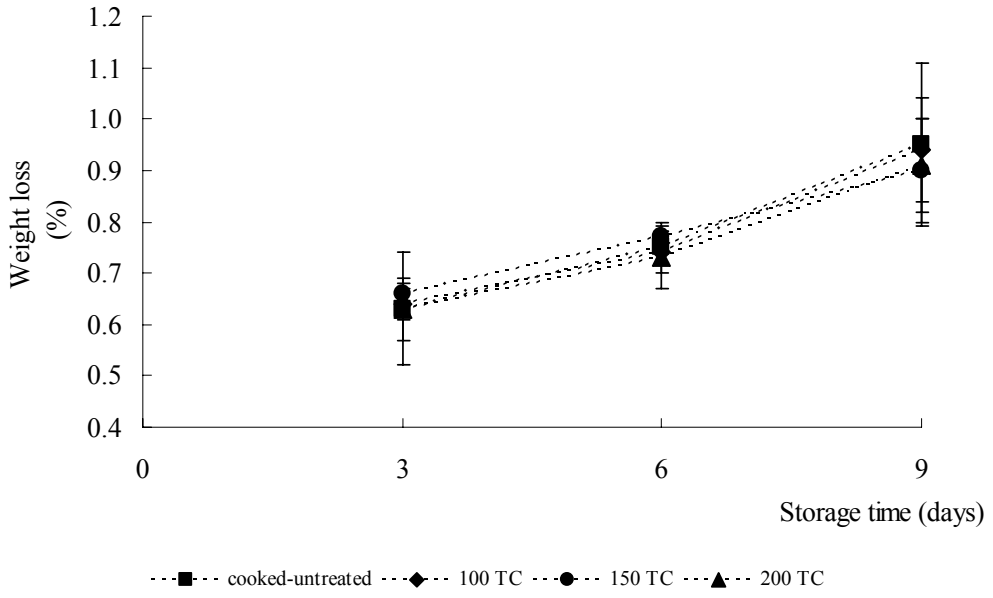


Figure 34. Changes in weight loss (%) during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle. Bars represent the standard deviations for four determinations.

6.2.2 Warner-Bratzler shear force values

The effect of TC on texture of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated Warner-Bratzler shear force values as shown in Figure 35. Adding TC had no effect on texture of spent hen *Pectoralis major* muscles during chilled storage as elucidated by no significant differences in Warner-Bratzler shear force values between all TC treated and untreated samples for both raw and cooked muscle samples throughout chilled storage ($P \geq 0.05$). In addition, no changes on texture were observed in both raw and cooked samples during storage ($P \geq 0.05$).

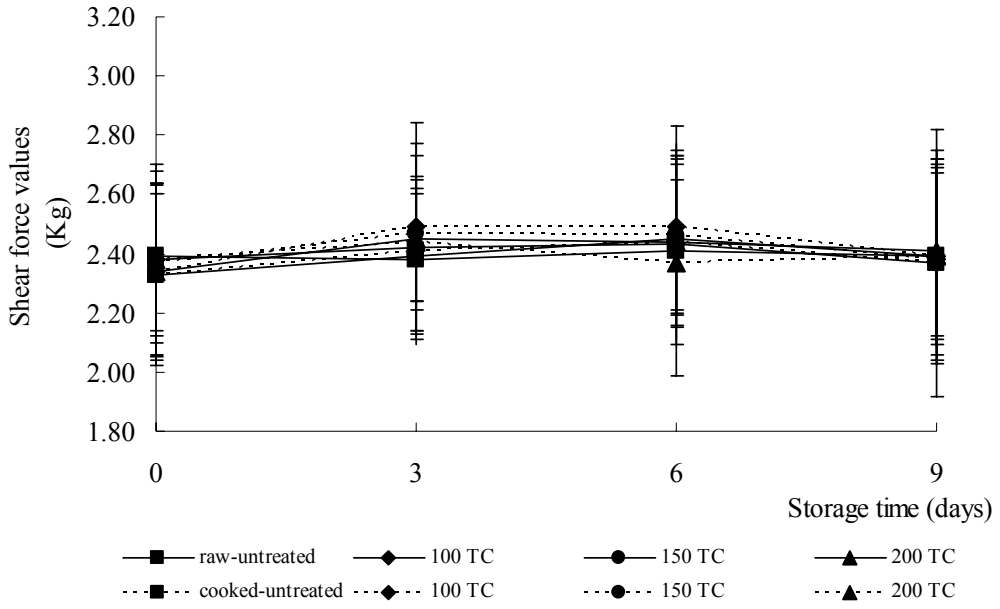


Figure 35. Changes in shear force values (kg) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 (mg/kg muscle). Bars represent the standard deviations for eight determinations.

6.2.3 Surface color

Changes in surface color of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle during chilled storage are presented in Figure 36. Treatment of TC had influence on L*, a*, and b* values of raw and cooked samples, especially in cooked meat. It showed darker color compared to untreated samples. TC used in the present study was a fine and water soluble powder, and was red-brown in color. Therefore, it was expected that spent hen muscle samples treated with TC had different color from untreated samples.

Discoloration of meat caused by adding TC have been reported in raw beef muscles by Zembayashi *et al*. (1999) and in meat patties (darker in color) by Mitsumoto *et al*. (2005). Tea catechins caused discoloration possibly by binding with the iron component of myoglobin (Mitsumoto *et al*., 2005). Conversely, a greatly improved color by adding TC have also reported in minced raw beef (Maher *et al*., 2002) and raw pork patties (Jo *et al*., 2003). This was probably caused by different kinds of raw material used. Mitsumoto *et al*. (2005) found that TC had greater influence on meat discoloration in meat patties prepared from white meat (chicken) than red meat (beef).

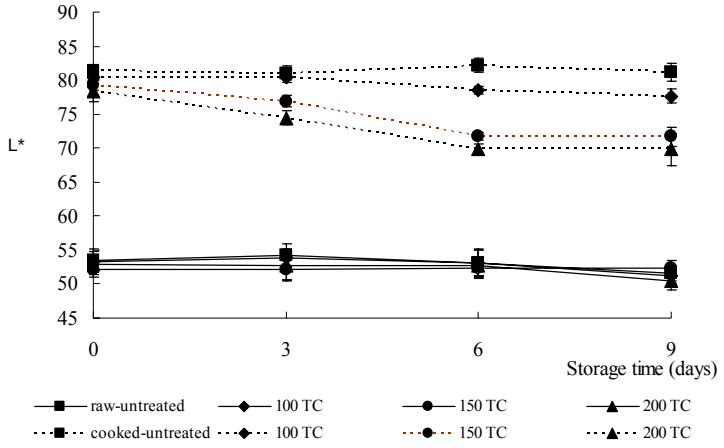
As shown in Figure 36a, treated raw spent hen meat with TC had no effect on L* values as indicated by no significant differences in L* values in all treated samples, compared to untreated samples throughout storage ($P \geq 0.05$). In contrast, adding TC had more influence on L* values of cooked samples. All TC treated samples showed significantly lower L* values, compared to untreated samples throughout chilled storage ($P < 0.05$). In addition, samples with increasing level of TC exhibited more decrease in L* values. Storage time had little effect on L* values of raw samples as indicated by slight decrease in these values either treated or untreated samples, especially at day 9 of storage ($P < 0.05$). For cooked samples, storage time had more pronounced influence on TC treated samples. As storage time increased from day 0 to day 6, adding TC at the level of 100 mg/kg muscle resulted in slight decrease in L* values, whereas dramatic decreases in these values were found in samples treated with TC at the level of 150 and 200 mg/kg muscle.

As shown in Figure 36b, adding TC had marked influence on a* values of both raw and cooked spent hen meat. Raw and cooked treated samples showed significantly higher a* values, compared to untreated samples throughout storage ($P < 0.05$), especially in cooked samples. The higher a* value in cooked samples in comparison with raw counterpart, might be affected by denatured myoglobin during heat treatment. Tea catechins might work as reducing agent, which could reduce metmyoglobin to oxymyoglobin to same degree. Lawrie (1991) noted that denaturation of red myoglobin and conversion to brown myohaemochromogen starts at 40^{o}C and is almost completely denatured between 80-85^{o}C. However, spent hen muscle had low content of myoglobin that resulted in negative a* value in raw muscle and changed to be positive a* value after cooking, indicating browner color. Tang *et*

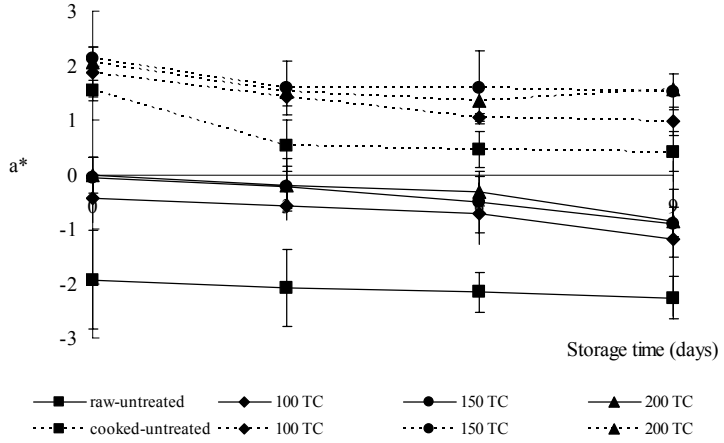
al., (2006) reported redder color in beef patties after TC treatment. The authors also suggested that TC polyphenol react readily with iron in meat to promote discoloration. However, this hypothesis requires for further study (Mitsumoto *et al*., 2005; Tang *et al*., 2006). During storage, treatment of TC at higher levels had no change on a* value of raw samples ($P \geq 0.05$). However, cooked meat treated with TC 100 mg/kg showed significant decrease in a* value ($P < 0.05$), while treatment of TC at 150 and 200 mg/kg muscle resulted in stability of a* value in cooked samples during 3 to 9 days of storage ($P \geq 0.05$). However, Zembayashi *et al*. (1999) found that dietary green tea (0.5 kg/day for 174 days before slaughter) decreased the iron content and a* value of raw beef muscles compared to controls, and they suggested that dietary tea reduced iron absorption in meat.

From Figure 36c, adding TC had more effect on cooked spent hen samples than raw samples. All cooked TC treated samples exhibited significantly lower b* values compared to untreated samples throughout storage ($P < 0.05$), while this phenomenon was less pronounced in raw samples during storage. In addition, adding TC at higher level in cooked meat resulted in lower b* values. Storage time had slight increase in b* of cooked untreated, while all TC cooked treated samples showed dramatic decrease in these values on day 0 to day 3 of storage. As the storage time increased, no changes in b* values for all TC treated were found.

Discoloration caused by TC might reduce the visual appearance and overall acceptability of spent hen meat, especially cooked spent hen meat products without adding other seasoning which consumers expect more white color. TC 200 was superior to TC150 for inhibiting lipid oxidation in both raw and cooked spent hen meat; however, it caused more discoloration which clearly reduced the visual appearance. Therefore, in current study, TC 150 was more suitable and was selected to study in the next sections.



(a)



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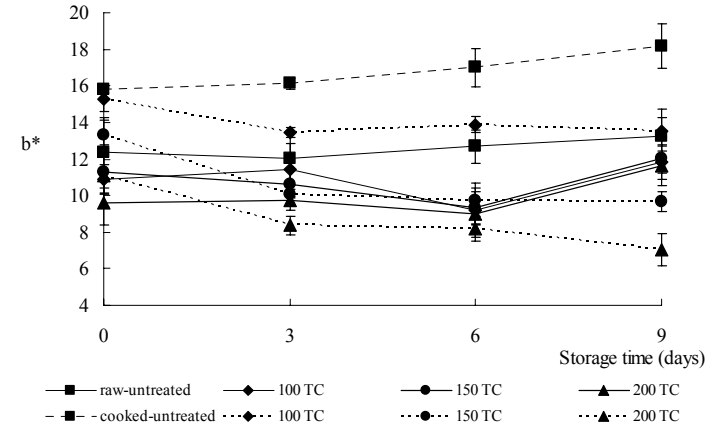


(c)

Figure 36. Changes in L* values (a), a* values (b), and b* values (c) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle. Bars represent the standard deviations for eight determinations.

6.3 Sensory changes

Sensory changes during chilled storage of cooked spent hen samples treated with TC were evaluated oxidized odor scores as shown in Table 17. As expected, oxidized odor scores increased as storage time increased for all TC treated and untreated samples ($P<0.05$). In addition, Panelists could just detected oxidized odor of both TC treated and untreated samples at the beginning time of storage. Thereafter, moderated oxidized odor scores of untreated and TC treated at the level of 100 mg/kg muscle were found, whereas moderate oxidized odor were observed on TC treated samples at the higher level. For longer time of storage all samples either treated or untreated samples showed strong oxidized odor scores, however, TC treated samples at the higher level slightly had better scores than that found in untreated and TC treated at 100 mg/kg muscle. This result was concomitant well with TBARS values (Figure 33). Therefore, it could be indicated the effectiveness of TC to retard lipid oxidation of spent hen meat.

Table 17. Oxidized odor scores during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150, and 200 mg/kg muscle.

Storage time (days)	Oxidized odor scores			
	0 (mg/kg muscle)	100 (mg/kg muscle)	150 (mg/kg muscle)	200 (mg/kg muscle)
0	$5.07\pm0.83^{a, x}$	$5.13\pm0.86^{a, x}$	$5.17\pm0.75^{a, x}$	$5.20\pm0.96^{a, x}$
3	$4.70\pm0.84^{a, x}$	$4.80\pm0.76^{a, x}$	$5.03\pm0.76^{a, x}$	$5.10\pm0.88^{a, x}$
6	$3.27\pm0.98^{b, y}$	$3.77\pm0.94^{b, y}$	$4.27\pm0.78^{b, x}$	$4.37\pm0.72^{b, x}$
9	$2.40\pm0.89^{c, x}$	$2.47\pm0.82^{c, x}$	$2.87\pm0.86^{c, x}$	$2.83\pm0.91^{c, x}$

Data are presented as mean ± standard deviation; n=30. (6=not detectable, 1=very strong).

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

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

7. Effect of combinations of NaCl, STPP, GP, and TC on quality changes of raw and cooked spent hen *Pectoralis major* muscles during chilled storage

As the resulted from section 5, treated spent hen meat with 2.5% GP solution at RT for 1 h was chosen for study in these section due to the lowest cooking loss and more tender meat compared to other samples. For retard lipid oxidation in meat samples, TC 150 mg/kg muscle was chosen due to more efficiency to reduce TBARS values and less effect on color values as described previously in section 6.

7.1 Changes in chemical properties

7.1.1 Protein Solubility

The influence of additives (2.5% GP at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on protein solubility of spent hen *Pectoralis major* muscle samples during chilled storage are presented in Figure 37. Both raw and cooked treated samples exhibited higher ($P<0.05$) protein solubility compared to untreated samples throughout storage. This result was confirmed an active of proteinase enzyme from GP on solubilized proteins as described previously. Storage time had little effect on protein solubility of untreated samples for both raw and cooked samples, but it did not show any effect on treated samples as elucidated by no changes on these values were found throughout chilled storage ($P\geq0.05$). The lower protein solubility of raw meat sample found in this section compared to section 4 was probably caused by the higher age of spent hen used. The lower protein solubility of chicken meat found in older Indigenous chicken (approximately 50%) compared to younger boiler (approximately 70%) was reported by Wattanachant (2003).

7.1.2 Soluble collagen content

Effect of additives (2.5% GP at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on soluble collagen content of spent hen *Pectoralis major* muscle samples during chilled storage are presented in Figure 38. Both raw and cooked treated samples exhibited higher ($P<0.05$) collagen solubility content compared to untreated samples from day 0 to day 9 of storage. This result was confirmed highly active against collagen and other connective tissue proteins of ginger powder as described previously. Storage time had no effect on soluble collagen

content of both raw and cooked spent hen meat either treated or untreated muscle samples as elucidated by no changes on this values for all samples was found throughout chilled storage (P≥0.05).

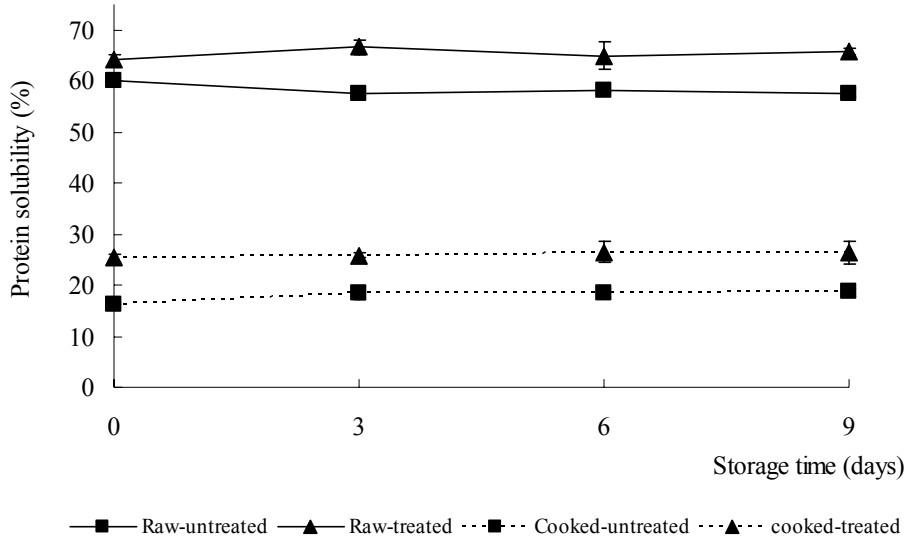


Figure 37. Changes in protein solubility (%) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

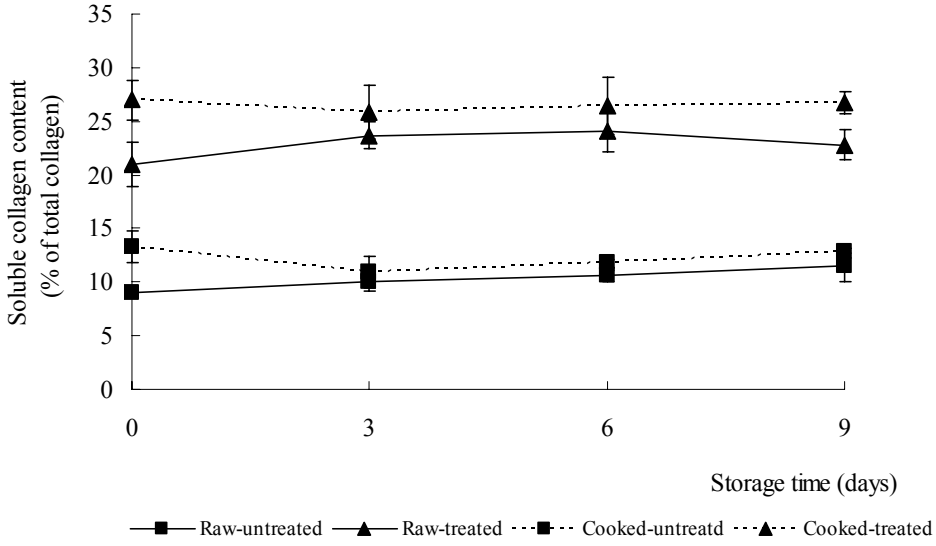


Figure 38. Changes in soluble collagen content (% of total collagen) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

7.1.3 TBARS

Effect of additives (2.5% GP solution at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on lipid oxidation of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated changes in TBARS values as presented in Figure 39. Storage time had effect on lipid oxidation of both raw and cooked spent hen meat either treated or untreated muscle samples. As storage time increased, a dramatic increase in TBARS values of cooked samples were found ($P<0.05$), whereas a slight increase in raw samples were observed throughout storage ($P<0.05$). In addition, cooked meat exhibited higher TBARS values than raw samples. However, adding additives had retarded lipid oxidation of both raw and cooked spent hen meat as elucidated by both raw and cooked treated samples showed significantly lower TBARS values compared to untreated samples throughout storage ($P<0.05$). This probably caused by both TC and STPP are antioxidant agent as previous described.

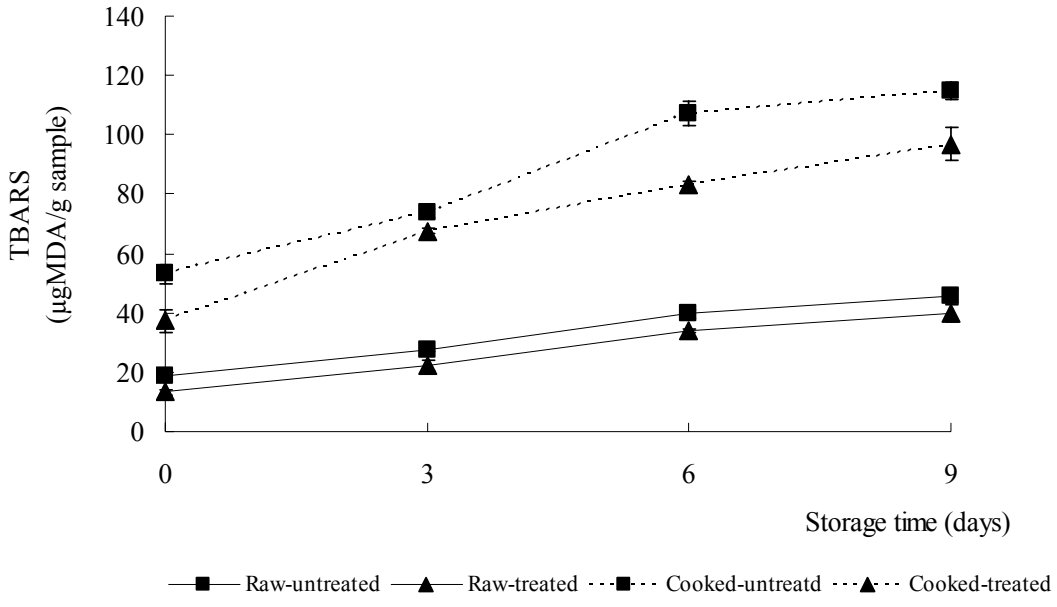


Figure 39. Change in TBARS (µg MDA/g sample) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for triplicate determinations.

7.2 Changes in physical properties

7.2.1 Drip loss, cooking loss, and weight loss

The effect of additives (2.5% GP solution at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on WHC of spent hen *Pectoralis major* muscle samples during chilled storage was evaluated drip loss/cooking loss of raw and weight loss of cooked samples as presented in Figure 40 and 41. As storage time increased from day 0 to day 9, both raw-treated and raw-untreated samples showed significant increase in all parameters that could elucidate the effect of storage time on WHC of spent hen meat during chilled storage. Adding additives could not improve WHC of raw samples as elucidated by raw treated samples showed higher cooking loss than untreated for all storage time ($P<0.05$). In addition, significant differences on drip loss between raw-treated and raw-untreated were not observed throughout storage ($P\geq0.05$). This probably caused by GP could enhance protein degradation in raw meat. As mention earlier, proteins degradation resulted in low property of proteins to hold water in muscles and exhibited higher cooking loss for raw-treated samples during chilled storage. However, adding additives could improve WHC of cooked samples as indicated by cooked treated samples exhibited significantly lower weight loss than untreated samples from day 0 to day 6 of storage ($P<0.05$), thereafter no significant differences between both samples was found ($P\geq0.05$). These results might be due to high content of soluble collagen in spent hen meat after treated with ginger powder was melt to become gelatin in cooked meat resulted in more holding water in muscles. In addition, NaCl and STPP solution could more penetrate into meat system when collagens become soluble form leading to more efficiency to hold water in muscles.

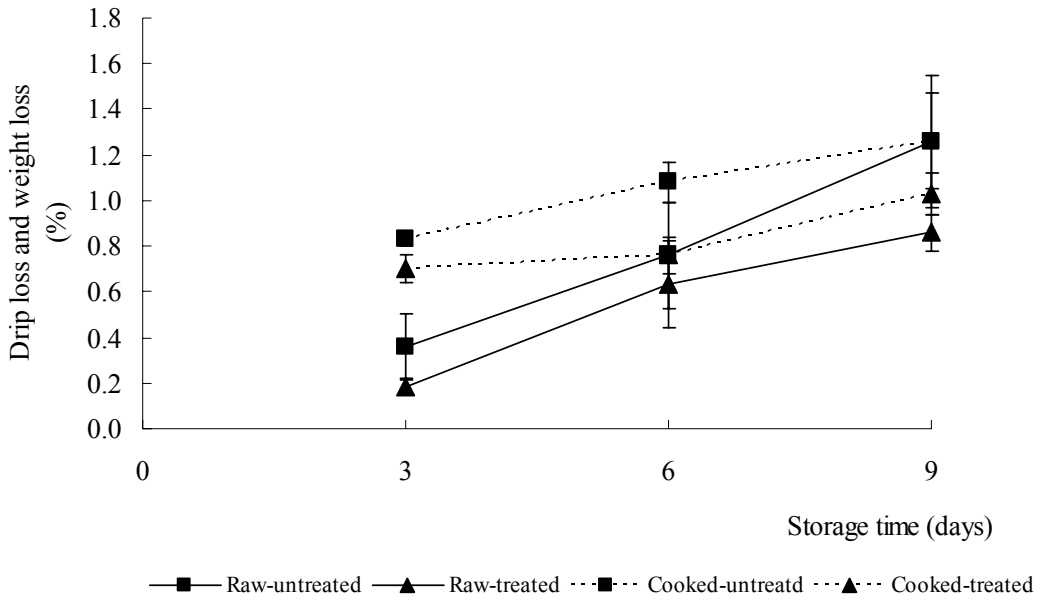


Figure 40. Changes in drip loss (%) of raw and weight loss during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for four determinations.

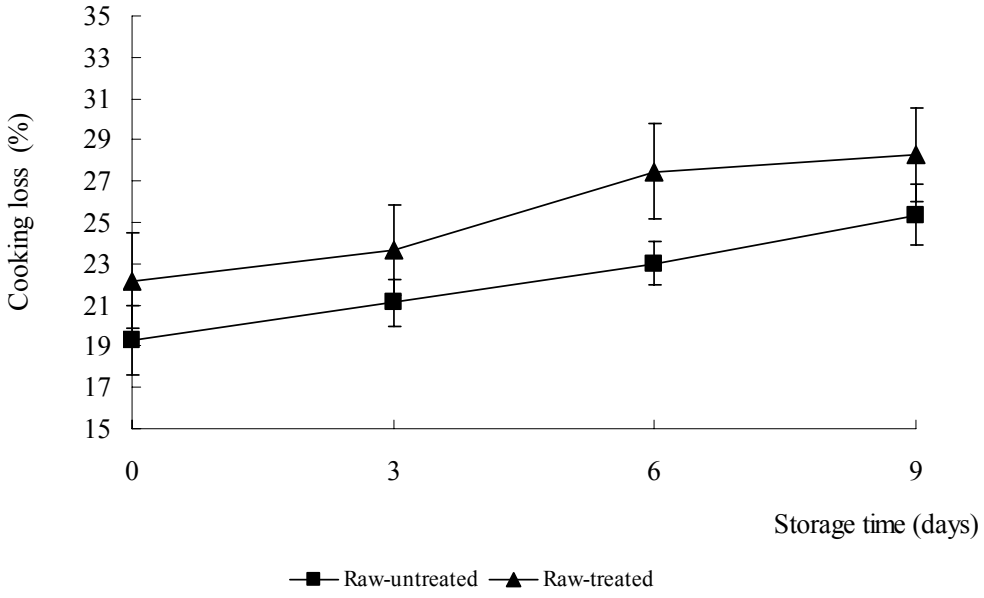


Figure 41. Changes in cooking loss (%) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for four determinations.

7.2.2 Warner-Bratzler shear force values

The effect of additives (2.5% GP solution at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) on Warner-Bratzler shear force values of spent hen *Pectoralis major* muscle samples during chilled storage are presented in Figure 42. Adding additives could improve texture of spent hen meat as elucidated by a statistically significant lower Warner-Bratzler shear force values of both raw and cooked treated with additive compared to untreated samples were found throughout storage. This was due to tenderizing effect of ginger protease enzyme as described previously. In addition, NaCl and STPP might have more efficiently to penetrate into meat after treated with enzyme leading to more water retention resulted in more tender meat.

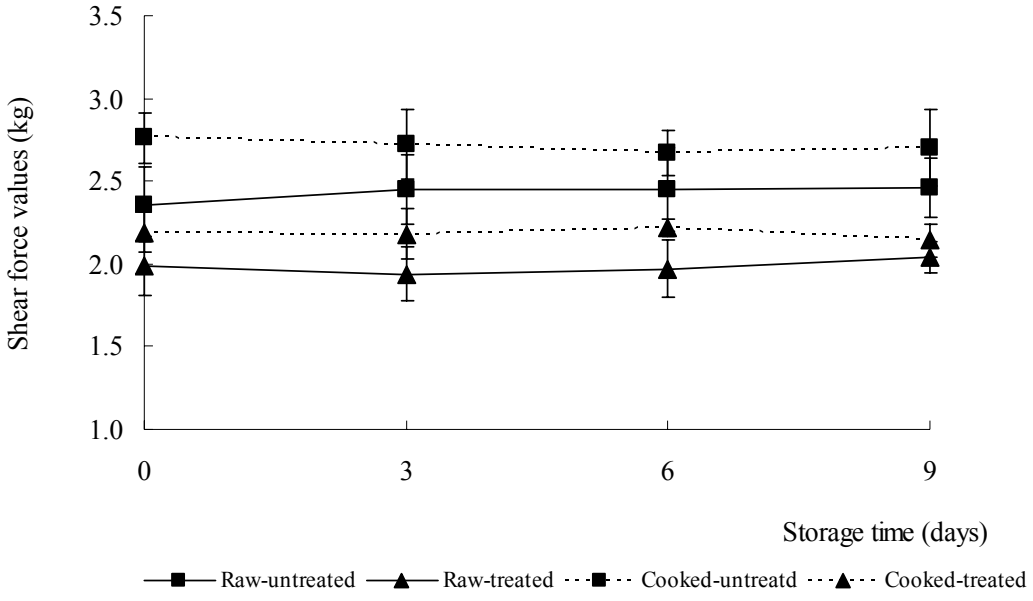
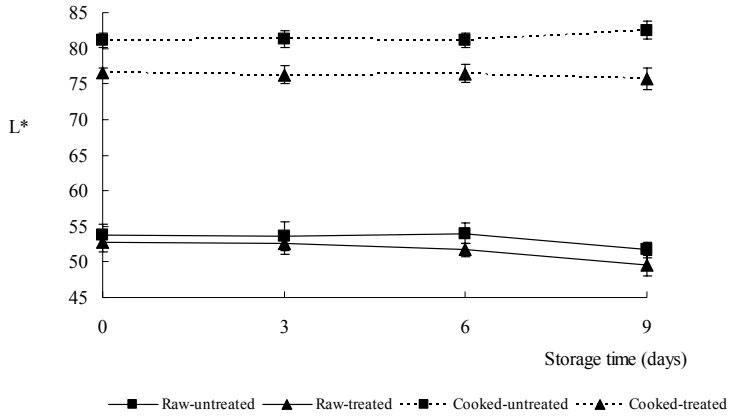


Figure 42. Changes in shear force values (kg) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP 15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for eight determinations.

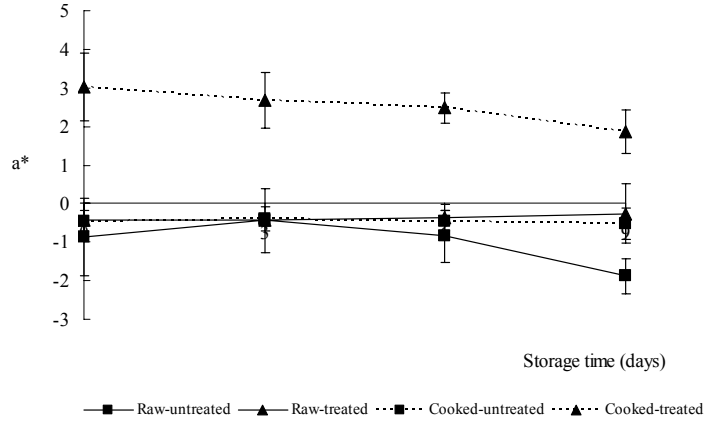
7.2.3 Surface color

Changes on surface color of spent hen *Pectoralis major* muscle treated with additives (2.5% GP solution at RT for 1 h, 1%NaCl+10% STPP for 15 sec, and TC 150 mg/kg muscle) and untreated samples during chilled storage were evaluated L*, a*, and b* values as shown in Figure 43. Treated raw spent hen meat with additives had little effect on L* values as indicated by no significant differences in L* values between treated samples and untreated samples was found from day 0 to day 3 of storage ($P \geq 0.05$), thereafter slightly lower L* values of treated samples were observed compared to untreated samples ($P < 0.05$) (Figure 43a). This phenomenal had more pronounce in cooked samples as indicated by all treated samples exhibited significantly lower L* values than untreated samples throughout storage ($P < 0.05$). Storage time had an effect on L* values of raw samples only on day 9 of storage and it showed decrease in this values either treated or untreated samples. This probably caused by higher pH of meat due to rapid growth of spoilage microorganism during long time of storage resulted in lower L* values.

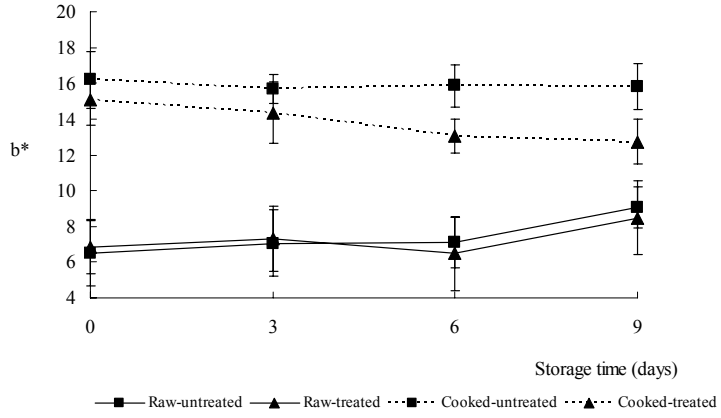
As shown in Figure 43b, adding additives had more influence on a* values of cooked than raw samples as indicated by cooked treated samples showed significantly higher a* values than untreated samples throughout storage ($P < 0.05$), whereas significant differences in a* values of raw-treated and raw-untreated samples were observed on day 9 of storage. In addition, cooked treated samples showed slight decrease in a* values as storage time increased ($P < 0.05$). As mention earlier, change in a* value was correlated well with Mb content that might undergo oxidation resulting in the release of iron content during chilled storage. The addition of STPP to meat has been shown to have antioxidation properties by chelating the heavy metal ions and keeping a part of the iron in the reduced stated (Stoick *et al*., 1991; Shahidi *et al*., 1987) resulted in no change in a* values of raw meat samples from day 0 to day 6 of storage. Discoloration was more pronounced in cooked meat probably due to great chelating effect of TC to bind with iron content which more generate during cooking as described previously. This phenomenal was clearly indicated different chelating effect of TC and STPP on a* values of raw and cooked spent hen meat.



(a)



(b)



(c)

Figure 43. Changes in L* values (a), a* values (b), and b* values (c) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for15 sec, and TC 150 mg/kg muscle compared to untreated samples. Bars represent the standard deviations for eight determinations.

As shown in Figure 43c, adding additives had more effect on b* of cooked than raw spent hen meat as elucidated by cooked treated samples showed significantly lower b* values than untreated samples on day 6 and day 9 of storage ($P<0.05$), whereas no significant differences in b* values between treated samples and untreated samples was found throughout storage ($P\geq0.05$). This probably caused by TC added and generated discoloration during cooking. This result indicated that there was discoloration in cooked spent hen meat than raw meat caused by additives treatments.

7.4 Sensory changes

The effect of additives on sensory characteristics of spent hen muscle samples were evaluated by flavor, color, and texture scores on day 0 of storage as presented in Table 17. Significant differences on color scores of both raw and cooked spent hen meat samples were found ($P<0.05$). For raw treated sample exhibited higher color scores than did found in untreated samples, in contrast cooked treated samples showed lower color scores when compared to untreated samples. This result could be elucidated that adding additives could enhance color of raw samples than did found in cooked samples which was probably affected by TC added as mentioned previously. Significant difference on flavor scores of both raw and cooked treated samples either untreated sample or treated samples were not found ($P\geq0.05$) elucidated that adding additives had no effect on flavor scores. On the other hand, both raw and cooked treated samples showed higher texture scores than did found in untreated samples. Therefore, it clearly indicated the combination of additives could improve texture of spent hen meat.

Table 18. Likeness scores of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC150 mg/kg muscle compared to untreated samples.

Sensory characteristics	Likeness scores			
	Raw		Cooked	
	Untreated	Treated	Untreated	Treated
Color	5.27±1.08^{y}	6.10±1.12^{x}	5.90±1.12^{x}	4.90±1.35^{y}
Flavor	5.90±1.03^{x}	5.93±0.83^{x}	5.53±0.97^{x}	5.77±1.01^{x}
Texture	5.10±0.96^{y}	5.63±1.19^{x}	5.00±1.02^{y}	6.57±1.10^{x}

Data are presented as mean ± standard deviation; n=30. (9=like extremely, 1=dislike extremely).

$^{x-z}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Conclusions

Spent hen *Pectoralis major* muscles had the similar nutritional value, compared to commercial broiler meat but higher content of stoma protein and higher fat content comprising of low cholesterol and high essential fatty acids.

Treatment of spent hen meat with NaCl and STPP was effective to improve yield of raw spent hen muscle and inhibited lipid oxidation of cooked spent hen muscle but could not improve texture, and color during chilled storage. Cooking was ineffectiveness to decrease toughness of the spent hen muscle.

Cooked meat by oven method had the highest cooking loss, the most toughness, and more influence on surface color than those found in other heating methods for all kinds of raw material used. Different raw materials cooked by moist heat exhibited lower shear force values and had higher texture scores than dry method. For all heating methods, treatment of spent hen meat with NaCl and STPP showed the highest protein solubility, the lowest cooking loss, and improved texture sensory scores than those found in other raw materials.

Ginger powder could decrease toughness of both raw and cooked spent hen muscles during chilled storage. On the other hand, it could effect on decrease in yield and more light color of raw meat. However, this phenomenon had less effect in cooked meat. Treated spent hen meat with ginger powder had increased proteolysis when extend storage times but no change on soluble collagen content and shear force value was observed throughout storage.

Adding TC had no effect on texture and yield of spent hen meat either raw or cooked samples during chilled storage. It could delay the accumulation of oxidation products in spent hen meat during chilled storage. However, discoloration caused by TC might reduce the visual appearance and overall acceptability of spent hen meat, especially cooked spent hen meat products without adding other seasoning which consumers expect more white in color.

Combination of additives could enhance texture and retard lipid oxidation in both raw and cooked spent hen meat during chilled storage. Although, adding additives showed increase in cooking loss of raw samples, it could improve yield of cooked samples during chilled storage. Discoloration was more pronounced in cooked spent hen meat than those found in raw meat during chilled storage.

Suggestions for future work:

1. Combination of additives should be applied for value added spent hen meat product which adding herbs or seasoning.

2. Reducing toughness of spent hen meat by using ginger powder could be applied for other value added products from spent hen meat.

3. Further research is needed to explore more purify of TC for cooked spent hen meat products to improve color deterioration of muscle.

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APPENDIX

Table 19. MetMb content (%) during chilled storage at 4°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (days)	MetMb content (%)	
	Raw-untreated	Raw-treated
0	$73.91\pm0.73^{a, x}$	$72.89\pm0.24^{a, x}$
3	$73.25\pm0.71^{a, x}$	$73.82\pm1.53^{a, x}$
6	$75.59\pm0.65^{b, x}$	$74.18\pm0.25^{ab, x}$
9	$75.73\pm0.82^{b, x}$	$75.41\pm0.15^{b, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

Table 20. TBARS (µg MDA/g sample) during chilled storage at 4°C of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (days)	TBARS (µg MDA/g sample)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$16.52\pm1.36^{a, x}$	$16.91\pm0.19^{a, x}$	$45.20\pm3.48^{a, x}$	$41.78\pm2.10^{a, x}$
3	$24.08\pm1.59^{b, x}$	$22.57\pm0.19^{b, x}$	$57.31\pm8.27^{b, x}$	$52.12\pm2.70^{b, x}$
6	$39.70\pm0.54^{c, y}$	$35.56\pm0.76^{c, x}$	$99.86\pm0.81^{c, y}$	$82.88\pm2.84^{c, x}$
9	$38.90\pm0.84^{c, x}$	$36.41\pm1.04^{c, x}$	$94.39\pm3.69^{c, y}$	$81.45\pm0.74^{c, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

Table 21. Cooking loss (%), drip loss (%), and weight loss (%) of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP during chilled storage at 4^{o}C.

Storage time (days)	Raw				Cooked	
	Cooking loss (%)		Drip loss (%)		Weight Loss (%)	
	untreated	treated	untreated	treated	untreated	treated
0	21.60±3.22^{a,x}	20.67±2.21^{a,x}	-	-	-	-
3	23.38±2.90^{ab,x}	24.37±1.76^{b,x}	0.35±0.02^{a,y}	0.24±0.06^{a,x}	0.51±0.11$^{a,\ x}$	0.73±0.08$^{a,\ y}$
6	25.89±1.83$^{bc\ ,y}$	22.25±2.02^{ab,x}	0.99±0.07^{b,y}	0.46±0.04^{b,x}	0.70±0.11$^{b,\ x}$	1.02±0.08$^{b,\ y}$
9	27.72±3.28$^{c,\ y}$	20.34±2.58^{a,x}	1.48±0.09^{c,y}	1.29±0.05^{c,x}	1.15±0.08$^{c,\ x}$	1.68±0.13$^{c,\ y}$

Data are presented as mean ± standard deviation from 8 determinations for cooking loss and 4 determinations of drip loss and weight loss.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 22. Shear force values (kg) during chilled storage at 4^{o}C of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (days)	Shear force values (kg)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$2.15\pm0.27^{a,x}$	$2.14\pm0.32^{a,x}$	$2.25\pm0.43^{b,x}$	$2.21\pm0.27^{b,x}$
3	$2.23\pm0.82^{ab,x}$	$2.18\pm0.38^{a,x}$	$2.02\pm0.15^{ab,x}$	$2.05\pm0.17^{ab,x}$
6	$2.82\pm0.46^{b,x}$	$2.79\pm0.70^{b,x}$	$2.58\pm0.15^{c,x}$	$2.93\pm0.31^{c,x}$
9	$2.52\pm0.81^{ab,x}$	$2.16\pm0.73^{a,x}$	$1.94\pm0.11^{a,x}$	$1.91\pm0.20^{a,x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 23. Surface Color (L*, a*, and b* values) during chilled storage at 4^{o}C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage Time (days)	Raw					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	52.04±1.77$^{ab, x}$	51.06±1.51^{b,y}	-2.42±0.71^{c,x}	-2.64±0.79^{c,x}	6.65±3.69^{a,x}	6.67±2.96^{a,x}
3	51.98±2.39^{ab,x}	51.30±1.79^{b,x}	-1.40±0.81^{ab,x}	-1.51±0.35^{b,x}	8.63±2.66^{a,x}	7.84±1.99^{a,x}
6	53.40±0.84^{a,x}	53.82±0.87^{a,x}	-0.82±0.46^{a,x}	-0.90±0.36^{a,x}	7.65±2.03^{a,x}	7.37±1.17^{a,x}
9	50.44±0.92^{b,y}	52.25±1.25^{b,x}	-1.68±0.63^{b,x}	-2.04±0.50^{b,x}	7.61±1.94^{a,x}	7.43±1.57^{a,x}

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 24. Surface Color (L*, a*, and b* values) during chilled storage at 4^{o}C of cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage Time (days)	Cooked					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	79.95±0.93$^{a, x}$	79.81±1.41$^{b, x}$	-0.21±0.32$^{a, x}$	-0.16±0.74$^{a, x}$	15.90±1.22 $^{a, x}$	15.74±1.99$^{a, x}$
3	81.26±1.15$^{a, x}$	80.84±1.33$^{ab, x}$	-0.85±0.31$^{c, x}$	-0.86±0.45$^{b, x}$	16.92±1.96$^{a, x}$	16.05±1.32$^{a, x}$
6	81.17±2.01$^{a, x}$	81.14±1.15$^{a, x}$	-0.44±0.17$^{ab, x}$	-0.52±0.42$^{ab, x}$	16.88±1.39$^{a, x}$	15.50±1.18$^{a, x}$
9	81.39±0.69$^{a, x}$	81.25±0.88$^{a, x}$	-0.56±0.26$^{b, x}$	-0.92±0.50$^{b, x}$	17.21±1.39$^{a, x}$	16.79±1.17$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 25. Protein solubility (%) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (months)	Protein solubility (%)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$76.36\pm1.10^{a, x}$	$79.24\pm0.61^{a, x}$	$26.09\pm1.34^{a, x}$	$27.71\pm1.22^{a, x}$
2	$71.80\pm2.31^{b, x}$	$74.07\pm2.23^{b, x}$	$22.01\pm1.62^{b, x}$	$23.67\pm2.45^{b, x}$
4	$61.77\pm1.51^{c, y}$	$66.63\pm0.55^{c, x}$	$22.32\pm1.79^{b, x}$	$24.14\pm0.48^{b, x}$
6	$59.53\pm1.26^{c, y}$	$63.11\pm0.88^{d, x}$	$22.18\pm2.07^{b, y}$	$23.28\pm2.00^{b, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 26. MetMb content (%) during frozen storage at -18°C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (months)	MetMb content (%)	
	Raw-untreated	Raw-treated
0	$74.60\pm0.57^{a, x}$	$74.58\pm0.16^{a, x}$
2	$75.82\pm0.04^{a, x}$	$75.72\pm0.48^{a, x}$
4	$76.74\pm0.15^{b, x}$	$76.82\pm0.18^{ab, x}$
6	$75.77\pm0.27^{b, x}$	$76.19\pm0.18^{b, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 27. TBARS (µg MDA/g sample) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (months)	TBARS (µg MDA/g sample)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	22.22±0.70$^{a, x}$	22.84±1.95$^{a, x}$	45.20±1.17$^{a, x}$	45.75±1.71$^{a, x}$
2	24.21±2.43$^{a, x}$	23.96±2.99$^{a, x}$	47.21±3.39$^{a, x}$	44.94±1.52$^{a, x}$
4	24.32±2.48$^{a, x}$	25.40±2.13$^{a, x}$	48.94±6.71$^{a, x}$	48.63±3.80$^{a, x}$
6	36.60±3.24$^{b, x}$	35.05±2.23$^{b, x}$	65.41±2.61$^{b, x}$	61.04±1.03$^{b, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 28. Cooking loss (%) and thawing loss (%) during frozen storage at -18°C of raw-untreated, raw-treated, cooked-untreated, and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (months)	Raw				Cooked	
	Cooking loss (%)		Thawing loss (%)		Thawing loss (%)	
	untreated	treated	untreated	treated	untreated	treated
0	**18.42±3.95$^{a, x}$**	**18.43±2.10$^{a, x}$**	-	-	-	-
2	**18.83±2.30$^{a, x}$**	**18.55±2.39$^{a, x}$**	**6.21±0.27$^{a, y}$**	**3.69±0.18$^{a, x}$**	**1.45±0.11$^{a, x}$**	**1.36±0.28$^{a, x}$**
4	**22.91±2.55$^{b, x}$**	**22.13±2.41$^{b, x}$**	**7.34±0.26$^{b, y}$**	**5.61±0.12$^{b, x}$**	**3.03±0.16$^{b, y}$**	**2.36±0.06$^{b, x}$**
6	**23.35±1.90$^{b, x}$**	**23.24±2.31$^{a, x}$**	**9.21±0.53$^{c, y}$**	**7.34±0.20$^{c, x}$**	**4.20±0.14$^{c, y}$**	**3.19±0.18$^{c, x}$**

Data are presented as mean ± standard deviation; n=8 for cooking loss and n=4 for thawing loss.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 29. Shear force values (kg) during frozen storage at -18^{o}C of raw-untreated, raw-treated, cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage time (months)	Shear force values (kg)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	2.21±0.54$^{ab, x}$	2.18±0.49$^{b, x}$	2.23±0.29$^{a, x}$	2.18±0.17$^{a, x}$
2	2.24±0.42$^{ab, x}$	2.22±0.20$^{b, x}$	2.63±0.10$^{b, x}$	2.63±0.26$^{b, x}$
4	2.57±0.27$^{b, x}$	2.70±0.31$^{c, x}$	2.90±0.13$^{c, x}$	2.92±0.24$^{c, x}$
6	1.88±0.45$^{a, x}$	1.81±0.10$^{a, x}$	2.37±0.23$^{a, x}$	2.30±0.20$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 30. Color (L*, a*, and b* values) during frozen storage at -18^{o}C of raw-untreated and raw-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage Time (days)	Raw					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	52.61±1.54$^{a, x}$	52.16±2.17$^{a, x}$	-1.47±0.43$^{b, x}$	-1.49±0.28$^{ab, x}$	7.79±1.84$^{a, x}$	7.63±1.95$^{a, x}$
3	51.45±1.73$^{a, x}$	51.29±0.84$^{ab, x}$	-1.51±0.45$^{b, x}$	-1.78±0.35$^{b, x}$	8.31±2.59$^{a, x}$	7.53±2.68$^{a, x}$
6	51.26±1.91$^{a, x}$	51.42±1.45$^{ab, x}$	-1.75±0.54$^{b, x}$	-1.38±0.33$^{a, x}$	7.71±1.39$^{a, x}$	7.81±1.21$^{a, x}$
9	51.16±1.94$^{a, x}$	50.12±1.38$^{b, x}$	-0.91±0.60$^{a, x}$	-1.24±0.32$^{a, x}$	8.56±3.35$^{a, x}$	7.35±3.46$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 31. Color (L*, a*, and b* values) during frozen storage at -18^{o}C of cooked-untreated and cooked-treated spent hen *Pectoralis major* muscles with NaCl and STPP.

Storage Time (days)	Cooked					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	80.43±1.29$^{a, x}$	80.20±0.82$^{a, x}$	-0.75±0.41$^{b, x}$	-0.37±0.41$^{b, x}$	14.90±0.73$^{c, x}$	15.52±1.09$^{a, x}$
3	77.88±1.68$^{c, x}$	77.26±2.07$^{b, x}$	-0.71±0.24$^{b, y}$	-0.13±0.42$^{ab, x}$	17.18±0.63$^{a, x}$	16.63±1.19$^{a, x}$
6	79.29±0.73$^{ab, x}$	78.49±0.81$^{b, x}$	-0.19±0.08$^{a, y}$	0.25±0.52$^{a, x}$	16.10±0.59$^{bc, x}$	15.63±0.90$^{a, x}$
9	78.10±1.13$^{bc, x}$	78.11±0.78$^{b, x}$	-0.10±0.29$^{a, x}$	-0.05±0.48$^{ab, x}$	16.62±0.84$^{ab, x}$	16.09±1.37$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 32. TCA soluble peptides (mmol tyrosine/g muscle) during chilled storage at 4°C of spent hen *Pectoralis major* muscles treated with 2.5 % GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples.

Storage time (days)	TCA soluble peptides (mmol tyrosine/g muscle)			
	untreated	1 h	2 h	24 h
0	$0.5933\pm0.1020^{c, z}$	$0.7932\pm0.0033^{d, y}$	$0.8797\pm0.0851^{b, x}$	$0.7790\pm0.0170^{d, y}$
3	$0.6477\pm0.0319^{c, z}$	$0.8993\pm0.0391^{c, x}$	$0.9434\pm0.1602^{b, x}$	$0.8609\pm0.0449^{c, x}$
6	$0.7943\pm0.0570^{b, z}$	$1.1277\pm0.0163^{b, x}$	$1.1926\pm0.1851^{a, x}$	$1.1525\pm0.0249^{b, x}$
9	$1.1094\pm0.0545^{b, x}$	$1.2904\pm0.0601^{a, x}$	$1.3632\pm0.0338^{a, x}$	$1.3018\pm0.0207^{a, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

Table 33. Protein solubility (%) during chilled storage at 4°C of spent hen *Pectoralis major* muscles treated with 2.5 % GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples.

Storage time (days)	Protein solubility (%)			
	untreated	1 h	2 h	24 h
0	$15.37\pm0.16^{a, z}$	$22.58\pm0.20^{a, x}$	$23.06\pm1.55^{b, x}$	$19.03\pm0.54^{a, y}$
3	$13.56\pm0.84^{a, z}$	$22.85\pm0.81^{a, x}$	$25.96\pm0.40^{a, w}$	$18.94\pm0.40^{a, y}$
6	$14.49\pm1.91^{a, z}$	$23.28\pm0.50^{a, x}$	$23.55\pm0.71^{b, x}$	$19.43\pm0.71^{a, y}$
9	$14.42\pm0.87^{a, x}$	$21.10\pm0.80^{b, x}$	$25.36\pm0.61^{a, w}$	$18.84\pm0.58^{a, y}$

Data are presented as mean ± standard deviation from triplicate determinations.

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

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

Table 34. Soluble collagen content (% of total collagen) during chilled storage at 4^{o}C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4^{o}C for 24 h compared to untreated samples.

Storage time (days)	Soluble collagen content (% of total collagen)							
	Raw				Cooked			
	untreated	1 h	2 h	24 h	untreated	1 h	2 h	24 h
0	6.21±0.41$^{a, z}$	18.31±1.99$^{a, x}$	12.15±1.51$^{a, y}$	10.91±1.74$^{a, y}$	11.59±0.12$^{a, z}$	26.76±0.62$^{a, x}$	15.43±1.35$^{a, y}$	17.21±1.77$^{a, y}$
3	5.76±0.88$^{ab, z}$	16.82±1.44$^{a, x}$	11.87±2.55$^{a, y}$	9.93±1.61$^{a, y}$	11.82±0.24$^{a, z}$	24.38±0.41$^{a, w}$	14.73±0.89$^{a, y}$	18.01±1.38$^{a, x}$
6	6.58±0.41$^{a, z}$	17.79±1.19$^{a ,x}$	10.58±0.95$^{a, y}$	10.94±3.04$^{a, y}$	11.39±1.51$^{a, z}$	24.53±0.27$^{a, w}$	16.30±0.24$^{a, y}$	18.55±0.62$^{a, x}$
9	4.86±0.36$^{b, z}$	17.15±1.53$^{a, x}$	9.41±1.92$^{a, y}$	10.55±1.32$^{a, y}$	12.56±0.46$^{a, z}$	24.73±2.06$^{a, x}$	16.49±0.89$^{a, y}$	16.49±0.89$^{a, y}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 35. Cooking loss (%) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples.

Storage time (days)	Cooking loss (%)			
	untreated	1 h	2 h	24 h
0	18.63±2.60$^{a, w}$	27.56±0.73$^{a, x}$	32.45±1.07$^{a, y}$	35.94±1.17$^{a, z}$
3	21.09±1.68$^{b, w}$	28.85±0.95$^{b, x}$	34.68±1.10$^{b, y}$	36.03±1.24$^{a, z}$
6	22.75±1.84$^{b, w}$	30.25±0.59$^{c, x}$	34.80±1.40$^{b, y}$	38.14±1.824$^{b, z}$
9	24.96±1.52$^{c, w}$	31.13±0.89$^{d, x}$	35.96±1.29$^{b, y}$	38.58±1.35$^{b, z}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{w-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 36. Weight loss (%) during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples.

Storage time (days)	Weight loss (%)			
	untreated	1 h	2 h	24 h
0	-	-	-	-
3	0.63±0.11$^{a, z}$	0.17±0.06$^{a, x}$	0.44±0.16$^{a, y}$	0.50±0.13$^{a, y}$
6	0.71±0.16$^{a, z}$	0.22±0.16$^{a, w}$	0.66±0.20$^{b, yz}$	0.49±0.22$^{a, xy}$
9	0.99±0.14$^{b, y}$	0.37±0.15$^{b, x}$	0.87±0.09$^{c, y}$	0.93±0.21$^{b, y}$

Data are presented as mean ± standard deviation from four determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{w-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 37. Shear force values (kg) during chilled storage at 4^{o}C of raw and cooked spent hen *Pectoralis major* muscles treated with 2 .5% GP solution at RT for 1, 2 h and treated at 4^{o}C for 24 h compared to untreated samples.

Storage time (days)	Shear force values (kg)							
	Raw				Cooked			
	untreated	1 h	2 h	24 h	untreated	1 h	2 h	24 h
0	2.61±0.12$^{a, x}$	2.40±0.22$^{a, y}$	2.32±0.18$^{a, y}$	2.41±0.17$^{a, y}$	2.76±0.25$^{a, x}$	2.13±0.15$^{a, z}$	2.21±0.18$^{a, yz}$	2.40±0.25$^{a, y}$
3	2.57±0.13$^{a, x}$	2.35±0.19$^{a, y}$	2.42±0.22$^{a, xy}$	2.40±0.16$^{a, xy}$	2.80±0.19$^{a, x}$	2.14±0.11$^{a, z}$	2.30±0.09$^{a, z}$	2.50±0.21$^{a, y}$
6	2.51±0.20$^{a ,x}$	2.37±0.20$^{a ,x}$	2.40±0.20$^{a, x}$	2.59±0.23$^{a, x}$	2.82±0.19$^{a, x}$	2.17±0.21$^{a, z}$	2.31±0.11$^{a, z}$	2.53±0.21$^{a, y}$
9	2.49±0.21$^{a, x}$	2.32±0.19$^{a, x}$	2.44±0.24$^{a, x}$	2.45±0.10$^{a, x}$	2.78±0.20$^{a, x}$	2.05±0.21$^{a, z}$	2.23±0.14$^{a, z}$	2.50±0.17$^{a, y}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 38. Color (L*, a*, and b* values) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1, 2 h and treated at 4°C for 24 h compared to untreated samples.

Storage time (days)	Raw				Cooked			
	untreated	1 h	2 h	24 h	untreated	1 h	2 h	24 h
	L*							
0	56.13±1.54$^{a, z}$	61.25±2.14$^{a, y}$	61.56±2.81$^{a, y}$	64.80±3.57$^{a, x}$	81.06±1.09$^{b, x}$	82.09±0.79$^{a, x}$	81.57±1.45$^{a, x}$	82.07±0.68$^{a, x}$
3	57.47±1.60$^{ab, z}$	61.34±2.56$^{a, y}$	61.76±2.17$^{a, y}$	64.96±1.81$^{a, x}$	80.58±1.14$^{b, z}$	82.31±0.78$^{a, x}$	81.43±0.73$^{a, y}$	82.13±0.49$^{a, xy}$
6	56.11±1.76$^{a, z}$	61.84±1.54$^{a, y}$	63.35±2.12$^{a, y}$	66.25±1.43$^{a, x}$	81.61±1.61$^{ab, x}$	81.80±0.61$^{a, x}$	81.43±1.01$^{a, x}$	82.18±0.38$^{a, x}$
9	59.03±1.77$^{b, z}$	62.11±1.66$^{a, y}$	62.87±1.48$^{a, y}$	65.82±1.90$^{a, x}$	82.47±0.66$^{a, x}$	82.36±0.58$^{a, x}$	81.80±0.98$^{a, x}$	81.59±0.81$^{a, x}$
	a*							
0	4.68±1.39$^{a, x}$	3.85±0.89$^{a, xy}$	3.28±0.75$^{a, y}$	3.98±0.74$^{a, xy}$	1.33±0.50$^{a, xy}$	0.97±0.39$^{a, y}$	1.58±0.46$^{a, x}$	0.99±0.35$^{a, y}$
3	3.70±1.65$^{a, x}$	3.72±1.16$^{a, x}$	2.81±0.74$^{a, x}$	3.57±0.79$^{ab, x}$	0.46±0.90$^{b, xy}$	0.91±0.45$^{a, x}$	0.23±0.57$^{b, y}$	0.88±0.30$^{a, x}$
6	3.61±0.89$^{a, x}$	3.44±1.22$^{ab, x}$	2.60±1.39$^{a, x}$	3.43±0.77$^{ab, x}$	0.45±0.87$^{b, x}$	0.13±0.33$^{b, x}$	0.30±0.61$^{b, x}$	0.18±0.69$^{b, x}$
9	2.10±0.98$^{b, x}$	2.35±1.04$^{b, x}$	2.36±0.75$^{a, x}$	2.73±0.85$^{b, x}$	0.09±0.39$^{b, x}$	0.17±0.32$^{b, x}$	0.28±0.56$^{b, x}$	0.09±0.30$^{b, x}$
	b*							
0	16.01±0.57$^{b, x}$	14.11±1.97$^{b, y}$	14.04±1.42$^{b, y}$	14.83±0.66$^{a, xy}$	16.45±0.74$^{a, x}$	15.20±1.14$^{b, y}$	13.99±1.56$^{b, y}$	14.47±0.90$^{c, y}$
3	17.42±0.89$^{a, x}$	15.45±1.96$^{ab, y}$	15.86±0.90$^{a, y}$	16.56±0.02$^{a, xy}$	16.83±1.17$^{a, x}$	15.42±1.21$^{b, y}$	14.87±0.72$^{ab, y}$	15.44±0.50$^{ab, y}$
6	16.73±0.79$^{ab, x}$	15.57±1.45$^{ab, xy}$	15.26±1.14$^{ab, y}$	15.17±1.81$^{a, y}$	17.15±1.50$^{a, x}$	17.10±1.27$^{a, x}$	15.38±1.12$^{a, y}$	15.17±0.64$^{bc, y}$
9	17.46±1.38$^{a, x}$	16.90±1.30$^{a, x}$	15.64±1.23$^{a, x}$	15.88±2.73$^{a, x}$	16.38±0.94$^{a, x}$	16.65±1.06$^{a, x}$	15.48±0.95$^{a, x}$	16.08±0.81$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{w-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 39. TBARS values (µg MDA/g sample) during chilled storage at 4^{o}C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150 and 200 mg/kg muscle.

Storage time (days)	TBARS values (µg MDA/g sample)							
	Raw				Cooked			
	0	100	150	200	0	100	150	200
	(mg/ kg muscle)	(mg/kg muscle)	(mg/kg muscle)	(mg/kg muscle)	(mg/kg muscle)	(mg/kg muscle)	(mg/kg muscle)	(mg/kg muscle)
0	17.88±1.30$^{a, y}$	15.72±1.67$^{a, y}$	11.90±1.03$^{a, y}$	10.45±2.75$^{a, x}$	58.36±6.68$^{a, z}$	45.46±2.07$^{a, y}$	33.37±2.02$^{a, z}$	34.59±0.80$^{a, x}$
3	35.21±1.42$^{a, z}$	23.74±1.43$^{b, y}$	16.47±3.28$^{b, y}$	15.31±1.16$^{b, x}$	84.73±5.72$^{b, z}$	77.74±4.83$^{b, y}$	74.52±4.75$^{b, xy}$	62.86±4.68$^{b, x}$
6	44.35±2.73$^{b, z}$	35.26±1.02$^{c, y}$	31.40±2.15$^{c, y}$	29.16±1.81$^{c, x}$	119.35±7.42$^{c, z}$	107.91±4.20$^{c, yz}$	97.88±6.43$^{c, y}$	82.05±6.65$^{c, x}$
9	46.07±1.61$^{b, z}$	42.39±1.82$^{d, y}$	40.11±1.31$^{d, xy}$	37.87±0.30$^{d, x}$	119.47±10.40$^{c, y}$	117.82±2.14$^{d, y}$	108.31±3.92$^{d, y}$	90.31±6.52$^{c, x}$

Data are presented as mean ± standard deviation from triplicates determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 40. Cooking loss (%) during chilled storage at 4°C of raw spent hen *Pectoralis major* muscles treated with TC 0, 100, 150 and 200 mg/kg muscle.

Storage time (days)	Cooking loss (%)			
	0 (mg/kg muscle)	100 (mg/kg muscle)	150 (mg/kg muscle)	200 (mg/kg muscle)
0	$17.43\pm2.05^{a,x}$	$16.99\pm1.72^{a,x}$	$17.77\pm1.80^{a,x}$	$17.39\pm2.09^{a,x}$
3	$21.09\pm0.87^{b,x}$	$21.62\pm0.90^{b,x}$	$21.07\pm0.83^{b,x}$	$20.79\pm0.76^{b,x}$
6	$23.29\pm1.22^{c,x}$	$22.26\pm0.85^{b,x}$	$22.95\pm1.62^{c,x}$	$22.34\pm2.60^{c,x}$
9	$25.61\pm2.01^{d,x}$	$23.96\pm2.13^{c,x}$	$24.05\pm1.57^{c,x}$	$23.94\pm2.60^{d,x}$

Data are presented as mean ± standard deviation from eight determinations.

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

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

Table 41. Weight loss (%) during chilled storage at 4°C of cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150 and 200 mg/kg muscle.

Storage time (days)	Weight loss (%)			
	0 (mg/kg muscle)	100 (mg/kg muscle)	150 (mg/kg muscle)	200 (mg/kg muscle)
3	$0.63\pm0.06^{a,x}$	$0.64\pm0.03^{a,x}$	$0.66\pm0.02^{a,x}$	$0.63\pm0.11^{a,x}$
6	$0.75\pm0.03^{a,x}$	$0.74\pm0.04^{a,x}$	$0.77\pm0.03^{a,x}$	$0.73\pm0.06^{a,x}$
9	$0.95\pm0.16^{b,x}$	$0.94\pm0.10^{b,x}$	$0.90\pm0.10^{b,x}$	$0.91\pm0.09^{b,x}$

Data are presented as mean ± standard deviation from four determinations.

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

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

Table 42. Shear force values (kg) during chilled storage at 4^{o}C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150 and 200 mg/kg muscle.

Storage time (days)	Shear force values (kg)							
	Raw				Cooked			
	0 (mg/kg muscle)	100 (mg/kg muscle)	150 (mg/kg muscle)	200 (mg/kg muscle)	0 (mg/kg muscle)	100 (mg/kg muscle)	150 (mg/kg muscle)	200 (mg/kg muscle)
0	2.39±0.29$^{a, x}$	2.38±0.32$^{a, x}$	2.33±0.31$^{a, x}$	2.34±0.29$^{a, x}$	2.33±0.30$^{a, x}$	2.34±0.30$^{a, x}$	2.37±0.23$^{a, x}$	2.38±0.26$^{a, x}$
3	2.38±0.24$^{a, x}$	2.42±0.18$^{a, x}$	2.39±0.26$^{a, x}$	2.45±0.21$^{a, x}$	2.41±0.32$^{a, x}$	2.49±0.35$^{a, x}$	2.47±0.26$^{a, x}$	2.44±0.33$^{a, x}$
6	2.41±0.32$^{a ,x}$	2.43±0.22$^{a ,x}$	2.45±0.25$^{a ,x}$	2.44±0.28$^{a ,x}$	2.44±0.33$^{a ,x}$	2.49±0.34$^{a ,x}$	2.46±0.27$^{a ,x}$	2.37±0.38$^{a ,x}$
9	2.39±0.36$^{a, x}$	2.37±0.45$^{a, x}$	2.39±0.30$^{a, x}$	2.41±0.29$^{a, x}$	2.37±0.27$^{a, x}$	2.39±0.28$^{a, x}$	2.38±0.34$^{a, x}$	2.39±0.33$^{a, x}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 43. Color (L*, a*, and b* values) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with TC 0, 100, 150 and 200 mg/kg muscle.

Storage time (days)	Raw				Cooked			
	0(mg/kg muscle)	100(mg/kgmuscle)	150(mg/kgmuscle)	200(mg/kgmuscle)	0(mg/kg muscle)	100(mg/kgmuscle)	150(mg/kgmuscle)	200(mg/kgmuscle)
	L*							
0	53.46±1.67$^{a, x}$	53.21±1.52$^{a, x}$	52.23±1.21$^{a, x}$	52.82±1.41$^{a, x}$	81.43±0.87$^{a, x}$	80.32±1.07$^{a, xy}$	79.27±0.78$^{a, yz}$	78.40±1.58$^{a, z}$
3	54.16±1.73$^{a, x}$	53.87±1.29$^{a, x}$	52.18±1.66$^{a, x}$	52.74±2.17$^{a, x}$	80.90±1.25$^{a, x}$	80.38±0.41$^{a, x}$	76.88±0.86$^{b, y}$	74.45±0.96$^{b, z}$
6	53.13±2.11$^{ab, x}$	53.09±1.88$^{a, x}$	52.26±1.35$^{a, x}$	52.64±1.44$^{a, x}$	82.16±1.10$^{a, w}$	78.52±0.64$^{b, y}$	71.75±0.58$^{c, y}$	69.93±0.73$^{c, z}$
9	51.57±1.06$^{b, xy}$	51.29±1.16$^{b, xy}$	52.26±1.29$^{a, x}$	50.40±1.35$^{b, y}$	81.13±1.29$^{a, w}$	77.65±0.99$^{c, x}$	71.69±1.39$^{c, y}$	69.81±2.31$^{c, z}$
	a*							
0	-1.93±0.90$^{a, y}$	-0.44±0.44$^{a, x}$	-0.06±0.37$^{a, x}$	-0.01±0.32$^{a, x}$	1.54±0.19$^{a, y}$	1.88±0.45$^{a, x}$	2.13±0.20$^{a, x}$	2.05±0.35$^{a, x}$
3	-2.08±0.70$^{a, y}$	-0.57±0.26$^{a, x}$	-0.23±0.39$^{a, x}$	-0.19±0.48$^{a, x}$	0.53±0.46$^{b, y}$	1.42±0.15$^{b, x}$	1.59±0.50$^{b, x}$	1.51±0.29$^{b, x}$
6	-2.16±0.37$^{a, y}$	-0.72±0.56$^{a, x}$	-0.50±0.57$^{ab, x}$	-0.31±0.27$^{a, x}$	0.46±0.32$^{b, z}$	1.04±0.07$^{c, y}$	1.59±0.67$^{b, x}$	1.35±0.34$^{b, xy}$
9	-2.26±0.39$^{a, y}$	-1.18±1.43$^{a, x}$	-0.90±0.62$^{b, x}$	-0.86±0.27$^{b, x}$	0.42±0.37$^{b, x}$	0.97±0.26$^{c, y}$	1.52±0.33$^{b, x}$	1.57±0.37$^{b, x}$
	b*							
0	12.34±1.92$^{a, x}$	10.88±0.83$^{a, yz}$	11.29±1.14^{ab,xy}	9.59±1.23$^{a, z}$	15.79±0.19$^{c, x}$	15.25±0.64$^{a, x}$	13.35±0.77$^{a, y}$	11.12±1.69$^{a, z}$
3	12.05±1.48$^{a, x}$	11.40±1.47$^{a, xy}$	10.63±0.76$^{b, yz}$	9.75±0.58$^{a, z}$	16.13±0.31$^{c, w}$	13.43±0.28$^{b, x}$	10.05±0.56$^{b, y}$	8.36±0.52$^{b, z}$
6	12.69±0.91$^{a, y}$	9.18±1.47$^{b, x}$	9.33±0.85$^{c, x}$	9.01±0.65$^{a, x}$	17.00±1.02$^{b, w}$	13.88±0.45$^{b, x}$	9.71±0.68$^{b, y}$	8.17±0.67$^{b, z}$
9	13.28±1.43$^{a, y}$	11.84±0.61$^{a, x}$	12.04±1.17$^{a, x}$	11.66±1.14$^{b, x}$	18.16±1.22$^{a, w}$	13.49±0.78$^{b, x}$	9.69±0.53$^{b, y}$	7.04±0.88$^{c, z}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{w-z}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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Table 44. TBARS values (µg MDA/g sample) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis majo*r muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage time (days)	TBARS values (µg MDA/g sample)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$18.68\pm1.82^{a, x}$	$13.34\pm0.81^{a, y}$	$53.24\pm3.27^{a, x}$	$37.24\pm3.56^{a, y}$
3	$27.64\pm0.63^{b, x}$	$22.32\pm1.53^{b, y}$	$73.62\pm1.65^{b, x}$	$67.60\pm0.68^{b, y}$
6	$39.63\pm1.39^{c, x}$	$33.91\pm0.81^{c, y}$	$107.28\pm3.99^{c, x}$	$83.08\pm1.44^{c, y}$
9	$45.74\pm2.31^{d, x}$	$40.02\pm3.00^{d, y}$	$115.00\pm2.90^{d, x}$	$96.78\pm5.60^{d, y}$

Data are presented as mean ± standard deviation from triplicate determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 45. Soluble collagen content (% of total collagen) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage time (days)	Soluble collagen content (% of total collagen)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$9.02\pm0.34^{a, y}$	$20.95\pm2.02^{a, x}$	$13.29\pm1.50^{a, y}$	$26.99\pm1.83^{a, x}$
3	$10.01\pm0.92^{a, y}$	$23.69\pm1.23^{a, x}$	$10.95\pm1.52^{a, y}$	$25.83\pm2.48^{a, x}$
6	$10.59\pm0.54^{a, y}$	$24.04\pm1.96^{a, x}$	$11.76\pm0.38^{a, y}$	$26.42\pm2.65^{a, x}$
9	$11.59\pm1.51^{a, y}$	$22.79\pm1.41^{a, x}$	$12.90\pm0.19^{a, y}$	$26.76\pm1.07^{a, x}$

Data are presented as mean ± standard deviation from triplicate determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 46. Drip loss (%) and cooking loss (%) of raw and weight loss (%) of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage time (days)	Raw				Cooked	
	drip loss (%)		cooking loss (%)		weight loss (%)	
	untreated	treated	untreated	treated	untreated	treated
0	-	-	$19.29\pm1.68^{a, y}$	$22.16\pm2.31^{a, x}$	-	-
3	$0.36\pm0.14^{a, x}$	$0.18\pm0.03^{a, x}$	$21.10\pm1.12^{b, y}$	$23.64\pm2.16^{a, x}$	$0.83\pm0.03^{a, x}$	$0.70\pm0.06^{a, y}$
6	$0.76\pm0.23^{b, x}$	$0.63\pm0.19^{b, x}$	$23.01\pm1.05^{c, y}$	$27.44\pm2.31^{b, x}$	$1.08\pm0.09^{b, x}$	$0.76\pm0.08^{a, y}$
9	$1.26\pm0.29^{c, x}$	$0.86\pm0.08^{c, x}$	$25.36\pm1.45^{d, y}$	$28.28\pm2.24^{b, x}$	$1.26\pm0.21^{b, x}$	$1.03\pm0.09^{b, x}$

Data are presented as mean ± standard deviation from eight determinations for cooking loss and four determinations for thawing loss.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

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Table 47. Shear force values (kg) during chilled storage at 4°C of raw and cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage time (days)	Shear force values (kg)			
	Raw		Cooked	
	untreated	treated	untreated	treated
0	$2.36\pm0.23^{a, x}$	$1.99\pm0.18^{a, y}$	$2.76\pm0.15^{a, x}$	$2.19\pm0.12^{a, y}$
3	$2.45\pm0.21^{a, x}$	$1.94\pm0.16^{a, y}$	$2.72\pm0.21^{a, x}$	$2.18\pm0.15^{a, y}$
6	$2.45\pm0.25^{a, x}$	$1.97\pm0.17^{a, y}$	$2.67\pm0.14^{a, x}$	$2.22\pm0.05^{a, y}$
9	$2.46\pm0.18^{a, x}$	$2.04\pm0.09^{a, y}$	$2.70\pm0.23^{a, x}$	$2.14\pm0.10^{a, y}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different ($P<0.05$).

$^{x-y}$ Means with differing superscripts in the same row are significantly different ($P<0.05$).

Table 48. Surface Color (L*, a*, and b* values) during chilled storage at 4^{o}C of raw spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage Time (days)	Raw					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	53.74±1.63$^{a, x}$	52.68±1.29^{a,x}	-0.86±1.00^{a,x}	-0.42±0.44^{a,x}	6.53±1.84^{a,x}	6.85±1.50^{a,x}
3	53.54±2.03$^{a, x}$	52.61±1.55^{a,x}	-0.44±0.13^{a,x}	-0.44±0.83^{a,x}	7.07±1.86^{a,x}	7.30±1.84^{a,x}
6	53.98±1.40$^{a, x}$	51.67±0.91^{a,y}	-0.85±0.67^{a,x}	-0.37±0.35^{a,x}	7.10±1.44^{a,x}	6.48±2.07^{a,x}
9	51.68±1.10$^{b, x}$	49.49±1.37^{b,y}	-1.88±0.46^{b,y}	-0.26±0.76^{a,x}	9.08±1.15^{b,x}	8.48±2.04^{a,x}

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

Table 49. Surface Color (L*, a*, and b* values) during chilled storage at 4^{o}C of cooked spent hen *Pectoralis major* muscles treated with 2.5% GP solution at RT for 1 h, 1.0%NaCl+10%STPP for 15 sec, and TC 150 mg/kg muscle compared to untreated samples.

Storage Time (days)	Cooked					
	L*		a*		b*	
	untreated	treated	untreated	treated	untreated	treated
0	81.16±1.02$^{b, x}$	76.57±0.72$^{a, y}$	-0.45±0.26$^{a, x}$	3.03±0.88$^{a, y}$	16.20±1.59$^{a, x}$	15.09±1.41$^{a, x}$
3	81.25±1.19$^{b, x}$	76.27±1.30$^{a, y}$	-0.40±0.32$^{a, x}$	2.68±0.72$^{a, y}$	15.67±0.81$^{a, x}$	14.36±1.70$^{ab, x}$
6	81.06±1.02$^{b, x}$	76.45±1.24$^{a, y}$	-0.45±0.26$^{a, x}$	2.48±0.39$^{ab, y}$	15.86±1.16$^{a, x}$	13.04±0.95$^{bc, y}$
9	82.53±1.26$^{a, x}$	75.73±1.46$^{a, y}$	-0.52±0.41$^{a, x}$	1.87±0.56$^{b, y}$	15.81±1.28$^{a, x}$	12.72±1.25$^{c, y}$

Data are presented as mean ± standard deviation from eight determinations.

$^{a-c}$ Means with differing superscripts in the same column are significantly different (P<0.05).

$^{x-y}$ Means with differing superscripts in the same row are significantly different (P<0.05).

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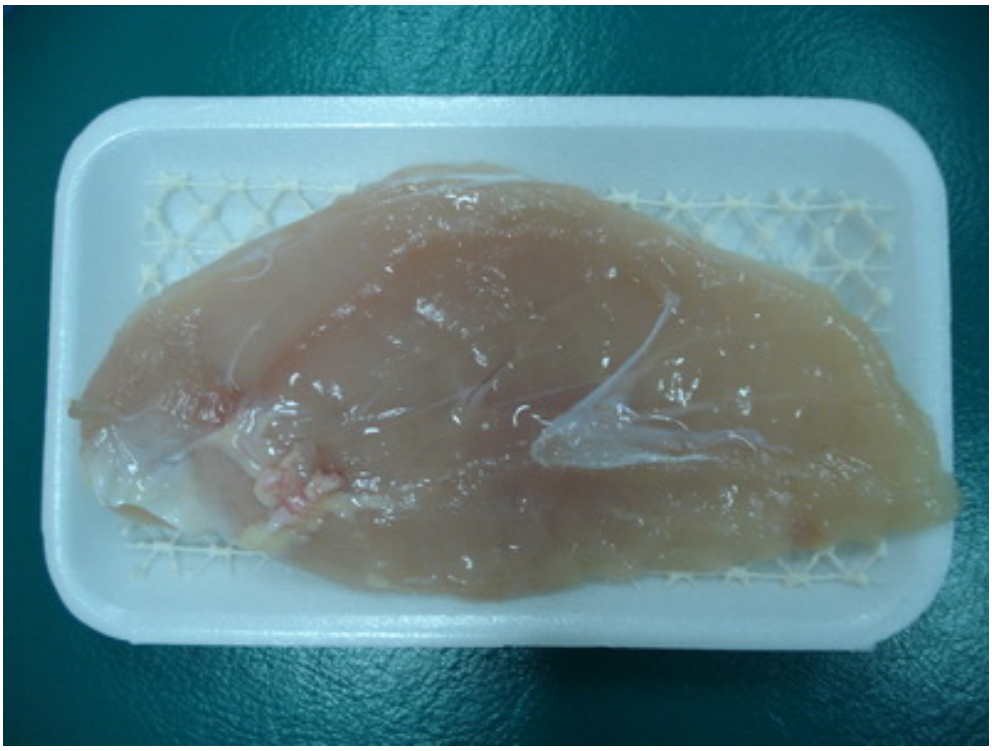

(a)

(b)

Figure 44. Spent hen *Pectoralis major* muscle: (a) raw meat and (b) cooked meat.

VITAE

Name MissAtchariya Chueachuaychoo

Student ID 4783009

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Sc. (Agro-Industry)	Prince of Songkla University	1998
M.Sc. (Food Technology)	Prince of Songkla University	2000

Work-Position and Address

Teacher Tinsulanonda Fisheris College, Institute of Vocational Education Songkhla, Thailand. Tambol Pawong, Muang district, Songkhla, Thailand.

List of Publication and Proceedings

1. Chueachuaychoo, A., Wattanachant S. and Benjakul S. 2010. Quality Characteristics of Raw and Cooked Spent Hen *Pectoralis Major* Muscles during Chilled Storage: Effect of Salt and Phosphate. International Food Research Journal. (accepted 18 August 2010).
2. Chueachuaychoo, A., Wattanachant S. and Benjakul S. 2011. Quality Characteristics of Raw and Cooked Spent Hen *Pectoralis Major* Muscles during Chilled Storage: Effect of Ginger Powder. (Submitted).
3. Chueachuaychoo, A., Wattanachant S. and Benjakul S. 2011. Quality Characteristics of Raw and Cooked Spent Hen *Pectoralis Major* Muscles during Chilled Storage: Effect of Tea Catechins. (Submitted).