VIBRATIONAL SPECTROSCOPY AND PHYSICOCHEMICAL PROPERTIES OF CHICKEN MEAT AS AFFECTED BY VARIED AGES AND THERMAL TREATMENTS OF DIFFERENT BREEDS

Sasikan Katemala

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สมบัติสเปกโทรสโกปีของการสั่นและเคมีกายภาพของเนื้อไก่ในช่วงอายุและการให้ความร้อนที่ต่างกันของไก่ต่างสายพันธุ์

นางสาวศศิกานต์ เกตุมาลา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

(Dr. Thanawit Kulrattanarak)
Chairperson

(Assoc. Prof. Dr. Jirawat Yongsawatdigul)
Member (Thesis Advisor)

(Assoc. Prof. Dr. Amonrat Molee)
Member

(Dr. Kanjana Thumanu)
Member

(Assoc. Prof. Dr. Visit Vaosoongnern)
Member

(Prof. Dr. Santi Maensiri)
Vice Rector for Academic Affairs and Internationalization

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

ศศิกานต์ เกตุมาลา : สมบัติสเปกโทรสโกปีของการสั่นและเคมีกายภาพของเนื้อไก่ในช่วงอายุและการให้ความร้อนที่ต่างกันของไก่ต่างสายพันธุ์ (VIBRATIONAL SPECTROSCOPY AND PHYSICOCHEMICAL PROPERTIES OF CHICKEN MEAT AS AFFECTED BY VARIED AGES AND THERMAL TREATMENTS OF DIFFERENT BREEDS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. จิรวัฒน์ ยงสวัสดิกุล, 171 หน้า.

วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาคุณภาพเนื้อของไก่โคราชที่ช่วงอายุต่างกันเปรียบเทียบกับไก่เนื้อทางการค้า รวมถึงศึกษาคุณภาพทางเคมี-กายภาพของเนื้อดิบส่วนอกตลอดจนศึกษาผลของอุณหภูมิการให้ความร้อนต่อการเปลี่ยนแปลงคุณภาพเนื้ออกของไก่ทั้งสามสายพันธุ์ คือไก่โคราช ไก่เนื้อทางการค้า และไก่พื้นเมืองเหลืองหางขาว และใช้เทคนิคฟูเรียร์ทรานสฟอร์มอินฟาเรดสเปกโทรสโกปีจากแหล่งแสงซินโครตรอนและฟูเรียร์ทรานสฟอร์มรามานสเปกโทรสโกปี ร่วมกับวิธีการวิเคราะห์องค์ประกอบหลักเพื่อหาความสัมพันธ์ระหว่างสเปกตราที่ได้จากเทคนิคเปกโทรสโกปีของการสั่นกับคุณภาพเนื้อ

ผลการศึกษาพบว่าอายุเป็นปัจจัยสำคัญต่อการเปลี่ยนแปลงคุณภาพเนื้อไก่โคราช ไก่โคราชที่อายุน้อย (8-10 สัปดาห์) มีลักษณะคุณภาพเนื้อเฉพาะคือปริมาณอิโนซีน 5′-โมโนฟอสเฟตสูงและค่าแรงเฉือนในเนื้อต่ำ ส่วนเนื้อไก่โคราชที่อายุมาก (16-20 สัปดาห์) นั้นมีปริมาณโปรตีนสูงค่าแรงเฉือนในเนื้อ รวมถึงปริมาณคอลลาเจนทั้งหมดเพิ่มขึ้น การวิเคราะห์องค์ประกอบหลักหาความสัมพันธ์ของสเปกตราที่ได้จากการวิเคราะห์ด้วยเทคนิคฟูเรียร์ทรานสฟอร์มรามานสเปกโทรสโกปีและคุณภาพทางเคมี-กายภาพสามารถแยกความแตกต่างระหว่างคุณภาพเนื้อของไก่เนื้อทางการค้าจากไก่โคราชได้อย่างชัดเจน โดยเนื้อส่วนอกของไก่โคราชที่อายุ 20 สัปดาห์สัมพันธ์เชิงบวกกับเอไมด์ I ของโครงสร้างเบต้าชีท และเอไมด์ III ของโครงสร้างคอลลาเจน นอกจากนี้ สเปกตราที่เลขคลื่น 3207 cm^{-1} สัมพันธ์กับปริมาณสัมพัทธ์ของโครงสร้างแอลฟา-ฮีลิกซ์แต่สัมพันธ์เชิงลบกับค่าแรงเฉือนในเนื้ออกไก่โคราช

คุณภาพเนื้อดิบส่วนอกของไก่โคราช ที่อายุทางการค้า (10 สัปดาห์) และไก่พื้นเมืองเหลืองหางขาว (16 สัปดาห์) มีปริมาณโปรตีนและสารประกอบคาร์บอนิลสูง ในขณะที่มีปริมาณไขมันต่ำกว่าไก่เนื้อทางการค้า (6 สัปดาห์) เนื้อของไก่เนื้อทางการค้ามีปริมาณความชื้น รวมถึงปริมาณอิโนซีน 5′-โมโนฟอสเฟต และกัวโนซีน 5′-โมโนฟอสเฟตสูงกว่าไก่โคราชและไก่พื้นเมืองเหลืองหางขาว ส่วนเนื้อไก่พื้นเมืองเหลืองหางขาวมีปริมาณอิโนซีนและคอลลาเจนที่ไม่ละลายน้ำสูง เนื้อของไก่เนื้อทางการค้าสัมพันธ์กับสเปกตราที่ได้จากการวิเคราะห์ด้วยเทคนิคฟูเรียร์ทรานสฟอร์มอินฟาเรดสเปกโทรสโกปีจากแหล่งแสงซินโครตรอนและฟูเรียร์ทรานสฟอร์มรามาน

สเปกโทรสโกปีที่แสดงถึงการสั่นแบบยืดของพันธะ O-H ในโมเลกุลของน้ำ พันธะ C-H ของโครงสร้างไขมันและ PO^{2-} ของโครงสร้างกรดนิวคลีอิก นอกจากนี้ลักษณะเด่นของโปรตีนในเนื้อไก่โคราชและไก่พื้นเมืองเหลืองหางขาวประกอบด้วยโครงสร้างทุติยภูมิแบบเบต้าเทอน (β-turn) และโครงสร้างที่ไม่มีรูปแบบ (random coil) ในขณะที่เนื้อไก่เนื้อทางการค้าส่วนใหญ่พบโครงสร้างแอลฟา-ฮีลิกซ์

ความร้อนที่อุณหภูมิสูง (121 °ซ) ไม่ส่งผลต่อค่าแรงเฉือนในเนื้ออกไก่โคราชและไก่พื้นเมืองเหลืองหางขาว นอกจากนี้ปริมาณสารส่งเสริมรสอร่อยคือ อิโนซีน 5′-โมโนฟอสเฟต และกัวโนซีน 5′-โมโนฟอสเฟต สามารถคงอยู่ในเนื้ออกไก่โคราชและไก่พื้นเมืองเหลืองหางขาวได้ดีกว่าไก่เนื้อทางการค้าเมื่อได้รับความร้อน การให้ความร้อนที่อุณหภูมิสูง (121 °ซ) ส่งผลให้ปริมาณคาร์บอนิลเพิ่มขึ้น ส่วนค่าการย่อยได้ของโปรตีนในเนื้อไก่ทั้งสามสายพันธุ์ลดลงเล็กน้อย เนื้อไก่ที่ได้รับความร้อนที่อุณภูมิ 70 °ซ มีความชื้นและโครงสร้างแอลฟา-ฮีลิกซ์สูง สัมพันธ์กับสเปกตราที่ได้จากการวิเคราะห์ด้วยเทคนิคฟูเรียร์ทรานสฟอร์มรามานสเปกโทรสโกปีที่แสดงถึงการสั่นแบบยืดของพันธะ O-H ในโมเลกุลของน้ำและเอไมด์ I ของโครงสร้างแอลฟา-ฮีลิกซ์ รวมถึงการให้ความร้อนที่อุณหภูมิ 90 และ 121 °ซ ทำให้โครงสร้างโปรตีนเปิดตัวเพิ่มขึ้นสัมพันธ์กับเลขคลื่นของสเปกตราที่แสดงถึงการสั่นแบบยืดของพันธะ C-H และ C-C ของโครงสร้างเบต้าชีท

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา ศศิกานต์ เกตุมาลา

ปีการศึกษา 2561 ลายมือชื่ออาจารย์ที่ปรึกษา

SASIKAN KATEMALA : VIBRATIONAL SPECTROSCOPY AND PHYSICOCHEMICAL PROPERTIES OF CHICKEN MEAT AS AFFECTED BY VARIED AGES AND THERMAL TREATMENTS OF DIFFERENT BREEDS. THESIS ADVISOR : ASSOC. PROF. JIRAWAT YONGSAWADIGUL, Ph. D., 171 PP.

SYNCHROTRON RADIATION FOURIER TRANSFORM INFRARED SPECTROSCOPY/FOURIER TRANSFORM RAMAN SPECTROSCOPY/ PRINCIPLE COMPONENT ANALYSIS/MEAT QUALITY/CHICKEN MEAT/HEAT TREATMENT

The objectives of this study were to evaluate meat quality of hybrid Korat chickens (KC) at various rearing periods in comparison with commercial broilers (CB). In addition, physico-chemical properties of raw and cooked chicken breast meat at different heating temperatures of KC, CB and Leung Hang Khao (Thai native chicken; NC) were evaluated. Synchrotron Radiation Fourier transform infrared (SR-FTIR) and Fourier transform Raman spectroscopy (FT-Raman) in combination with principle component analysis (PCA) were used to determine the correlation between wavenumbers of vibrational spectra and various meat quality traits.

KC meat quality is greatly affected by age. KC at younger ages (8-10 weeks old) were characterized by higher inosine 5'-monophosphate (IMP) with softer texture, while older KC (16-20 weeks old) exhibited higher protein content and firmer texture. PCA revealed that the meat quality of CB was greatly different from KC meat. High shear force values of KC meat at 20 weeks old were well correlated with

an increase in β-sheet structure (amide I) and amide III of collagen, detected by FT-Raman. Moreover, Raman spectra at 3207 cm^{-1} and relative α-helical content were negatively correlated with shear force values of KC breast meat.

At their market age, raw breast meat of KC (10 weeks old) and NC (16 weeks old) showed higher protein and carbonyl content, and lower lipid content than CB (6 weeks old). CB contained higher moisture content, IMP, and guanosine 5'-monophosphate (GMP). NC meat exhibited high contents of inosine and insoluble collagen content. The CB meat showed dominant regions of O-H stretching of water, C-H stretching of lipid, and PO_2^- stretching of nucleic acids based on FT-Raman and SR-FTIR spectra. The β-turn and random coil were found predominant in KC and NC meat, while mainly α-helix was found in CB meat.

High heat treatment (121 °C) had no effect on shear force of KC and NC breast meat. Taste enhancing compounds, including IMP and GMP, showed higher retention in KC and NC breast meat than did in CB breast meat upon thermal treatment. High heat treatment at 121 °C increased protein carbonyl, while slightly decreased protein digestibility of 3 chicken breeds. Cooked chicken meat at 70 °C contained higher moisture content and predominant α-helix structure, corresponding to O-H stretching of water, amide I α-helix on FT-Raman. Heating at 90 and 121 °C increased unfolding protein structure and β-sheet conformation as evidenced by FT-Raman bands of C-H stretching and C-C stretching of β-sheet structure.

School of Food Technology Student's Signature ศศิกานต์ เกตุมาลา

Academic Year 2018 Advisor's Signature

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
wk	=	Week
HPLC	=	High performance chromatography
g	=	Relative centrifugal fields
rpm	=	Revolutions per minute
M	=	Molar
mM	=	Millimolar
mg	=	Milligram
µg	=	Microgram
ml	=	Milliliter
µl	=	Microliter
U	=	Unit activity
min	=	Minute
s	=	Second
mm	=	Millimeter
µm	=	Micrometer
nm	=	Nanometer
mW	=	Milliwatt
KC	=	Korat chickens
NC	=	Thai native chickens (Leung Hang Khao sires)
CB	=	Commercial broilers

CHAPTER I

INTRODUCTION

1.1 Introduction

Chicken meat is “white meat” which is well accepted by health concious consumers because of relatively low fat and cholesterol when compared to red meat (Jaturasitha, Srikanchai, Kreuzer, and Wicke, 2008). Most commercial chicken meat is derived from fast-growing broiler strains with the age within 5 to 6 weeks (Choe, Nam, Jung, Kim, Yun, and Jo, 2010; Jaturasitha et al., 2008). Typically, native chicken meat in different countries has a unique taste and texture that are preferred by some domestic consumers than commercial broilers, including Thai native chickens (Jaturasitha et al., 2008; Wattanachant, Benjakul, and Ledward, 2004), Korean native chicken (Choe et al., 2010; Jeon, Choe, Jung, Kruk, Lim, and Jo, 2010), Chinese native chicken (Zhao, Cui, Liu, Zheng, Chen, and Wen, 2011) and Japanese native chicken (Saegusa, Hirano, Ozawa, Goda, Shimada, and Saito, 1987). Production of these chickens is limited due to lower growth rate, poor feed efficiency, and lower lean muscle gaining ability (Choe et al., 2010; Jeon et al., 2010). Korat crossbred chicken (KC) between Leung Hang Khao sires (Thai native chicken) and SUT 101 chickens dams have been developed to improve growth performance of native chicken. The SUT 101 chicken is a crossbreed between broiler and layer chicken. The KC meat is considered much more palatable with unique taste and chewier

texture as compared to broiler's meat. Thus far, little scientific information is available regarding the quality attributes of the KC meat.

Meat quality attributes and composition are greatly affected by several factors, either intrinsic (genetics/breeds, sex, slaughtering ages, and muscle types) or extrinsic (feeding, environmental factors, slaughtering process, post mortem biochemical changes, and processing conditions). As an animal grow older, composition of body and muscle changes. The effect of age on chicken meat quality is unclear and could be varied with breed. Indigenous chickens generally have a slower growth rate than commercial broilers, which may contribute to differences in their meat quality. Protein and fat contents increase, while moisture content decreases (Rikimaru and Takahashi, 2010). Wattanachant et al. (2004) reported that Thai indigenous chicken muscles contained higher protein contents, but lower fat and ash contents compared to broiler muscles. Myoglobin content increases with age of animals, as resulting in an increase in redness (a*) (Wattanachant et al., 2004). Wattanachant et al. (2004) also reported that lightness (L*), redness (a*), and yellowness (b*) of Thai indigenous chicken muscles were higher than those of broiler muscles. On the other hand, Debut et al. (2003) found that breast and thigh meat of the fast-growing chickens were lighter than the slow-growing chickens. Collagen content varies between age and breed. The collagen cross-linking and shear values also increased, while soluble collagen decreased with age (Pearson and Young, 1989). Higher collagen-content with higher crosslinkings of connective tissue was reported for older and slower growing birds (Fletcher, 2002). The important taste-active nucleotides including inosine 5'-monophosphate (IMP) and quanosine 5'-monophosphate (GMP) are flavor enhancer providing umami taste (Sasaki, Motoyama, and Mitsumoto, 2007;

Yamaguchi, 1991). The relationship between chicken age and IMP content is still not clear. Rikimaru and Takahashi (2010) reported that IMP content increases as age increases, on the other hand, Chen et al. (2002) observed that IMP content decreases with age. Regarding to chicken breeds, Jayasena et al. (2014) reported that Korean native chicken showed higher concentrations of IMP than commercial broiler in both breast and leg meat. Furthermore, cholesterol content is important from the viewpoint of human nutrition. Komprda, Zelenka, Tieffová, Štohandlová, and Foltýn (1999) reported that cholesterol content tended to decrease with the increasing growth intensity in breast and thigh of broiler meat. Broiler breasts contained higher cholesterol than those of hybrid and indigenous chickens (Intarapichet, Suksombat, and Maikhunthod, 2008; Kalita, Sultana, Roy and Bharali, 2018). However, Abdul Rehman, Akhter, Khan, and Anjum (2016) observed non-significant difference in cholesterol content of breast meat between Fayoumi and commercial White Leghorn breeds. Variation of meat quality greatly depends on age and breeds of chicken. Meat quality of Korat chicken has scarcely been studied. Understanding uniqueness of meat quality of Korat chicken at each rearing period and finding the quality characteristics of Korat chicken and Leung Hang Khao in comparison with commercial broilers would be vital information for more efficient utilization of meat of these chickens for more value-added food products.

Cooking meat improves microbiological quality and sensory properties. Heating temperature is one of main factors affecting the physico-chemical changes of meat and the final quality of meat products. Many studies reported the changes of meat quality by heating. During cooking, meat color changes due to degradation of myoglobin through oxygenation, oxidation and reduction reactions (Lorenzo,

Cittadini, Munekata, and Domínguez, 2015). The connective tissues are solubilized by heat, leading to an improvement of meat tenderness. At the same time, heat denaturation of myofibrillar proteins causes meat toughening (Palka and Daun, 1999). Denaturation of myofibrillar proteins begin between 40 and 50 °C, a further increase is observed between 60 and 70 °C (shrinkage of intramuscular collagen at 65 °C), and shrinkage and dehydration of the actomyosin occur between 70 and 90 °C (Bailey and Light, 1989). Cooking loss is related to denaturation of myofibrillar proteins and affects the loss of nutritional composition in meat. Moreover, protein oxidation which occurs during heating affects meat quality (Santé-Lhoutellier, Engel, Aubry, and Gatellier, 2008; Sun, Cui, Zhao, Zhao, and Yang, 2011). Protein oxidation products have been linked to loss of water holding capacity (WHC) and changes in texture and color in meat (Filgueras et al., 2010; Melody, Lonergan, Rowe, Huiatt, Mayes, and Huff-Lonergan, 2004; Zakrys-Waliwander, O'Sullivan, O'Neill, and Kerry, 2012). Thermal treatments of meat also affect protein digestibility. At 70 °C, protein denaturation enhances speed and increases pepsin accessibility, whereas above 100 °C, aggregation of protein from oxidation reduces pepsin digestion (Bax et al., 2012). However, the overall *in vitro* digestibility is improved in cooked meat (Bax et al., 2012). Moreover, Kaur, Maudens, Haisman, Boland, and Singh (2014) suggested that cooked beef at 100 °C for 30 min resulted in formation of “limited peptides” which were not further digested by digestive enzymes and not be bioavailable. Few data related to digestibility of chicken meat proteins as affected by cooking are available. It is important to understand the changes of meat quality during heating so that the optimal process can be developed.

Infrared (IR) and Raman spectroscopy are fast and nondestructive techniques for molecular level analysis of food samples which has a great advantage over conventional methods as it can meet the demand for in situ, on-line monitoring, and multiple analysis without any additional sample preparation (Boyaci et al., 2015). These techniques have gained interest and great potential for analysis of food components over the past few decades. IR and Raman spectroscopy are complementary techniques. Both methods are useful to fingerprint different samples. Generally, the polar molecules absorbing actively in IR are not scattering-active, after laser light excitation (Raman). Alternatively, the non-polar but polarizable molecules being scattering-active in Raman spectroscopy (Santos, Gerbino, Tymczyszyn, and Gomez-Zavaglia, 2015). Different chemical bonds in biomolecules present a distinct vibrational spectrum as a chemical 'fingerprint'. IR and Raman spectroscopy has been used to assess meat quality, including changes of protein secondary structure during heating (Berhe, Engelsen, Hviid, and Lametsch, 2014; Calabrò and Magazù, 2012), fatty acids composition (Beattie, Bell, Borgaard, Fearon, and Moss, 2006; Rohman, Sismindari, Erwanto, and Che Man, 2011), and water content in meat and meat products (Herrero, Carmona, Cofrades, and Jiménez-Colmenero, 2008; Perisic, Afseth, Ofstad, and Kohler, 2011). Synchrotron radiation- based Fourier-transform infrared spectroscopy (SR-FTIR) is an infrared spectroscopy that used synchrotron light in the range of infrared radiation as a source to irradiate samples. Synchrotron light is produced from a circular accelerator of charged particles, such as electrons. As electrons accelerate around each bend in the ring, they are guided by powerful magnets and give off energy in the form of light (Marinkovic and Chance, 2006). With the advantages of synchrotron light brightness which is usually 100-1000 times

brighter than a conventional globar source, it is capable of exploring within the microstructures of samples in small area of specimen with a high signal-to-noise ratio (S/N) at ultraspatial resolutions (Pascolo et al., 2014). SR-FTIR technique can extract more detailed structural information on microscales of biomolecules. These techniques in combination with principle component analysis (PCA) have been developed to extract information of a large data set (Berhe et al., 2014; Perisic et al., 2011). Correlation between the specific wavenumbers and physico-chemical properties could help to characterize the uniqueness of meat quality and reveal the valuable information for further application.

1.2 Research objectives

The objectives of this study were:

1. To investigate changes of chicken breast and thigh meat from Korat chickens at various rearing periods in comparison with commercial broilers.
2. To determine the effect of cooking temperatures on physicochemical properties of chicken breast meat from Korat chickens and Leung Hang Khao sires (Thai native chicken) in comparison with commercial broilers.
3. To investigate quality of raw and cooked Korat chicken meat and Leung Hang Khao sires (Thai native chicken) at various cooking temperatures in comparison with commercial broilers using SR-FTIR and FT-Raman spectroscopy in combination with principle component analysis (PCA).

1.3 Research hypotheses

Age of animal would affect physicochemical properties of meat. Breast and thigh muscle also exhibit different meat quality. Moreover, the cooking temperatures have direct impact on changes of muscle composition and *in vitro* digestibility. The degree of protein denaturation and oxidation varies with cooking temperatures. In addition, SR-FTIR and FT-Raman spectroscopy can be used to monitor changes of proteins, lipids, and water in muscle of chicken at various ages and those induced by temperatures. PCA could be applied to correlate between vibrational spectra (SR-FTIR and FT-Raman) and meat quality traits.

1.4 Scope of the study

The effect of age on quality of breast and thigh meat of male Korat chickens reared at 8, 10, 12, 16, and 20 weeks was evaluated and compared with the commercial broiler. Proximate analyses were determined. Raw meat samples were subjected to determine pH at 24 h postmortem. WHC, shear force values, and color were measured. The fatty acids composition, collagen, nucleotides, cholesterol, and purine compounds were also investigated.

The effect of breeds on chicken meat quality was investigated in raw breast meat of male KC, Leung Hang Khao sires (Thai native chicken), and commercial broilers at their respective market age. Proximate compositions were analyzed. pH at 24 h postmortem, WHC, and color were determined. Protein oxidation measurement was conducted based on carbonyl content. The collagen and nucleotides were investigated.

The effect of various cooking temperatures on chicken meat quality was investigated. The breast meat of male Korat chickens, Leung Hang Khao sires (Thai native chicken), and commercial broilers at their respective market age were cooked at 70, 90, and 121 °C for 40 min. The cooking loss, WHC, color, and shear force values were measured. The *in vitro* protein digestion, protein oxidation, collagen, and taste-active nucleotides of cooked meat samples were investigated.

Furthermore, FT-Raman spectroscopy in combination with PCA was employed to monitor changes of muscle composition in relation with chicken ages. SR-FTIR and FT-Raman spectroscopy in combination with PCA were applied to determine the meat quality of raw chicken meat from different breeds as well as cooked chicken meat obtained from different cooking temperatures.

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CHAPTER II

LITERATURE REVIEWS

2.1 Chicken meat quality

Chicken meat quality traits consist of 3 categories: appearance (i.e., color), physical (i.e., pH, water-holding capacity, drip loss, cooking loss, tenderness, juiciness of meat), chemical (i.e., protein, fat and ash contents, amino acids and fatty acids compositions), and functionality. The meat quality influences a consumer's perception of meat and meat products such as color, taste, and texture. Moreover, consumers have more concerns on healthier meat with the attention on protein and fat content, fatty acid compositions, cholesterol and purine content. Meat quality depends on a number of factors including intrinsic factors (genetic/breed/stain, sex, age, and muscle type) and extrinsic factors (diet, storage and processing condition). Differences in growth rate among chicken breeds will result in different muscle development and muscle composition that affects meat quality.

2.2 Proximate composition

Proximate composition is important indices for meat quality. There is an increasing trend for consumers to favor healthier meat products with lower in fat and higher protein content. Typically, the protein and fat content increased and moisture content decrease with age of animals (Díaz, Rodríguez, Torres, and Cobos, 2010; Janisch, Krischeck, and Wicke, 2011). The breast meat protein content

increased with the age of the broiler (Janisch et al., 2011). The crude protein contents were higher in the breast meat than in the thigh meat (Choe et al., 2010; Wattanachant, Benjakul, and Ledward, 2004). There was a genotype effect for the protein content of chicken meat. The slow birds had higher protein than fast which may be related to the slow birds were older than the fast at harvesting (Fanatico, Pillai, Emmert, and Owens, 2007).

The older birds had more fat in the meat than the younger birds (Díaz et al., 2010; Rikimaru and Takahashi, 2010). On the other hand, Zelenka, Fajmonová, and Komprda (2001) did not observe any increase in breast fat content in fast-growing chickens depending on their age. The thigh and drumstick meat showed higher lipid content than breast meat (Choe et al., 2010; Díaz et al., 2010). Muscle type by chicken breast muscle is mainly composed of glycolytic white fibers, while drumstick meat is composed of more oxidative red fibers (Jaturasitha, Srikanchai, Kreuzer, and Wicke, 2008). The glycolytic (anaerobic) pathway through which pyruvate is converted into lactic acid in the sarcoplasmic, and the oxidative (aerobic) pathway through which pyruvate is oxidized by the mitochondria. Oxidative fibers exhibit higher intramyocellular lipid content than fast glycolytic fibers (Essén-Gustavsson, Karlsson, Lundström, and Enfält, 1994). Genotype (breed and strain) plays a major role in carcass fatness. Differences in meat fat content between genotypes have been attributed to differences in the degree of animal maturity (Fanatico, et al., 2007). Meat from indigenous chickens contained lower fat content than those of general boilers (Choe et al., 2010; Jayasena et al., 2013). This confirms that in some cases the genotype effect on fat deposition could go beyond age influence (Tang et al., 2009).

Furthermore, less-intensive fattening is expected to result in leaner carcasses (Khantaprab, Nikki, and Nobukuni, 1997).

2.3 pH and functional properties

2.3.1 pH

The pH decreased with the age of the birds. Meat of older chickens exhibits lower pH than younger chickens. Díaz et al. (2010) reported that breast and drumstick meat of older slow growing chicken (Mos breed) exhibit lower pH value than that of younger. Moreover, Glamoclija et al. (2015) found that pH values of m. pectoralis major muscle in older broilers lower than younger ones. In general, meat of older animals exhibit lower pH value than younger animals, which is attributed to greater muscle glycogen content in the former (Kadim et al., 2006). Wattanachant et al. (2004) also found higher pH of biceps femoris muscle than pectoralis muscle. In drumstick meat, the pH values were higher than the breast meat. This was probably due to different type of muscles in drumstick (oxidative muscles vs. glycolytic muscles in the breast) (Díaz et al., 2010). The pH of the Korean native chicken breast muscles was lower than that of the commercial breast muscles, but no differences in thigh meat (Choe et al., 2010). Different pH values among types of chicken may be attributed to the pre-slaughter stress, which affects muscle glycogen content (Berri et al., 2007). The indigenous strains are more aggressive and alert behavior than the imported breeds (Jaturasitha, Chaiwang, and Kreuzer, 2016).

2.3.2 Color

Color is the most common quality indicator used by consumers to evaluate meat and meat products. Meat color varies according to the concentration

of pigments (myoglobin and hemoglobin). However, when animal is stunned and bled, most of blood is removed from carcass, leaving myoglobin (Mb) as the main meat color pigment. The meat color varies with the amount and type of Mb contained in the muscle tissue. The variation in meat color is from different species, breeds, age, muscle type, as well as processing conditions. The meat of the youngest chickens showed higher lightness (L*) than that of the older ones (Díaz et al., 2010). The myoglobin content increases with the age of the animals, leading to an increase in redness (a*) in chicken meat (Wattanachant et al., 2004). Jaturasitha et al. (2008) reported that the ratio of red and intermediate to white fibers was higher in the thigh muscle. Choe et al. (2010) found that Korean native chickens were brighter than the commercial broilers which was in consistent with Wattanachant et al. (2004) who reported that lightness (L*), redness (a*), and yellowness (b*) of Thai indigenous chicken muscles were higher than those of broiler muscles.

The color of cooked meat is one of critical quality parameters. Heat treatments contribute to changes in meat color. During cooking, oxidation and denaturation of deoxymyoglobin and oxymyoglobin to metmyoglobin are noted (García-Segovia, Andrés-Bello, and Martínez-Monzó, 2007). Heating causes denaturation of the globin, which then precipitates with other meat proteins. Denaturation of myoglobin and other proteins begins between 55 °C and 65 °C in meat (Hunt, Sorheim, and Slinde, 1999). As the globin is denatured, metmyoglobin forms the brown globin hemichromogen, also known as ferrihemochrome. Tissues with more heme pigment will appear darker when cooked (Lyon and Lyon, 2002). Meat from older animals turns brown quicker than young animals during cooking (Hague et al., 1994).

2.3.3 Water holding capacity (WHC)

WHC is the ability of meat to retain its water when external forces are applied. Much of the water (about 95%) in meat is classified as “free water” which exists in myofibrils by surface tension or capillary action, and about 5% of water is classified as “chemically bound” which binds to the hydrophilic groups of amino acids in muscle proteins. The “free water” is related to WHC determination. After slaughter, WHC decreases (Zhang, Mittal, and Barbut, 1995). The WHC of meat is important due to water loss is economically undesirable and the WHC influences the textural attributes such as juiciness. An improved capacity for WHC with age was observed. The meat of the youngest animals showed lower WHC, while higher drip loss than that of the older ones (Díaz et al., 2010). The leg muscle shows a higher cooking loss than does the breast muscle, which might be associated with its higher lipid content because part of the lipid could be lost during cooking. However, Jaturasitha et al. (2008) found WHC of breast and thigh meat mostly were comparable among genotypes. Tang et al. (2009) have demonstrated lower cooking loss of meat Chinese native chickens (slow-growing genotypes) when compared with broiler line (fast-growing genotypes). Fanatico et al. (2007) has described higher drip losses in slow growing broilers than in fast-growing animals. However, no difference in WHC was noted between the Korean native chicken and commercial broilers (Choe et al, 2010).

2.4 Fatty acid composition

Fatty acid composition is also a factor that influences meat quality. Saturated fatty acids are considered harmful to human health and, on the contrary,

polyunsaturated fatty acids (rich in n-6 and n-3) play a favorable role in the prevention of some human diseases (cancer, obesity and cardiovascular diseases). In addition, essential fatty acids including n-6 fatty acids such as linoleic acid (C18:2) and arachidonic acid (C20:4) are important, because they cannot be biogenerated in the living body and must be supplied in diet. Chicken meat contains the principal source of polyunsaturated fatty acids (PUFA) with high concentration of n-3 PUFA (Howe, Meyer, Record, and Baghurst, 2006).The major fatty acids in chicken meat are oleic acid (C18:1), palmitic acid (C16:0), and linoleic acid (C18:2) (Choe et al., 2010; Jayasena et al., 2013). The percentage of oleic and linoleic acids were higher in the leg meat than in the breast meat of chicken. The breast meat had significantly higher levels of arachidonic acid and DHA than did the leg meat (Jayasena et al., 2013; 2014). The breed of chicken had a dominating effect on the fatty acid composition at their respective market ages. Wattanachant et al. (2004) reported that Thai indigenous chicken muscles contained a higher percentage of saturated fatty acids and a lower percentage of polyunsaturated fatty acids than broiler chicken muscles. There was no difference in total monounsaturated fatty acids between muscles. Choe et al. (2010) and Jayasena et al. (2014) also repoted the higher content of linoleic acid, arachidonic acid, and DHA in KNC was markedly higher than those in commercial broiler. This result was possibly caused by the differences in diets and feeding behavior. The indigenous chickens tend to scratch while eating and have been observed, to pick up feed particles more selectively than broilers (Van Marle-Koster and Webb, 2000).

2.5 Cholesterol

Cholesterol content in meat is being great concern among health conscious consumer. By contrast, high cholesterol ingestion is a risk factor for coronary diseases, particularly atherosclerosis (Komprda, Zelenka, Tieffová, Štohandlová, and Foltýn, 1999). Komprda et al. (1999) reported that cholesterol content trended to decrease with the increasing growth intensity in breast and thigh broiler meat as it is an integral constituent of the cell membranes. Besides, cholesterol content reduced in aged rats (Choi and Sugano, 1987). The reduction is largely attributed to an impairment of cholesterol synthesis enzymes in adult rats which largely attributable to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Choi and Sugano, 1987). Cholesterol content may be positively related to content of fat deposition in carcass (Haque, Ahammad, Howlider, and Chowdhury, 2016). There was difference in cholesterol contents between breast and thigh muscles. The cholesterol content in thigh meat from White Leghorn and Fayoumi (Pakistan) chickens was comparatively higher than in breast portion (Abdul Rehman, Rehman, Akhter, Khan, and Anjum, 2016). There have been reported that crossbred chicken had lowest cholesterol content as compared with fast growing chickens (Komprda et al., 2000).

2.6 Purine

Purine is natural substances found in chicken meat and becoming widely concerned because excess intake of purine increases the risk of hyperuricemia and gout. Gout is a painful inflammatory arthritis due to the accumulation of monosodium urate crystals within joints and other soft tissues (Choi, Liu, and Curhan, 2005).

Although purines are needed in human cells, there is normally little need for dietary purines due to purines can be synthesized via de novo and salvaged from body and reused. In human, purines are metabolized to uric acid. However, overproduction of uric acid correlated with high serum uric acid levels, which can cause gout and could be a risk factor for cardiovascular disease, kidney disease, and metabolic syndrome (Kaneko, Aoyagi, Fukuuchi, Inazawa, and Yamaoka, 2014). Therefore, frequent and high intake of purine-rich foods reportedly enhance in the serum uric acid level is closely associated with negative effect for health especially results in gout or a recurrent gout attacks. Total purine bases consist of adenine, guanine, hypoxanthine, and xanthine, only adenine and hypoxanthine significantly increased serum uric acid levels (Young, 1982). Young (1982) reported that the level of hypoxanthine is present in major amount in chicken meat. A high-protein diet typically contains large quantities of purines. Therefore, meat and meat products are categorized as purine-rich foods. Hence, avoiding or reducing amount of purine-rich foods consume may help reduce the risk of gout attacks (Choi et al., 2005).

Changes in the level of total purines and the release of free bases have been found to occur during heat treatments of chicken meat. Hypoxanthine was lost from the meat into the cooking juices. The cooking juices were found to contain high levels of hypoxanthine and only trace amounts of adenine and guanine (Young, 1982). It appears that the effect of cooking can improve meat quality by losing purine in meat.

2.7 Nucleotides

Taste is an important eating quality of meat. Umami taste is one of the basic tastes of meat. The predominant taste-active nucleotides in meat ,5´-ribonucleotides, including inosine monophosphate (IMP), guanosine monophosphate (GMP), and adenosine monophosphate (AMP) which are flavor enhancers providing umami taste in chicken meat (Yamaguchi and Ninomiya, 2000). In addition, IMP is a major nucleotide in chicken muscle (Yamaguchi and Ninomiya, 2000; Aliani and Farmer, 2005). IMP is produced by degrading adenosine triphosphate (ATP) via enzymatic hydrolysis after slaughter. ATP degradation produces a series of compounds: ATP to adenosine diphosphate (ADP) to adenosine monophosphate (AMP) to IMP. This compound is gradually transformed into inosine (HxR), ribose and hypoxanthine (Hx) in the meat (Vani, Modi, Kavitha, Sachindra, and Mahendrakar, 2006). The amount of IMP content decreased during the aging period as it was converted to hypoxanthine through inosine.

The variation of IMP and GMP among different chicken age, breeds and its muscle types as well as heating process have been detected. Bird age only had minor effect on the level of IMP (Jayasena et al., 2015). However, IMP content of some chickens showed an increase with increasing age, including Chinese native chicken (Lueyang black-bone) (Zhang, Lu, Wang, Yin, and Yang, 2018), Korean native chicken (Jung et al., 2011), and broilers (Rikimaru and Takahashi, 2010). By contrast, Chen et al. (2002) reported that IMP content in chicken meat decrease as the age of the chicken increases. The difference in IMP content of muscle types was also found. Breast meat had higher level of IMP than leg or thigh meat (Jayasena et al., 2013; Rikimaru and Takahashi, 2010). These may be explained by the distinct composition

of muscle fibers that breast meat is composed of more than 90% type IIB muscle fibers (white fibers), whereas leg meat is mainly composed of type I muscle fibers (red fibers) (Jaturasitha et al., 2008). The type II muscle fibers exhibit higher accumulation of IMP than do type I muscle fibers in rat skeletal muscle (Arabadjis, Tullson, and Terjung, 1993). Furthermore, type I muscle fibers exhibit greater 5'-nucleotidase activity, the enzyme that catalyzes the degradation of IMP to inosine compared to type II muscle fibers (Tullson and Terjung, 1999). Different chicken breeds have different muscle development that may have a large effect on IMP metabolism. The breast and leg meat from Korean native chickens (100-day-old) showed significant higher IMP content than commercial broilers (Ross) (Jayasena et al., 2014). The breast and leg meat from Chinese native chicken (Wenchang and Xianju) also had higher IMP concentration than broilers (Tang et al., 2009). The chickens with slow-growing genotypes have been characterized by higher in IMP concentration from chicken with rapid-growing genotypes. This is due to IMPDH expression levels were reduced in free-range breeding, resulting in a reduction of transformation by IMPDH. On the other hand, when the animals exercise, ATP consumption gradually increases in the muscle, and degradation rate is greater than the synthesis one, leading to an increase in ADP, and finally IMP (Zhang et al., 2018). Therefore, degradation rate of nucleotides may be rapid in commercial broiler, contributing to lower IMP content than native chicken. Heat treatment depletes umami-active compounds because they are leached from meat to cooking juice (Sasaki, Motoyama, and Mitsumoto, 2007). In addition IMP is degraded to inosine and hypoxanthine (Kavitha and Modi, 2007).

2.8 Textural properties

Meat texture is one of the most important meat traits for consumer satisfaction. Meat texture depends on several factors such as myofibrillar proteins, muscle fiber composition (connective tissue, fat), morphology of muscle structures (thickness of perimysium, number of muscle fiber, fiber diameter or fiber area, sarcomere length) (Palka and Daun, 1999). Typically, shear force value is related to the tenderness of meat. Connective tissue plays an important role in shaping meat tenderness. Collagen is the principle fibrous protein in connective tissue. Variations in the amount, arrangement and thermal stability of collagen vary with muscle type, age, and genotype/breed of animals. The meat of younger animals showed lower shear force value than the older ones because of an increase of the connective tissue within the muscle tissue with increasing age (Díaz et al., 2010; Janisch et al., 2011). Animal maturity is associated with increased thermal and mechanical stability of intramuscular connective tissue (Bailey and Light, 1989; Janisch et al., 2011). Replacement of divalent crosslinks with mature crosslinks has been linked to a reduction in the heat solubility of collagen (Purslow, 2018). Collagen cross-linking increases with the age and is often associated with increased toughness (Fletcher, 2002). In addition, the increase in muscle fiber diameter and density was paralleled by an increase in endomysium and perimysium thickness as well as shear force value with age of animal increasing (Berri et al., 2007). Thigh meat contained higher soluble and total collagen contents than the breast whereas the corresponding Warner-Bratzler shear force value in thigh meat was higher (Chen et al., 2016). Moreover, Wattanachant, Benjakul, and Ledward (2005) reported that perimysium of pectoralis muscle were thinner than those of the biceps femoris muscle. This may be

correlated with lower collagen content in breast meat than thigh meat. The different texture from different chicken breeds may be caused by different age and genetic factors (muscle type, structure, and composition, such as the content and nature of intramuscular collagen). Native chicken meat usually has a unique texture of firmer texture than commercial broilers. This might be related to higher total collagen but less soluble collagen contents (Wattanachant et al., 2004) as well as thicker perimysium (Wattanachant et al., 2005). Wattanachant et al. (2004) reported that total collagen contents of Thai indigenous (Gallus domesticus) pectoralis and biceps femoris muscles were 5.09 and 12.85 mg/g, respectively, which were higher than those found in broiler (commercial breed, CP707) pectoralis (3.86 mg/g) and biceps femoris muscles (8.70 mg/g). Differences in the collagen contents between the slow- and fast-growing chicken could be attributed to differences in the ages of the birds at slaughter. It has also been shown that heat solubility of collagen decreases with increased collagen cross-linking as an animal grows older (Pearson and Young, 1989). Therefore, older indigenous chickens had higher total with more highly crosslinked collagen content compared with younger broilers. Furthermore, faster growing muscles could be expected to have a greater newly synthesized collagen, immature crosslinks, and so contained higher of heat soluble collagen content (Purslow, 2018). The improvement of tenderness in meat is mainly caused by solubilizing of connective tissues during heating, while heat denaturation of myofibrillar proteins generally causes meat toughening (Palka and Daun, 1999).

2.9 Protein oxidation

Oxidation of protein, which is a major component in meat and meat products, has received growing interest due to its responsible for protein structure modifications that impact on quality of meat and meat products. Muscle proteins are susceptible to oxidative reaction by different oxidation initiators. Protein oxidation is induced either 1.) directly by reactive oxygen species (ROS) either free radical (•OH, O_2•-, RS•, ROO•) or non-free radical (H_2O_2, ROOH), reactive aldehydes and ketones, 2.) indirectly by reaction with secondary products of oxidative stress, and 3.) reactive nitrogen species (RNS) cause a covalent modification of protein (Soladoye, Juárez, Aalhus, Shand, and Estévez, 2015). Meat contains various endogenous initiators or catalysts of oxidation, such as ferric heme pigments, transition metal ions, and oxidative enzymes. Furthermore, processing and storage of meat is likely to intensify oxidative degradation (Rysman, Hecke, Poucke, Smet, and Royen, 2016). The oxidative damage in proteins can cause modification of backbones and side chains, which leads to structural changes at the levels of primary, secondary, and tertiary structures of proteins. These structural changes from the oxidative damage leading to impair the conformational of protein including an increase of protein surface hydrophobicity (Santé-Lhoutellier, Astruc, Marinova, Grève, and Gatellier, 2008), protein fragmentation and aggregation (Decker, Xiong, Calvert, Crum, and Blanchard 1993; Santé-Lhoutellier et al., 2008), protein polymerization and generation of disulfide bonds resulting in the formation of cross-linked proteins (Korzeniowska, Króliczewska, and Kopeć, 2015), and functional modifications of proteins, as well as nutritional modification of protein, including loss of essential amino acids, decreased protein digestibility and amino acid bioavailability such as

decreased thiol and tyrosine groups by forming disulfide and dityrosine bridges (Santé-Lhoutellier et al., 2008). Furthermore, development of protein oxidation in meat system also negatively impact on meat quality such as meat tenderness (Estévez, Ventanas, and Cava, 2005), juiciness (Huff-Lonergan and Lonergan, 2005), and decrease water holding capacity (Decker et al., 1993). These modifications can be involved in the regulation of fresh meat quality and influence the processing properties of meat products.

2.10 Protein digestibility

Digestibility of meat and meat products is important to ensure nutritional value of meat. Accumulation of nonhydrolyzed or partially hydrolyzed proteins in large intestine could be a cause of colon cancer by extensively fermenting, leading to formation of mutagenic products (Santé-Lhoutellier, Aubry, and Gatellier, 2007). The efficiency of protein digestibility depends on the structure and physicochemical state of proteins. Oxidation between ROS and proteins during meat processing induces on changes of protein structure. A relatively mild oxidative conditions will induce slight modifications and partial unfolding of the protein structure, thus yields oxidized proteins of enhancing susceptibility to proteases, whereas severe protein oxidation leads to cross-linking and massive aggregation as well as modification of protease-active sites, resulting in a reduced susceptibility to digestive enzymes (Soladoye et al., 2015). Heating also acts on the macrostructure and microstructure of the meat that has been shown to affect protein digestibility. Heat treatment causes denaturation of protein in the fibers, resulting in shrinkage and hardening of the tissue (Kaur, Maudens, Haisman, Boland, and Singh 2014). As cooking continues, proteins

coagulate; the tightly coiled polypeptide chains unfold, aggregates and results in intermolecular cross-links at high temperatures which can reduce protein digestibility, thus affecting amino acid release and bioavailability (Kaur et al., 2014). The mode of action of digestive enzymes was different for raw and cooked meat myofibrils. Enzymes acted randomly and uniformly on raw meat myofibrils, whereas the enzyme action was observed to start from the edges of the cooked meat myofibrils and then move towards the center as the digestion progressed. Cooking disrupts the basic structure of protein resulting in the formation of a fused matrix and more dense protein structure, which is resistant to further enzyme digestion (Kaur et al., 2014). Filgueras, Gatellier, Zambiazi, and Santé-Lhoutellier (2011) reported that cooking decreased the myofibrillar protein susceptibility to pepsin activity, while after cooking, the proteolysis rate by pancreatic enzymes increased. Bax et al. (2012) reported that above 100 °C, aggregation induced by oxidation slowed pepsin digestion, but improved overall digestibility in an in vitro system. Wen et al. (2015) reported that cooking led to a reduction of pork protein digestibility. Heating alters amino acid residues of primary protein structure of protein via carbonylation, modification of aromatic residues, and the formation of Maillard reaction products affecting bioavailability of amino acids. Furthermore, collagen fibers are more resistant than other proteins to proteolytic enzymes and is considered to be only partly digestible. The low digestibility of collagen is due to naturally-occurring crosslinks. Denatured collagen is quite resistant to hydrolysis by proteases because of high levels of proline and hydroxyproline. These amino acids reportedly prevent the flexibility of the protein backbone that facilitates binding to the active site of the enzyme (Kaur, Rutherfurd, Moughan, Drummond, and Boland, 2010).

2.11 Vibrational spectroscopy

In recent years, vibrational techniques growing interest as tools for meat quality assessment. Although some traditional methods have been proven to be suitable and accuracy for determination meat quality such as fatty acid composition, water holding capacity, instrumental texture methods, iodine values, peroxide values, sensory evaluation, and microbiological analysis. These methods have the disadvantage as they are being invasive, destructive, costly, time consuming, involving laborious wet chemistry for sample preparation. Information they provided is limited, labor-intensive, and complex process of sample preparation (Herrero, 2008a, 2008b). It has been shown that vibrational spectroscopy data are related to the results obtained with traditional methods and could be used to evaluate meat quality which is valuable for quantitative and qualitative analyses. In this respect, vibrational spectroscopy including, Fourier transform infrared spectroscopy (FT-IR) and Fourier transform Raman spectroscopy (FT-Raman) are becoming an attractive alternative to the existing traditional techniques in quality assessment of meat because they have several advantages compared to traditional methods. These include direct, rapid, non-destructive, non-invasive, and providing information about different food components at the same time, *in situ* investigation. In addition, only small portions of samples are required without complicated sample preparation and tedious procedures, and environment-friendly analytical techniques without chemicals as well as suitable for routine or online analysis (Li-Chan, 1996).

Vibrational spectroscopy measures the oscillations of atoms in molecules and is a useful technique to identify substances and monitor changes in chemical bonding at molecular level. These techniques have provided structural changes of the main

components in meat including proteins, water and lipids that occur during handling, processing and storage. Raman spectroscopy like IR spectroscopy measures fundamental molecular vibrations on stretching and deformation modes, whereas absorption of fundamental vibrational modes makes the basis for IR. The Raman spectrum is obtained by a change in the polarizability during vibration, while the IR spectrum is obtained by a change in the molecular dipole moment during vibration. Thus, Raman is complementary to IR with respect to the sensitivity of different types of molecular vibrations. Certain atomic bonds within the molecules that do not produce strong vibrational responses in the mid-IR region produce a polarizability response that is measure in Raman spectra. In general, Raman is more sensitive to backbone structure of macromolecules, while the functional side groups are sensitive in IR. Therefore, the polar groups such as C=O, N-H, and O-H showed a strong absorption across IR spectrum. Nonpolar groups such as C=C, C-C and S-S showed strong Raman scattering (Li-Chan, 1996). The main advantage of Raman spectroscopy over IR spectroscopy especially when applied in meat products containing approximately 75 % water is that spectra are not dominated by molecular vibrations of water. However, Raman spectroscopy has limitation of being sensitive to sample fluorescence (Li-Chan, 1996).

2.11.1 Fourier -transform IR spectroscopy (FT-IR)

IR spectroscopy, the energy for these transitions is provided by radiation in the IR region of the electromagnetic spectrum. Mid infrared region (4000-400 cm^{-1}) of electromagnetic spectra contain mainly fundamental vibration bands where the vibration results in a change in the dipole moment of the molecule. Organic molecules possess bonds and functional groups. When IR radiation is applied

to organic molecules (functional groups), it breaks down the molecule's equilibrium (position) state, causing two energy transitions in a molecule. It promotes transitions in a molecule between rotational and vibrational energy. Transitions between rotational and vibrational energy levels cause a net change in the dipole moment, leading to IR absorbion of a molecule. Therefore, an IR absorption profile is unique to a specific molecular vibration frequency. When IR passes through a sample, some of IR is absorbed by the sample and some is passed through (transmitted). The resulting spectrum represents the molecular absorption/transmission, which creates a molecular fingerprint of the sample. Identification of molecular functional groups is the major application of IR spectrometry (Yu, 2006).

2.11.1.1 Synchrotron radiation-based Fourier-transform IR spectroscopy (SR-FTIR)

Synchrotron radiation is an extremely bright light generated from a synchrotron which is a facility consist of giant particle accelerator that turns electrons into light. The advantage of synchrotron light brightness and small effective source size makes it capable of exploring the molecular chemical makes up within intact microstructures of biological tissue at ultra-spectial resolution (Yu, 2006). When a synchrotron light (IR) source, FTIR spectroscopy and microscopy are combined together, it is called "synchrotron radiation-based FTIR microspectroscopy (SR-FTIR). This technique takes advantage of synchrotron light brightness which is usually 100–1000 times brighter than conventional globar source (conventional thermal IR). It is capable of exploring the molecular chemistry within microstructures of biological samples with high signal to noise ratio at ultraspatial resolutions as fine

as 3–10 μm and can encompass a wider spectral range so that more accuracy and precision as well as more detailed structural information can be extracted (Yu, 2006).

2.11.1.2 IR fingerprint bands

The IR band region between 3600-3100 cm^{-1} provides information about the O-H and N-H stretching vibrations. The region of the spectra where –C-H stretches observed at the region 3100-2800 cm^{-1} is distinctive in lipids. The characteristic bands at 2960 cm^{-1} and 2875 cm^{-1}, asymmetric and symmetric stretching vibrations of C-H of aliphatic CH_3; bands at 2920 and 2850 cm^{-1}, asymmetric and symmetric stretching vibrations of C-H of aliphatic CH_2. Lipid show a distinctive IR band near 1750 cm^{-1} is mostly to the C=O stretching vibration (Marinkovic and Chance, 2006).

The characteristic of protein structure is unique in peptide bond. The peptide bond contains C=O, C-N, and N-H. The spectral region to protein secondary structural components is the amide bands corresponding to the amide I, amide II, and amide III, which can be used to characterize conformation of protein secondary structure. The amide I (1700-1600 cm^{-1}) vibration absorb near 1650 cm^{-1}, arises almost entirely to the C=O stretching vibration of the peptide linkages (approximately 80%) with minor contributions from the out-of-phase C-N stretching vibration. The amide I band components are found to be correlated closely to the secondary structure of the backbone and is therefore the amide I vibration that is most commonly used for secondary structure analysis. α-Helice give rise to a main absorption band close to 1655 cm^{-1}. Antiparallel β-sheets exhibit a strong band near 1630 cm^{-1} and a weaker band near 1685 cm^{-1}. The amide II band, in contrast, at 1550 cm^{-1} is the out-of-phase combination of the N-H in plane bending

(40-60% of potential energy) and from the C-N stretching vibration (18-40%) with smaller contributions from the C=O in plane bend and the C-C and N-C stretching vibrations, showing much less protein conformational sensitivity than its amide I counterpart. The amide III band between 1400-1200 cm^{-1} is the in-phase combination of the N-H bending and the C-N stretching vibration with small contributions from the C-O in plane bending, C-C stretching vibration (Barth, 2007). Bands of lipids related to C-H scissoring vibration at 1470 cm^{-1} and C−O−C stretching vibration, around 1060 cm^{-1}. Finally, the group of features in the region 1200-1000 cm^{-1} present in the spectra of C-O stretching from carbohydrates (Marinkovic and Chance, 2006).

2.11.2 Fourier -transform Raman spectroscopy (FT-Raman)

In Raman spectroscopy, samples are excited with a strong monochromatic laser light that may be in the ultraviolet (UV), visible (VIS), or near-infrared (NIR) regions of the electromagnetic spectrum which reacted with a molecule in the sample. Most of the photons are scattered from the sample with the same energy level as the incident light called “elastically scattering” (Rayleigh scattering) whereas very few photons are scattered with a lower or higher frequency compared with the frequency of the incident light called “inelastically scattering” (Stokes or anti-Stokes scattering). The difference in frequency of the incident light and inelastically scattered light is called the Raman shift ($\Delta\nu$ in cm^{-1}) or the Raman spectrum (Berhe et al., 2014; Li-Chan, 1996).

2.11.2.1 Raman fingerprint bands

Raman spectra exhibit bands of fundamental vibrational transitions, thus providing molecular structure information of several compounds. Raman spectroscopy provides information mainly about secondary and tertiary

structure and variation in local environment of proteins including polypeptide backbone, microenvironment of amino acid side chains (tyrosyl doublets, tryptophan residues, aliphatic amino acids) and disulfide bonds. It has proven to be a powerful technique for investigating the structure of lipids, water, and carbohydrates. Raman bands assigned for water structure comprise two O-H stretching bands in the 3600-3000 cm^{-1} region. Change in secondary structure mainly analyzing amide region and C-C stretching bands. Raman bands assign for protein including amide I (1685-1645 cm^{-1}) and amide III (1350-1200 cm^{-1}) bands which were the most useful Raman bands for determining the secondary structure of meat protein (α-helix, β-sheet, turns, unordered or random coil structures) (Herrero, 2008a, 2008b). Proteins with high α-helical content show more intense Raman band centered around 1658-1645 cm^{-1} assigned unambiguously to the amide I vibrational mode which involves mainly C=O stretching with a lesser contributions of C-N and N-H stretching, N-H in-plane bending of peptide groups (Beattie, Bell, Farmer, Moss, and Patterson, 2004; Li-Chan, 1996). The amide I band with predominantly β-sheet structures show the band between 1680-1665 cm^{-1}, 1640-1612 cm^{-1} and a high proportion of unordered structure (random coil) is attributable to proteins with an amide I band centered at 1665-1660 cm^{-1} (Herrero, 2008a, 2008b; Pelton and McLean, 2000). Amide III band is another Raman band that provides information about secondary structure of proteins (Herrero, 2008a, 2008b; Li-Chan, 1996). The amide III region involve C-N stretching and N-H in-plane bending vibrations of the peptide bond as well as contributions from Cα-C stretching and C=O in-plane bending. The amide III band is widely used to confirm the results obtained from the amide I band, but is difficult to interpret the exact position of conformations by

the fact that proteins produce a complex pattern of bands and has some overlap between α-helix, β-sheet, turn, and random coil (Herrero, 2008a, 2008b). The intensity of the amide III band for α-helix structure appears around 1300–1260 cm^{-1} which overlaps with the region assigned to turns. The frequency ranges that are characteristics of α-helix (1300-1265 cm^{-1}), β-sheet (1245–1230 cm^{-1}) and random coil (1255–1240 cm^{-1}) are overlap to some extent (Beattie, Bell, Borggaard, and Moss, 2008). Another way to look at the secondary structure of protein, and confirming the protein structure elucidated by amide I band, is by using the C-C stretching bands: C-C stretching vibrations in the 1060-890 cm^{-1} range are characteristic of α-helix (945-890 cm^{-1}) and β-sheet (1060-1020 cm^{-1}) structures (Herrero, 2008a, 2008b).

Modifications in protein local environment of muscle foods, aliphatic hydrophobic residues assigned at 2860, 2935, and 2970 cm^{-1} (C-H stretching) and at 1450 cm^{-1} (CH_2 and CH_3 bending vibrations) bands. A reduction in intensity of aliphatic amino acid bands possibly results from hydrophobic interactions of aliphatic residues (Herrero, 2008a, 2008b). Aromatic amino acids show several Raman characteristics which are useful to monitor the polarity of the micro-environment. Tryptophan (Trp) residues and tyrosine (Tyr) doublet bands provide information about local environment and hydrophobic interactions of proteins, giving information about proteins tertiary structure. Trp vibrations are visible at 544, 577, 760, 879, 1014, 1340, 1363, 1553 and 1582 cm^{-1} (Li-Chan, 1996). Modifications of the tertiary structure can be accompanied by exposure of buried Trp residues in hydrophobic micro-environment to the polar aqueous solvent, which corresponds to a decrease in Trp intensity.

Furthermore, a high ratio of intensity I_{1360}/I_{1340} indicates a hydrophobic environment. A low ratio indicated Trp is involved more in hydrogen bonding of a hydrophilic environment (Herrero, 2008b). The ratio of the tyrosyl doublet at 850 and 830 cm^{-1} is involved in hydrogen bonding of the phenolic hydroxyl group by acting both hydrogen acceptor and donor in a polar environment. Moreover, the tyrosine doublet ratio has been proposed for determining if the tyrosine exposed or buried in protein network (Herrero, 2008a).

Disulfide and sulfhydryl groups can be detected by the S-S stretching and S-H stretching bands in the 560-510 cm^{-1} and 2580-2550 cm^{-1} region, respectively (Berhe et al., 2014). Proteins and peptides with disulfide bonds (C-C-S-S-C-C group) of cystine show bands centered at 510, 525, and 540 cm^{-1}, which are assigned to gauche-gauche-gauche, gauche-gauche-trans, and trans-gauche-trans conformations, respectively (Li-Chan, Nakai, Hirotsuka, 1994).

2.11.3 Application of vibrational spectroscopy in meat quality

Vibrational spectroscopy including both IR and Raman spectroscopy has been widely adopted as an analytical technique for the determination quality in meat and meat products. It provides the information about the structural changes of meat composition such as proteins, lipids, water, and carbohydrates in relation to meat quality. Its properties as fingerprint technique can be used for both qualitative and quantitative analysis. Quantitative information about the secondary structure of proteins can be obtained from the amide I spectral profile. Spectra analysis technique in Gaussian and Lorentzian multi-component peak modeling/fitting method is employed to estimate the relative content of protein secondary structure (Yu, 2006).

Changes in the frequencies, half-widths, and intensities of the Raman and IR bands of chemical groups of these compounds are indicative of structural changes.

2.11.3.1 Chemometric analysis

IR and Rama spectra consist of several thousands of data points. Chemometrics methods is the use of mathematical and statistical methods that designed to treat such multivariate data, including principle component analysis (PCA), partial squares regression (PLS), and partial squares-discrimination analysis (PLS-DA). Chemometric tools help to gain a deeper insight and complete interpretation of spectroscopic data. Among the multivariate analysis techniques, PCA is the most frequently used because it is a starting point in the process of data minimizing. PCA is to transform data comprising measurements of variates into a new data set of much more manageable size. The transformed variates are known as principal component (PC) "scores" and are ordered such that the first few contain most of the information that was spread across all of the original data. An important feature of PCA is plots of the scores and loadings. The score plots depict the covariance between samples and provide data overview. Line plot of loadings illustrate the importance of the original variables of each PC.

The implementation of vibrational spectroscopy in conjunction with principle component analysis (PCA) is usually adopted to qualitatively classify meat quality differences among samples groups including monitoring the structural changes of meat components (protein, fat, and water) during processing as well as classification between samples to solve adulteration problems. Al-Jowder, Kemsley, and Wilson (1997) indicated that IR spectroscopy can readily distinguish between fresh and frozen-thawed turkey, chicken, and pork, based on PCA

analysis. IR spectra bands assigned for protein (1650 and 1550 cm^{-1}) and lipid (1740 cm^{-1}) are important factors for the discrimination of meat types. Beattie, Bell, Borgaard, Fearon, and Moss (2006) used Raman spectra at region from 1900-270 cm^{-1}, indicating C–C, C=C, C–O and C=O, stretches and the C–H bends to predict the fatty acid composition of unextracted adipose tissue Longisimus dorsi of pork, beef, lamb, and breast chicken meat. It was found that the bulk unsaturation parameters could be predicted successfully for cis unsaturation (R^2 = 0.97, root mean square error of prediction (RMSEP) = 4.6%), PUFA (R^2 = 0.97 and RMSEP = 4.0%). Campos et al. (2014) identified the frankfurters type (chicken, turkey and mixed meat) based on Raman spectra and PCA. The separation of the samples is based on the fat and moisture correlated with Raman spectra region of fat and lipid at 2900 cm^{-1} (C-H stretching), 1750 cm^{-1} (C=O stretching), 1450 cm^{-1} (CH_2 scissoring) and water at 3200 cm^{-1} (O-H stretching).

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CHAPTER III

MEAT QUALITY AND RAMAN SPECTROSCOPIC CHARACTERIZATION OF KORAT HYBRID CHICKEN OBTAINED FROM VARIOUS REARING PERIODS

3.1 Abstract

Meat quality attributes vary with chicken age. Understanding the relationship between poultry age and the quality of the meat would be beneficial for efficient poultry farming to meet market needs. The Korat hybrid chicken (KC) is a new crossbred chicken whose meat quality is distinct from that of commercial broiler (CB) chickens and has not been well characterised. In this study, we characterised the physico-chemical properties of KC meat and correlate the findings with Raman spectral data. The protein content of KC breast and thigh meat increased with age. The pH of thigh meat decreased, while the water holding capacity of breast meat increased as the age of the chickens increased. The amount of cholesterol in breast meat decreased as the rearing period was extended. Inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) of breast meat decreased as KC grew older. The shear force values of meat from older birds increased concomitantly with an increase in total collagen. Principle component analysis (PCA) revealed that the meat quality of CB was greatly different from KC meat. High shear force values of KC meat at 20 weeks old were well correlated with an increase in the β-sheet structure (amide I) and amide III of collagen. Raman spectra at 3207 cm^{-1} and relative α-helical

content were negatively correlated with shear force values of KC breast meat. These could be used as markers to evaluate KC meat quality.

Keywords: meat quality, age, chicken, Fourier transform Raman spectroscopy, principle component analysis

3.2 Introduction

Chicken meat is considered a good source of protein with lower fat and cholesterol compared to other red meats (Jaturasitha, Srikanchai, Kreuzer, and Wicke, 2008). Commercial chicken meat is derived from a fast-growing broiler strain that can grow within 5-6 weeks (Choe, Lee, and Jo, 2009). An alternative source is native chicken, which is considered a delicacy, particularly in Asian food cultures (Guan et al., 2013; Jaturasitha, Chaiwang, and Kreuzer, 2017; Wattanachant, Benjakul, and Ledward, 2004) However, native chicken production is commercially limited due to its slow growth rate and lean muscle-gaining ability (Jaturasitha, Kayan, Wike, 2008; Wattanachant et al., 2004). It typically takes about 14–23 weeks to reach a marketable size (Jaturasitha et al., 2017). For this reason, hybrid chicken has been developed in various regions throughout the world (Batkowska, Brodacki, Zieba, Horbanczuk, and Lukaszewicz, 2015; Huang et al., 2011; Jaturasitha, Kayan, et al., 2008; Łukasiewicz, Niemiec, Wnuk, and Mroczek-Sosnowska, 2014; Miguel et al., 2011; Park et al., 2010). Korat chicken (KC) is a crossbreed between Leung Hang Khao sires (Thai native chickens) and SUT 101 chicken dams (a crossbreed between broiler and layer chickens, which has demonstrated better growth performance than its

sires (Maliwan, Khempaka, and Molee, 2017). Thus far, little scientific information is available regarding the meat quality of KC.

The age of the animal plays an important role in meat quality. Połtowicz and Doktor (2012) reported that the water holding capacity (WHC) of leg muscles of hybrid chickens tended to improve with age. Meat from older animals was also darker in colour because levels of myoglobin increased with age (Wideman, O'Bryan, and Crandall, 2016). Crosslinked collagen increases with age, resulting in tougher texture of the meat from older animals (Weston et al., 2002). Inosine 5'-monophosphate (IMP) is one of the key compounds contributing to umami taste. The relationship between chicken age and IMP content appears to vary with species and/or rearing system. Rikimaru and Takahashi (2010) reported that IMP content in thigh meat of broilers increased with age, but Chen et al. (2002) observed that IMP content in the pectoralis major and musculus peronaeus of Taihe Silkies chickens (Chinese native chickens) decreased with age. The effect of age on meat quality and muscle composition of KC is still unknown. Understandings this relationship would reveal the uniqueness of meat quality at each period of rearing.

Raman spectroscopy has an advantage over conventional analytical methods because it directly measures components at the molecular level with a relatively short analysis time and small sample size. Moreover, Raman spectra showed weak background scattering from water (Li-Chan, 1996) therefore, it is ideal for monitoring the quality of chicken meat *in situ* where the moisture content is around 78%. Raman spectroscopy reveals the specific bands or fingerprint of the chemical bonds in a molecule, providing structural changes of food components, including proteins, lipids, and water. Thus, Raman spectroscopy could be a promising technique for meat

quality evaluation. Beattie, Bell, Farmer, Moss, and Patterson (2004) suggested that the ratio of α-helices to β-sheets of proteins and the hydrophobicity of the myofibrillar environment are important factors influencing shear force, tenderness, texture, and overall acceptability of beef. Principle component analysis (PCA) is used to minimise Raman spectral data and provides an insightful relationship between spectra and results from physical and chemical analyses. The relationship between Raman spectra and KC meat quality characteristics at each rearing period could lead to wavenumber marker(s) that could be used to evaluate the meat quality of KC and commercial broilers. Therefore, the objective of this study was to investigate changes in the physico-chemical properties of breast and thigh meat from KC at various ages in comparison with commercial broilers. In addition, Raman spectroscopy, in combination with PCA, was used to identify the distinct KC meat quality characteristics based on the slaughtering age.

3.3 Materials and methods

3.3.1 Animals and sample preparation

All procedures were approved by the Animal Ethics Committees of Suranaree University of Technology (SUT). A total of 150 one-day-old KC were randomly distributed into three pens (50 chicks/pen) and reared indoors under the same environmental conditions at Suranaree University of Technology (SUT) Farm (Nakhon Ratchasima, Thailand). Stocking density was 8 birds/m^2. Feed and water were provided *ad libitum*. Birds were fed a commercial diet for starter (0-4 weeks old), grower (5-6 weeks old), and finisher (7-20 weeks old) containing 21, 19, and 17% crude protein, respectively. Birds had no access to the outdoor environment.

At each rearing period of 8, 10, 12, 16, and 20 weeks, five KC males were randomly selected from each pen, fasted for 18 h, and then brought to a commercial chicken slaughterhouse (Nakhon Ratchasima, Thailand). Birds were exsanguinated by stunning with electrocution before a conventional neck cut, bled, and plucked according to Genesis GAP chicken production standards. Then, the carcass was manually eviscerated and washed, immediately packed in a polystyrene box filled with ice and transported to the SUT laboratory within 1 h. Breast and thigh meat of 6-week-old male commercial broilers (CB) were obtained from a chicken meat processing company (Charoen Pokphand Foods (Thailand) Public Company Limited, Nakhon Ratchasima, Thailand). Broiler meat samples were stored at 0-2˚C for 3 h before being transported to the SUT laboratory within 1 h. Both CB and KC meat was chilled at 4 °C for 24 h. Subsequently, KC breast and thigh meat were excised from carcasses, and skin, fat, and connective tissues were removed. The pH was determined 24 h post-mortem for all meat samples. The water holding capacity (WHC) and shear force of the meat were measured at 24 h post-mortem. Colour was also monitored within 48 h. The remaining meat from each sample was immediately minced using a meat grinder, vacuum-packed, and stored at -80 °C for Raman spectroscopy measurement and chemical analyses within 1 and 4 months, respectively. Before chemical analyses, frozen samples were thawed in a 4 °C refrigerator for 12-18 h.

3.3.2 Proximate composition

KC and CB breast and thigh meat were analysed for moisture, ash, and protein content according to the Association of Official Analytical Chemists (AOAC) (2010). Total lipids were measured according to Folch, Lees, and Sloane-Stanley (1957).

3.3.3 Physical properties

Meat samples (1 g) were homogenised with 5 mL of deionised water and the pH of the homogenates was measured using a pH meter (Wattanachant et al., 2004). WHC was determined according to Ryoichi, Degychi, and Nagata (1993) by centrifugation at 6,710×g, at 25 °C for 10 min. The surface meat colour was measured using a colorimeter (Hunter Associates Laboratory, Reston, VA., USA) with a D65 light source. An average value taken from three different locations on each sample is presented in Table 3.2.

Samples were heated in a water bath at 80 °C for 10 min, and cut into 2.0 × 3.0 × 0.5 cm pieces for shear force measurements using a Texture Analyser (TA.XT. Plus, Stable Micro Systems, UK) equipped with a Warner-Bratzler (Wattanachant et al., 2004). Nine replicates were measured for each sample.

3.3.4 Fatty acids composition

Lipids were extracted according to Folch et al. (1957) using a chloroform-methanol mixture (2:1 v/v). The extracted lipid samples were added to heptadecanoic acid (17:0) as an internal standard and fatty acid methyl esters (FAME) was performed by methylation using boron trifluoride (BF_3)-methanol, followed by separation in a gas chromatograph (HP-7890, Agilent Technologies, Palo Alto, State. USA) equipped with a flame ionisation detector (FID) according to the method of Bostami, Mun, and Yang (2017). The injection port temperature was set at 240 °C and the detector temperature was 250 °C. Identification and quantification of fatty acids were performed using external standards (Supelco 37 FAME, Sigma–Aldrich Co., St. Louis, MO).

3.3.5 Cholesterol analysis

Cholesterol extraction was performed according to Rowea, Macedob, Visentainer, Souzaa, and Matsushita (1999) with slight modifications. Cholesterol was quantified using a gas chromatograph (GC)-flame ionisation detector equipped with a HP-5 column (30 m × 0.32 mm; film thickness, 0.25 μm; Agilent Technologies, Palo Alto, USA). α-Cholestane was used as an internal standard. The injection port temperature was set at 260 °C and the detector temperature was 255 °C. Cholesterol identification was done by comparing the relative retention time of the sample with the standard (Carlo Erba Reagents, Milan, Italy).

3.3.6 Purine analysis

Meat samples (0.5 g) were hydrolysed with 70% perchloric acid for 1 h at 95 °C according to the method of Kaneko, Aoyagi, Fukuuchi, Inazawa, and Yamaoka (2014). Purine bases were separated on an Asahipak GS-320 HQ column (7.5 × 300 mm, 6 μm particles; Showa Denko America, Inc., USA) equipped with a HPLC system (HP1260, Agilent Technologies, USA). Detection was monitored at a wavelength of 260 nm. The quantity of adenine, guanine, hypoxanthine, xanthine, and uric acid were determined by comparing the peak area with that of the external standards.

3.3.7 Changes of nucleotides

The amounts of nucleotides from meat samples were measured according to Burns and Ke (1985) with slight modifications. The extracted nucleotides were analysed using an HPLC (HP 1260, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a Hypersil ODS C18 reverse-phase column (4.6 × 150 mm, 3 μm particles; Thermo Scientific, Waltham, MA, USA). The mobile phase A was 150 mM

potassium dihydrogen phosphate (KH_2PO_4) and 150 mM potassium chloride (KCl, pH 6) and the mobile phase B was the mobile phase A mixed with 20% acetonitrile. The gradient flow rate was set at 0.5 ml/min. The mobile phase was 3% B for 0-5 min; it was increased to 9% B for 5-10 min, and reached 20% B at 15 min, and was finally increased to 100% B at 20 min, which was maintained for 5 min. The column temperature was maintained at 25 °C, and detection was monitored at 254 nm. The amount of inosine monophosphate (IMP), guanosine monophosphate (GMP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine, and hypoxanthine were quantified by comparing the peak area with that of the external standards (Sigma–Aldrich Co., St. Louis, MO).

3.3.8 Collagen content

Total collagen content was determined by alkaline hydrolysis as described by Reddy and Enwemeka (1996) with some modifications. Samples were hydrolysed with 7 M NaOH at 120 °C for 40 min. The hydrolysate was neutralised with 3.5 M sulfuric acid (H_2SO_4), filtered, and reacted with chloramine T solution and Ehrlich's reagent. The absorbance was measured at 550 nm using hydroxyproline (Sigma–Aldrich Co., St. Louis, MO) as a standard. Total collagen content was calculated using a conversion factor of 7.25 (Bergman and Loxley, 1963). The insoluble collagen content of dried residues was determined according to Liu, Nishimura, and Takahashi (1996).

3.3.9 Fourier-transform Raman spectroscopy (FT-Raman)

Thawed samples were homogenized and packed into the holder pocket. Raman spectra were collected on a Bruker Vertex 70 FT-Raman spectrometer (Bruker, Karlsruhe, Germany) over the range of 4000-400 cm^{-1} at a resolution of

4 cm^{-1} and 256 scans. Sulphur was used to calibrate the Raman frequency. A diode-pumped Nd:YAG laser at 1064 nm with 500 mW of laser power was the excitation source. A Ge detector used liquid nitrogen as the coolant. Instrument control and spectral acquisition were performed using OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) software. In each treatment, 10 spectra were collected for each replication to obtain a total of 30 spectra. Spectra were processed with the unscramble X 10.5 (Camo, Oslo, Norway) software by considering the third-order polynomial using the Savitzky-Golay algorithm with 17 points of smoothing, allowing the minimisation of the effects of variable baselines. The baseline correction was made by offsetting and then extending the multiplicative signal correction (EMSC). The intensity values of Raman bands were determined. Then, protein secondary structures were determined as percentages of α-helix, β-sheet, β-turn, and random coil conformations based on curve fitting in the 1700-1600 cm^{-1} (amide I) range using the appropriate Gaussian and Lorentzian functions in the OPUS 7.2 software (Bruker Optics Ltd, Ettlingen, Germany).

PCA of the Raman spectra in a range of 3801-2704 and 1803-399 cm^{-1} were processed as previous described and analysed using the unscramble X 10.5 software (Camo, Oslo, Norway), using three spectra per treatment. Wavenumbers with high loadings were selected for multivariate analysis with physico-chemical data. All variables were weighted using a standard deviation weighting process. The most common weighting used was 1/SDev when investigating relationships with other variables. The correlation between selected Raman spectra and the physico-chemical properties was determined by Pearson's correlation.

3.3.10 Statistical analysis

All experiments were conducted with three independent replications. Data were analysed for degree of variation and significant difference between groups of age of chickens using analysis of variance (ANOVA). Tukey's multiple range test was used to compare differences among mean values ($P < 0.05$). Mean values and standard deviation (SD) are reported. The difference between muscle types (breast vs. thigh) at the same age was analyzed using a t-test. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3.4 Results and discussion

3.4.1 Proximate composition

The moisture content of KC breast meat decreased with the age of birds ($P < 0.05$, Table 3.1). Crude protein content of KC breast and thigh meat increased until 10 weeks of rearing and remained constant afterwards. KC breast and thigh meat at 10-20 weeks old contained higher crude protein content than that of commercial broilers ($P < 0.05$), while the total lipid content of KC meat was lower. These results are in agreement with those reported by Wattanachant et al. (2004). Zheng et al. (2016) reported that native chicken showed higher levels of liver proteins involved in lipid degradation. These data could, therefore, imply that lipid metabolism in native and/or hybrid chickens occurred to a greater extent than that in broiler chickens, which could be one of reasons for the lower lipid content in KC. Age had no effect on total lipid and ash content of KC until up to 20 weeks of rearing ($P > 0.05$).

Table 3.1 Proximate composition (%) of breast and thigh meat from different ages of Korat chicken (mean ± standard deviation).

Muscle type	Chicken breeds	Age (wk)	Moisture (%)	Crude protein (% db)	Total lipid (% db)	Ash (% db)
Breast	CB	6	76.91 ± 0.38^{a}	84.76 ± 2.89^{b}	5.95 ± 1.06^{a}	4.93 ± 0.05^{a}
	KC	8	75.54 ± 0.15^{b}	86.00 ± 0.54^{b}	3.59 ± 0.68^{b}	4.94 ± 0.16^{a}
		10	75.80 ± 0.11^{b}	90.84 ± 1.50^{a}	3.88 ± 0.77^{b}	4.35 ± 0.14^{b}
		12	75.33 ± 0.56^{bc}	92.65 ± 1.77^{a}	3.08 ± 0.32^{b}	4.40 ± 0.09^{b}
		16	74.79 ± 0.23^{cd}	90.02 ± 1.39^{a}	3.77 ± 0.58^{b}	4.85 ± 0.10^{a}
		20	74.43 ± 0.22^{d}	90.16 ± 1.32^{a}	3.90 ± 0.52^{b}	4.82 ± 0.17^{a}
Thigh	CB	6	75.41 ± 0.92^{b}	75.36 ± 3.77^{b}	16.87 ± 1.72^{a}	4.38 ± 0.24^{ab}
	KC	8	76.63 ± 0.39^{a}	75.84 ± 1.34^{b}	9.98 ± 2.29^{c}	4.38 ± 0.37^{ab}
		10	76.71 ± 0.46^{a}	81.74 ± 6.04^{ab}	10.57 ± 1.32^{bc}	4.12 ± 0.14^{b}
		12	76.82 ± 0.41^{a}	79.91 ± 4.36^{ab}	13.13 ± 1.12^{b}	4.39 ± 0.05^{ab}
		16	76.03 ± 0.83^{ab}	80.68 ± 2.59^{ab}	10.81 ± 1.38^{bc}	4.53 ± 0.21^{a}
		20	76.13 ± 0.88^{ab}	83.28 ± 2.79^{a}	8.85 ± 1.47^{c}	4.74 ± 0.11^{a}

$^{a-d}$ Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type. db; dry basis.

3.4.2 Physicochemical properties

The pH values of KC breast and thigh meat decreased with increasing age ($P < 0.05$, Table 3.2). Older birds appeared to have more glycogen storage in both the breast and thighs, leading to a greater decrease in the post-mortem pH (Díaz et al., 2010). In general, the pH value of breast meat was lower than thigh meat. Breast meat is composed of type IIB fibres, containing higher glycogen content (Listrat et al., 2016). Therefore, lactic acid accumulation post-mortem was higher in breast meat. Higher WHC was noticed in breast meat obtained from older KC. No differences in pH and WHC were observed between 10-week-old KC and 6-week-old CB, which is their market age. Changes in meat colour were subtle with age (Table 3.2). Lower meat pH values in older KC birds would lead to a greater extent of myoglobin denaturation, which would increase light scattering and meat paleness (Mir, Rafiq, Kumar, Singh, and Shukla, 2017). Surface colour did not show any differences between the two strains at their market age (10 weeks for KC vs 6 weeks for CB). Overall, KC breast meat showed a darker appearance than CB meat, while the thigh colour was comparable. The slow-growing birds normally have redder meat than fast-growing birds because they are typically older with higher contents of haem pigments (Wideman et al., 2016).

Table 3.2 Physico-chemical properties of breast and thigh meat from Korat chicken at different ages (mean ± standard deviation).

Muscle type	Chicken	Age (wk)	pH	WHC (%)	L*	a*	b*
Breast	CB	6	5.92 ± 0.19^{ab}	61.56 ± 5.19^{c}	64.87 ± 5.49^{ab}	3.05 ± 1.67^{b}	12.65 ± 2.96^{a}
	KC	8	5.76 ± 0.01^{bc}	64.00 ± 9.91^{bc}	60.29 ± 5.34^{cd}	2.39 ± 1.00^{bc}	10.38 ± 1.65^{b}
		10	6.00 ± 0.12^{a}	72.56 ± 3.25^{abc}	55.64 ± 2.10^{e}	2.29 ± 0.74^{bc}	13.03 ± 1.71^{a}
		12	5.77 ± 0.04^{bc}	71.43 ± 12.35^{abc}	57.39 ± 3.09^{de}	2.29 ± 1.19^{bc}	11.18 ± 1.77^{b}
		16	5.76 ± 0.08^{bc}	76.58 ± 5.58^{ab}	62.63 ± 3.23^{bc}	1.85 ± 0.96^{c}	8.17 ± 1.49^{c}
		20	5.66 ± 0.17^{c}	81.55 ± 7.56^{a}	67.81 ± 4.08^{a}	3.14 ± 0.80^{b}	7.30 ± 2.36^{c}
Thigh	CB	6	6.56 ± 0.19^{a}	72.57 ± 6.90^{bc}	52.76 ± 3.42^{c}	4.16 ± 1.53^{b}	10.31 ± 3.59
	KC	8	6.36 ± 0.08^{b}	70.62 ± 6.28^{bc}	58.65 ± 3.08^{a}	4.59 ± 1.47^{b}	8.54 ± 3.61
		10	6.40 ± 0.07^{ab}	81.04 ± 4.73^{b}	53.79 ± 2.55^{bc}	4.87 ± 1.13^{b}	8.03 ± 3.82
		12	6.27 ± 0.04^{b}	75.05 ± 7.48^{bc}	55.86 ± 2.29^{abc}	3.39 ± 1.38^{b}	8.14 ± 4.21
		16	6.24 ± 0.03^{b}	68.25 ± 6.33^{c}	56.32 ± 2.97^{ab}	4.10 ± 1.12^{b}	7.50 ± 3.81
		20	5.99 ± 0.05^{c}	73.30 ± 2.95^{bc}	58.26 ± 5.34^{a}	7.04 ± 2.62^{a}	8.76 ± 3.07

$^{a-c}$ Mean values with different superscripts differ significantly ($P < 0.05$) within the same muscle type.

3.4.3 Fatty acids and cholesterol

No differences in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) content were observed in KC breast meat at varied ages ($P > 0.05$, Table 3.3). In contrast, the contents of these fatty acids decreased in thigh meat of 16-20-week-old KC ($P < 0.05$, Table 3.3). Thigh meat of KC at 10 weeks and of CB contained the highest proportion of PUFA, while the highest proportion of MUFA was found in CB meat from both breast and thigh. For both strains, palmitic acid, oleic acid, and linoleic acid were the predominant fatty acids. KC meat expressed higher levels of n-3 fatty acids, particularly docosahexaenoic acid (DHA; C22:6). Differences in fatty acid content reflected strain differences. In previous studies, Jaturasitha, Srikanchai, et al. (2008) reported that Thai chickens had higher proportions of n-3 fatty acids compared with broiler chickens.

Table 3.3 Fatty acid compositions of breast and thigh meat from different ages of Korat chicken.

Muscle type	Fatty acids*	Fatty acids content (mg/g dry sample)					
		CB	KC (wk)				
		(6 wk)	8	10	12	16	20
Breast	C14:0 (Myristic acid)	0.14	0.06	0.08	0.06	0.08	0.07
	C16:0 (Palmitic acid)	7.33	3.05	3.68	2.98	3.84	3.23
	C18:0 (Stearic acid)	2.40	1.50	1.67	1.34	1.72	1.43
	SFA	**9.87^{a}**	**4.61^{b}**	**5.42^{b}**	**4.39^{b}**	**5.65^{b}**	**4.73^{b}**
	C16:1 (Palmitoleic acid)	1.14	0.07	0.15	0.11	0.13	0.11
	C18:1n9c (Oleic acid)	10.07	2.55	3.26	2.98	3.93	3.26
	C20:1 (cis-11-Eicosenic acid)	0.45	0.08	0.12	0.11	0.17	0.12
	C24:1 (Nervonic acid)	0.36	0.39	0.40	0.25	0.32	0.28
	MUFA	**12.02^{a}**	**3.08^{b}**	**3.93^{b}**	**3.46^{b}**	**4.55^{b}**	**3.77^{b}**
	C18:2n6c (Linoleic acid)	8.36	3.35	3.97	3.25	4.32	3.31
	C18:3n3 (Linolenic acid)	0.14	0.04	0.04	0.04	0.05	0.05
	C20:2 (cis-11,14-Eicosadienoic acid)	0.16	0.09	0.10	0.06	0.08	0.07
	C20:3n3 (cis-11,14,17-Eicosatrienoic acid)	0.90	1.48	1.67	1.03	1.50	1.41
	C20:3n6 (cis-8,11,14-Eicosatrienoic acid)	0.29	0.14	0.66	0.12	0.12	0.09
	C22:6n3 (cis-4,7,10,13,16,19-Docosahexaenoic acid)	0.11	0.34	0.36	0.23	0.27	0.33
	PUFA	**9.96^{a}**	**5.43^{b}**	**6.32^{b}**	**4.73^{b}**	**6.35^{b}**	**5.26^{b}**

*SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

[a-d] Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

Table 3.3 Fatty acid compositions of breast and thigh meat from different ages of Korat chicken (Continued).

Muscle type	Fatty acids*	Fatty acids content (mg/g dry sample)					
		CB	KC (wk)				
		(6 wk)	8	10	12	16	20
Thigh	C14:0 (Myristic acid)	0.49	0.60	0.74	0.51	0.39	0.23
	C16:0 (Palmitic acid)	25.15	12.67	18.98	14.34	12.17	8.13
	C18:0 (Stearic acid)	6.70	5.44	7.43	5.83	5.20	4.80
	SFA	**32.34^{a}**	**18.70^{cd}**	**27.15^{ab}**	**20.69^{bc}**	**17.77^{cd}**	**13.15^{d}**
	C16:1 (Palmitoleic acid)	5.27	0.92	2.10	1.34	0.96	0.45
	C18:1n9c (Oleic acid)	39.20	18.58	25.73	19.40	15.42	10.46
	C20:1 (cis-11-Eicosenic acid)	1.77	1.09	1.44	1.01	0.87	0.48
	C24:1 (Nervonic acid)	0.43	0.51	0.74	0.54	0.47	0.48
	MUFA	**46.67^{a}**	**21.10^{bc}**	**30.00^{b}**	**22.30^{bc}**	**17.73^{cd}**	**11.87^{d}**
	C18:2n6c (Linoleic acid)	26.23	22.90	27.86	20.33	17.33	12.87
	C18:3n3 (Linolenic acid)	0.50	0.32	0.38	0.31	0.24	0.21
	C20:2 (cis-11,14-Eicosadienoic acid)	0.24	0.22	0.27	0.20	0.22	0.17
	C20:3n3 (cis-11,14,17-Eicosatrienoic acid)	1.24	2.12	3.53	2.01	2.26	2.25
	C20:3n6 (cis-8,11,14-Eicosatrienoic acid)	0.39	0.22	0.32	0.26	0.21	0.16
	C22:6n3 (cis-4,7,10,13,16,19-Docosahexaenoic acid)	0.14	0.43	0.63	0.39	0.36	0.40
	PUFA	**28.75^{ab}**	**26.2^{abc}**	**32.98^{a}**	**23.51^{bcd}**	**20.61^{cd}**	**16.06^{d}**

*SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

$^{a-d}$ Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

The cholesterol content in KC breast meat decreased with age with a minimum value of 177.9 mg/100 g dry basis (db) in breast meat at 20 weeks old ($P < 0.05$, Figure 3.1). In contrast, changes in the cholesterol content in thigh meat were subtle ($P > 0.05$, Figure 3.1). The reduction of cholesterol with advancing age has been reported by Choi et al. (1987) who found an impairment in enzymes relevant for cholesterol synthesis in adult rats, including acetoacetyl-CoA synthetase, acetoacetyl-CoA thiolase, 3-hydroxyl-3-methylglutryl-coenzyme A (HMG-CoA) reductase, HMG-CoA synthase, mevalonate kinase, and cholesterol 7α-hydroxylase. Moreover, thigh meat showed higher cholesterol content than breast meat in both breeds. Thigh meat mainly consists of oxidative red muscles containing higher fat and cholesterol content (Dinh et al., 2011). It should be pointed out that 10-week-old KC and 6-week-old CB contained comparable cholesterol content at market size.

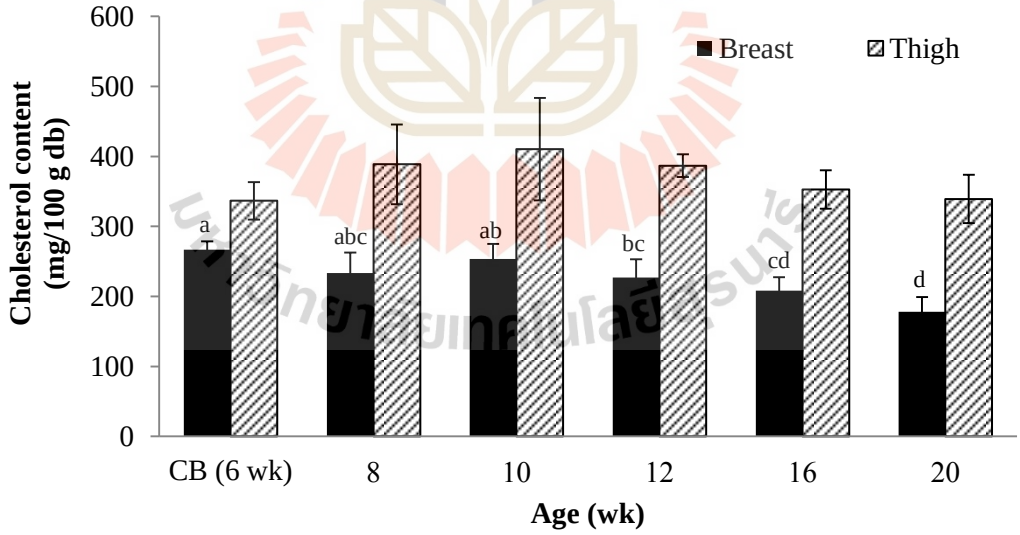


Figure 3.1 Cholesterol content in breast and thigh meat from Korat chickens of different ages. [a-d] Means with different superscripts differ significantly ($P < 0.05$).

3.4.4 Purines and nucleotides

High-purine food intake could trigger gout attacks. Chicken meat is known to be a high-purine food item. The total purine content of KC breast meat remained stable during the course of rearing between 8-20 weeks old, but that of the thigh meat slightly decreased with increasing age ($P > 0.05$) (Figure 3.2). The main purine content of chicken meat differed depending on breeds and muscle parts. KC breast meat contained lower purine content than that of CB ($P < 0.05$). Breast meat showed higher purine content than thigh meat irrespective of the bird's age. Breast meat is mainly composed of type IIB, fast-twitch muscle fibres, which exhibit a lower capacity for purine nucleotide degradation during muscle contraction than red muscle thigh meat containing type I, slow-twitch muscle fibres (Arabadjis, Tullson, and Terjung, 1993).

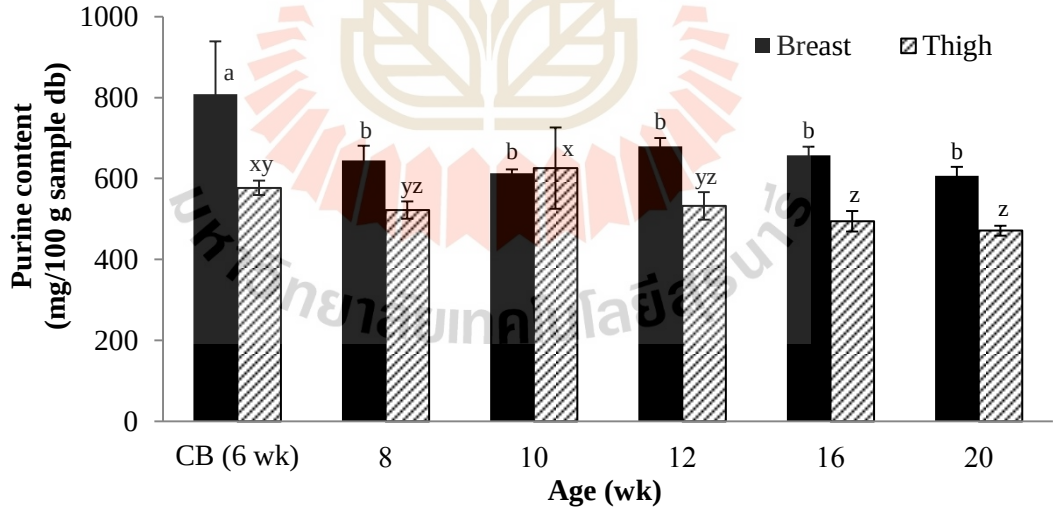


Figure 3.2 Total purine content in breast and thigh meat from Korat chickens of different ages. Means with different superscripts differ significantly ($P < 0.05$) as $^{a-b}$ within breast, $^{x-z}$ within thigh.

IMP and GMP of KC breast meat decreased with age ($P < 0.05$, Table 3.4). IMP is a major taste active nucleotide in chicken meat that is approximately 20-30 times higher than GMP in breast meat and 10-20 times higher than GMP in thigh meat. Breast meat contained higher IMP content than thigh meat, while the latter showed higher GMP content. Thigh meat is mainly composed of type I fiber with greater activity of 5'-nucleotidase, an enzyme catalysing the degradation of IMP (Jayasena et al., 2014). This could explain the lower values of IMP in thigh meat. At 10 weeks of rearing, KC meat contained higher IMP than CB meat. This result is consistent with previous studies reporting that the IMP content of native chicken meat is higher than that of broilers (Jung et al., 2014). This could imply that KC thigh meat would tend to have more umami than its CB counterparts. As inosine is a degradation product of IMP, higher inosine content implies higher degradation of IMP in CB than KC at the market age.

Table 3.4 Changes of nucleotides and their degradation products in chicken muscle at various rearing periods (mean ± standard deviation).

Muscle type	Compound	Content (µg/g dry sample)					
		CB	Rearing period (weeks)				
		(6 weeks)	8	10	12	16	20
Breast	GMP	179.5±26.2^{a}	156.1±7.2^{ab}	127.1±9.7^{bc}	119.9±8.3^{c}	128.8±33.4^{bc}	116.5±16.00^{c}
	IMP	3766.0±147.1^{bc}	5254.7±246.1^{a}	4465.6±350.2^{b}	2722.5±383.9^{d}	3756.0±915.1^{bc}	3196.1±576.3^{cd}
	ADP	451.3±106.6^{ab}	453.9±12.5^{ab}	452.5±41.3^{ab}	480.8±40.1^{a}	481.0±76.6^{a}	376.5±20.8^{b}
	AMP	114.7±8.7^{b}	91.5±4.1^{c}	109.9±10.9^{b}	130.9±9.6^{a}	123.3±12.1^{ab}	119.5±5.2^{ab}
	Inosine	1058.5±46.8^{c}	811.1±130.2^{c}	1045.6±197.1^{c}	1885.4±212.0^{a}	1616.8±226.3^{ab}	1458.2±225.0^{b}
Thigh	GMP	239.0±15.2^{a}	112.0±38.8^{c}	197.7±16.5^{b}	185.3±7.2^{b}	162.3±7.4^{b}	166.5±48.3^{b}
	IMP	1585.9±504.6^{c}	2419.8±427.2^{ab}	2591.2±262.6^{a}	1789.1±351.4^{c}	1973.7±201.2^{bc}	1637.6±314.0^{c}
	ADP	437.4±20.7^{c}	492.1±42.6^{ab}	450.1±31.2^{c}	530.5±48.4^{a}	469.7±60.9^{ab}	444.9±28.3^{c}
	AMP	195.4±10.9^{b}	181.5±9.4^{b}	189.8±13.8^{b}	228.4±16.1^{a}	186.8±25.9^{b}	200.4±21.8^{b}
	Inosine	764.4±106.6^{b}	596.3±41.5^{c}	594.6±64.0^{c}	884.7±155.4^{ab}	991.4±51.4^{a}	1018.9±55.5^{a}

$^{a-d}$ Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

3.4.5 Textural properties

Shear force values of KC thigh meat increased with an extended rearing period ($P < 0.05$, Figure 3.3). An increase in shear force values corresponded to an increased collagen content (Figure 3.4a). Thigh meat showed higher shear force values than breast meat. Moreover, breast meat of 10-week-old KC exhibited higher shear force values than those of CB ($P < 0.05$), resulting in a tougher and chewier texture. Moreover, crosslinking of collagen increases with age (Fletcher, 2002), which would likely occur to a greater extent in KC at 10 weeks old than CB. A softer texture in CB was correlated with a lower insoluble collagen content (Figure 3.4b). Our results demonstrated that the texture of the hybrid chicken, KC, was distinctively different from broilers, which was attributed to the different collagen content between these two strains.

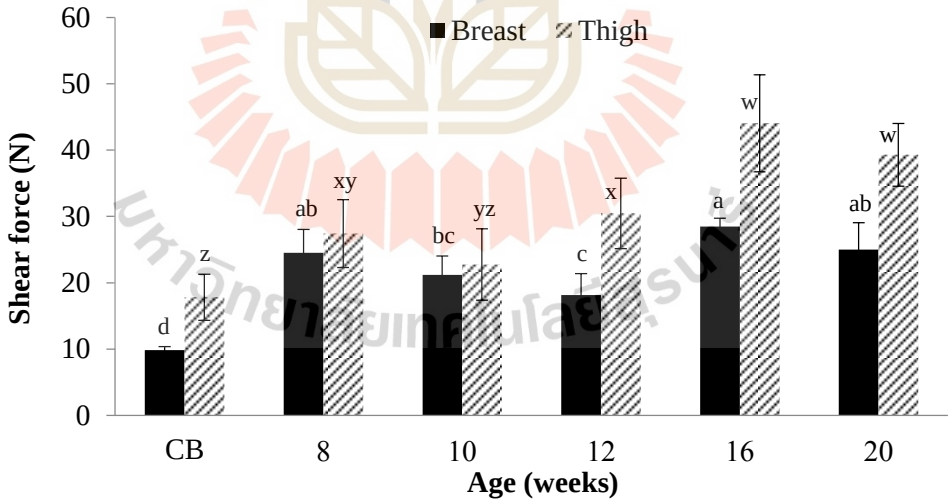


Figure 3.3 Shear force values of breast and thigh meat from Korat chicken of different ages. Means with different superscripts differ significantly ($P < 0.05$) as $^{a-d}$ within breast, $^{w-z}$ within thigh.

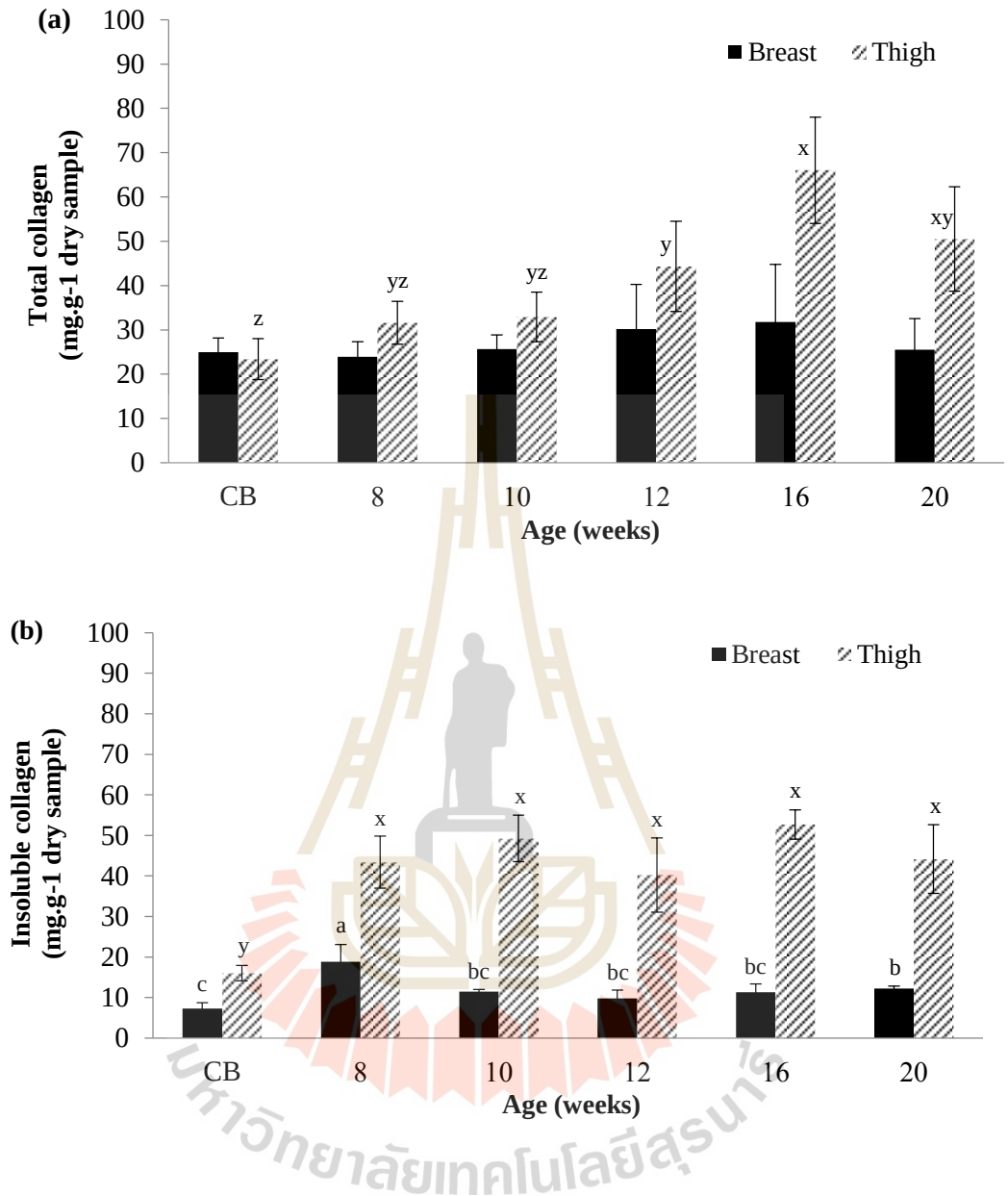


Figure 3.4 Total collagen (a) and insoluble collagen (b) content in breast and thigh meat from Korat chickens of different ages. Means with different superscripts differ significantly ($P < 0.05$) as $^{a-c}$ within breast, $^{x-y}$ within thigh.

3.4.6 Raman spectroscopy

KC meat at various ages and CB meat showed distinct Raman spectra (Figure 3.5). The normalised intensity of a Raman band at 3200 cm^{-1} indicated that water OH stretching was higher in CB breast meat (Table 3.5). In contrast, OH stretching of KC thigh meat appeared to be higher than its CB counterpart. These changes were correlated with moisture content (Table 3.1). KC meat showed a decrease in Raman intensity of C-H stretching at 2935 cm^{-1} and CH_2 bending at 1450 cm^{-1} as the rearing period was extended. The Raman intensity at 1320 cm^{-1} from C-H bending of KC thigh meat also decreased with increasing age. These changes indicated increased hydrophobic interactions via aliphatic residues in thigh meat with respect to rearing period. In addition, the lowest Tyr doublet ratio (I_{857}/I_{827}) observed in thigh meat of 20 weeks old KC indicated an increase in buried Tyr residues within the protein network with age. Such changes were less obvious in breast muscle. Both C-H bending and Tyr doublet suggested a greater extent of hydrophobic interactions via aliphatic and Tyr residues, respectively, of thigh muscle proteins with increasing age. In addition, C-C stretching at 935 cm^{-1}, corresponding to an α-helix structure in breast meat, decreased as KCs grew older. Proteins with disulfide bonds show Raman bands near 510, 520, and 540 cm^{-1}, corresponding to the conformation of “gauche-gauche-gauche”, “gauche-gauche-trans” and “trans-gauche-trans”, respectively (Li-Chan, Nakai, and Hirotsuka, 1994). The intensity of S-S stretching at 530 cm^{-1} of “gauche-gauche-trans” conformation was highest in the thigh meat of 20-week-old KC. This might also implied that disulfide cross-linkings of proteins predominantly occurred in the thigh meat of relatively older chicken. Moreover, KC tended to have higher band intensity of disulphide bonds than CB

meat, suggesting higher stability of proteins structure in KC meat (Kang, Li, He, Ma, and Song, 2017).

When secondary structure was analyzed using the amide I spectral profile, α-helical structure (1657-1645 cm^{-1}) decreased with increasing age of KC (Table 3.6). It should be noted that thigh meat exhibited higher α-helical structure than breast meat in both breeds. Beattie et al. (2004) showed a positive correlation between α-helical band and tenderness. In contrast, hydrophobic interactions and β-sheet structure were positively correlated with meat toughness (Beattie et al, 2004). It was evident that the overall structural information of muscle proteins between CB and KC was different.

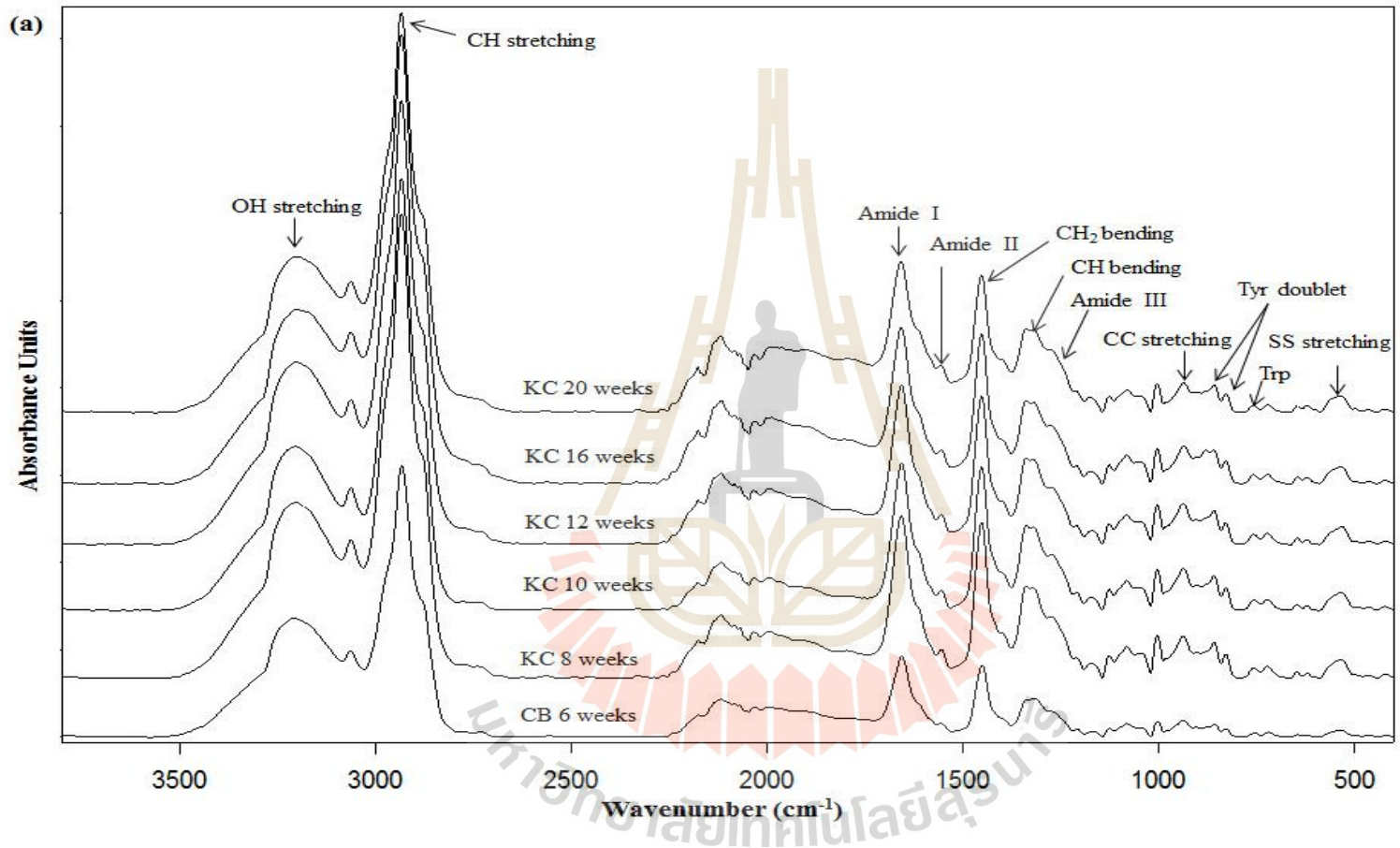


Figure 3.5 Raman spectra of breast (a) and thigh (b) meat from Korat chickens (KCs) and commercial broiler (CB) chickens of different ages at a wavenumber of 3800-400 cm^{-1}.

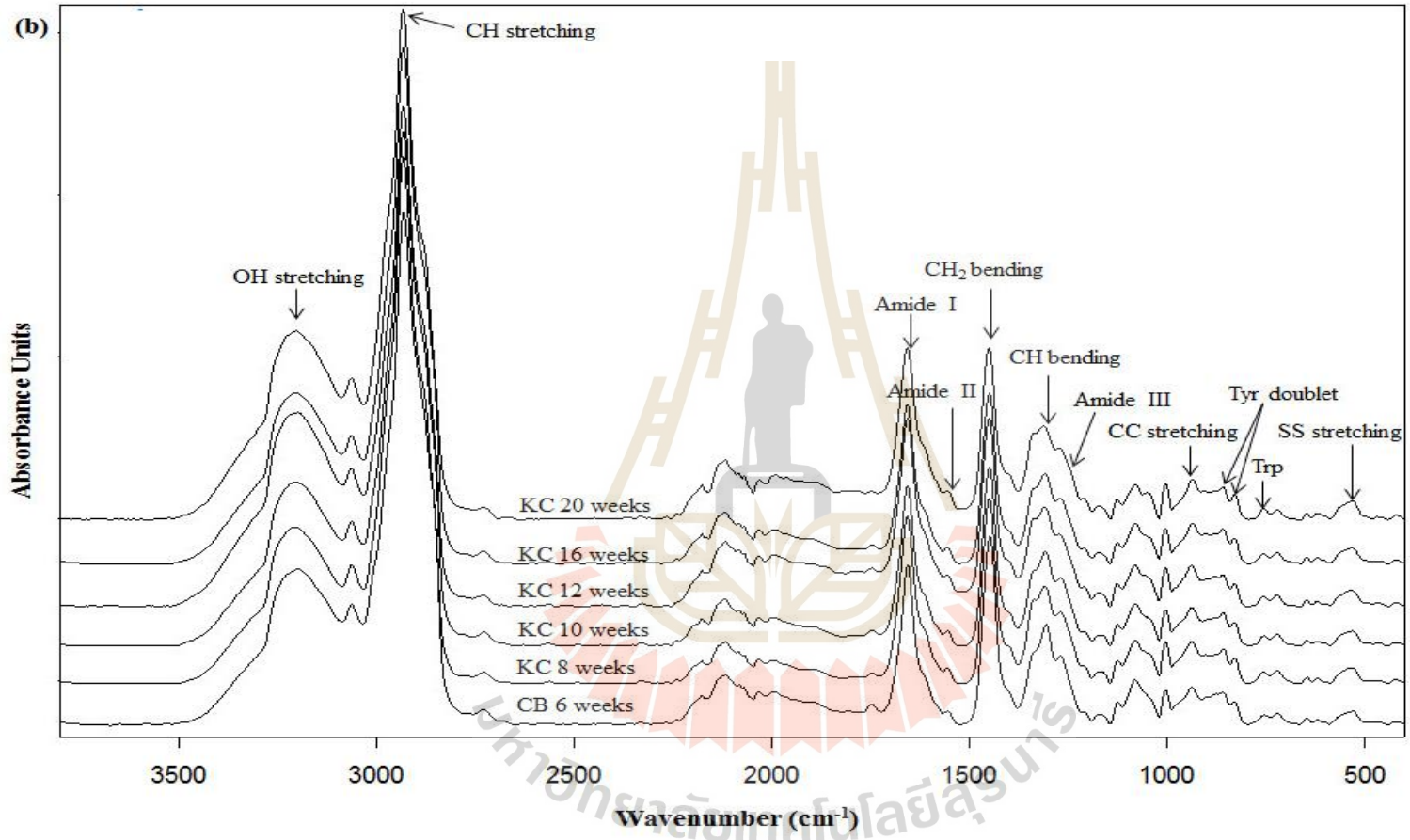


Figure 3.5 Raman spectra of breast (a) and thigh (b) meat from Korat chickens (KCs) and commercial broiler (CB) chickens of different ages at a wavenumber of 3800-400 cm^{-1} (Continued).

Table 3.5 Normalized intensities of Raman bands of Korat chicken breast and thigh meat from different ages (mean ± standard deviation).

Muscle type	Chicken breed	Age (weeks)	Normalized intensities of the Raman bands ($x10^{-3}$)						
			O-H stretching (3200 cm^{-1})	**C-H stretching** (2935 cm^{-1})	**CH_2 bending** (1450 cm^{-1})	**C-H bending** (1320 cm^{-1})	**C-C stretching** (935 cm^{-1})	**Tyr doublet** (I_{857}/I_{827})	**S-S stretching** (530 cm^{-1})
Breast	**CB**	6	0.89 ± 0.03^{a}	3.95 ± 0.03^{ab}	1.07 ± 0.01^{d}	0.07 ± 0.01^{a}	0.14 ± 0.01d	1.11 ± 0.05	0.11 ± 0.01^{b}
	KC	8	0.76 ± 0.00^{b}	3.94 ± 0.06^{ab}	1.36 ± 0.04^{ab}	0.07 ± 0.01^{b}	0.19 ± 0.01^{ab}	1.00 ± 0.04	0.17 ± 0.01^{a}
		10	0.78 ± 0.02^{b}	4.01 ± 0.06^{a}	1.40 ± 0.01^{a}	0.08 ± 0.01^{b}	0.22 ± 0.00^{a}	1.08 ± 0.03	0.18 ± 0.00^{a}
		12	0.79 ± 0.03^{b}	3.82 ± 0.09^{b}	1.32 ± 0.03^{b}	0.07 ± 0.01^{b}	0.20 ± 0.10^{a}	0.99 ± 0.14	0.17 ± 0.00^{a}
		16	0.74 ± 0.05^{b}	3.79 ± 0.10^{b}	1.31 ± 0.02^{b}	0.06 ± 0.00^{b}	0.17 ± 0.01^{bc}	0.88 ± 0.19	0.16 ± 0.01^{a}
		20	0.72 ± 0.04^{b}	3.83 ± 0.06^{b}	1.17 ± 0.04^{c}	0.04 ± 0.00^{b}	0.16 ± 0.02^{cd}	0.91 ± 0.15	0.16 ± 0.01^{a}
Thigh	**CB**	6	0.62 ± 0.04^{b}	4.00 ± 0.04^{ab}	1.58 ± 0.05^{a}	0.26 ± 0.04^{a}	0.13 ± 0.03	0.76 ± 0.04^{ab}	0.10 ± 0.01^{b}
	KC	8	0.65 ± 0.07^{ab}	4.08 ± 0.09^{a}	1.54 ± 0.02^{ab}	0.18 ± 0.01^{b}	0.15 ± 0.01	0.85 ± 0.08^{a}	0.12 ± 0.01^{b}
		10	0.67 ± 0.03^{ab}	3.97 ± 0.03^{ab}	1.49 ± 0.02^{b}	0.20 ± 0.01^{b}	0.14 ± 0.00	0.96 ± 0.08^{a}	0.11 ± 0.00^{b}
		12	0.80 ± 0.04^{a}	3.89 ± 0.07^{b}	1.51 ± 0.02^{b}	0.16 ± 0.01^{ab}	0.17 ± 0.01	0.80 ± 0.07^{a}	0.13 ± 0.01^{b}
		16	0.68 ± 0.03^{ab}	4.01 ± 0.03^{ab}	1.43 ± 0.02^{c}	0.17 ± 0.01^{b}	0.15 ± 0.01	0.87 ± 0.01^{a}	0.12 ± 0.00^{b}
		20	0.70 ± 0.09^{ab}	3.91 ± 0.04^{b}	1.40 ± 0.02^{c}	0.13 ± 0.01^{c}	0.15 ± 0.02	0.59 ± 0.13^{b}	0.16 ± 0.00^{a}

[a-c] Mean values in the same row with different superscripts differ significantly ($P < 0.05$) within the same muscle type.

Table 3.6 Relative content of secondary structures of Korat chicken breast and thigh meat from different ages (mean ± standard deviation).

Muscle type	Chicken breeds	Age (wk)	Relative content (%)			
			α-helix (1657-1645 cm^{-1})	**β-sheet** (1680-1665, 1640-1612 cm^{-1})	**Random coil** (1665-1660 cm^{-1})	**β-turn** (1690-1680 cm^{-1})
Breast	CB	6	46.34±0.78[a]	25.33±1.26	16.79±0.67	11.54±0.24
	KC	8	45.10±0.85[ab]	26.24±0.84	17.01±0.33	11.65±1.08
		10	43.11±1.44[ab]	28.15±1.49	17.05±1.07	11.69±1.25
		12	43.12±0.30[ab]	28.08±1.22	17.03±0.25	11.77±1.58
		16	42.37±1.70[b]	26.89±1.21	17.00±1.01	13.74±2.36
		20	42.17±1.76[b]	27.86±0.72	17.80±0.56	12.17±1.19
Thigh	CB	6	47.46±0.33[a]	26.40±0.04	15.85±0.47	10.30±0.19
	KC	8	47.37±0.42[a]	25.01±0.84	17.27±0.08	10.35±0.61
		10	47.75±0.77[a]	24.89±1.64	16.23±0.44	11.14±1.60
		12	47.53±0.14[a]	25.34±0.79	16.44±0.41	10.70±0.71
		16	46.19±0.55[ab]	26.02±1.32	16.99±0.14	10.80±0.84
		20	44.91±1.23[c]	26.37±1.03	17.19±1.15	11.53±1.06

[a-b] Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type.

The two principle components (PC) explained about 57% and 53% of the total variability of all data obtained from breast and thigh meat, respectively. In breast meat, the first PC discriminated KC and CB, while different age groups of KC were separated on the second PC (Figure 3.6A). Raman bands with high correlation loading are displayed in Table 3.7 (Beattie et al., 2004; Beattie, Bell, Borggaard, and Moss, 2008; Fowler, Ponnampalam, Schmidt, Wynn, and Hopkin, 2015; Herrero, 2008a; 2008b; Herrero, Hernandez, Jimenez-Colmenero, and Perez, 2017; Li-Chan, 1996; Li-Chan et al., 1994; Ngarize, Herman, Adams, and Howell, 2004; Phongpa-Ngan, Aggrey, Mulligan, and Wicker, 2014). Shear force values and Raman spectra at 1670 cm^{-1} and 1612 cm^{-1} (amide I of β-sheet structure), 1558 cm^{-1} (amide II), and 1277 cm^{-1} (amide III of collagen) were characteristics of older KC breast meat, especially KC at 20 weeks old. Based on the Pearson correlation coefficient, the shear force value of KC breast meat was negatively correlated with α-helix structure and O-H stretching at 3207 cm^{-1} with coefficient values of -0.88 ($P < 0.01$). In contrast, CB breast meat showed high moisture content, corresponding to O-H stretching at 3207 cm^{-1}, with a positive correlation ($P < 0.01$). In addition, CB breast meat exhibited distinct lipid component characteristics, including cholesterol, total lipid, SFA, MUFA, and PUFA, corresponding to 3068 cm^{-1} (CH=CH stretching of unsaturated fatty acids) and C-H stretching of fatty acids (2895, 2914 cm^{-1}, Figures 3.6A, B). Moreover, the second PC revealed that the KC breast meat of 8-10 week olds was highly correlated with the C-C stretching of the α-helix structure (959 cm^{-1}, Figures 3.6A, B), suggesting predominant α-helices in young KC birds.

In thigh meat, the first PC discriminated various ages of KC and CB (Figure 3.6C). Younger KC thigh meat (8-10 weeks old) had quality characteristics

similar to CB thigh meat. High shear force as well as high intensity at 1672, 1639 cm^{-1} (amide I of β-sheet structure), 1240 cm^{-1} (amide III of β-sheet and random coil structure) were distinct features of KC at 20 weeks old. CB thigh meat exhibited a higher α-helix structure, pH and fatty acids (SFA, MUFA, PUFA) and high intensity of Raman bands at 3011, 2895, 2854 cm^{-1} (C-H stretching), and 974 cm^{-1} (C-C stretching) (Figure 3.6D). The high intensity of the Raman spectra at regions of C-H stretching appeared to correlate with the high fatty acid content in CB thigh meat. The second PC highlighted that KC thigh meat at 8-10 weeks old showed distinct IMP content characteristics. Raman spectroscopy was shown to be a powerful technique and provided insightful information on muscle protein structure between these two breeds.

Table 3.7 Raman bands assignment of observed spectral of chicken meat.

Wavenumber (cm^{-1})	**Vibrational mode**
3207	O-H stretching
3011, 2914, 2895, 2854	C-H stretching
3068	CH=CH stretching of unsaturated fatty acids
1657-1645	Amide I (a-helix)
1680-1665, 1640-1610	Amide I (β-sheet)
1665-1660	Amide I (random coil)
1690-1680	Amide I (β-turn)
1558	Amide II
1277	Amide III (collagen)
1240	Amide III (β-sheet and random coil)
974	C-C stretching
959	C-C stretching (α-helix)

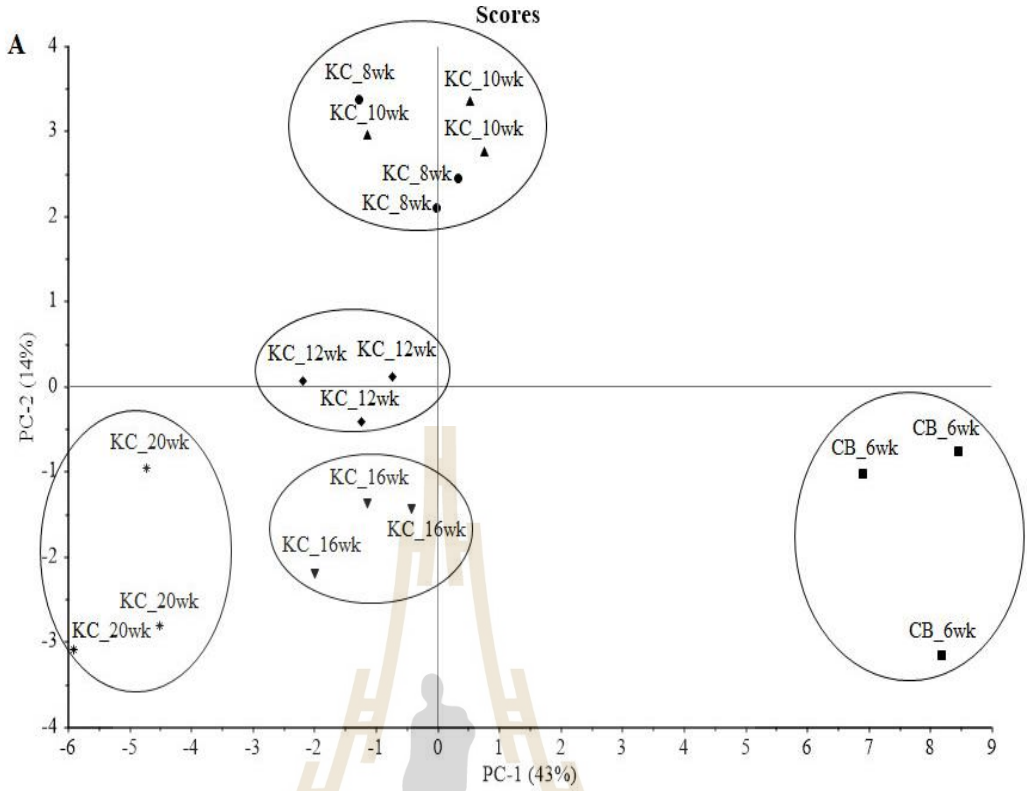


Figure 3.6 PC score plot and correlation loading plot (PC-1 vs PC-2) of Raman spectra and physico-chemical properties of breast (A and B) and thigh (C and D) meat from KCs of different ages. CB; commercial broiler chicken (6 weeks old), KC; Korat crossbreed chicken (8, 10, 12, 16, 20 weeks old). WHC; water holding capacity, SFA; saturated fatty acid, MUFA; monounsaturated fatty acid; PUFA; polyunsaturated fatty acid, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate.

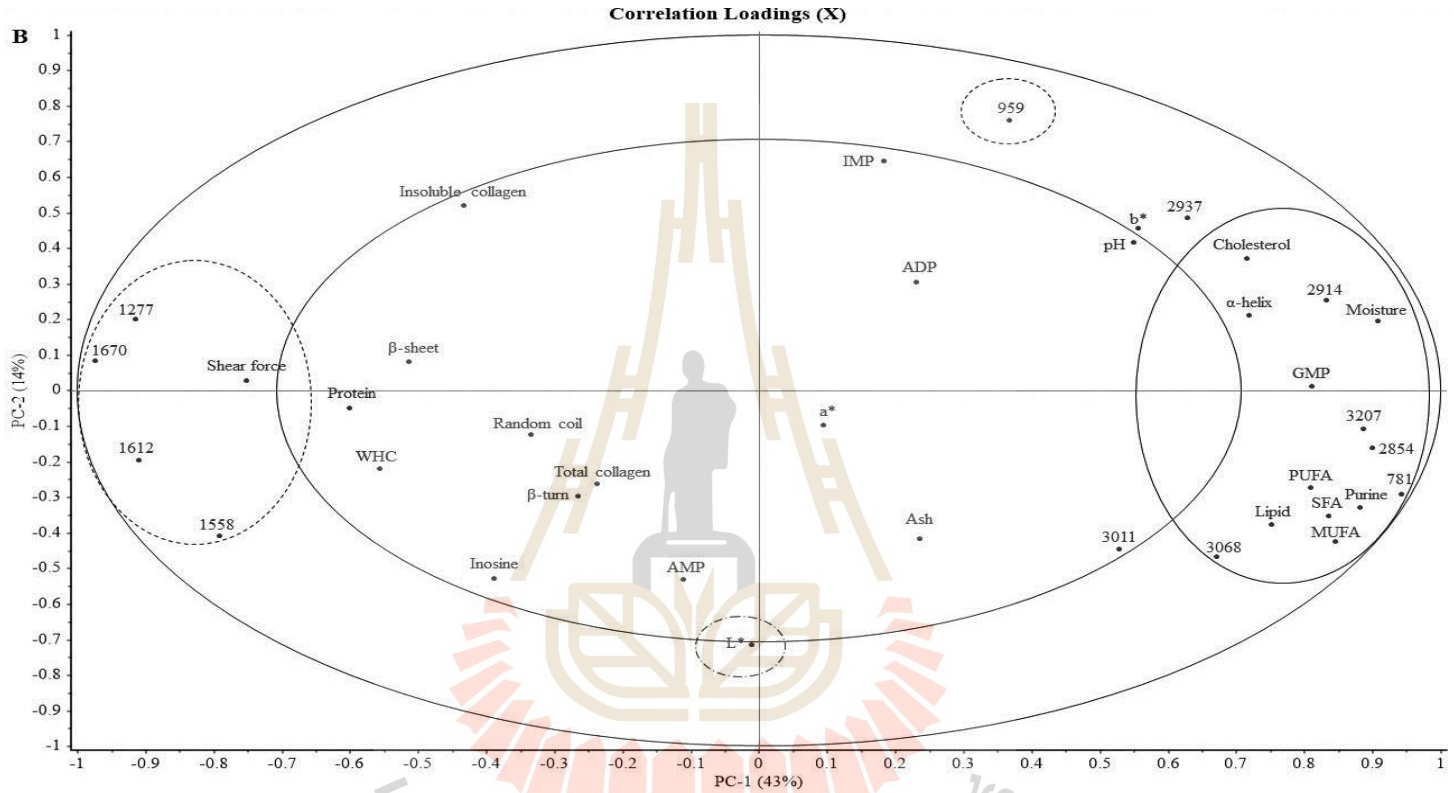
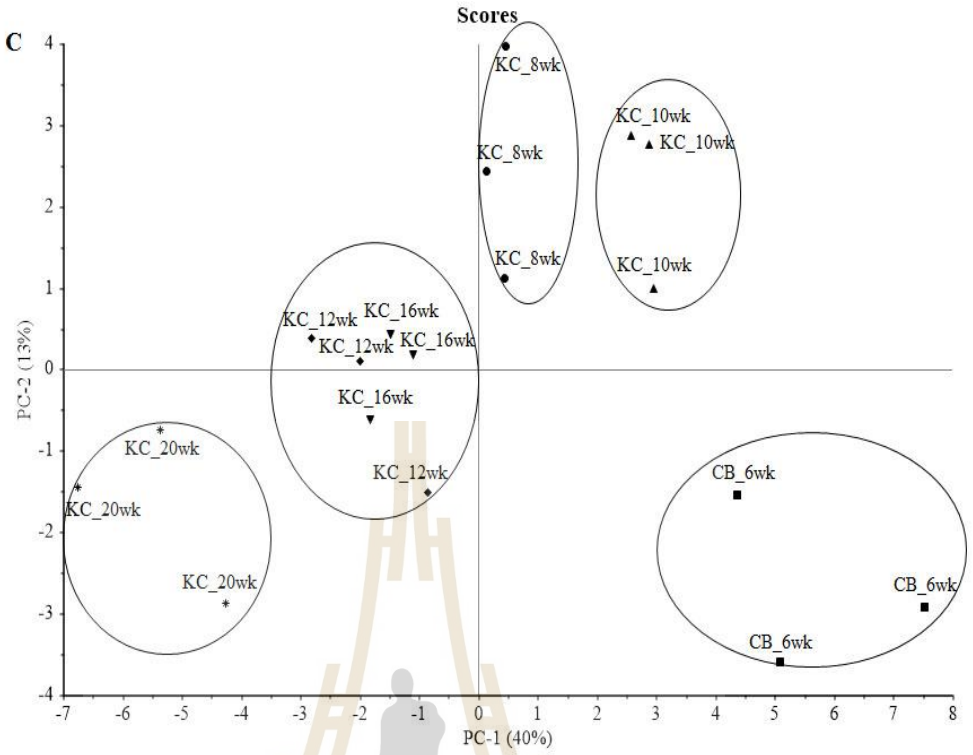


Figure 3.6 PC score plot and correlation loading plot (PC-1 vs PC-2) of Raman spectra and physico-chemical properties of breast (A and B) and thigh (C and D) meat from KCs of different ages. CB; commercial broiler chicken (6 weeks old), KC; Korat crossbreed chicken (8, 10, 12, 16, 20 weeks old). WHC; water holding capacity, SFA; saturated fatty acid, MUFA; monounsaturated fatty acid; PUFA; polyunsaturated fatty acid, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate (Continued).



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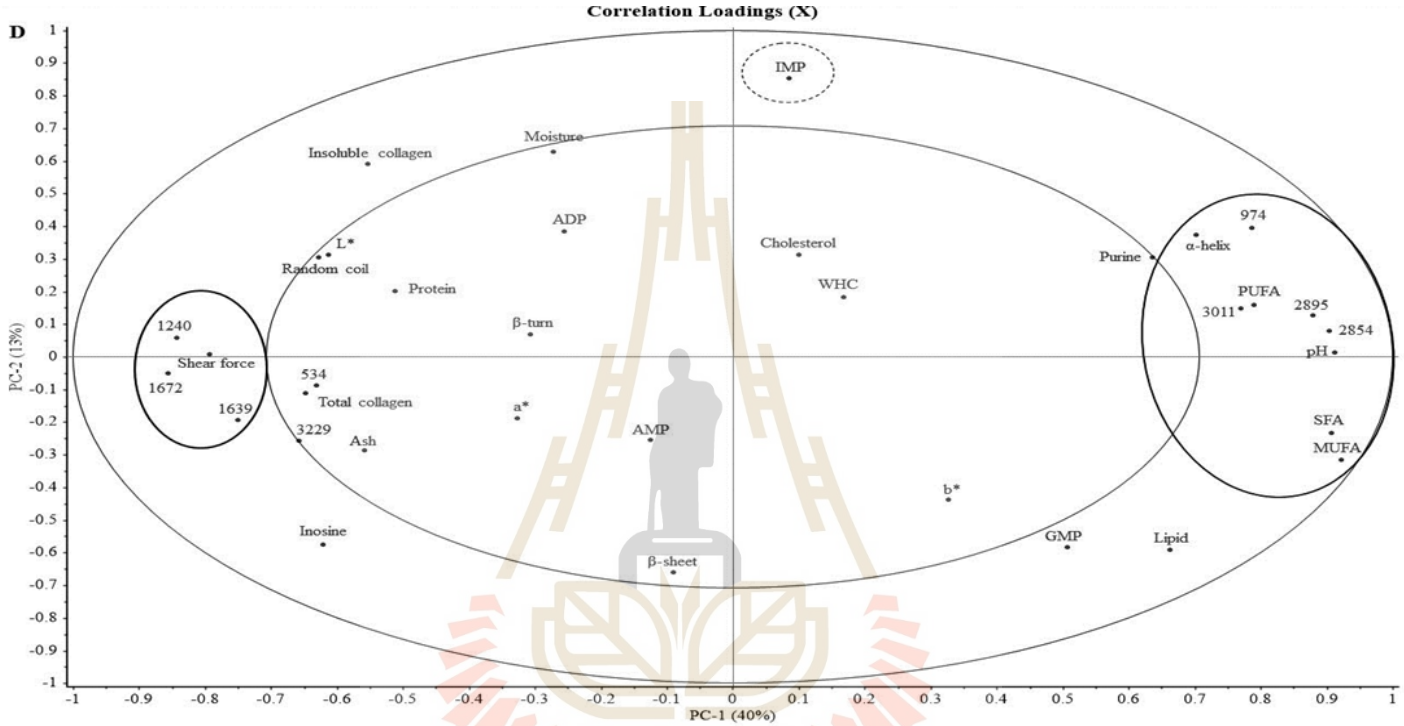

Figure 3.6 PC score plot and correlation loading plot (PC-1 vs PC-2) of Raman spectra and physico-chemical properties of breast (A and B) and thigh (C and D) meat from KCs of different ages. CB; commercial broiler chicken (6 weeks old), KC; Korat crossbreed chicken (8, 10, 12, 16, 20 weeks old). WHC; water holding capacity, SFA; saturated fatty acid, MUFA; monounsaturated fatty acid; PUFA; polyunsaturated fatty acid, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate (Continued).

3.5 Conclusions

In conclusion, KC meat exhibited distinct characteristics at varied rearing periods. The α-helical structure decreased with KC age, while the extent of hydrophobic interactions and disulphide bonds increased. KC meat showed higher shear force values and higher β-sheet structure content than CB meat. Based on PCA, KC meat quality was clearly distinguished from CB meat. Shear force values of older KC meat (16-20 weeks old) were well correlated with changes in the β-sheet structure of amide I regions as well as amide III of collagen. In addition, the shear force of KC breast meat was negatively correlated with the Raman spectra of OH stretching at 3207 cm^{-1} and relative α-helical content. CB meat exhibited a high content of α-helix structure and fatty acids, correlating with Raman C-H stretching. This is the first report demonstrating the potential of Raman spectroscopy to differentiate qualities of KC chicken meat at different ages.

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CHAPTER IV

A COMPARATIVE STUDY ON MEAT QUALITY AND VIBRATIONAL SPECTROSCOPIC PROPERTIES OF DIFFERENT CHICKEN BREEDS

4.1 Abstract

Quality of breast meat from commercial broiler (CB), Thai native chicken Leung Hang Khao (NC), and the crossbred called Korat chicken (KC), was investigated. KC and NC meat showed higher protein than CB meat ($P < 0.05$). Water holding capacity (WHC) was highest in KC meat ($P < 0.05$). The highest lightness (L*) was found in CB meat. The CB meat also contained higher moisture and lipid content as well as nucleotides, inosine 5'-monophosphate (IMP), gaunosine 5'-monophosphate (GMP), and adenosine monophosphate (AMP) ($P < 0.05$). NC meat contained the highest inosine and insoluble collagen content ($P < 0.05$). KC and NC meat showed higher carbonyl content than CB meat. Based on principal component analysis (PCA) of spectra obtained from SR-FTIR, FT-Raman, and physico-chemical properties, chicken meat was clearly separated by breeds. High moisture, lipid and nucleotides content corresponding to distinct wavenumbers obtained from FT-Raman spectra of O-H stretching of water (3203 cm^{-1}), C-H stretching of lipid (2854 cm^{-1}) and SR-FTIR spectra of PO_2^- stretching of nucleic acids (1240 cm^{-1}), respectively. Different chicken breeds have varied secondary

structural proteins in muscle, predominant of β-turn and random coil were found in KC and NC meat, while mainly α-helix was found in CB meat.

Keywords: meat quality, chicken breeds, broiler, Thai native chicken, Korat crossbred chicken, Synchrotron Radiation-Fourier transform infrared spectroscopy, Fourier-transform Raman spectroscopy

4.2 Introduction

Chicken meat consumption has been increased as it is deemed as healthy white meat with low fat and high protein (Choe et al., 2010). Fast growing broiler strains are mainly used to produce commercial chicken meat with 5-6 weeks rearing period (Choe, Lee, and Jo, 2009). Native chicken meat has gained consumer acceptance, particularly in Asian countries, due to its unique taste and texture as well as perception of healthier than commercial broiler (Wattanachant, Benjakul, and Ledward, 2004). However, native chicken meat has not been produced in sufficient numbers to meet the consumer demand because of their lower growth rate, poor feed efficiency, and lower lean-muscle gaining ability (Jeon et al., 2010). Consequently, the crossbred chickens have been developed for genetic improvement in better production capacity. Physico-chemical properties of meat greatly vary with breeds. Variations in level of glycogen and genetic control of glycolytic potential in the muscle of different breeds determine the rate of pH decline after slaughter and the ultimate pH (Le Bihan-Duval et al., 2008). Meat color is also different among poultry breeds. Typically, slow-growing chickens have a redder meat color than fast-growing chicken because they are older with higher myoglobin (Lonergan, Deeb,

Fedler, and Lamont, 2003). Different in taste and texture of chicken meat were noted between breeds. Native and crossbred chicken meat samples from Korea, Japan, and Thailand, were deemed to be more tasty and chewier texture compared to broilers (Jayasena et al., 2015; Rikimaru and Takahashi, 2010; Wattanachant et al., 2004). The umami taste mainly depends on level of taste-active nucleotides, namely inosine monophosphate (IMP) and guanosine monophosphate (GMP) in muscle (Yamaguchi and Ninomiya, 2000). Moreover, genetics influences on muscle growth rate, resulting in different myofiber characteristics that play an important role in the shear force value of meat (Chen, Ma, Tang, and Ji, 2007). Currently, protein oxidation of meat has gained interest due to its influences on meat quality and human nutrition. Protein oxidation in meat impairs the protein structure, affecting denaturation and functionality, including tenderness, water-holding capacity (WHC), juiciness of meat, and susceptibility to proteolysis (Bhattacharya, Kandeepan, and Vishnuraj, 2016; Zhang, Xiao, and Ahn., 2013). The extent of protein oxidation is affected by reactive oxygen species during meat maturation, which is likely to be varied with breeds. Leung Hang Khao is one of indigenous chickens in Thailand, whose scientific information on meat quality is limited. Korat chicken is a crossbred between Leung Hang Khao sires and SUT 101 chicken dams (a crossbreed between broiler and layer chicken, which is developed by Suranaree University of Technology and showed improved growth performance as compared to native chicken (Maliwan, Khempaka, and Molee, 2017). However, meat quality of Korat chicken has not been systematically studied. Elucidation of meat quality of Leung Hang Khao and Korat crossbred chicken in comparison with commercial broilers would lead to valorization of native and crossbred chicken.

Vibrational spectroscopy including Fourier transform infrared (FT-IR) and Raman (FT-Raman) spectroscopy has been used to identify or predict quality of meat and meat products (Argyri et al., 2013; Papadopoulou, Panagou, Tassou, and Nychas, 2011). These measurements are performed in direct contact with meat samples and provide structural conformation changes at the molecular level within intact cells. IR and Raman spectroscopy are complementary techniques. Polar groups occur as strong IR stretching bands, while non-polar groups are intense bands in Raman. IR spectra represent the transmitted, reflected or dispersed radiation which was originated from changes in molecular dipoles associated with vibrations and rotations. In addition, Raman spectra originate from inelastic scattering of the incident light and depend on changes in the polarizability of functional groups when atoms vibrate (Abbas, Dardenne, and Baeten, 2012). Synchrotron radiation (SR) is an electromagnetic wave that is emitted from relativistic charged particles i.e. photon or electron moving near a speed of light under acceleration. It takes advantage of extremely intense and the beam can focus to a spot with a diameter ≤ 10 μm, making it capable of exploring within the microstructures of biological tissues (Miller and Dumas, 2006). Synchrotron-based Fourier transform infrared spectroscopy (SR-FTIR) provides higher accuracy and precision with low signal to noise ratio as compared to a benchtop (globar) source FTIR (Marinkovic and Chance, 2006). The use of IR and Raman spectroscopy in combination with chemometrics such as principle component analysis (PCA) has been successfully developed to differentiate quality of meat and meat products (Perisic, Afseth, Ofstad, and Kohler, 2011). Correlation between wavenumbers of vibrational spectra and meat quality traits among chicken breeds would be used as an indicator for determine quality of different chicken breeds.

Objectives of this study was to evaluate meat quality of Korat crossbred chicken (KC) and Leung Hang Khao (Thai native chicken; NC) in comparison with commercial broiler (CB), based on physico-chemical properties and SR-FTIR and FT-Raman spectroscopy. Correlations of spectroscopic data and meat quality were established to investigate the specific wavenumber for characterization the quality of each chicken breeds and use the vibrational information for further implication of vibrational spectroscopy as a rapid and nondestructive techniques for determine chicken meat quality.

4.3 Materials and methods

4.3.1 Animals and sample preparation

A total of 120 of each genotype, 1-day-old mixed sex KC and NC were randomly distributed into pen (40 chicks/pen/5 m^2; 3 replications) in indoor facility and raised under the same condition at Suranaree University of Technology Farm (Nakhon Ratchasima, Thailand). Birds were fed ad libitum with the same commercial diet for starter (0-4 weeks old), grower (5-6 weeks old), and finisher (7-16 weeks old) containing 21, 19, and 17% crude protein, respectively. Birds had free access to water and no access to the outdoor environment. When animals reached the market age at 10 weeks old for KC and 16 weeks old for NC, thirty six male (12 chicks/pen) of each breed were randomly selected and subject to a total feed withdrawal of 12-15 h, weighted (KC; 1.40-1.82 kg and NC; 1.40-2.04 kg) and slaughtered in a commercial slaughterhouse (Nakhon Ratchasima, Thailand). Birds were processed under commercial conditions using electrocution as the stunning system, conventional neck cut, bled, scalded, plucked, and eviscerated. Then, the carcasses were packed in

ice box and brought to the laboratory within 1 h. The breast meat samples were collected after 24 h postmortem in a 4 °C chiller. Skin, bone, visible connective tissue and fat were removed. Breast meat samples of male commercial broiler (CB) at 6 weeks old with live weight of 2.9-3.0 kg were obtained from commercial chicken meat processing company (Charoen Pokphand Foods (Thailand) Public Company Limited, Nakhon Ratchasima, Thailand). A 24-h postmortem pH was determined. WHC was measured within 24 h. Color of meat was determined within 48 h. Samples were also allocated for subsequent SR-FTIR and FT-Raman spectroscopy measurement. The remaining samples were minced, vacuum-packed, and stored at -80 °C until subsequent further analysis. Before analysis, frozen samples were thawed in a refrigerator at 4°C for 12-18 h.

4.3.2 Proximate composition and physico-chemical properties

Moisture content, crude protein and ash were determined according to AOAC (2010). Moisture content was measured by drying the samples (2 g) at 105 °C. Crude protein content was measured by the Kjeldahl method. Ash was measured by heating the sample (2 g) in a furnace at 550 °C. The chloroform/methanol (1:2 v/v) extraction method described by Folch, Lees, and Sloane-Stanley (1957) was used to determine total lipid content.

At 24 h post mortem, pH values were measured according to Wattanachant et al. (2004) using a pH meter (MP220, Mettler-Toledo, Schwerzenbach, Switzerland). Approximately 1 g of minced meat was homogenized in 5 ml of distilled water for 30 s in an ultraturrax homogenizer (Ultra turrax T25, Ika, WerkeGmbh & Co., Staufen, Germany). The mean values of 3 measurements from each sample were presented.

Color of breast meat was measured using a colorimeter (Hunter Associates Laboratory, Reston, VA., USA), which was standardized using a light trap (black hole) and white tiles. Color for each sample was expressed in terms of Commission international del'Eclairage values for lightness (L*), redness (a*), and yellowness (b*), using a light source of D65 (daylight, 65° light angle). Three measurements taken from different locations on the meat surface were conducted.

Water holding capacity (WHC) was measured using a modification of the method used by Ryoichi, Degychi, and Nagata (1993). Briefly, 2 g sample of breast meat was placed in a filter paper (No. 4, Whatman International Ltd., Maidstone England) and centrifuged at 6,710 × g, 25 °C for 10 min. WHC was calculated as a percentage of weight of absorbed moisture in the filter paper to moisture content of the original meat sample.

4.3.3 Nucleotides

Nucleotide content of meat samples was measured according to Kim, Ku, Joo, Lee, and Jang (2012) with slight modifications. After thawing, samples (5 g) were homogenized with 50 ml of 7.5% cold perchloric acid in an ultraturrax homogenizer (Ultra turrax T25, Ika, WerkeGmbh & Co., Staufen, Germany), centrifuged at 2,000 × g for 5 min at 4 °C, and the supernatant was collected. The extract was then mixed with 0.6 M neutralizing buffer (pH 7.6; KH_2PO_4+K_2HPO_4). After 10 min, the supernatant was filtered through a 0.45-µm nylon filter and analyzed using HPLC (HP 1260, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a C18 reverse-phase column (Hypersil™ ODS, 4.6 × 150 mm, 3 µm particles) (Thermo Scientific, Waltham, MA, USA).

The injection volume was 10 µl and elution time was 30 min using mobile phase A (150 mM KH_2PO_4 and 150 mM KCl, pH 6) and mobile phase B (mobile phase A mixed with 20% acetonitrile) at a flow rate of 0.5 ml/min. A gradient started with 97% A for 5 min, reduced to 91% A for 5 min, 80% A for 10 min, and finally 100% B for 10 min. The column temperature was maintained at 25 °C, and detection was monitored at a wavelength of 254 nm. Quantity of inosine 5'- monophosphate (IMP), guanosine monophosphate (GMP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine, and hypoxanthine were calculated using the external standards (Sigma–Aldrich Co., St. Louis, MO, USA).

4.3.4 Collagen content

Total collagen content was determined by alkaline hydrolysis as described by Reddy and Enwemeka (1996). Samples were hydrolyzed with 7 M sodium hydroxide (NaOH), then heated at 120 °C for 40 min. The hydrolysate was neutralized with 3.5 M sulfuric acid (H_2SO_4), filtered, reacted with chloramine T solution and Ehrlich's reagent. Absorbance was measured at 550 nm using a spectrophotometer. The amount of hydroxyproline was determined and total collagen content was calculated using a factor of 7.25 (Palka and Daun, 1999).

Insoluble collagen content was determined according to the method of Liu, Nishimura, and Takahashi (1996). Meat samples were homogenized with 25% Ringer's solution. Homogenates were heated at 77 °C for 70 min in a water bath and centrifuged at 2,300 × g at 4 °C for 30 min. The extraction was repeated twice and residues were dried overnight at 105 °C. The insoluble collagen content of residues was determined and calculated as described above.

4.3.5 Protein carbonyl

Protein oxidation as measured by total carbonyl content, was evaluated using 2, 4-dinitrophenylhydrazine (DNPH) according to Mercier, Gatellier, and Renerre (2004) with slight modifications. The raw meat was thawed, minced and homogenized 1:10 (w/v) in 20 mM phosphate buffer (pH 6.5) using an ultraturrax homogenizer (Ultra turrax T25, Ika, WerkeGmbh & Co., Staufen, Germany) for 30 s. The homogenates were precipitated with 0.5 ml of 20% trichloroacetic acid (TCA). The precipitate was collected to determine protein carbonyl compounds by react with the DNPH to form a 2, 4-dinitrophenyl hydrazone. The precipitate of DNP hydrazones was washed with 5 ml of 1:1 ethanol:ethyl acetate (v/v) until clear supernatant was obtained. Then, it was dried under flushing N2 gas and dissolved in 2 ml of 20 mM sodium phosphate buffer (pH 6.5) containing 6 M guanidine hydrochloride. The absorbance was measured at 370 nm and expressed as nmol of carbonyl per mg of protein using the adsorption coefficient for the protein hydrazones (21.0 mM^{-1} cm^{-1}). Protein concentration was analyzed in a control sample (without added DNPH) spectrophotometrically at 595 nm using bovine serum albumun (BSA) as standard.

4.3.6 Vibrational spectroscopic measurement

4.3.6.1 Synchrotron Radiation Fourier transform infrared (SR-FTIR)

The experiment was performed at the Synchrotron Light Research Institute (SLRI) (Nakhon Ratchasima, Thailand). Spectra were collected at room temperature using a FT-IR (Hyperion 2000, Bruker Opics, Ettlingen, Germany) coupled with an Infrared microscopy 15x objective equipped with MCT

D315 detector cooled with liquid nitrogen at an infrared microspectroscopy of beamline (BL4.1 Infrared Spectroscopy and Imaging). Synchrotron radiation was collected from the BM4 of the 1.2 GeV storage ring at the SLRI to use for infrared radiation source. Samples were embedded in optimal cutting temperature (OCT) compound and then snap-frozen in liquid N_2. The samples were stored at -80 °C until cryo-sectioning to 6-µm sections by a cryo-microtome (Microm HM525; Thermo Fisher Scientific, Walldorf, Germany) and put on barium fluoride (BaF_2) windows for SR-FTIR microspectroscopy in a transmission mode. Measurement was performed in the mapping mode over the wavenumber range of 4000–800 cm^{-1}, using an aperture size 10 × 10 µm with a spectral resolution of 4 cm^{-1} and 64 scans. Spectral acquisition and instrument control were performed using OPUS Software 7.2 (Bruker Opics Ltd., Ettlingen, Germany).

4.3.6.2 Fourier transform Raman (FT-Raman)

Thawed minced samples were equilibrated to room temperature before Raman spectroscopy measurement. Raman spectra were collected on a Bruker Vertex 70 FT-Raman spectrometer (Bruker, Karlsruhe, Germany) over the range number 4000-400 cm^{-1} at spectral resolution of 4 cm^{-1} and 256 scans. Sulfur was used to calibrate the Raman frequency. A diode-pumped Nd:YAG laser at 1064 nm with an output 500 mW of laser power was used as the excitation source. FT-Raman spectral acquisition and instrument control were performed using OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) software. A total 30 spectra were collected per sample.

4.3.6.3 Spectra processing and chemometric analysis

To further evaluate the changes in amide I, spectra were curve fitted in the 1700-1600 cm^{-1} region, using appropriate Gaussian and Lorentzian functions in OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) to estimate secondary structure. IR and Raman spectra were preprocessed by smoothing, baseline correction, and normalization to enhance resolution of superimposed bands and to minimize problems from unavoidable baseline shifts. PCA of IR and Raman spectra ranging 3801-2704, 1802-899 cm^{-1} and 3801-2704, 1803-399 cm^{-1}, respectively, were analyzed using the unscramble X 10.5 (Camo, Oslo, Norway). Wavenumbers with high loading were selected along with data of secondary structure, and physico-chemical properties for PCA analysis using the Unscrambler software X 10.5.1 (Camo, Oslo, Norway). The weighting method used for PCA analysis was 1/SDev when investigating relationships with other variables.

4.3.7 Statistical analysis

Comparative meat quality of CB, KC, and NC meat was conducted using a completely random design. All analytical experiments were made in triplicate. A one-way ANOVA was used to analyze the effects of chicken breeds on meat quality. Mean comparisons were carried out using Tukey's test. Statistical significance was accepted at $P < 0.05$. All statistical analyses were performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) in order to compare means derived from each chicken breeds within each analysis, and were expressed as mean ± standard deviation (SD).

4.4 Results and discussion

4.4.1 Proximate composition and physico-chemical properties

KC and NC meat contained higher protein content but lower moisture and total lipid content than CB meat ($P < 0.05$, Table 4.1). Ash content of KC meat was lower than that of CB and NC meat ($P < 0.05$). KC and NC meat showed lower pH than CB meat. KC meat also showed the highest WHC ($P < 0.05$). Regarding meat color, KC meat showed the lowest L* ($P < 0.05$), while a* and b* were comparable among all three breeds ($P > 0.05$). Higher protein content of native and crossbred chicken meat as compared to CB has been reported in Korean, Japanese, and Thai chickens (Jung et al., 2014; Rikimaru and Takahashi, 2010; Wattanachant et al., 2004). Higher fat content was also found in fast-growing broilers than the slow-growing birds (Jung et al., 2014; Wattanachant et al., 2004). Fast-growing chickens possessing high muscle development and growth also showed higher intramuscular fat content than slow-growing counterparts (Ismail and Joo, 2017). Lipid biosynthesis appeared to occur in a greater extent in commercial broilers, while local chicken breed showed higher levels of lipid degradation (Zheng et al., 2016). Such physiological differences might explain higher lipid content in broiler chicken meat. KC meat was darker than CB meat, which was likely due to higher amount of myoglobin and heam pigments. KC and NC meat showed lower pH than CB meat, which was in agreement with previous studied which reported that fast-growing birds showed higher the ultimate pH value (Berri et al., 2005; Wattanachant et al., 2004). Meat of fast-growing birds exhibited lower glycogen content compared to slow-growing birds. Conversion of glycogen to lactic acid after death, thus, occurred to a lesser extent (Berri et al., 2005; Talpur et al., 2018). Moreover, Berri et al. (2005)

reported that slow-growing chickens were more active and struggled more intensively on the shackle line, leading to higher acidification of muscle. Our studies showed that meat quality greatly varied with among these 3 breeds.

Table 4.1 Proximate composition and physico-chemical properties of breast meat of three chicken breeds (mean ± standard deviation).

Parameters*	**CB**	**KC**	**NC**
Moisture (%)	76.98 ± 0.45[a]	75.22 ± 0.27[b]	75.10 ± 0.34[b]
Crude protein (%, db)	85.02 ± 3.28[b]	95.61 ± 0.85[a]	96.21 ± 0.91[a]
Crude fat (%, db)	6.08 ± 0.75[a]	2.49 ± 0.64[b]	3.22 ± 0.10[b]
Ash (%, db)	4.94 ± 0.07[a]	4.49 ± 0.06[c]	4.63 ± 0.07[b]
pH	5.92 ± 0.19[a]	5.65 ± 0.04[b]	5.83 ± 0.22[ab]
WHC (%)	61.35 ± 4.77[b]	66.80 ± 2.62[a]	59.50 ± 2.35[b]
L*	63.71 ± 4.64[a]	57.53 ± 4.69[b]	60.81 ± 7.34[ab]
a*	2.56 ± 2.38	2.39 ± 3.11	3.30 ± 2.31
b*	10.90 ± 5.32	11.72 ± 5.89	12.70 ± 4.16

[a-b] Mean value ± standard deviation in the same row with different letters differ significantly ($P < 0.05$). * db; dry basis.

4.4.2 Nucleotides

IMP was a major nucleotide in breast meat of all 3 species. CB meat contained the highest IMP and GMP content, while NC meat showed the lowest ($P < 0.05$, Table 4.2). IMP and GMP are nucletotides contributing to umami. Meat typically contained higher content of IMP than GMP (Yamaguchi and Ninomiya, 2000). IMP was the most abundant nucleotide, while GMP was detected at

lower concentration in meat have been reported in the literature for pork meat (Flores, Armero, Aristoy, and Toldra, 1999), beef meat (Hwang, Ismail, and Joo, 2020) as well as chicken meat (Aliani and Farmer, 2005). 5´-purinemononucleotides including, IMP, GMP, and AMP are umami compounds that contribute to the umami taste, alone or conjugated with monosodium glutamate for synergistic effects (Kawai, Okiyama, and Ueday, 2002). Our result suggests that CB meat could have higher umami compared to others (Table 4.2). The variation of IMP concentration was related to genes that are regulated IMP metabolism (Ma et al., 2015). Compared to ADP and AMP, ATP showed relatively low content in 3 breeds and NC meat contained negligible ATP. AMP content was also found to be the lowest in NC meat. In addition, NC meat contained the highest inosine content. Hypoxanthine was not detected in breast meat of all breeds. Low ATP content in all 3 chicken breeds indicated that ATP was almost completely depleted after slaughter, and being degraded to ADP, AMP, IMP, and other derivatives. The rate of ATP reduction in NC appeared to be highest (Table 4.2). These differences may reflect the effect of stress of pre-slaughter and at slaughter on the rate of post-mortem glycolysis. The slow-growing line KC and NC being more reactive and struggling than the fast-growing line CB hastened the pH drop and depletion of ATP also occurs (Ali, Kang, and Joo, 2008). Variation of pH and fiber type composition among chicken breeds affected different the breakdown of IMP into inosine. IMP is pH-dependent due to residence of weak chemical bonds. Vani, Modi, Kavitha, Sachindra, and Mahendrakar (2006) reported that the degradation rate of IMP at acidic pH was greater than at neutral or alkaline pH. Consequently, lower pH in NC and KC meat (Table 4.1) must be expected to influence IMP degradation through

aging process than CB meat. Moreover, native chickens contain more oxidative fibers (type I fiber) compared to domesticated birds (Jaturasitha, Chaiwang, and Kreuzer, 2017). Activity of 5´-nucleotidase catalyzing degradation of IMP to inosine was higher in type I muscle fiber than in type II muscle fiber in rat skeletal muscle (Tullson and Terjung, 1999). This would explain higher inosine content in KC and NC.

4.4.3 Collagen content

Total collagen contents of 3 chicken breeds were comparable ($P > 0.05$, Table 4.2). NC exhibited the highest insoluble collagen content followed by KC meat and NC meat, respectively ($P < 0.05$). Collagen content influences the texture characteristic of chicken meat. Differences in collagen contents observed in this study could be attributed to breed and age differences. Maturation of crosslinks in collagen increases with age, resulting in higher insoluble collagen content observed in the breast meat 16-week-old NC. CB at 6 weeks of age showed the lowest content. This was in agreement with Intarapichet, Suksombat, and Maikhunthod (2008) who reported that older slow-growing birds exhibit higher insoluble collagen content than the fast-growing birds. However, total collagen content did not show significant differences among the 3 different breeds. However, total collagen content was comparable among 3 breeds. Jaturasitha et al. (2008) demonstrated that shear force of breast meat was positively correlated with collagen content, which gives the toughness to meat.. These results reflect the unique textural properties of each chicken breeds. Besides, the textural characteristic of KC meat was intermediate between NC and CB meat.

4.4.4 Protein carbonyl

Protein oxidation of native and hybrid chicken appeared to be higher than the commercial broiler as evidenced by protein carbonyl content (Table 4.2). The variations in protein carbonylation between chicken breeds can be ascribed to the different in concentration of myoglobin and composition of muscles fiber type. Thai native chicken muscles contain higher proportions of oxidative metabolism (Type I fiber, slow-twitch oxidative) and intermediate metabolism (Type IIA fiber, fast oxidative glycolytic), with lower proportion of glycolytic metabolism type (Type IIB fiber) than muscles of fast-growing chickens (Jaturasitha et al., 2008). Oxidative muscles are more susceptible to oxidation than glycolytic ones, which can be attributed to the differences in myoglobin concentration (Silva et al., 2018). Protein oxidation can be induced by free radicals generated from heame-iron catalyzed reaction. Free radicals then react with certain amino acid residues in the protein structure, leading to protein oxidation (Estévez, 2011). Moreover, lower pH of KC and NC meat resulted in higher concentration of H^+, which would favor the redox cycle of myoglobin and promote its pro-oxidation (Estévez, 2015). Higher protein oxidation in KC and NC also suggested the older birds undergo greater extent of muscular oxidation. (Cui, Kong, and Zhang, 2012; Del vesco et al., 2017). The higher protein oxidations could be responsible for a deterioration of meat quality, such as an increase in toughness (Lund, Heinonen, Baron, and Estévez, 2011), loss of WHC (Delles, Xiong, True, Ao, and Dawson, 2015) as well as impair protein digestibility (Sante-Lhoutellier, Aubry, and Gatellier, 2007).

Table 4.2 Chemical parameters related to meat quality of chicken breast meat from three different breeds (mean ± standard deviation)**.**

Parameters*	**CB**	**KC**	**NC**
Nuclotides (μg/g db)			
IMP	3776.51 ± 131.08[a]	3335.62 ± 391.48[b]	3239.55 ± 211.16[c]
GMP	180.13 ± 26.88[a]	129.50 ± 20.14[b]	101.73 ± 5.37[b]
ATP	93.40 ± 5.58[a]	79.69 ± 12.09[b]	N.D.
ADP	452.20 ± 105.01	509.28 ± 24.83	532.43 ± 19.77
AMP	115.06 ± 8.82[a]	126.79 ± 2.36[b]	99.32 ± 4.28[c]
Inosine	1061.78 ± 51.39[c]	1575.19 ± 82.82[b]	1828.92 ± 79.36[a]
Hypoxanthine	N.D.	N.D.	N.D.
Total collagen (mg/g db)	19.59 ± 2.96	20.19 ± 2.48	24.14 ± 6.91
Insoluble collagen (mg/g db)	8.19 ± 1.42[c]	11.76 ± 1.37[b]	19.25 ± 1.48[a]
Carbonyl content (nmol/mg protein)	3.24 ± 0.51[b]	5.30 ± 1.32[a]	4.58 ± 1.41[ab]

[a-c] Mean value in each bar with different superscript letters differ significantly ($P < 0.05$), *db; dry basis. N.D.; Not detected.

4.4.5 Vibrational spectra and chemometric analysis

Representative SR-FTIR and FT-Raman spectra of meat samples are shown in Figure 4.1a, b, respectively. Distinct IR spectra at 1240 cm^{-1} was observed only in the CB breast meat (Figure 4.1a), corresponding to asymmetric ($\upsilon_{as}PO_2^-$) stretching vibration of nucleic acids. Moreover, the FT-Raman spectrum in range of 650-500 cm^{-1} were observed a shift wavenumber in CB meat with band located at 539 cm^{-1}, while KC and NC meat with bands located at 532 cm^{-1} (Figure 4.1c). Assignments of peaks in IR and Raman spectra follow previous literatures as shown

in Table 4.3 (Barth, 2007; Beattie, Bell, Borggaard, and Moss., 2008; Böcker et al., 2007; Herrero, 2008a, b; Kang, Li, He, Ma, and Song, 2017; Lamyaa, 2013; Li-Chan, Nakai, and Hirotsuka, 1994; Li-Chan and Nakai, 1991; Ngarize et al., 2004; Phongpa-Ngan, Aggrey, Mulligan, and Wicker, 2014; Sinanoglou, Cavouras, Xenogiannopoulos, Proestos, and Zoumpoulakis, 2018; Wong et al., 1991; Yu, 2006). Characteristic peaks of SR-FTIR and Raman spectra of three chicken breeds are summarized in Table 4.4. IR spectra at 1240 cm^{-1} corresponding to asymmetric ($\upsilon_{as}PO_2^-$) stretching vibration of asymmetric stretching vibrations of PO_2^- which is present in both nucleic acids and phospholipids. However, the SR-FTIR spectra of all chicken meats showed weak shoulder of 1745 cm^{-1} (Figure 4.1a), the peak intensity is negligible. Therefore, the absorbance ratio A 1745 cm^{-1}/A 1240 cm^{-1} is below the range of 1.5-2.0. This means that the absorption band at 1240 cm^{-1} in this study is associated with phosphodiester groups in nucleic acids (Wong et al., 1991; Lamyaa, 2013). This asymmetric ($\upsilon_{as}PO_2^-$) stretching vibration of nucleic acids showed the highest intensity in CB meat (Figure 1a). It has been reported that PO_2^- of ATP absorbs near 1230 cm^{-1} (Thoenges and Barth, 2002). Thus, this wavenumber can be used as a marker differentiating CB from the other two breeds. In addition, SR-FTIR spectra showed the highest absorption band at 1543 and 1302 cm^{-1} in CB meat which were assigned to α-helix at amide II and amide III region, respectively. These results also corresponded with the highest the relative content of α-helix as demonstrated in Table 4.5. Moreover, high N-H stretching at 3291 cm^{-1} of SR-FTIR spectra in NC meat was found to coincide with the highest protein content (Table 4.1).

In Raman spectra, CB and KC meat exhibited high intensity at 3203 cm^{-1} (O-H stretching of water), correlating with high moisture contents (Table 4.1).

High intensity at 1451 cm^{-1} (CH_3, CH_2, CH bending), 1126 cm^{-1} (C-C, C-N, C-O stretching), and 934 cm^{-1} (C-C stretching; α-helix) showed a positive relationship with WHC in KC meat (Table 1). Intensities at wavenumbers of 1448, 1031-1124, 936 cm^{-1} were higher for beef loins that had higher juiciness scores (Fowler et al., 2018). Furthermore, this study revealed that the Raman spectra of KC and NC meat exhibited high intensity bands at 1337 cm^{-1} (Tyrosine; Tyr) and 1174 cm^{-1} (Trptophan; Trp), indicating more exposure of aromatic hydrophobic residues of protein structure in KC and NC meat. Additionally, KN and NC meat with band at 532 cm^{-1} of S-S stretching in gauche–gauche-trans conformation, while CB showed band at 539 cm^{-1} of S-S bonds in trans-gauche-trans conformation. This denoted that different stretching or aliphatic chain vibrations of S–S bonds among 3 chicken breeds. The S-S stretching of disulfide bonds conformations was also found to be the highest in KC and NC meat, which would reflect higher disulfide cross-linkings in these 2 breeds. Raman spectra of these bands provide characteristic of a more hydrophobic environment, gauche–gauche-trans disulfide bonds conformations with higher cross-linking contribute to the stability of proteins structure in KC and NC meats than CB meat. Moreover, an increase in hydrophobicity of protein environment correlated with tough meat (Beattie et al., 2004). It should be noted that the protein conformation of KC meat appear to be the same as NC more than CB.

Quantitative estimation of the protein secondary structure obtained from amide I of SR-FTIR spectra revealed that α-helix was the main conformation maintaining the structure of chicken meat proteins. CB meat contained highest relative content of α-helix structure (Table 4.5). The highest of β-turn structure was found in the NC meat. The variation in protein structures influences their meat

quality. Advanced SR-FTIR has been developed mostly for medical research; however, application for meat quality investigation has been limited so far. This is the first study revealed that, with SR-FTIR, the inherent secondary structures of protein amoung chicken breeds can be differentiated at cellular level.

Table 4.3 IR and Raman bands assignment of characteristics bands of three chicken breeds.

Wavenumber (cm^{-1})		Vibrational mode
IR	**Raman**	
3500-3100	3500-3200	O-H, N-H stretching
	3000-2800	C-H stretching
1657-1648	1657-1645	Amide I (α-helix)
1695-1674,1640-1610	1680-1665, 1640-1610	Amide I (β-sheet)
1657-1642	1665-1660	Amide I (random coil)
1686-1662	1690-1680	Amide I (β-turn)
	1337	Tryptophan
1543		Amide II (α-helix)
	1451	CH_3, CH_2, CH bending
1302		Amide III (α-helix)
1240		Asymmetric ($\upsilon_{as}PO_2^-$) stretching
	1174	Tyrosine
	1126	C-C, C-N, C-O stretching
	936, 934	C-C stretching (α-helix)
	650-500	S-S stretching (disulfide bonds)
	539	trans-gauche-trans
	532	gauche-gauche-trans

Table 4.4 Integral area of SR-FTIR bands and normalized intensities of Raman bands of three chicken breeds (mean ± standard deviation).

	Normalized bands ($x10^{-2}$)		
Bands assignment	**CB**	**KC**	**NC**
SR-FTIR bands			
N-H stretching ($3291cm^{-1}$)	0.57 ± 0.02^{b}	0.61 ± 0.02^{b}	0.66 ± 0.01^{a}
Amide II (α-helix) ($1543\ cm^{-1}$)	1.75 ± 0.04^{a}	1.70 ± 0.11^{ab}	1.56 ± 0.03^{b}
Amide III (α-helix) ($1302\ cm^{-1}$)	0.17 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.13 ± 0.01^{b}
Asymmetric PO_2^- stretching ($1240cm^{-1}$)	0.41 ± 0.05^{a}	0.19 ± 0.04^{b}	0.18 ± 0.03^{b}
Raman bands			
O-H stretching ($3205\ cm^{-1}$)	0.84 ± 0.02^{a}	0.83 ± 0.03^{a}	0.65 ± 0.06^{b}
Tryptophan ($1337cm^{-1}$)	0.10 ± 0.01^{b}	0.16 ± 0.01^{a}	0.13 ± 0.02^{a}
CH_3, CH_2, CH bending ($1451\ cm^{-1}$)	1.16 ± 0.06^{b}	1.35 ± 0.08^{a}	1.15 ± 0.07^{b}
Tyrosine ($1174\ cm^{-1}$)	0.06 ± 0.01^{b}	0.08 ± 0.00^{a}	0.07 ± 0.00^{ab}
C-C, C-N, C-O stretching ($1126\ cm^{-1}$)	0.09 ± 0.00^{b}	0.13 ± 0.01^{a}	0.10 ± 0.01^{b}
C-C stretching (α-helix) ($934\ cm^{-1}$)	0.15 ± 0.01^{b}	0.21 ± 0.03^{a}	0.16 ± 0.01^{b}
S-S stretching (disulfide bonds) ($539, 532\ cm^{-1}$)	0.12 ± 0.01^{b}	0.17 ± 0.07^{a}	0.16 ± 0.01^{a}

[a-b] Mean values in the row with different superscripts differ significantly ($P < 0.05$).

Table 4.5 Relative content (%) of protein secondary structures of three chicken breeds obtained from amide I profile of SR-FTIR spectra (mean ± standard deviation).

Protein secondary structures	Relative content (%)		
	CB	KC	NC
SR-FTIR			
α-Helix	45.95±4.45[a]	38.32±1.59[b]	39.13±1.84[b]
β-Sheet	26.85±2.40	26.40±1.54	27.52±3.19
Random coil	12.41±2.04	16.22±1.45	12.83±2.39
β-Turn	14.79±1.85[b]	19.06±1.75[ab]	20.53±2.35[a]
FT-Raman			
α-Helix	44.54±1.41	44.54±1.41	43.53±0.73
β-Sheet	27.81±0.51	27.26±0.07	28.01±0.77
Random coil	16.51±0.57	16.21±1.30	18.28±0.51
β-Turn	11.50±1.25	11.99±1.18	10.19±0.83

[a.b] Mean values with different superscripts in the same column within each vibrational spectroscopy technique differ significantly ($P < 0.05$).

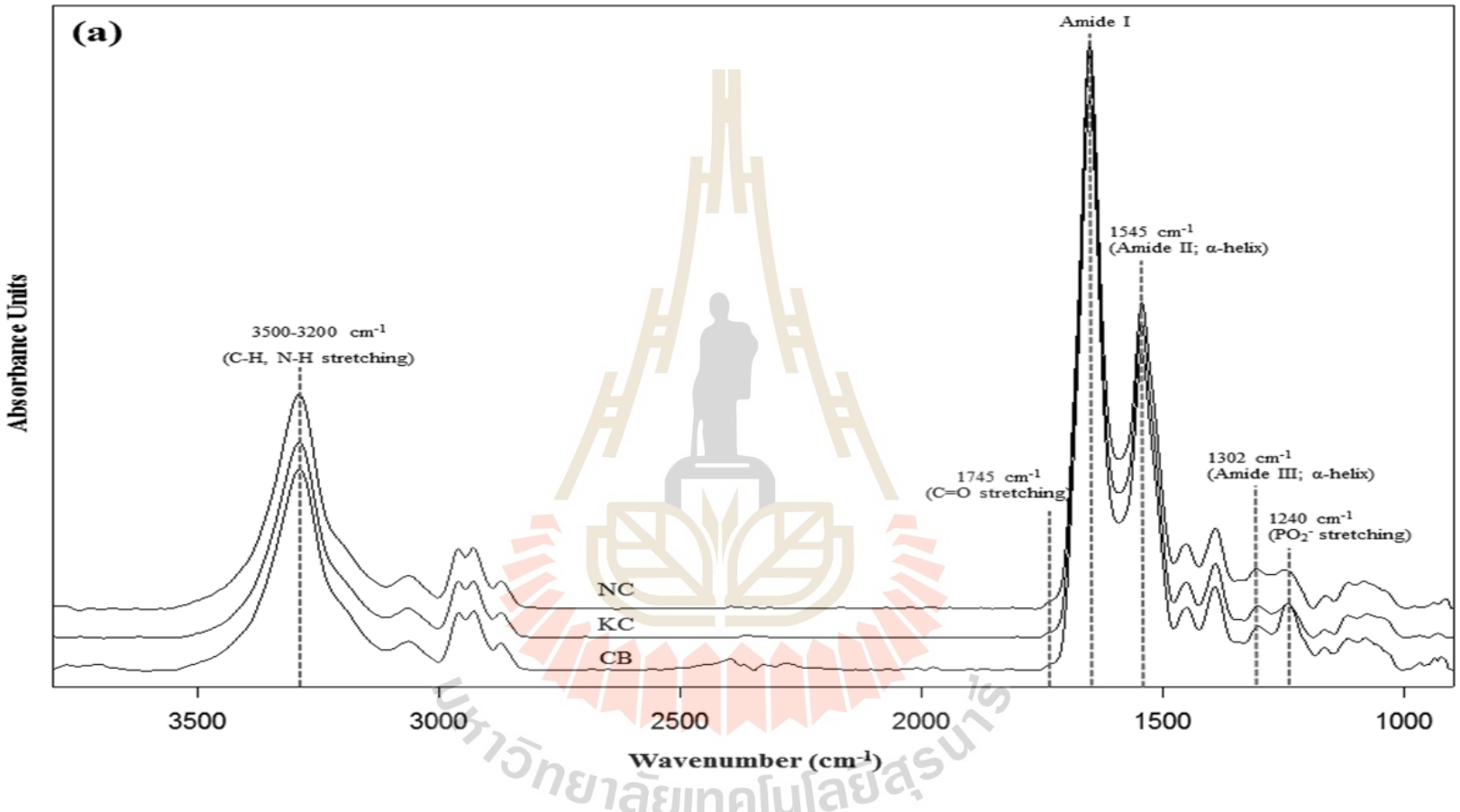

Figure 4.1 Average of preprocessed SR-FTIR (a) and FT-Raman spectra (b) and the disulfide bonds region of FT-Raman spectra (c) of three chicken breeds. CB; Commercial broiler (6 weeks of age), KC; Korat crossbred chicken (10 weeks of age), NC; Thai native chicken (16 weeks of age).

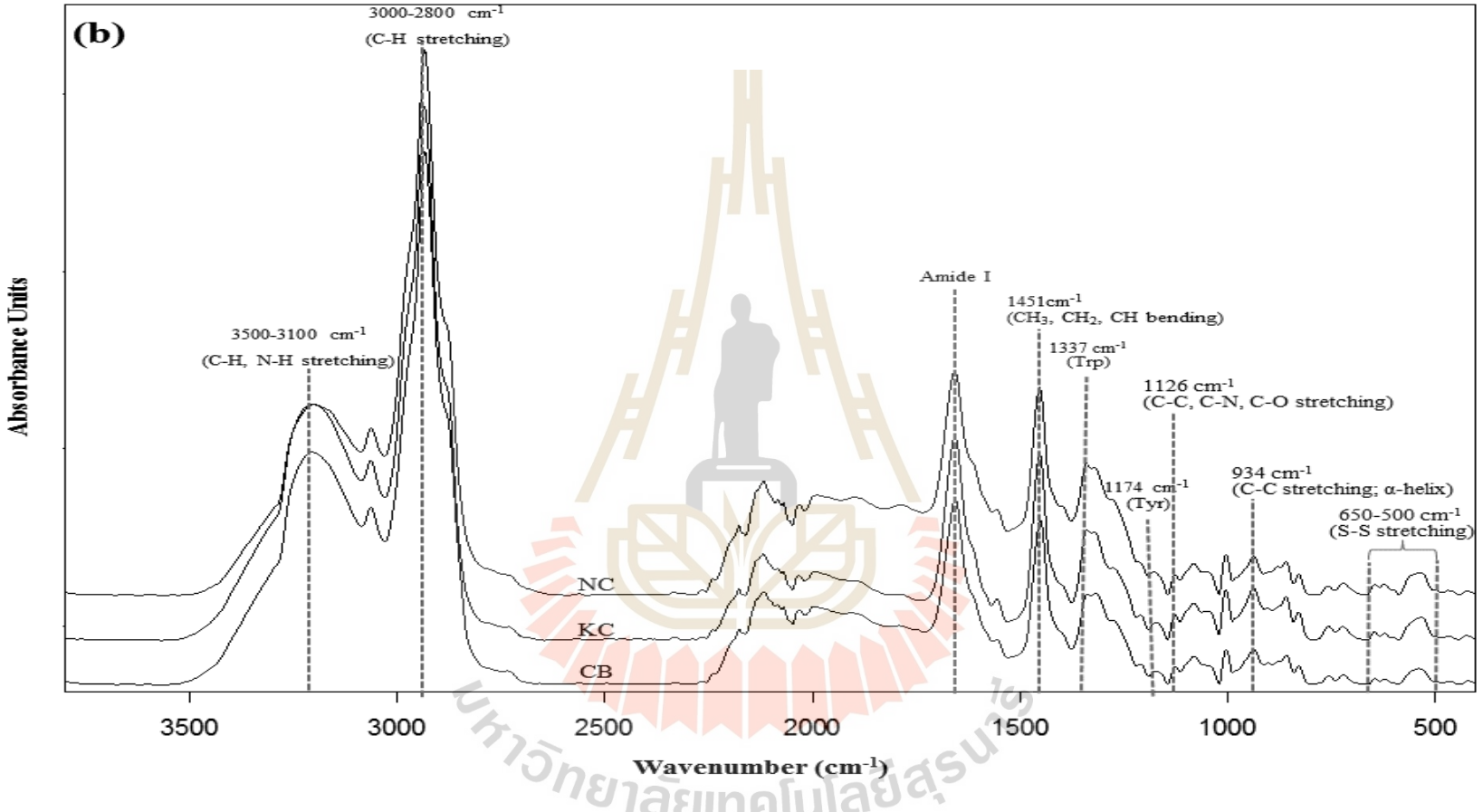

Figure 4.1 Average of preprocessed SR-FTIR (a) and FT-Raman spectra (b) and the disulfide bonds region of FT-Raman spectra (c) of three chicken breeds. CB; Commercial broiler (6 weeks of age), KC; Korat crossbred chicken (10 weeks of age), NC; Thai native chicken (16 weeks of age) (Continued).

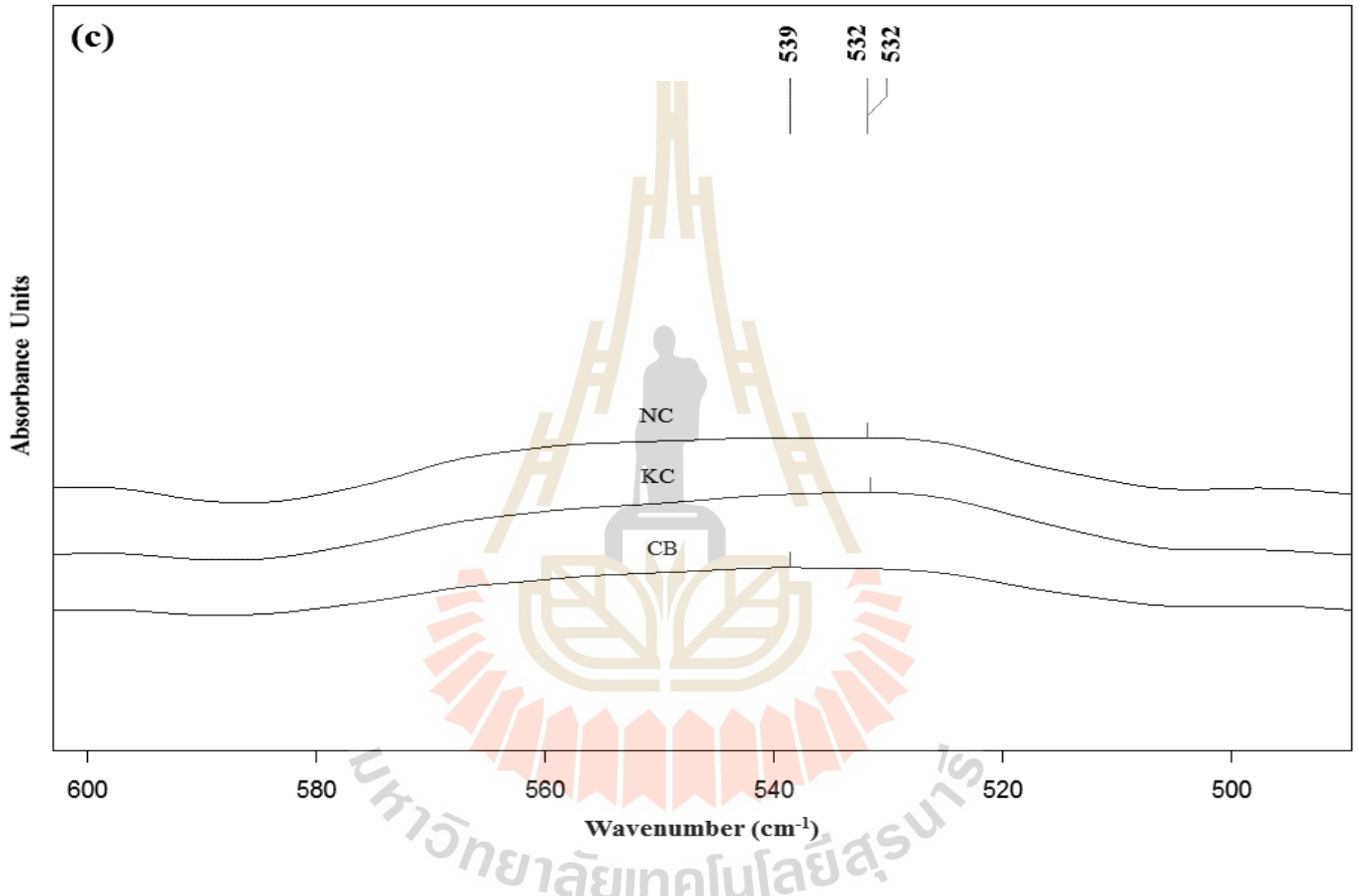

Figure 4.1 Average of preprocessed SR-FTIR (a) and FT-Raman spectra (b) and the disulfide bonds region of FT-Raman spectra (c) of three chicken breeds. CB; Commercial broiler (6 weeks of age), KC; Korat crossbred chicken (10 weeks of age), NC; Thai native chicken (16 weeks of age) (Continued).

In order to classify the differences in meat quality among chicken breeds, PCA was performed on selected high loading wavenumbers of SR-FTIR and FT-Raman spectra, as well as physico-chemical properties. Score plots of the first and second principle components (PCs) explained about 65 % of total variability (Figure 4.2). The PCA-score plot shows three chicken breeds clearly distinguished based on meat quality measured and spectra from vibrational spectroscopy. Moreover, the correlation loading plot (PC-1 vs PC-2) of average SR-FTIR, FT-Raman spectra and physico-chemical analysis of three chicken breeds was shown in Figure 4.3, indicates the distinction of meat quality parameters and spectra separating each chicken breeds from others. CB meat was separated from NC meat along PC-1, while KC was separated from others by PC-2 (Figure 4.2). CB breast meat was characterized as high moisture, lipid and taste-active nucleotides (IMP and GMP) content (Figure 4.3). This was also positively correlated O-H stretching (3203 cm^{-1}), C-H stretching of methyl and methylene groups stretching of lipid (2916, 2854 cm^{-1}) obtained from FT-Raman. The distinct characterisitics of SR-FTIR spectra of CB include amide II of α-helix structure (1543 cm^{-1}) and PO_2^- asymmetric stretching of nucleic acids (1240 cm^{-1}) (Figure 4.3). High signal from O-H stretching from FT-Raman appeared to correlate with high moisture content in CB meat (Table 4.1). In addition, high C-H stretching, and PO_2^- asymmetric stretching of nucleic acids correlated well with high lipid and nucleotides content of CB. In contrast, NC meat aligned in the negative direction of PC-1, which was characterized as high protein, inosine, and insoluble collagen content as well as dominant β-turn and random coil structure. KC aligned in positive direction of PC-2 with distinct characteristics of higher WHC, AMP, carbonyl content and random coil

structure, but lower ash and lightness (L*). It should be mentioned that intensity of C-C stretching of Raman band at 934 cm^{-1} was correlated with WHC in KC meat. This study revealed that conformation of muscle protein varies with breed. Protein structure of KC and NC appeared to be in the unfolding to a greater extent than that of CB. Herrero, Cambero, Ordóñez, de la Hoz, and Carmona (2009) reported that increase of turns and unordered structure (random coil) correlated with increasing hardness, springiness and breaking force of meat protein structures. This unique protein structure could contribute to tougher texture of KC and NC meat. Our study opens new insights on the structural information of meat protein from these 3 different chicken breeds.

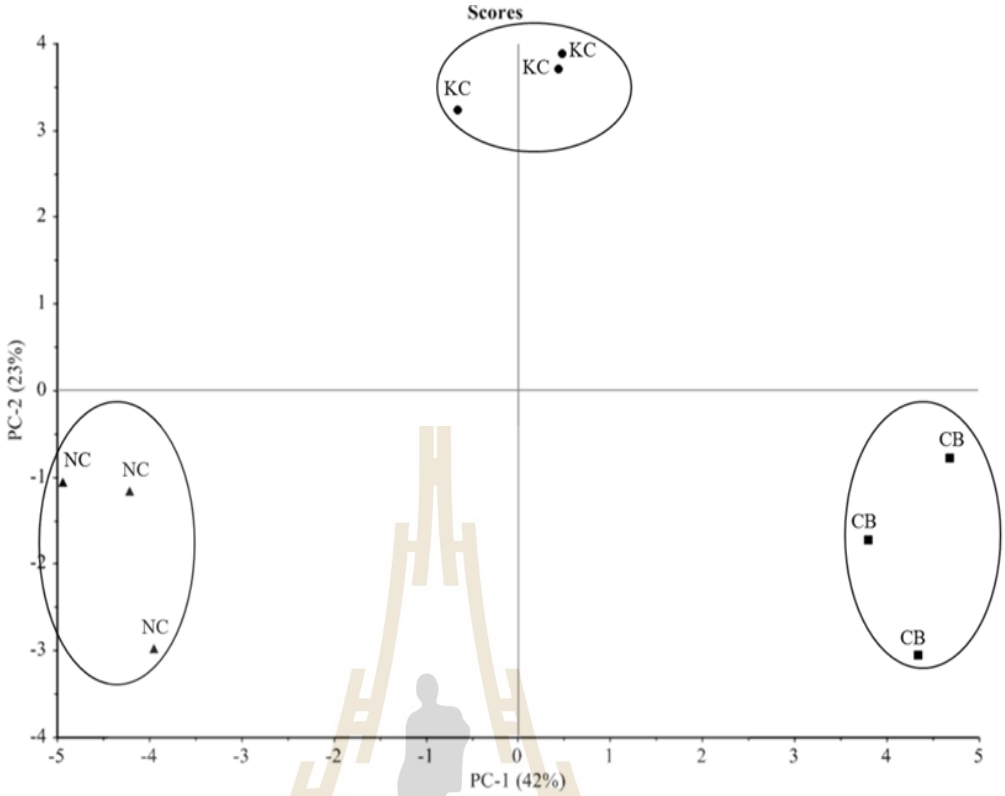

Figure 4.2 Principle component (PC) score plots (PC-1 vs PC-2) of average SR-FTIR, FT-Raman spectra and physico-chemical analysis of three chicken breeds. CB; Commercial broiler (6 weeks old), KC; Korat crossbred chicken (10 weeks old), NC; Native chicken (16 weeks old).

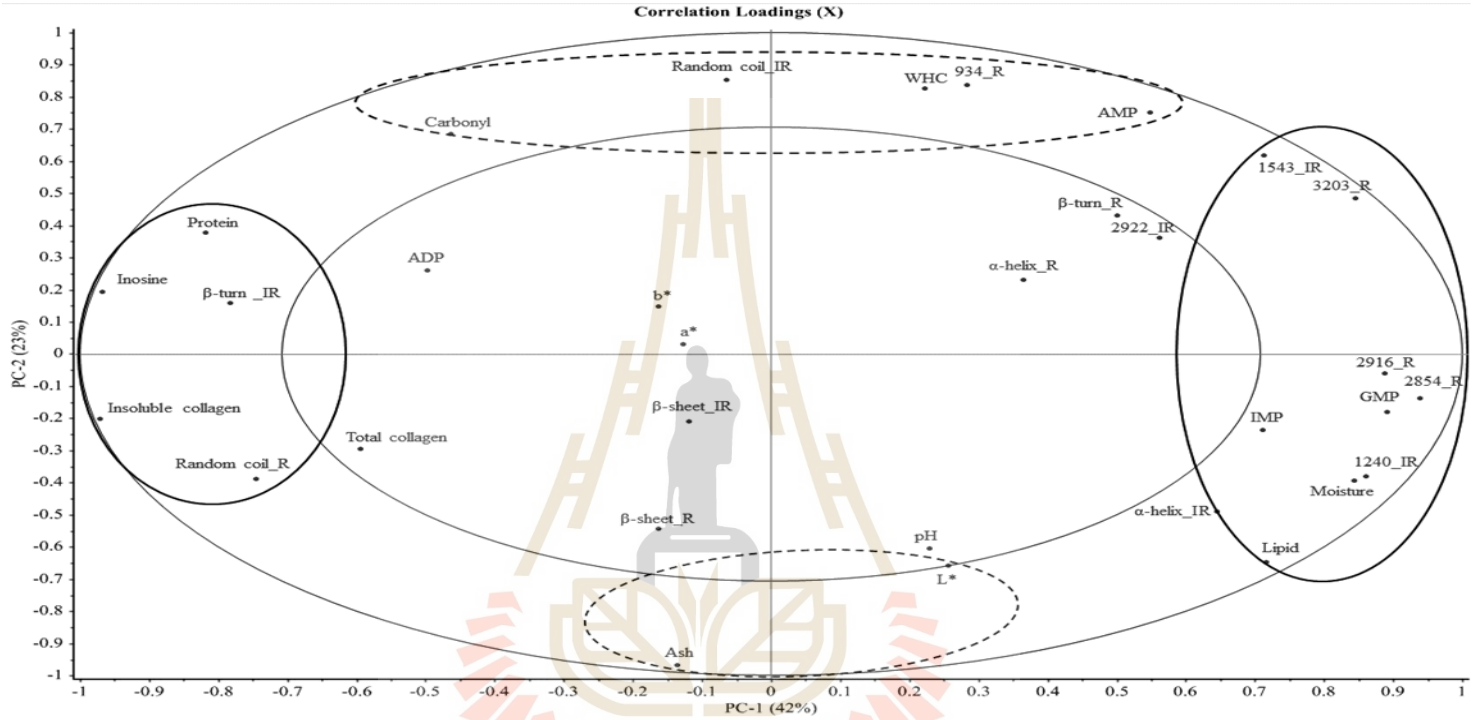

Figure 4.3 Correlation loading plot (PC-1 vs PC-2) of spectra and physico-chemical properties of three chicken breeds. WHC; Water Holding Capacity, SFA; Saturated Fatty Acid, MUFA; Monounsaturated Fatty Acid; PUFA; Polyunsaturated Fatty Acid, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate. IR; SR-FTIR spectra, R; FT-Raman spectra.

Our results demonstrated that SR-FTIR and FT-Raman spectroscopy are complementary techniques that can be used to detect differences of chicken meats quality and protein conformation. SR-FTIR is strong dipomoment structure in a powerful technique for monitoring protein conformation in amide regions and phosphodiester groups in nucleic acids, while FT-Raman non polar covalent bonds revealed local environments of protein (tyrosine, tryptophan residues), lipids, and disulfide bonds. A few previous works have been applied FT-IR in combination with Raman spectroscopy for assessment meat quality such as beef meat spoilage (Argyri et al., 2013), water-holding capacity (WHC) of porcine meat (Pedersen et al., 2003). Thus, this study was used SR-FTIR coupled with FT-Raman spectroscopy and demonstrate for the first time that these 2 complementary techniques can be used to classify meat quality and provide signatures of wavenumber for each chicken breeds.

4.5 Conclusions

In conclusions, these three chicken breeds showed distinct physico-chemical properties and protein structure. CB breast meat showed unique features in high content of moisture, lipid, taste-active nucleotides (IMP and GMP) along with high α-helical content and disulfide bonds. High protein, inosine and insoluble collagen content and β-turn were observed in NC meat. KC meat showed high WHC. NC and KC meats demonstrated high β-turn and random coil structure. KC was characterised by high WHC. Our results provide in-depth molecular information as related to chicken meat quality, which can be applied for further chicken meat product processing of these breeds. In addition, SR-FTIR and FT-Raman spectroscopy are promising non-destructive techniques that provide the insightful information for

differentiating meat quality among these 3 breeds. It is noteworthy that wavenumbers at 3203 cm^{-1} (FT-Raman), 2854 cm^{-1} (FT-Raman) and 1240 cm^{-1} (SR-FTIR), 934 cm^{-1} (FT-Raman) could be used as markers to characterize the moisture, lipid, and WHC traits, respectively of meat from these 3 different chicken breeds.

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CHAPTER V

CHANGES IN MEAT QUALITY AND VIBRATIONAL SPECTROSCOPIC PROPERTIES OF CHICKEN MEAT AS INFLUENCED BY HEATING TEMPERATURES

5.1 Abstract

Heat treatments applied in meat processing normally induce changes in quality of meat product. The objective of this study was to determine the effect of heat treatments (70, 90, and 121 °C for 40 min) on meat quality of breast meat of Korat chicken (KC) in comparison with commercial broiler (CB) and Leung Hang Khao (Thai native chicken; NC). The high heat treatment (121 °C) caused the meat from KC and NC become darker than CB meat. Heat treatments affect to increase WHC and cooking loss, while decrease in moisture and insoluble collagen content. Taste enhancing compounds including, is inosine-5'-monophosphate (IMP) and guanosine-5'-monophosphate GMP showed higher retained in KC and NC meat than CB meat. Shear force values of KC and NC meat were not affected by high heating temperatures. High heat treatment (121 °C) increase protein carbonyl, while decreased the protein digestibility. Principle component analysis (PCA) revealed that low heating temperature (70 °C) accompanied by high moisture content and predominant of α-helix structure in chicken meat correlated with FT-Raman spectra at 3217 cm^{-1} (O-H stretching of water), and 1651 cm^{-1} (amide I; α-helix), respectively. Heating temperature 90 and 121 °C, cooked chicken meat expose of protein structure

contributes to increase β-sheet correlated with FT-Raman bands at 2968, 2910, 2860 cm^{-1} (C-H stretching), 1020, 990 cm^{-1} (C-C stretching; β-sheet). Moreover, cooked KC and CB meat from NC meat were contained high α-helical structure, while cooked NC meat was characterized with β-sheet and random coil structures.

Keywords: Heat treatments, physico-chemical properties, Synchrotron radiation Fourier transform infrared spectroscopy, Fourier transform Raman spectroscopy

5.2 Introduction

Heat treatments are generally applied in meat processing to achieve microbiological safety and palatability. Improper heat treatments may deteriorate the appearance and taste, and contribute to tough texture as well as reduce nutritional value of chicken meat products (Dawson, Sheldon, and Miles, 1991; Kaur, Maudens, Haisman, Boland, and Singh, 2014). Hence, the effective thermal processes and proper control is crucial. Changes of color of cooked meat were due to degradation of myoglobin through oxygenation, oxidation and reduction (Lorenzo, Cittadini, Munekata, and Domínguez, 2015). Heat treatment affect to fade of taste-active nucleotides in chicken meat including inosine-5'-monophosphate (IMP) and gaunosine-5'-monophoshphate (GMP) content in meat due to leaching, degradation, and reaction with other compounds (Jo, Cho, Chang, and Nam, 2012; Sasaki, Motoyama, and Mitsumoto, 2007; Shi and Ho, 1994). Many researches have been reported that nucleotides content in native chicken meat higher than broiler chicken

meat (Jung et al., 2011; Rikimaru and Takahashi, 2010; Tang et al., 2009). However, little information available on changes of taste-active nucleotides content in different chicken breeds upon heating. Transversal and longitudinal shrinkage of muscle fibers and, denaturation of myofibrillar proteins and sarcoplasmic proteins as well as solubilization of connective tissue lead to modification of textural properties of cooked meat (Christensen, Bertram, Aaslyng, and Christensen, 2011; Palka, 1999). Heat treatments generate free radicals which induce protein oxidation affect to deteriorate meat quality (Traore et al., 2012). Protein oxidation also impaired their digestibility and also limited bioavailability of amino acids in meat products (Kaur et al., 2014). The meat composition among chicken breeds and subject into different cooking temperatures may be diversity in their meat digestibility. The fermentation products of undigested portion of food protein in the colon have been reported harmful for human health (Hughes, Magee, and Bingham, 2000). Thus, the digestibility is one of the crucial aspects in meat quality that need to consider.

Vibrational spectroscopy including, infrared (IR) and Raman spectroscopy are complementary techniques which have been used to provide information about protein structural changes, leading to an understanding of meat quality changes during heating. Furthermore, Synchrotron-based Fourier transform infrared spectroscopy (SR-FTIR) has been used to reveal the protein secondary structure changes as affected by heating process within intact tissue at cellular level at with high ultraspatial resolution (Yu, 2005). Using advanced SR-FTIR to exploring the structural changes at the molecular level in microstructure could takes advantage in differentiate the cooked meat quality. Combined with advanced statistical in chemometric methods

such as principle component analysis (PCA), vibrational spectroscopy has potential to extract vital information and help assessment the changes of meat quality during heating (Berhe, Engelsen, Hviid, and Lametsch, 2014). Therefore, studies on structural changes of meat composition related to quality of cooked chicken meat using SR-FTIR and FT-Raman spectroscopic coupled with PCA has potential to characterized cooked meat quality at each heating temperature.

Korat chicken (KC) is a crossbred between Leung Hang Khao sires (Thai native chicken; NC) and SUT 101 dams (a crossbreed between broiler and layer chicken). According to our previous study, KC and NC had unique texture with high nutritional quality as compared to broiler's meat. Thus far, information regarding the changes in quality attributes of the KC and NC meat during heating is unknown. The selection heating temperature is one of the factors that need to consider in order to development the high quality of meat products. Therefore, the present study aimed to investigate, the effect of heat treatments on the meat quality of Korat crossbred chicken (KC) breast meat in comparison with commercial broiler (CB) and Leung Hang Khao (Thai native chicken; NC), and using vibrational spectroscopic techniques including SR-FTIR and FT-Raman spectroscopy to detect the changes of chicken meat quality after heat treatments and its relation with vibrational spectral data.

5.3 Materials and methods

5.3.1 Animals, sample preparation and heating procedure

A total of 120 of each genotype, 1-day-old mixed sex Korat crossbred chicken (KC) and Thai native chicken (White Tail-Yellow Cock; NC) were raised

under the same condition in three separated pens (40 birds/pen/5m^2) at Suranaree University of Technology Farm (Nakhonratchasima, Thailand). The birds were fed ad libitum with the same commercial diet for starter (0-4 weeks old), grower (5-6 weeks old), and finisher (7-16 weeks old) containing 21, 19, and 17% crude protein, respectively. Birds had free access to water and no access to the outdoor environment. When birds reached the commercial size of 10 weeks for KC and 16 weeks for NC, thirty six male (12 chicks/pen) of each breeds were randomly selected and subject to a total feed withdrawal of 12-15 h, weighted (KC; 1.40-1.82 kg and NC; 1.40-2.04 kg) and slaughtered in at a commercial slaughterhouse (Nakhon Ratchasima, Thailand). The birds were processed under commercial conditions using electrocution as the stunning system, conventional neck cut, bled, scalded, plucked, and eviscerated. Then, the carcasses were packed in ice box and brought to the laboratory within 1 h. The breast meat samples were collected after 24 h postmortem in a 4 °C chiller and then skin, bone, visible connective tissue and fat were removed. The breast meat samples of male broiler (CB) at 6 weeks old with live weight 2.90-3.00 kg obtained from a chicken meat processing company (CPF (Thailand) Public Company Limited, Nakhon Ratchasima, Thailand) was used as a control. A 24-h postmortem pH was determined. All chicken meat samples were cut into 4 × 4 × 0.5 cm and heated in a water bath set at 70 and 90 °C for 40 min. For high temperature treatments, samples were heat in an autoclave set at 121 °C for 40 min. Subsequently, cooked samples were then immediately placed on ice for 10 min and determined for cooking loss. Shear force values were measured at the same day of pH. Color was determined within 48 h. The separated samples were removed for subsequent SR-FTIR and FT-Raman spectroscopy measurement.

Each treatment was conducted in 3 replicates. The remained samples were minced, vacuum packed, blast frozen and stored at -80 °C until subsequent further analysis. Before analysis, the frozen meats were thawed in a refrigerator at 4 °C for 12-18 h.

5.3.2 Color, WHC, and cooking loss

Color was recorded using a colorimeter (Hunter Associates Laboratory, Reston, VA., USA). All measurements were made in the CIE L*a*b* color space using the D65 illuminant. The instrument was standardized with the light trap (black hole) and white tiles before measurement. The color values were expressed as L* (lightness), a* (redness/greenness) and b* (yellowness/blueness).

WHC was determined according to Ryoichi, Degychi, and Nagata (1993) with some modification. Absorbed moisture in the filter paper was determined. The minced sample (2 g) was placed into a centrifugation tube with a filter paper (No. 4, Whatman International Ltd., Maidstone England), then centrifuged at 6,710 × g, 25 °C for 10 min. Absorbed moisture in the filter paper was determined. WHC was calculated as a percentage of the initial moisture of meat.

Cooking loss was calculated from differences in the weight of raw and cooked meat after heat treatments. Subsequently, samples were immediately placed on ice and weighed.

5.3.3 Taste-active nucleotides

To determine the levels of nucleotides, the cooked meat samples were measured according to the method described by Kim et al. (2012) with slight modifications. Briefly, nucleotides were extracted by homogenizing meat samples (5 g) with 50 ml of 7.5% cold perchloric acid. The mixture was centrifuged at 2,000 × g for 5 min at 4 °C. The extract was then mixed with 0.6 M neutralizing

buffer (pH 7.6; KH_2PO_4+K_2HPO_4) for 10 min and filter through a 0.45-µm nylon filter. Samples were separated on C18 reverse-phase column (Hypersil™ ODS C18, 4.6 × 150 mm, 3 µm particles, Thermo Scientific, Waltham, MA, USA) equipped with HPLC system (HP 1260, Agilent Technologies, Inc., Santa Clara, CA, USA). The injection volume was 10 µl and was eluted with a mobile phase A containing 150 mM potassium dihydrogen phosphate (KH_2PO_4) and 150 mM potassium chloride (KCl), pH 6 and mobile phase B containing mobile phase A mixed with 20% acetonitrile at a flow rate of 0.5 ml/min. A gradient elution mode of 97% A for 5 min, after that reduced to 91% A for 5 min, 80% A for 10 min, and finally 100% B for 10 min. The column temperature was maintained at 25 °C, and detection was monitored at 254 nm. The quantity of inosine 5'- monophosphate (IMP), guanosine monophosphate (GMP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine, and hypoxanthine are calculated using the external standards (Sigma–Aldrich Co., St. Louis, MO, USA). The % retention of IMP and GMP was calculated from the following formula;

$$(\%)\ \text{Retention} = \left[\frac{A}{B}\right] \times 100$$

where, A is nucleotide content in sample after heating, B is nucleotide content in sample before heating.

5.3.4 Textural properties and collagen content

Shear force of cooked samples with the size of 1.0 × 2.0 × 0.5 cm was evaluated using a Texture Analyzer (TA.XT. Plus, Stable Micro Systems, City, UK) equipped with a Warner-Bratzler shear apparatus attached to a 25-kg load cell at a 2 mm/s crosshead speed (Wattanachant, Benjakul, and Ledward, 2005). The force

required to move the blade to shear through the longitudinal axis of muscle fibers was measured. The mean value of the 9 replicates for each treatment was presented.

Total collagen content was determined by alkaline hydrolysis as described by Reddy and Enwemeka (1996). Samples were hydrolyzed with 7 M sodium hydroxide (NaOH) at 120 °C for 40 min. The hydrolysate was neutralized with 3.5 M sulfuric acid (H_2SO_4), filtered, reacted with chloramine T solution and Ehrlich's reagent. Absorbance was measured at 550 nm using a spectrophotometer. The amount of hydroxyproline was determined and total collagen content was calculated using a coefficient of 7.25 (Palka, 1999).

Insoluble collagen content was determined according to the method of Liu, Nishimura, and Takahashi (1996). Meat samples were homogenized with 25% Ringer's solution. The homogenates were heated at 77 °C for 70 min in a water bath and centrifuged at 2,300 × g at 4 °C for 30 min. The extraction was repeated twice and then the residues were dried overnight at 105 °C. Hydroxyproline content of the dried residue was determined and collagen content was calculated as describe above to represent insoluble collagen content.

5.3.5 Protein oxidation measurement

Protein oxidation was measured by the method of Mercier, Gatellier, and Renerre (2004) with minor modifications. Briefly, cooked samples (1 g) were homogenized in 10 ml of 20 mM phosphate buffer (pH 6.5) using an Ultraturrax homogenizer at 10000 rpm for 30 s. (Ultra turrax T25, Ika, WerkeGmbh & Co., Staufen, Germany). Carbonyl groups were detected by reactivity with 2, 4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. Two equal aliquots of meat homogenate (0.5 ml) was precipitated with 0.5 ml of 20% trichloroacetic acid

(TCA) and centrifuged at 4,500 × g for 5 min at 4 °C. One pallet was treated with 2 ml of 0.2% (w/v) 2, 4-dinitrophenylhydrazine (DNPH) dissolved in 2 M HCl, and the other with 2 M HCl as a blank. Both samples were kept in dark at room temperature and vortex-mixed every 15 min. Subsequently, samples were further centrifuged for 5 min at 4,500 rpm at 4 °C. DNPH was removed by washing with 5 ml of 10% TCA and then 5 ml of the mixture of ethanol:ethyl acetate (1:1) untilclear solution. The pellets were dried with N_2 gas and finally solubilized in 2 ml of 20 mM sodium phosphate buffer (pH 6.5) containing 6 M guanidine hydrochloride. Protein concentration was analyzed spectrophotometrically at 595 nm. The amount of carbonyl was read at 370 nm for protein hydrazone and expressed as nanomol of carbonyl per mg protein using the extinction coefficient of 21.0/mM/cm). Bovine serum albumin dissolved in 6M guanidine with 20 mM sodium phosphate buffer pH 6.5 was used as a protein standard. Carbonyl content was expressed as nanomoles of DNPH fixed per milligram of protein.

5.3.6 *In vitro* protein digestibility

Protein digestibility was assessed according to Minekus et al. (2014) with slight modifications. Cooked meat samples (2 g) were homogenized in 4 ml of simulated gastric fluid (SGF). The homogenate was adjusted to pH 3.0 and then pepsin solution (454.44 units/mg solid, porcine gastric mucosa; P7000, Sigma, St. Louis, MO, USA) and $CaCl_2$ was added to achieve 2000 U/ml and 75 µM of the final mixture. The mixture was incubated in a shaking water bath at 37 °C and 150 rpm for 2 h. Digestion was ceased by adjusting pH to 7.0. The gastric digested sample (~5 ml, pH 7.0) was mixed with 5 ml simulated intestinal fluid (SIF). The pH was re-adjusted to 7.0 using 1 M NaOH prior to the addition of 100 µl of pancreatin

(5.57 U TAME/mg solid) (P7545, 8 × USP specifications, Sigma-Aldrich Co., St Louis, USA) was added to achieve 100 U/ml of final digestion mixture and then CaCl2 was added to reach 0.3 mM. Intestinal digestion was performed in the shaking water bath at 37 °C, 150 rpm for 2 h, and stopped by heating in water bath at 95 °C for 10 min, placed in an ice bath for 10 min, and centrifuged at 4,500 × g at 4 °C for 35 min. The final volume of digested samples was recorded. Supernatants were stored at -20 °C. The digests samples were analyzed for α-amino groups produced during digestion using 2, 4, 6 - trinitrobenzene sulfonate (TNBS) method as described by Alder-Nissen (1979). The control (blank) was treated in the similar manner but used deionized water instead meat sample. Total α-amino group was determined by hydrolyzing samples with 6 N HCl at 120 °C for 24 h before analysis of α-amino groups. The in vitro protein digestibility (%) was calculated using the following formula;

$$\text{Digestibility (\%)} = \left[\frac{\text{ANs - ANb}}{\text{ANt}}\right] \times 100$$

where, AN_s is α-amino group of digested samples, AN_b is α-amino group content of blank, and AN_t is total α-amino group content in sample.

5.3.7 Vibrational spectroscopy

5.3.7.1 Synchrotron Radiation Fourier transform infrared (SR-FTIR) measurement

Infrared spectra were collected at room temperature using a SR-FFTIR (Hyperion 2000, Bruker Opics, Ettlingen, Germany). In brief, cooked meat samples were cut into sectioned (4 μm) by a Cryostat (Microm HM525; Thermo Fisher Scientific, Walldorf, Germany) and put on Barium fluoride (BaF_2) windows,

and dried in a vacuum chamber for overnight. Samples were measured in transmission mode under an infrared microscopy 15x objective equipped with MCT D315 detector cooled with liquid nitrogen at an infrared microspectroscopy at beamline BL4.1 (Infrared Spectroscopy and Imaging) of the Synchrotron Light Research Institute (SLRI) (Nakhonratchasima, Thailand) connected with Synchrotron radiation entered the interferometer via an instrument port designed for IR emission. The measurement was performed in the mapping mode over the wavenumber range of 4000–800 cm^{-1}, using an aperture size of 10 × 10 μm, co-adding 64 interferograms at a norminal resolution of 4 cm^{-1}. The spectra were subtracted against background air spectrum in each scan. Spectral acquisition and instrument control were performed using OPUS Software 7.2 (Bruker Opics Ltd., Ettlingen, Germany)

5.3.7.2 Fourier transform Raman (FT-Raman) measurement

Raman data were acquired using a Bruker Vertex 70 FT-Raman spectrometer (Bruker, Karlsruhe, Germany). Frozen minced meat in raw and cooked samples were thawed at 4 °C overnight and equilibrated to room temperature before packed in a sample holder for Raman spectroscopy measurement. Spectra were recorded over the range of 4000-400 cm^{-1} at resolution of 4 cm^{-1} and 256 scans. Sulfur was used to calibrate the Raman frequency. Spectra were excited with the 1064 nm Nd:YAG laser line with 500 mW of laser power. FT-Raman spectral acquisition and instrument control were performed using OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) software. A total 30 spectra were collected per sample.

The secondary structure of proteins were determined as percentage of α-helix, β-sheet, β-turn, and random coil, curve fitting in 1700-1600 cm^{-1} (amide I region) of SR-FTIR and FT-Raman spectra was carried out

using appropriate Gaussian and Lorentzian functions in OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) software.

5.3.7.3 PCA analysis

Principle component analysis (PCA) (with a spectral range of 3800-2704 cm^{-1} and 1800-899 cm^{-1}) were performed using the Unscramble software (Version 10.5, Camo, Oslo, Norway) for identification of a significant variation between the data set. Spectral data were initially submitted to preprocess by taking the third polynomial order using the Savitzky-Golay algorithm (with eight point of smoothing allow to minimization of the effects of variable baselines) and normalized with extended multiplicative signal correction (EMSC). Seven principle component (PCs) were chosen for analysis. Subsequently, the selected high loading wavenumbers obtained from loading plot, secondary protein structure (%), and physico-chemical data were subjected into the Unscrambler software X 10.5.1 package (Camo, Oslo, Norway) to perform principal component analysis (PCA) with the weighting of standardization. Full cross-validation was used as the validation method. Correlations between wavenumbers of SR-FTIR and FT-Raman spectra and secondary protein structure (%) and meat quality traits were analyzed.

5.3.8 Statistical analysis

All analytical experiments were made in triplicate. Data were evaluated statistically using the SPSS version 16.0 program (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was conducted to determine the significance of the main effects of the breed and heating temperature on the parameters determined. Differences between treatments means were analyzed for significance ($P < 0.05$) using Tukey's test.

5.4 Results and discussion

5.4.1 Color, WHC, and cooking loss

High heat treatment at 121 °C led to a decrease of L* (lightness) value and an increase of a* (redness) in all three chicken breeds (Table 5.1). The change of chicken meat color during heating might be related to different chemical state of heme pigment. Myoglobin is almost completely denatured between 80-85 °C (Lawrie, 1991). Heating at 121 °C affect to loss of the globin function of protecting heme and then favor oxidation of myoglobin to metmyoglobin which has a low color intensity (Walters, 1975). High heat treatment at 121 °C, CB and NC meat showed lighter and lower redness as compared to KC meat. In addition, KC and NC is more yellow (lower b*-value) in comparison with CB meat after heating at 121°C. In terms of WHC, all 3 chicken breeds showed increasing in WHC with heating temperature. At mild heating condition (70-90 °C), CB meat showed the highest WHC. It could be postulated that higher degree of myofibrillar proteins and collagen denaturation in KC and NC meat might lead to higher amounts of water expulsion. An increase in cooking loss as a function of heating temperature is in agreement with previous studies (Christensen et al., 2011; Palka and Daun, 1999). Cooking loss of CB meat was higher than that of KC and NC meat ($P < 0.05$) at cooking condition of 70-90 °C, whereas breed showed no effect on the cooking loss ($P > 0.05$) at 121 °C.

Table 5.1 Cooking qualities of breast meat from various breeds under different temperatures (mean ± standard deviation).

Cooking temperature (°C)	Chicken Breeds	Moisture	L*	a*	b*	WHC (%)	Cooking loss (%)
70	CB	72.51 ± 0.95^{a}	80.73 ± 2.88^{a}	2.31 ± 0.54^{cd}	16.23 ± 1.02^{bc}	57.55 ± 1.71^{bc}	20.90 ± 2.99^{c}
	KC	72.61 ± 0.11^{a}	79.67 ± 1.57^{a}	2.33 ± 0.82^{cd}	14.56 ± 1.39^{d}	47.78 ± 1.51^{d}	5.59 ± 0.33^{e}
	NC	72.09 ± 0.09^{a}	79.34 ± 0.76^{a}	1.82 ± 0.59^{de}	15.30 ± 1.55^{cd}	47.44 ± 1.28^{d}	11.67 ± 0.49^{d}
90	CB	67.90 ± 0.52^{b}	79.43 ± 2.46^{a}	2.38 ± 0.74^{cd}	16.25 ± 1.43^{bc}	63.00 ± 2.02^{a}	32.87 ± 0.89^{a}
	KC	68.51 ± 0.20^{b}	78.69 ± 2.19^{a}	1.51 ± 0.47^{e}	14.59 ± 1.40^{d}	56.50 ± 0.80^{bc}	23.00 ± 1.57^{c}
	NC	68.23 ± 0.29^{b}	79.25 ± 2.18^{a}	0.54 ± 0.55^{f}	15.44 ± 1.04^{cd}	54.06 ± 2.95^{c}	24.47 ± 0.87^{c}
121	CB	66.07 ± 0.62^{c}	76.09 ± 2.81^{b}	2.95 ± 0.50^{bc}	17.11 ± 1.45^{b}	62.57 ± 3.12^{a}	37.15 ± 0.91^{a}
	KC	65.74 ± 0.26^{cd}	72.11 ± 2.55^{c}	4.35 ± 0.97^{a}	21.39 ± 1.97^{a}	61.62 ± 1.34^{a}	32.50 ± 0.67^{a}
	NC	64.99 ± 0.30^{d}	74.38 ± 1.53^{b}	3.62 ± 0.93^{b}	21.75 ± 1.98^{a}	59.49 ± 1.39^{ab}	33.59 ± 1.01^{ab}

$^{a-e}$ Mean value in the same column with different superscripts differ significantly ($P < 0.05$).

5.4.2 Taste-active nucleotides

IMP of KC and NC meat remained unchanged at heating temperatures studied (Figure 5.1a). In contrast, approximately 60% retention of IMP was observed in cooked CB meat at 3 conditions. GMP of KC and NC increased upon heating at 121 °C, while that of CB remained unchange as compared to heating at lower temperature at 70 and 90 °C (Figure 5.1b). This result different from previous reported (Matoba, Kuchiba, Kimura, and Hasegawa, 1988), indicated that IMP and GMP are thermal degradation upon heating at 100 °C, thus further clarification is need. Overall, KC and NC meat trend to retain more compounds contributing to umami flavor than CB meat.

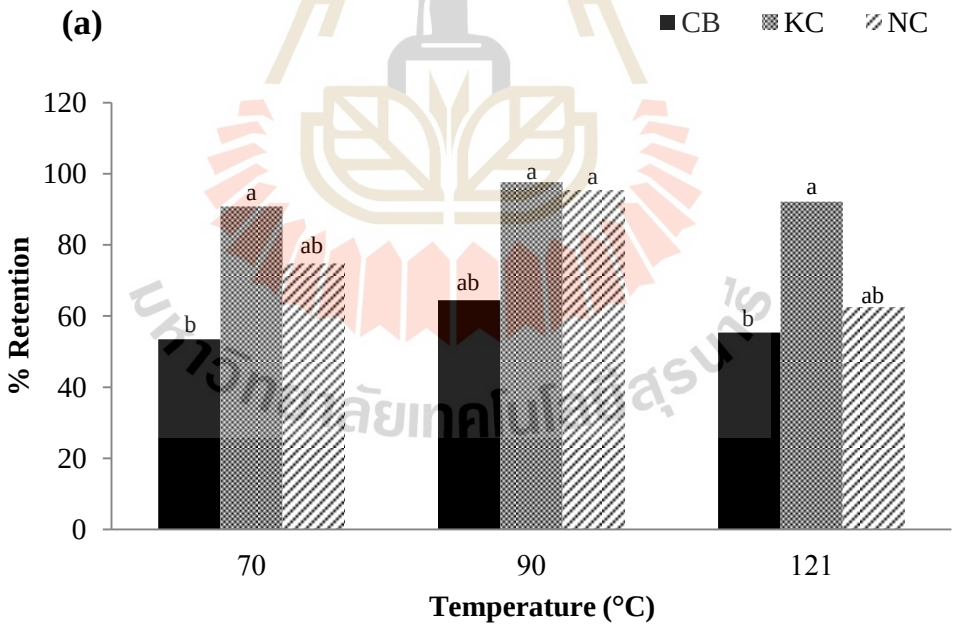

Figure 5.1 IMP (a) and GMP (b) retention (%) of cooked breast meats at different temperatures. $^{a-b}$ of Different superscripts indicates differences in mean values ($P < 0.05$).

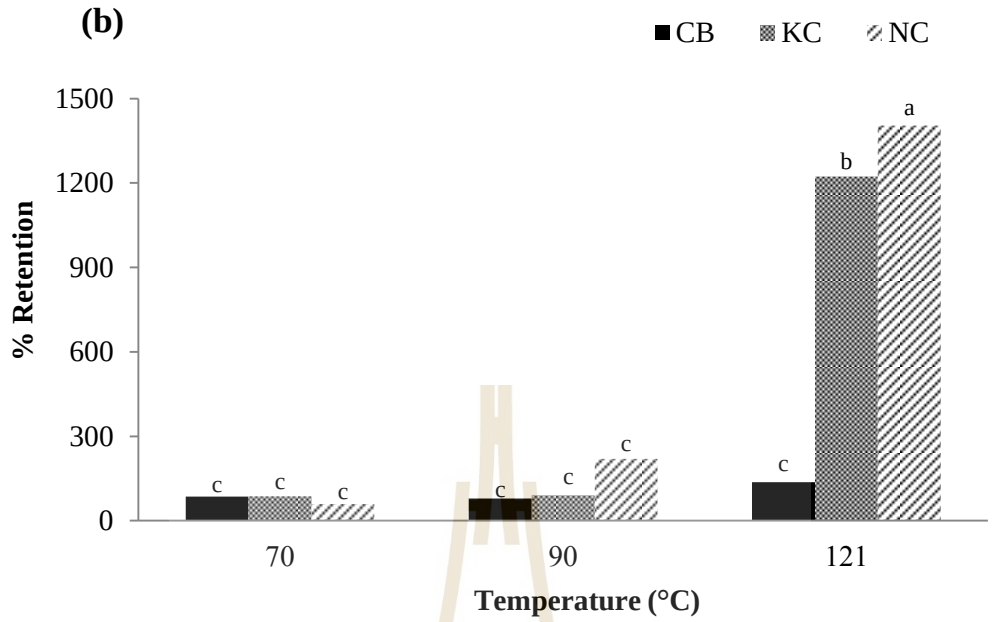

Figure 5.1 IMP (a) and GMP (b) retention (%) of cooked breast meats at different temperatures. [a-c] of Different superscripts indicates differences in mean values ($P < 0.05$) (Continued).

5.4.3 Textural properties

Total collagen content of cooked NC meat decreased with temperature, while that of CB and KC appeared to be constant upon heating (Figure 5.2a). KC and NC meat contained higher amounts of total collagen and insoluble collagen content than did CB meat at all temperatures tested except at 121 °C. High heat treatment at 121 °C resulted in the highest loss of insoluble collagen content ($P < 0.05$). This result confirms that cleavage of mature cross-links in collagen occurs at high temperature at 121°C (Bailey and Light, 1989). Moreover, Yu, Morton, Clerens, and Dyer (2016) reported that skeleton muscle proteins are not able to maintain their native structures above 100 °C. The higher amount of insoluble collagen content in

KC and NC meat indicated higher amount of cross-linked collagen. This could be due to the breed and/or age variation. Solubility of collagen decreases with increasing age of birds. KC and NC have 3 and 11 weeks, respectively older than CB, so they contained higher stable cross-linked collagen related to higher insoluble collagen at heating temperature 70 and 90 °C (Foegeding and Lanier, 1996; Pearson and Young, 1989). However, high heating temperature (121 °C), NC showed highest insoluble collagen content ($P < 0.05$).

At 70 °C, shear force values of all 3 species were comparable ($P > 0.05$, Figure 5.3). When heating temperature increased to 90 °C, shear force values of cooked KC and NC meat were higher than CB meat ($P < 0.05$). KC and NC contained more stable cross-linked collagen than CB (Figure 5.2b) and denaturation of meat connective collagen takes place at temperature 60-70 °C (Yu et al., 2016). So, after heating at 90 and 121 °C the cooked meat become stronger shrinkage from connective tissue affect to toughen meat and thus make a greater contribution to high shear force value than CB meat. When heating at 121 °C was applied, textural degradation of CB was observed, while NC and KC meat show comparable shear force values to those cooked at 90 °C. This result can explain by lower insoluble collagen content has been linked to an increase in the heat solubility of collagen in CB than KC and NC meat, effect to higher loss of collagen content to support the textural structure. Moreover, Wattanachant et al. (2005) reported that he perimysium and endomysium of broiler muscles melted after cooking at 80 °C, however, only slight disintegration was observed in these tissues in the indigenous chicken muscles. Muscle structures of the KC and NC chicken muscles appeared to be more stable than those of the CB muscles.

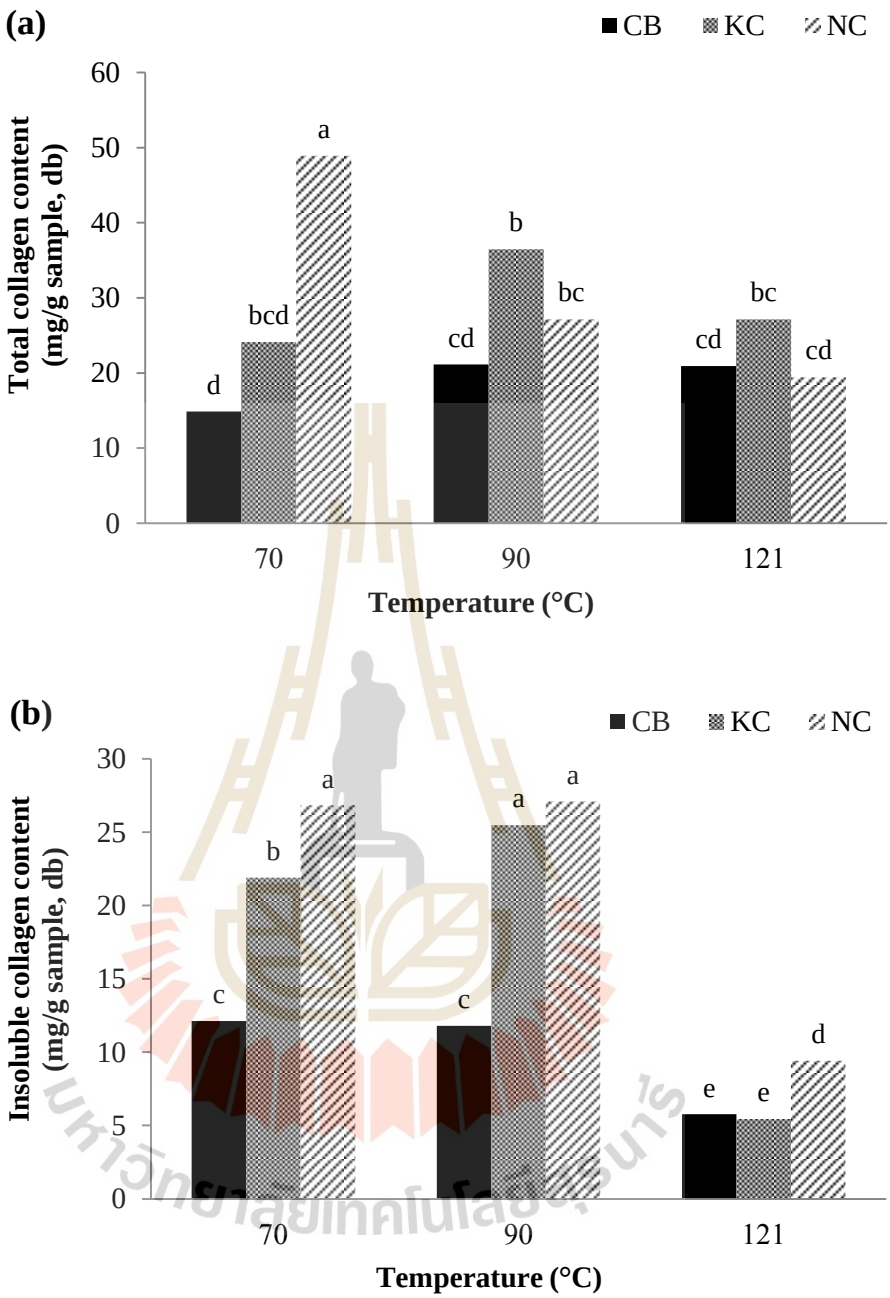

Figure 5.2 Total collagen (a) and insoluble collagen (b) content of cooked breast meats at different temperatures. [a-e] of Different superscripts indicates differences in mean values ($P < 0.05$).

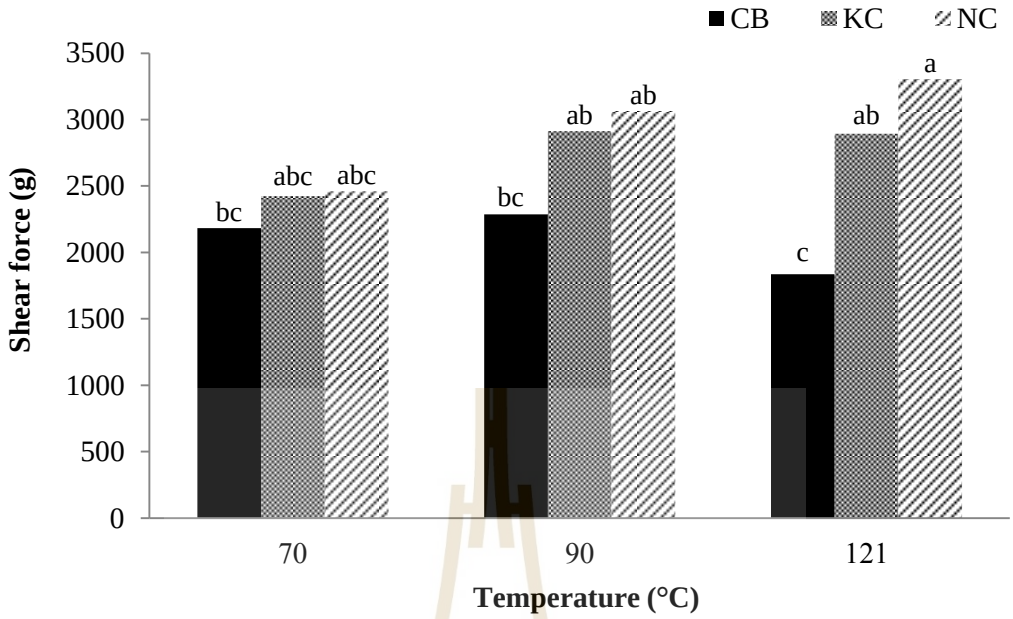

Figure 5.3 Shear force value of cooked breast meats at different temperatures**.** [a–c] of Different superscripts indicates differences in mean values ($P < 0.05$).

5.4.4 Protein oxidation

Generation of carbonyls is the most common indicative for oxidized proteins. Carbonyl content of cooked meat increased with heating temperatures in all three breeds (Figure 5.4), indicating protein oxidation induced by thermal treatment. Heat treatments generate free radicals and decrease antioxidant protection lead to oxidation (Traore et al., 2012). Higher carbonyl group formation during heating was observed in KC and NC rather than CB meat. This could be related to the extent of lipid oxidation and heme iron. From our previous study, KC meat contained higher proportion of polyunsaturated fatty acids (PUFA), while CB meat contained high proportion of monounsaturated fatty acids (MUFA). Also, cooking meat enhances fatty acid oxidation. Therefore, KC meat tends to have higher lipid oxidation due to

higher degree of unsaturation. The products of lipid oxidation including carbonyl compounds can react with protein structure induced protein oxidation (Zhang, Xiao, and Ahn, 2013). Furthermore, heating leads to myoglobin denaturation and oxidative cleavage of hematin pigment lead to release of iron from the heme molecules which promote the formation of free radicals involved in lipid and protein oxidation (Traore et al., 2012). Higher protein oxidation in KC and NC might be due to contained higher heme as they exhibited darker color (lower L*, Table 5.1), which can catalyze lipid oxidation and protein oxidation to a greater extent.

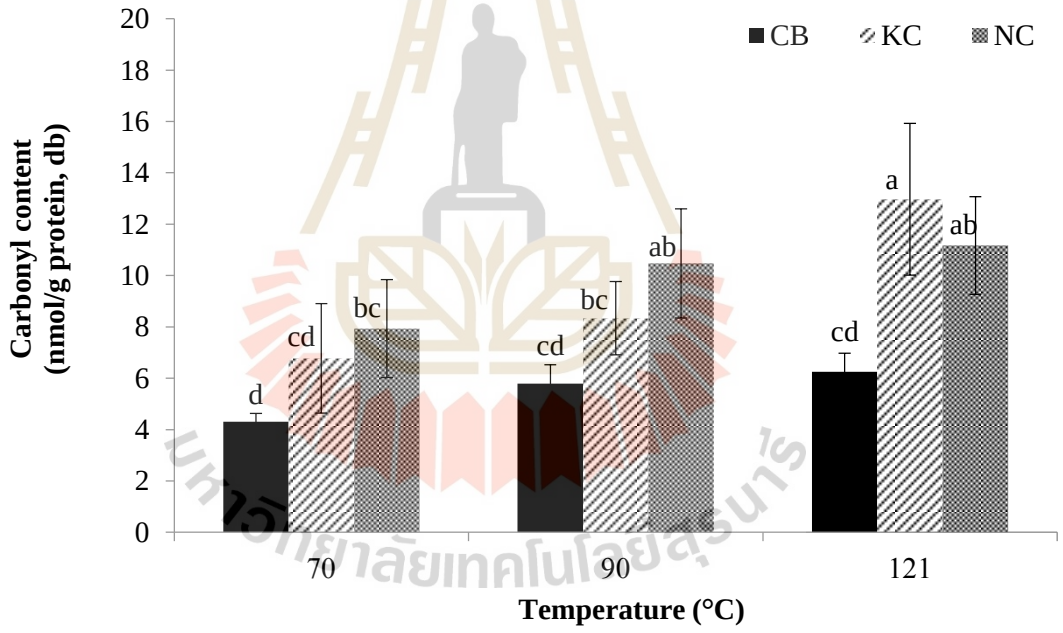

Figure 5.4 Carbonyl content of cooked breast meats at different temperatures. $^{a–d}$ of Different superscripts indicates differences in mean values ($P < 0.05$).

5.4.5 *In vitro* protein digestibility

Heat treatments affected the susceptibility of protein digestion by modification the structural of proteins. Protein digestibility appeared to decease as heating temperature increased (Figure 5.5). High heating temperature (121 °C) affect to lowest digestibility, while heating at 70 and 90 °C result in a greater digestibility in cooked meat of all three chicken breeds. Thermal denaturation at mild condition exposes cleavage sites to digestive enzymes, and increases susceptibility to proteases (Bax et al., 2013). At the extreme heating condition, high level of protein oxidation leads to cross-linking and aggregation, reducing proteolysis (Bax et al., 2013; Rysman, Van Hecke, Van Poucke, De Smet, and Van Royen, 2016). The agreement was found in our results that high heating temperature exhibited high protein oxidation (Figure 5.4). In addition, cooked NC meat at heating temperature (121 °C) showed lowest digestibility might be explained by higher insoluble collagen in NC could be a prime factor of low proteolysis. According to Kaur et al. (2014), insoluble collagen consists of cross-links bonds that stabilize the helical structure of collagen, thus, restrict to expose of the structure for protease attack. Moreover, heating also induce shrinkage of protein allows inter- and intra-protein interactions, resulting in more dense protein structure (Straadt, Rasmussen, Andersen, and Bertram, 2007) which can reduce protein susceptibility to enzymatic proteolysis (Kaur et al., 2014). These results suggesting that the appropriate processing, cooked KC and NC meat at 90 °C as well as cooked CB at 70 °C due to contribute the highest protein digestibility.

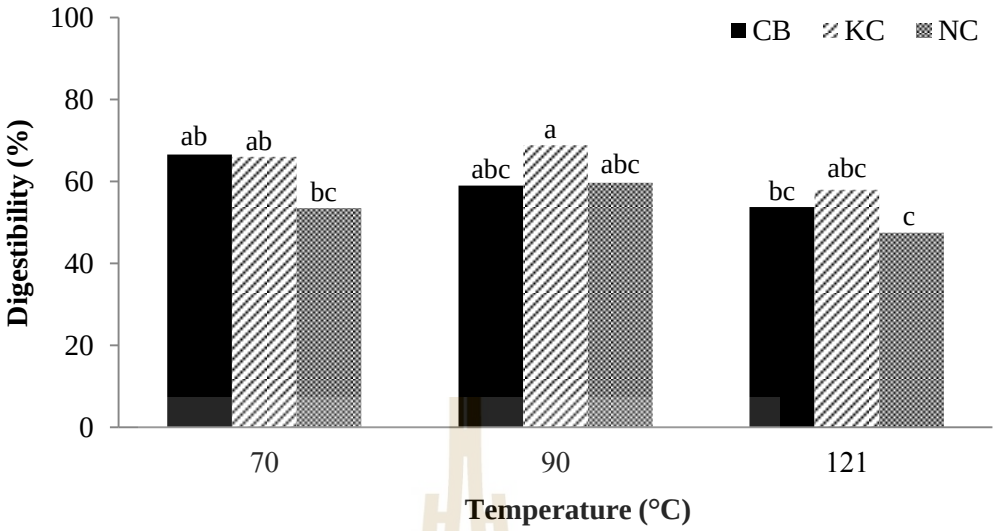

Figure 5.5 Digestibility of cooked breast meats at different temperatures. $^{a–c}$ of Different superscripts indicates differences in mean values ($P < 0.05$).

5.4.6 SR-FTIR and FT-Raman spectroscopy

Quantitative information about the secondary structure of protein obtained from the amide I profile of SR-FTIR and FT-Raman spectra showed in Table 5.2. β-sheet is the main protein secondary structures that found in cooked chicken meat, followed by α-helix structure. In addition, the percentage of both random coil and β-turn which found in low proportion in chicken meat was not different after heating. However, heating temperature from 70 to 121 °C does not have effect on significant changes of β-sheet and α-helix structures. This may because completely denaturation of meat proteins at heating temperature 70 °C. NC showed lowest percentage of α-helix, while highest in percentage of β-sheet at all heating temperature.

Table 5.2 Changes of secondary structures of cooked chicken meat from three different breeds at various heat treatments (mean ± standard deviation).

Cooking temperature (°C)	Chicken Breeds	Secondary structures (%)			
		α-Helix	β-Sheet	Random coil	β-Turn
SR-FTIR					
70	CB	27.29 ± 0.94^{a}	44.71 ± 1.39^{cd}	13.44 ± 0.60	14.55 ± 0.09
	KC	25.69 ± 0.63^{ab}	45.21 ± 1.26^{bcd}	13.91 ± 0.70	15.19 ± 0.07
	NC	23.65 ± 1.73^{bc}	47.61 ± 2.12^{abc}	15.35 ± 1.72	13.39 ± 2.23
90	CB	26.26 ± 0.96^{a}	44.64 ± 1.32^{cd}	13.87 ± 0.63	15.24 ± 0.36
	KC	26.18 ± 0.33^{a}	44.92 ± 0.90^{bcd}	14.01 ± 0.13	14.89 ± 1.11
	NC	23.44 ± 0.52^{bc}	48.02 ± 0.37^{ab}	12.79 ± 0.41	15.75 ± 0.36
121	CB	26.70 ± 0.54^{a}	43.69 ± 0.53^{d}	14.04 ± 0.29	15.58 ± 0.18
	KC	27.22 ± 0.33^{a}	43.78 ± 1.04^{d}	13.52 ± 1.28	15.48 ± 0.61
	NC	22.36 ± 1.28^{c}	49.99 ± 1.19^{a}	13.04 ± 1.80	14.61 ± 0.50
FT-Raman					
70	CB	26.86 ± 1.24^{abc}	34.84 ± 0.24	20.72 ± 0.61	17.58 ± 1.73
	KC	29.84 ± 1.94^{a}	32.99 ± 2.30	20.07 ± 0.55	17.10 ± 0.26
	NC	29.01 ± 0.93^{ab}	33.68 ± 0.27	20.79 ± 0.05	16.52 ± 0.85
90	CB	27.52 ± 1.22^{abc}	34.13 ± 1.38	20.79 ± 1.40	17.56 ± 0.98
	KC	26.94 ± 2.11^{abc}	34.04 ± 1.43	21.43 ± 0.73	17.59 ± 0.80
	NC	27.11 ± 0.33^{abc}	34.75 ± 1.50	19.90 ± 0.19	18.24 ± 1.16
121	CB	24.87 ± 1.67^{c}	35.33 ± 0.70	20.38 ± 0.67	19.42 ± 0.47
	KC	25.58 ± 0.65^{bc}	35.11 ± 0.89	21.61 ± 1.16	17.71 ± 1.04
	NC	24.69 ± 0.99^{c}	35.54 ± 1.06	20.05 ± 1.06	19.71 ± 2.57

$^{a-d}$ Mean values with different superscripts in the same column within each vibrational spectroscopy technique differ significantly ($P < 0.05$).

Principle component analysis (PCA) was carried out to study the relationship between vibrational spectra (SR-FTIR and FT-Raman) and quality of cooked chicken meat at different heating temperature. The results showed that PCA clearly distinguish among different heating temperatures and also in types of three chicken breeds (Figure 5.6a). Two main principle components (PC) were extracted that accounted for 69% of the total variability (PC-1 explains 48% variance and PC-2 is 21% (Figure 5.6a). Heating temperature at 90 and 121 °C and are separated from low (70 °C) heating temperature along PC-1, while the differences of NC from CB and KC meat is visible in PC-2 (Figure 5.6a). PC-1 showed the stepwise structural changes in meat cooked at 70 to 121 °C. In order to assess specific spectral features that are classification of the chicken meat samples presented in PCA score plots, the correlation loading plot are displayed in Figure 5.6b. Corresponding, a summary of tentative assignments of the IR and Raman bands, which is in accordance with literature are given in Table 5.3. (Beattie, Bell, Borggaard, and Moss, 2008; Ellis, Broadhurst, Clarke, and Goodacre, 2005; Herrero, 2008a; Herrero, 2008b; Kang, Li, He, Ma, and Song, 2017; Marinkovic and Chance, 2006; Schmidt, Scheier, and Hopkins, 2013; Sinanoglou, Cavouras, Xenogiannopoulos, Proestos, and Zoumpoulakis, 2018). The correlation loading plot (Figure 5.6b) for PC-1 reveals that cooked chicken meats at low temperature (70 °C) were positively correlated with higher moisture content, lightness (L*), ADP, AMP, and inosine content as well as α-helical structure obtained from FT-Raman spectra. These quality parameters correlated with Raman spectra of O-H stretching of water (3217 cm^{-1}) which can infer to moisture content in chicken cooked at 70 °C, which was higher than those cooked at 90 and 121 °C. Moreover, high intensity of Raman spectra at 1651 cm^{-1} (α-helix

structures) was assigned to amide I region and 527 cm^{-1} are indicated that α-helix conformation and S-S stretching of disulfide bonds in the "gauche-gauche-trans" conformation was predominant in meat cooked upon heating at low temperature (70 °C). A positive correlation was found between cooked chicken meat at 90 and 121 °C and WHC, cooking loss, yellowness (b*), GMP and hypoxanthine content. These quality parameters showed correlation with Raman bands at region of C-H stretching vibrations of methyl and methylene groups at 2968, 2910, 2860 cm^{-1}, the peaks arising from 1020, 990 cm^{-1} could be ascribed to C-C stretching vibrations of β-sheet structure. The present study provides information that heating temperature at 90 and 121 °C had a significant effect of unfolding protein structure and increase in β-sheet structure. Moreover, the high intensity of Raman bands at 891 and 843 cm^{-1} corresponded to tryptophan and tyrosine, indicated that buried residues the environment of trypthophan is more hydrophobic and involvement of hydrogen bonding, respectively. High hydrophobic and hydrogen bonding was correlated with tougher texture has previously been described (Herrero, 2008a)

Cooked NC meat and cooked KC, CB meat is differentiated by PC-2. The cooked KC and CB meat were characterized by high ATP. The observed SR-FTIR spectra at 1668 cm^{-1} is assigned to amide I of turns structure, and 1564 cm^{-1} is assigned to amide II of α-helix structure. The peaks at 1462 cm^{-1} attributed to C=O ester of fat, Moreover, the characteristic group frequency at 1414 cm^{-1} due to C–N stretching from amides and amines, respectively. This may be because of higher changes amides and amines during heating KC, CB meat than NC meat. The cooked NC meat correlated with β-sheet and random coil structure obtained from SR-FTIR spectra as well as SR-FTIR spectra C-H stretching (2914, 2864 cm^{-1}), which indicated

high expose of protein structure correlated with contained high β-sheet and random coil structure. Further, there was noticed that FT-Raman spectra mostly used differentiate meat quality from different heating temperature, while SR-FTIR spectra used to identify the chicken meat from different breeds.

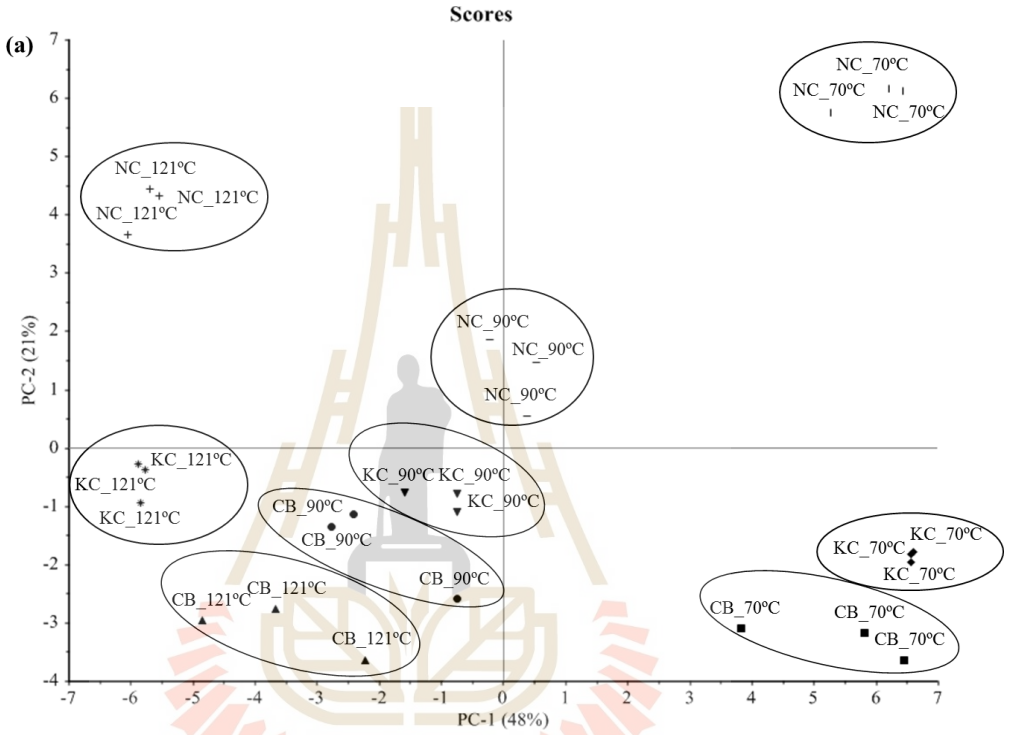

Figure 5.6 Plots of scores (a) and correlation loading (b) of Principle Component (PC)1 versus PC2 from average spectra of cooked breast meats from 3 chicken breeds (KC; Korat crossbred chicken, CB; Commercial broiler, NC; Native chicken) at different temperatures (70, 90, 121 °C). IR; SR-FTIR spectra, R; FT-Raman spectra.WHC; Water holding capacity, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate, L*; lightness, a*; redness, b*; yellowness.

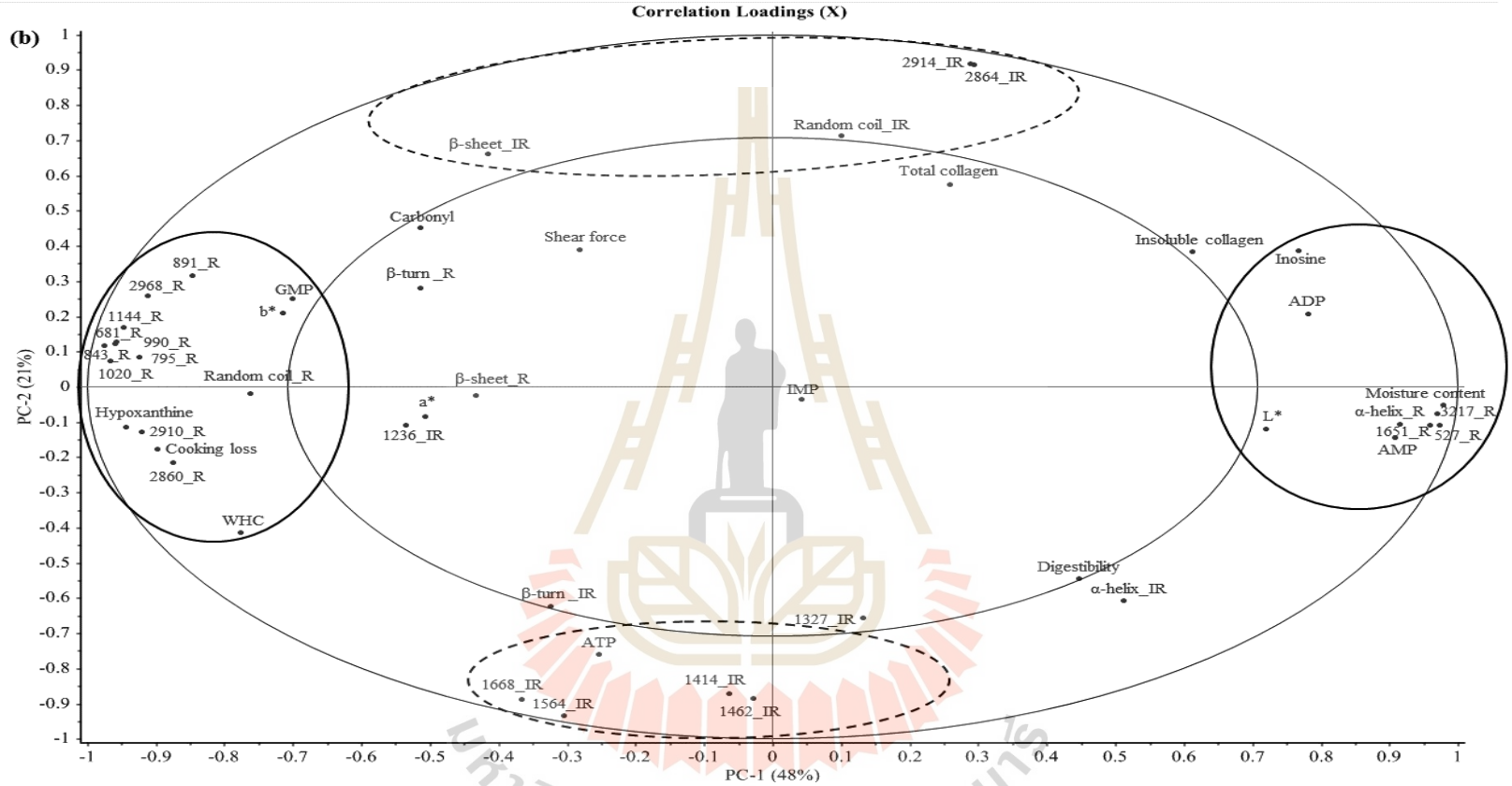

Figure 5.6 Plots of scores (a) and correlation loading (b) of Principle Component (PC)1 versus PC2 from average spectra of cooked breast meats from 3 chicken breed (KC; Korat crossbred chicken, CB; Commercial broiler, NC; Native chicken) at different temperatures (70, 90, 121 °C). IR; SR-FTIR spectra, R; FT-Raman spectra.WHC; Water holding capacity, IMP; inosine monophosphate, GMP; guanosine monophosphate, ATP; adenosine triphosphate, ADP; adenosine diphosphate, AMP; adenosine monophosphate, L*; lightness, a*; redness, b*; yellowness (Continued).

Table 5.3 Bands assignment of vibrational spectral of cooked CB, KC, and NC breast meat at different heat treatments (70, 90, and 121 °C).

Wavenumber (cm^{-1})		Vibrational mode
IR	**Raman**	
	3217	O-H stretching
	2968, 2910, 2860	C-H stretching
2914, 2864		C-H stretching
1668		Amide I (β-turn)
	1651	Amide I (α-helix)
1564		Amide II (α-helix)
1462		C=O stretching
1414		C-N stretching from amines
	1020, 990	C-C stretching (β-sheet)
	891	Tryptophan
	843	Tyrosine
	527	S-S stretching (gauche-gauche-trans)

5.5 Conclusions

The high heat treatment (121 °C) caused the meat from KC and NC become darker than CB meat. Heat treatments affect to increase WHC and cooking loss, while decrease in moisture content of all three chicken breeds. Taste enhancing compounds including, IMP and GMP showed higher retained in KC and NC meat than CB meat. The KC and NC meat were tougher correlated with higher shear force value than the CB meat at all heating temperatures. However, significant loss of total collagen content was found in NC meat. High heating temperature (121 °C) resulted in the highest loss of insoluble collagen content ($P < 0.05$). Heat treatment induced protein oxidation in all breeds. High heat treatment (121 °C) decreased the protein digestibility, while high protein digestibility was found at cooked chicken meat heating at 70-90 °C ($P > 0.05$). Application of PCA between SR-FTIR, FT-Raman spectra and physico-chemical properties revealed the spectra that high correlated with quality of cooked chicken meats. Low temperature (70 °C) cooked chicken meat accompanied by high moisture content and predominant in α-helix structure. These observed correlated with FT-Raman spectra at region of 3217 cm^{-1} (O-H stretching of water), and 1651 cm^{-1} (amide I; α-helix), respectively. Heating temperature at 90 and 121 °C revealed an expose of protein structure contribute to increase β-sheet and random coil structures, transform strong correlation with FT-Raman bands at region of C-H stretching (2968, 2910, 2860 cm^{-1}), and C-C stretching of β-sheet structure (1020, 990 cm^{-1}). Besides, changes in the microenvironment of tryptophan (891 cm^{-1}) and tyrosine (843 cm^{-1}) residues, which may lead to tough texture. Moreover, PCA can accomplish the classification the quality of cooked meat from different chicken breeds. The quality characteristics that

used to differentiate cooked KC and CB meat from NC meat were contained high α-helical structure, amides and amines correlated with SR-FTIR spectra at 1564 cm^{-1} (amide II; α-helix), and 1414 cm^{-1}, respectively. Cooked NC meat was characterized with β-sheet and random coil structures as well as SR-FTIR spectra of C-H stretching (2914, 2864 cm^{-1}), which indicated high expose of protein structure. These vibrational spectroscopic results have led to a further extension of applications of vibrational spectroscopy as an in-depth scientific with non-destructive and rapid tool for determine quality of cooked chicken meats.

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CHAPTER VI

SUMMARY

It has been demonstrated that protein content of KC meat increased with age of bird. The pH of thigh meat decreased, while the water holding capacity of breast meat increased as the age of chickens increased. Breast meat developed lighter appearance, while thigh meat was darker with increasing age. Cholesterol content of KC breast meat and fatty acids of thigh decreased with age. Inosine 5'-monophosphate (IMP) of breast meat decreased as KC grew older. Guanosine 5'-monophosphate (GMP) of breast meat also decreased, whereas increased in thigh meat. Higher shear force value corresponded with high total collagen content in older KC. Raman spectroscopy revealed that the α-helical structure decreased, while hydrophobic interactions and disulphide bonds in thigh meat increased with KC age. Moreover, PCA showed that KC meat quality was clearly distinguished from CB meat. Shear force values of older KC meat (16-20 weeks old) were well correlated with changes in the β-sheet structure of amide I regions as well as amide III of collagen. In addition, the shear force of KC breast meat was negatively correlated with the Raman spectra of O-H stretching at 3207 cm^{-1} and relative α-helical content. Besides, CB meat exhibited a high content of α-helix structure and fatty acids, correlating with Raman C-H stretching

At their market age, KC (10 weeks old) and NC (16 weeks old) meat showed higher protein and carbonyl content than CB meat (6 weeks old), while CB showed

higher moisture, lipid content, lightness (L*) and taste-active nucleotides (IMP and GMP) than formers. Furthermore, NC meat showed higher inosine and insoluble collagen content. KC meat exhibited highest water holding capacity (WHC). Based on PCA of spectra obtained from SR-FTIR, FT-Raman, and physico-chemical properties, chicken meat was clearly separated by breeds. Different chicken breeds have varied secondary structural proteins in muscle, predominant of β-turn and random coil were found in KC and NC meat, while mainly α-helix was found in CB meat. High moisture, lipid, and nucleotides content in CB meat corresponding to distinct wavenumbers obtained from FT-Raman spectra of O-H stretching of water (3203 cm^{-1}), C-H stretching of lipid (2854 cm^{-1}) and SR-FTIR spectra of PO_2^- stretching of nucleotides (1240 cm^{-1}), respectively. Furthermore, the C-C stretching of α-helix structure at 934 cm^{-1} obtained from FT-Raman correlated with high WHC in KC meat.

When meat from 3 breeds were heated at 70, 90, and 121 °C for 40 min, the cooked meat contained higher cooking loss and WHC, while lower in moisture content and insoluble collagen content with increase heating temperature. The high heat treatment (121 °C) caused the meat from KC and NC become darker than CB meat. IMP and GMP showed higher retention in KC and NC meat than CB meat. High heat treatment (121 °C) increased protein carbonyl, but decreased protein digestibility. PCA revealed that the cooked chicken meat at 70 °C correlated with FT-Raman spectra at region of O-H stretching of water (3500-3100 cm^{-1}), and amide I of α-helix structure (1651 cm^{-1}). Heating at 90 and 121 °C correlated with FT-Raman bands at C-H stretching (3100-2800cm^{-1}), C-C stretching of β-sheet structure (1020, 990 cm^{-1}).

BIOGRAPHY

Sasikan Katemala was born in October 9, 1981, at Nakhon Ratchasima, Thailand. She studied for her high school diploma at Satreesetthabudbumpen School, Bangkok, Thailand (1993-1999). In 2003, she received Bachelor's degree of Science (Chemistry) from Thammasat University and then received Master's degree of Science (Food Technology) from Chulalongkorn University in 2007. In 2013, she decided to study her Ph.D. program in Food Technology under support of Suranaree University of Technology and Thailand Research Fund.

She presented oral and poster presentation including: 1) The 19th Food Innovation Asia Conference (Bangkok, Thailand, 15-17 June 2017) with the honorable mention of poster presentation in title of "Physicochemical changes of Korat crossbred chicken meat in relation with ages", 2) The 20th Food Innovation Asia Conference (Bangkok, Thailand, 14-16 June 2018) with the poster presentation in title of "Influence of heat treatment on physicochemical changes and *in vitro* protein digestibility of Korat crossbred chicken meat", 3) The 6th Meat Science and Technology Conference (Bangkok, Thailand, 18-19 June 2018) with the oral presentation in title of "Infrared spectroscopy and textural properties of meat from 3 chicken breeds subjected to various thermal treatments" and poster presentation in the title of "Effect of age on physico-chemical properties of Korat chicken meat", and 4) The 21st Food Innovation Asia Conference (Bangkok, Thailand, 13-15 June 2019) with the poster presentation in title of "A comparative study of physicochemical properties and vibrational spectroscopy of chicken meat from different breeds".