Meat quality and Raman spectroscopic characterization of Korat hybrid chicken obtained from various rearing periods

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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 characterized. In this study, we characterized 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 and guanosine 5′-monophosphate 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 revealed that the meat quality of CB was greatly different from that of KC meat. High shear force values of KC meat at 20 wk of age were well correlated with an increase in the β-sheet structure (amide I) and amide III of collagen. Raman spectra at 3,207 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.

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

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INTRODUCTION

Chicken meat is considered a good source of protein with lower fat and cholesterol than other red meats (Jaturasitha et al., 2008b). Commercial chicken meat is derived from a fast-growing broiler strain that can grow within 5 to 6 wk (Choe et al., 2009). An alternative source is native chicken, which is considered a delicacy, particularly in Asian food cultures (Wattanachant et al., 2004; Guan et al., 2013; Jaturasitha et al., 2017) However, native chicken production is commercially limited because of its slow growth rate and lean muscle-gaining ability (Wattanachant et al., 2004; Jaturasitha et al., 2008a). It typically takes about 14 to 23 wk to reach a marketable size (Jaturasitha et al., 2017). For this reason, hybrid chicken has been developed in various regions throughout the world (Jaturasitha et al., 2008a; Park et al., 2010; Huang et al., 2011; Miguel et al., 2011; Łukasiewicz et al., 2014; Batkowska et al., 2015). Korat chicken (**KC**) is a crossbreed between Leung Hang Khao sires (Thai native chickens) and Suranaree University of Technology (**SUT**) 101 chicken dams (a crossbreed between broiler and layer chickens), which has demonstrated better growth performance than its sires. (Maliwan et al., 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 breast and leg muscles of hybrid chickens tended to improve with age. Meat from older animals was also darker in color because levels of myoglobin increased with age (Wideman et al., 2016). Crosslinked collagen increases with age, resulting in tougher texture of the meat from older animals

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(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 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 peroneus 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 et al. (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 minimize 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 (**CB**). 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 CB. In addition, Raman spectroscopy, in combination with PCA, was used to identify the distinct KC meat quality characteristics based on the slaughtering age.

MATERIALS AND METHODS

Animals and Sample Preparation

All procedures were approved by the Animal Ethics Committees of SUT. A total of 150 one-day-old KC were randomly distributed into 3 pens (50 chicks/pen) and reared indoors under the same environmental conditions at the 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 wk old), grower (5–6 wk old), and finisher (7–20 wk 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 wk, 5 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 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°C to 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 postmortem for all meat samples. The WHC and shear force of the meat were measured at 24 h postmortem. Color 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 mo, respectively. Before chemical analyses, frozen samples were thawed in a 4°C refrigerator for 12 to 18 h.

Proximate Composition

KC and CB breast and thigh meat were analyzed for moisture, ash, and protein content according to the Association of Official Analytical Chemists (AOAC) (2010). Total lipids were measured according to the study by Folch et al. (1957).

Physical Properties

Meat samples (1 g) were homogenized 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 the study by Ryoichi et al. (1993) by centrifugation at 6,710 × *g*, at 25°C for 10 min. The surface meat color was measured using a colorimeter (Hunter Associates Laboratory, Reston, VA) with a D_{65} light source. An average value taken from 3 different locations on each sample are presented.

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.

Fatty Acids Composition

Lipids were extracted according to the study by 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 was prepared by methylation using boron trifluoride (BF_3)-methanol, followed by separation in a gas chromatography (HP-7890; Agilent Technologies, Palo Alto, CA) equipped with a flame ionisation detector according to the method of Bostami et al. (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).

Cholesterol Analysis

Cholesterol extraction was performed according to the study by Rowea et al. (1999) with slight modifications. Cholesterol was quantified using a gas chromatograph-flame ionisation detector equipped with a HP-5 column (30 m × 0.32 mm; film thickness, 0.25 μm; Agilent Technologies, Palo Alto, CA). 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 performed by comparing the relative retention time of the sample with the standard (Carlo Erba Reagents, Milan, Italy).

Purine Analysis

Meat samples (0.5 g) were hydrolyzed with 70% perchloric acid for 1 h at 95°C according to the method of Kaneko et al. (2014). Purine bases were separated on an Asahipak GS-320 HQ column (7.5 × 300 mm, 6-μm particles; Showa Denko America, Inc., NY) equipped with a HPLC system (HP1260; Agilent Technologies). 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.

Changes of Nucleotides

The amounts of nucleotides from meat samples were measured according to the study by Kim et al. (2012) with slight modifications. The extracted nucleotides were analyzed using an HPLC (HP 1260; Agilent Technologies, Inc., Santa Clara, CA) equipped with a Hypersil ODS C18 reverse-phase column (4.6 × 150 mm, 3-μm particles; Thermo Scientific, Waltham, MA). The mobile phase A was 150-mmol potassium dihydrogen phosphate (KH_2PO_4) and 150-mmol potassium chloride (KCl), pH 6. 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 to 5 min; it was increased to 9% B for 5 to 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).

Collagen Content

Total collagen content was determined by alkaline hydrolysis as described by Reddy and Enwemeka (1996) with some modifications. Samples were hydrolyzed with 7M NaOH at 120°C for 40 min. The hydrolysate was neutralised with 3.5M sulphuric 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 the study by Liu et al. (1996).

Fourier-Transform Raman Spectroscopy

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 4,000-400 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 the 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 using 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 minimization of the effects of variable baselines. The baseline correction was made by offsetting and then extending the multiplicative signal correction. 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 1,700-1,600 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 3,801-2,704 and 1,803-399 cm^{-1} were processed as previous described and analyzed using the unscramble X 10.5 software (Camo, Oslo, Norway), using 3 average 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

Table 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^{b,c}	92.65 ± 1.77^{a}	3.08 ± 0.32^{b}	4.40 ± 0.09^{b}
		16	74.79 ± 0.23^{c,d}	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^{a,b}
	KC	8	76.63 ± 0.39^{a}	75.84 ± 1.34^{b}	9.98 ± 2.29^{c}	4.38 ± 0.37^{a,b}
		10	76.71 ± 0.46^{a}	81.74 ± 6.04^{a,b}	10.57 ± 1.32^{b,c}	4.12 ± 0.14^{b}
		12	76.82 ± 0.41^{a}	79.91 ± 4.36^{a,b}	13.13 ± 1.12^{b}	4.39 ± 0.05^{a,b}
		16	76.03 ± 0.83^{a,b}	80.68 ± 2.59^{a,b}	10.81 ± 1.38^{b,c}	4.53 ± 0.21^{a}
		20	76.13 ± 0.88^{a,b}	83.28 ± 2.79^{a}	8.85 ± 1.47^{c}	4.74 ± 0.11^{a}

$^{a\text{-}c}$Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type.
Abbreviations: CB, commercial broiler; db; dry basis; KC, Korat hybrid chicken.

variables. The correlation between selected Raman spectra and the physico-chemical properties was determined by Pearson's correlation.

Statistical Analysis

All experiments were conducted with 3 independent replications. The effect of age of chickens in each measured parameter was analyzed using ANOVA. Tukey's multiple range test was used to compare differences among mean values ($P < 0.05$). Mean values and 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).

RESULTS AND DISCUSSION

Proximate Compositions

The moisture content of KC breast meat decreased with the age of birds ($P < 0.05$; Table 1). Crude protein content of KC breast and thigh meat increased until 10 wk of rearing and remained constant afterwards. KC breast and thigh meat at 10 to 20 wk of age contained higher crude protein content than that of CB ($P < 0.05$), 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 the reasons for the lower lipid content in KC. Age had no effect on total lipid and ash content of KC until up to 20 wk of rearing ($P > 0.05$).

Physicochemical Properties

The pH values of KC breast and thigh meat decreased with increasing age ($P < 0.05$; Table 2). Older birds appeared to have more glycogen storage in both the breast and thighs, leading to a greater decrease in the postmortem pH (Díaz et al., 2010). In general, the pH value of breast meat was lower than that of thigh meat. Breast meat is composed of type IIB fibers, containing higher glycogen content (Listrat et al., 2016). Therefore, lactic acid accumulation postmortem 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-

Table 2. Physico-chemical properties of breast and thigh meat of Korat chicken from different ages (mean ± standard deviation).

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

$^{a\text{-}e}$Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type.
Abbreviations: CB, commercial broiler; KC, Korat hybrid chicken; WHC, water holding capacity.

Table 3. Fatty acid compositions of breast and thigh meat of Korat chicken from different ages.

		Fatty acids content (mg/g dry sample)					
			KC (wk)				
Muscle type	Fatty acids	CB (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}
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^{c,d}	27.15^{a,b}	20.69^{b,c}	17.77^{c,d}	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^{b,c}	30.00^{b}	22.30^{b,c}	17.73^{c,d}	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^{a,b}	26.20^{a,b,c}	32.98^{a}	23.51^{b,c,d}	20.61^{c,d}	16.06^{d}

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

Abbreviations: CB, commercial broiler; KC, Korat hybrid chicken; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

week-old KC and 6-week-old CB, which is their market age. Changes in meat color were subtle with age (Table 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 et al., 2017). Surface color did not show any differences between the 2 strains at their market age (10 wk for KC vs. 6 wk for CB). Overall, KC breast meat showed a darker appearance than CB meat, while the thigh color was comparable. The slow-growing birds

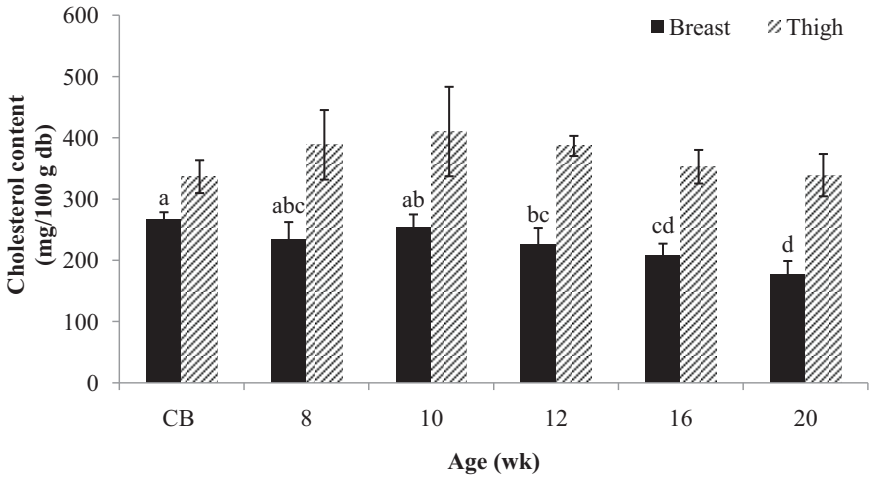

Figure 1. Cholesterol content in breast and thigh meat of Korat chickens from different ages. Means with different superscripts differ significantly ($P < 0.05$).

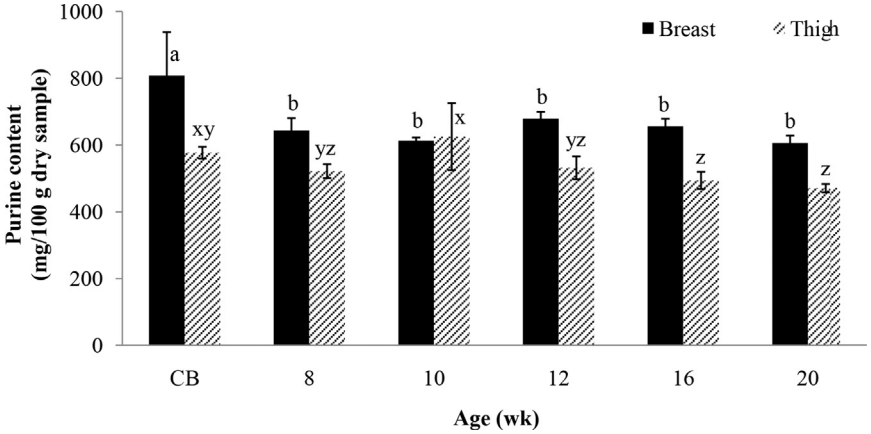

Figure 2. Total purine content in breast and thigh meat of Korat chickens from different ages. Means with different superscripts differ significantly ($P < 0.05$) as [a-b]within breast and [x-z]within thigh.

normally have redder meat than fast-growing birds because they are typically older with higher contents of haem pigments (Wideman et al., 2016).

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). In contrast, the contents of these fatty acids decreased in thigh meat of 16- to 20-week-old KC ($P < 0.05$; Table 3). Thigh meat of KC at 10 wk 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 (C22:6). Differences in fatty acid content reflected strain differences. In previous studies, Jaturasitha et al. (2008b) reported that Thai chickens had higher proportions of n-3 fatty acids than broiler chickens.

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 wk of age ($P < 0.05$; Figure 1). In contrast, changes in the cholesterol content in thigh meat were subtle ($P > 0.05$; Figure 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.

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- and 20-week-old birds, but that of the thigh meat slightly decreased with increasing age ($P > 0.05$) (Figure 2).

Table 4. Changes of nucleotides and their degradation products in chicken muscle at various rearing periods.

		Content (μg/g dry sample)					
			Rearing period of KC (wk)				
Muscle type	Compounds	CB (6 wk)	8	10	12	16	20
Breast	GMP	179.5 ± 26.2[a]	156.1 ± 7.2[a,b]	127.1 ± 9.7[b,c]	119.9 ± 8.3[c]	128.8 ± 33.4[b,c]	116.5 ± 16.00[c]
	IMP	3,766.0 ± 147.1[b,c]	5,254.7 ± 246.1[a]	4,465.6 ± 350.2[b]	2,722.5 ± 383.9[d]	3,756.0 ± 915.1[b,c]	3,196.1 ± 576.3[c,d]
	ADP	451.3 ± 106.6[a,b]	453.9 ± 12.5[a,b]	452.5 ± 41.3[a,b]	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[a,b]	119.5 ± 5.2[a,b]
	Inosine	1,058.5 ± 46.8[c]	811.1 ± 130.2[c]	1,045.6 ± 197.1[c]	1,885.4 ± 212.0[a]	1,616.8 ± 226.3[a,b]	1,458.2 ± 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	1,585.9 ± 504.6[c]	2,419.8 ± 427.2[a,b]	2,591.2 ± 262.6[a]	1,789.1 ± 351.4[c]	1,973.7 ± 201.2[b,c]	1,637.6 ± 314.0[c]
	ADP	437.4 ± 20.7[c]	492.1 ± 42.6[a,b]	450.1 ± 31.2[c]	530.5 ± 48.4[a]	469.7 ± 60.9[a,b]	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[a,b]	991.4 ± 51.4[a]	1,018.9 ± 55.5[a]

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

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; CB, commercial broiler; GMP, guanosine monophosphate; IMP, inosine monophosphate; KC, Korat hybrid chicken.

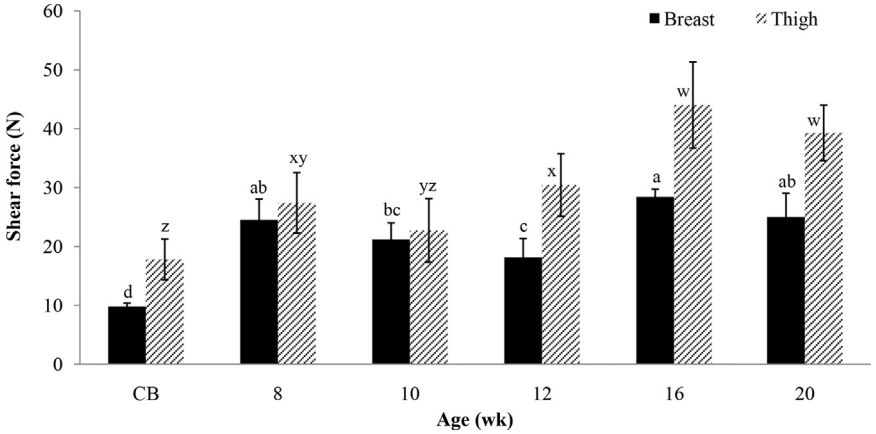

Figure 3. Shear force values of breast and thigh meat of Korat chicken from different ages. Means with different superscripts differ significantly ($P < 0.05$) as [a-d]within breast and [w-z]within thigh.

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 fibers, which exhibit a lower capacity for purine nucleotide degradation during muscle contraction than red muscle thigh meat containing type I, slow-twitch muscle fibers (Arabadjis et al., 1993).

IMP and GMP of KC breast meat decreased with age ($P < 0.05$; Table 4). IMP is a major taste active nucleotide in chicken meat that is approximately 20 to 30 times higher than GMP in breast meat and 10 to 20 times

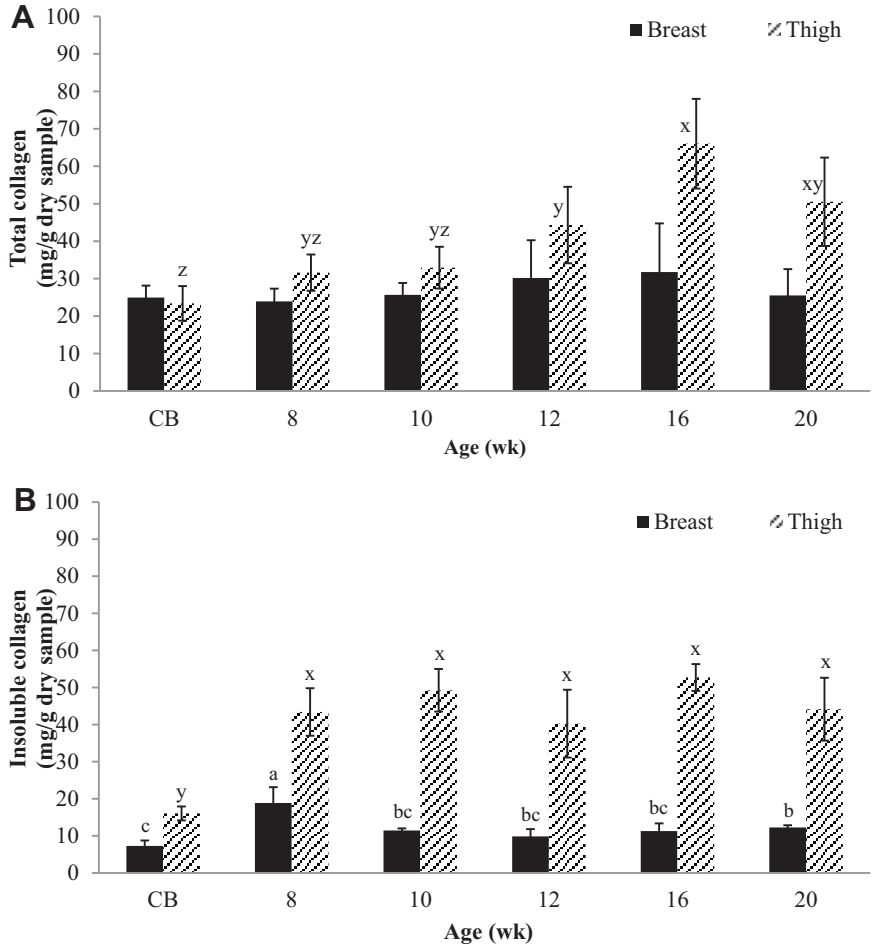

Figure 4. Total collagen (A) and insoluble collagen (B) content in breast and thigh meat of Korat chickens from different ages. Means with different superscripts differ significantly ($P < 0.05$) as [a-c]within breast and [x-y]within thigh.

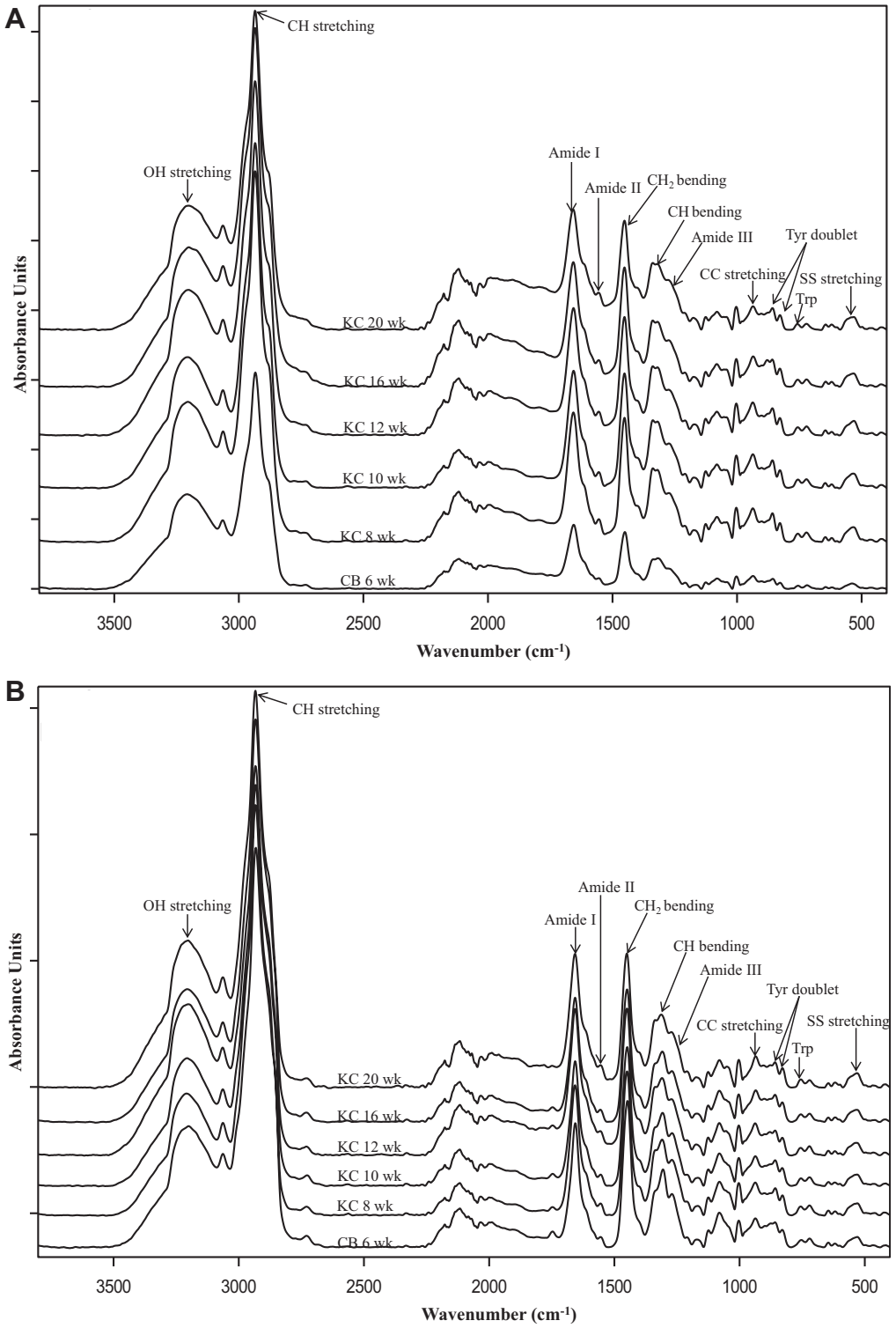

Figure 5. Raman spectra of breast (A) and thigh (B) meat of Korat chickens (KC) and commercial broiler (CB) chickens from different ages at a wavenumber of 3,800-400 cm^{-1}.

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 fibers with greater activity of 5′-nucleotidase, an enzyme catalyzing the degradation of IMP (Jayasena et al., 2014; Tullson and Terjung, 1999). This could explain the lower values of IMP in thigh meat. At 10 wk 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 5. Normalized intensities of Raman bands of Korat chicken breast and thigh meat from different ages (mean ± standard deviation).

Muscle type	Chicken breed	Age (wk)	Normalized intensities of the Raman bands ($\times 10^{-3}$)						
			O-H stretching (3,200 cm^{-1})	C-H stretching (2,935 cm^{-1})	CH_2 bending (1,450 cm^{-1})	C-H bending (1,320 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}	$3.95 \pm 0.03^{a,b}$	1.07 ± 0.01^{d}	0.07 ± 0.01^{a}	0.14 ± 0.01^{d}	1.11 ± 0.05	0.11 ± 0.01^{b}
	KC	8	0.76 ± 0.00^{b}	$3.94 \pm 0.06^{a,b}$	$1.36 \pm 0.04^{a,b}$	0.07 ± 0.01^{b}	$0.19 \pm 0.01^{a,b}$	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}	$0.17 \pm 0.01^{b,c}$	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}	$0.16 \pm 0.02^{c,d}$	0.91 ± 0.15	0.16 ± 0.01^{a}
Thigh	CB	6	0.62 ± 0.04^{b}	$4.00 \pm 0.04^{a,b}$	1.58 ± 0.05^{a}	0.26 ± 0.04^{a}	0.13 ± 0.03	$0.76 \pm 0.04^{a,b}$	0.10 ± 0.01^{b}
	KC	8	$0.65 \pm 0.07^{a,b}$	4.08 ± 0.09^{a}	$1.54 \pm 0.02^{a,b}$	0.18 ± 0.01^{b}	0.15 ± 0.01	0.85 ± 0.08^{a}	0.12 ± 0.01^{b}
		10	$0.67 \pm 0.03^{a,b}$	$3.97 \pm 0.03^{a,b}$	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}	$0.16 \pm 0.01^{a,b}$	0.17 ± 0.01	0.80 ± 0.07^{a}	0.13 ± 0.01^{b}
		16	$0.68 \pm 0.03^{a,b}$	$4.01 \pm 0.03^{a,b}$	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	$0.70 \pm 0.09^{a,b}$	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-d]Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type.
Abbreviations: CB, commercial broiler; KC, Korat hybrid chicken.

Textural Properties

Shear force values of KC thigh meat increased with an extended rearing period ($P < 0.05$; Figure 3). An increase in shear force values corresponded to an increased collagen content (Figure 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 that of CB ($P < 0.05$), resulting in a tougher and chewier texture. Moreover, crossl-inking of collagen increases with age (Fletcher, 2002), which would likely occur to a greater extent in KC at 10 wk of age than CB. A softer texture in CB was correlated with a lower insoluble collagen content (Figure 4B). Our results demonstrated that the texture of the hybrid chicken, KC, was distinctively different from that of broilers, which was attributed to the difference in collagen content between these 2 strains.

Raman Spectroscopy

KC meat at various ages and CB meat showed distinct Raman spectra (Figure 5). The normalized intensity of a Raman band at 3,200 cm^{-1} indicated that water O-H stretching was higher in CB breast meat (Table 5). In contrast, O-H stretching of KC thigh meat appeared to be higher than that in its CB counterpart. These changes were correlated with moisture content (Table 1). KC meat showed a decrease in Raman intensity of C-H stretching at 2,935 cm^{-1} and CH_2 bending at 1,450 cm^{-1} as the rearing period was extended. The decrease of intensity of these bands may have resulted from hydrophobic interactions of aliphatic residues (Lippert et al., 1976). The Raman intensity at 1,320 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 tyrosine (Tyr) doublet ratio (I_{857}/I_{827}) observed in thigh meat of 20-week-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 KC grew older. Proteins with disulfide bonds show Raman bands near 510, 520, and 540 cm^{-1}, corresponding to the conformation of

Table 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 (1,65-8-1,645 cm^{-1})	β-sheet (1,680–1,665, 1,640–1,610 cm^{-1})	Random coil (1,665–1,660 cm^{-1})	β-turn (1,690–1,680 cm^{-1})
Breast	CB	6	46.34 ± 0.78^{a}	25.33 ± 1.26	16.79 ± 0.67	11.54 ± 0.24
	KC	8	$45.10 \pm 0.85^{a,b}$	26.24 ± 0.84	17.01 ± 0.33	11.65 ± 1.08
		10	$43.11 \pm 1.44^{a,b}$	28.15 ± 1.49	17.05 ± 1.07	11.69 ± 1.25
		12	$43.12 \pm 0.30^{a,b}$	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	$46.19 \pm 0.55^{a,b}$	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-c]Mean values in the same column with different superscripts differ significantly ($P < 0.05$) within the same muscle type.
Abbreviations: CB, commercial broiler; KC, Korat hybrid chicken.

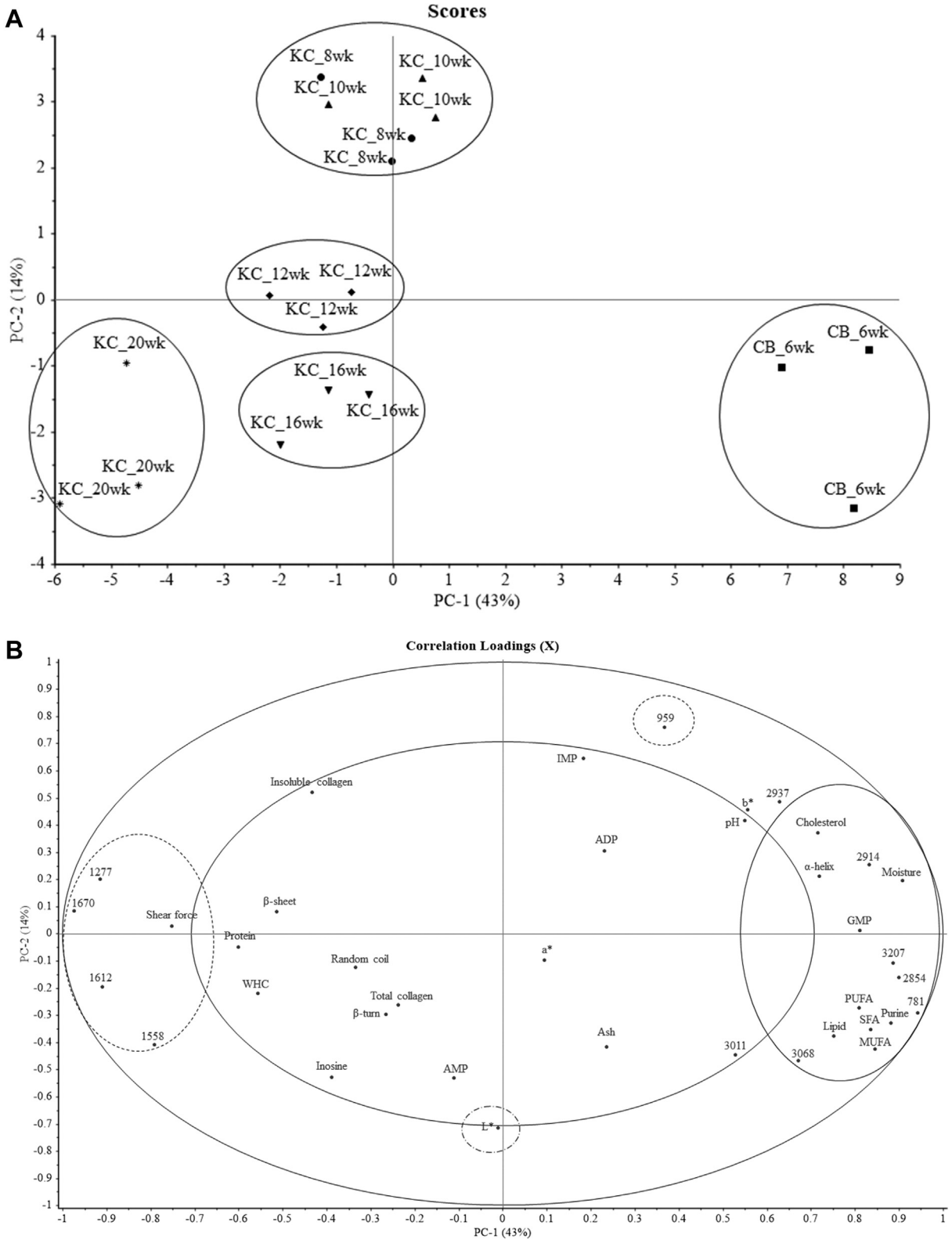

Figure 6. PC score plot and correlation loading plot (PC-1 vs. PC-2) of Raman spectra and physico-chemical properties of breast (A and B) and thigh (C and D) meat from KC of different ages. Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CB, commercial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

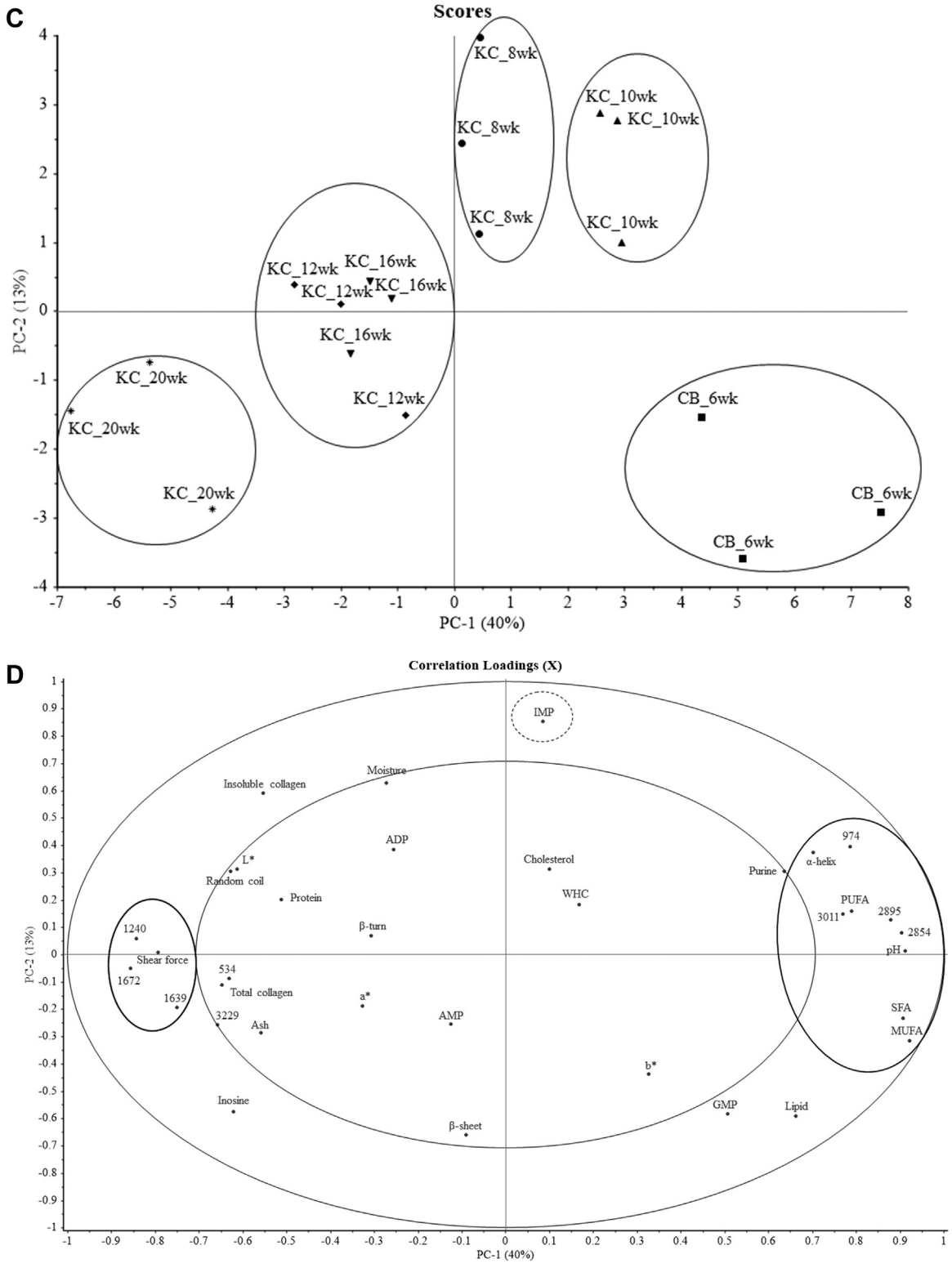

Figure 6. Continued

"gauche-gauche-gauche", "gauche-gauche-trans", and "trans-gauche-trans", respectively (Li-Chan et al., 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 imply 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 et al., 2017).

When secondary structure was analyzed using the amide I spectral profile, α-helical structure (1,657-1,645 cm^{-1}) decreased with increasing age of KC (Table 6). It should be noted that thigh meat exhibited higher α-helical structure than breast meat in both

Table 7. Raman bands assignment of observed spectral of chicken meat.

Wavenumber (cm^{-1})	Vibrational mode
3,207	O-H stretching
3,011, 2,914, 2,895, 2,854	C-H stretching
3,068	CH=CH stretching of unsaturated fatty acids
1,658-1,645	Amide I (α-helix)
1,680-1,665, 1640-1,610	Amide I (β-sheet)
1,665-1,660	Amide I (random coil)
1,690-1,680	Amide I (β-turn)
1,558	Amide II
1,277	Amide III (collagen)
1,240	Amide III (β-sheet and random coil)
974	C-C stretching
959	C-C stretching (α-helix)

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.

The 2 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 6A). Raman bands with high correlation loading are displayed in Table 7 (Li-Chan et al., 1994; Li-Chan, 1996; Beattie et al., 2004, 2008; Ngarize et al., 2004; Herrero, 2008a, 2008b; Phongpa-Ngan et al., 2014; Fowler et al., 2015; Herrero et al., 2017). Shear force values and Raman spectra at 1,670 cm^{-1} and 1,612 cm^{-1} (amide I of β-sheet structure), 1,558 cm^{-1} (amide II), and 1,277 cm^{-1} (amide III of collagen) were characteristics of older KC breast meat, especially KC at 20 wk of age. 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 3,207 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 3,207 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 3,068 cm^{-1} (CH=CH stretching of unsaturated fatty acids) and C-H stretching of fatty acids (2,895 and 2,914 cm^{-1}; Figures 6A and 6B). Moreover, the second PC revealed that the KC breast meat of 8- to 10-week-olds was highly correlated with the C-C stretching of the α-helix structure (959 cm^{-1}; Figures 6A and 6B), suggesting predominant α-helices in young KC birds.

In thigh meat, the first PC discriminated various ages of KC and CB (Figure 6C). Younger KC thigh meat (8–10 wk old) had quality characteristics similar to CB thigh meat. High shear force as well as high intensity at 1,672, 1,639 cm^{-1} (amide I of β-sheet structure), and 1,240 cm^{-1} (amide III of β-sheet and random coil structure) were distinct features of KC at 20 wk of age. CB thigh meat exhibited a higher α-helix structure, pH, and fatty acids (SFA, MUFA, PUFA) and high intensity of Raman bands at 3,011, 2,895, 2,854 cm^{-1} (C-H stretching), and 974 cm^{-1} (C-C stretching) (Figure 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 to 10 wk of age showed distinct IMP content characteristics. Raman spectroscopy was shown to be a powerful technique and provided insightful information on muscle protein structure between these 2 breeds.

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 wk 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 3,207 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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DISCLOSURES

Authors declare no conflict of interest.

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