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Synchrotron fourier transform infrared microspectroscopy and scanning electron microscopy assessment of key physical meat properties of Thai native chickens for selection in breeding programs

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Abstract

In this study, synchrotron-based Fourier transform infrared spectroscopy (S-FTIR) microspectroscopy and scanning electron microscopy (SEM) were employed to examine the key physical meat properties of the muscle texture and secondary structure of protein from the chicken breast meat in Thai native chickens (TNCs), Thai synthetic chickens (TSCs), and Thai native crossbred chickens (TNC crossbreds) compared to commercial broilers (BRs). In total, 500 one-day-old chickens of all breeds were raised until the slaughter weight of each breed was reached. The experiment was a completely randomized design, and breast meat samples were collected from each breed at the market weight. SEM analysis was employed to identify the texture characteristics of chicken meat among different groups. Compared to BR birds, TNCs, TSCs, and TNC crossbreds exhibited higher numbers of muscle fibers and lower muscle fiber diameters. S-FTIR analysis revealed differences between the average original spectra and normalized spectra, reflective of the absorbance of the protein secondary structure and lipids among the groups. TNC meat contained protein with an increased content of α-helix and lower lipid content; therefore, TNC breeds can potentially provide chicken meat with a good texture and be suitable to be functional chicken meat; in addition, it can be beneficial for designing breeding programs for subsequent breed development.

Keywords: Synchrotron-based FTIR microspectroscopy, Scanning electron microscopy, α-helix secondary protein, β-turn secondary protein, Thai native chicken

1. Introduction

Currently, slow-growing chicken breeds are the global industry standard in terms of chickens raised for meat, largely prompted by concerns about quality and sustainability as well as increased concerns about animal health. Therefore, currently, the global market preference for chicken meat, as well as their raising, has changed to slow-growing chicken breeds in France, the United Kingdom, the Netherlands, and Germany, as well as in Asian countries [1-3]. For this reason, currently, animal breeding is focused on the introduction of new chicken breeds that are compatible with native chickens as a method to respond to the global market demand for chicken meat exhibiting optimal nutritional characteristics, healthy consumption, food safety, as well as raising systems, as all these features can affect the consumer preference and acceptance [4]. However, texture characteristics of chicken are the key issue in terms of the physical properties of meat; especially, its chewiness, juiciness, and taste are some of the main attributes that consumers appreciate, leading to the preference for slow-growing chicken breeds over fast-growing commercial breeds [4,5].

Characteristics of chicken meat originate from its distinct physical properties. The chewy, juicy, firm, and tender texture of native chicken meat has become increasingly important in the global market as a result of changing consumer preferences [6,7]. Muscle fibers constitute one of the most significant factors that affect these characteristics and play a critical role in the quality of chicken meat via the effects of tenderness and taste, as well as meat eating qualities [6]. The ability to characterize muscle fibers of native chicken breeds has improved, which is crucial for animal breeding because it creates a new breeding goal for improving chicken meat quality to meet the current market trends and consumer desires.

Over the past few years, the Network Center for Animal Breeding and Omics Research, Faculty of Agriculture, Khon Kaen University, Thailand, has been successful in improving the morphological and production traits of Thai native chickens (TNCs) with selection indices that include growth efficiency, egg production, and chest circumference. For example, the Pradu Hang Dam Mor Kor 55 (PD) chicken has demonstrated good growth performance, while the Chee KKU 12 (CH) chicken has demonstrated excellent egg production [8]. As terminal crosses, Thai synthetic chickens (TSCs) have incorporated the TNC features of the CH breed to produce four Thai synthetic chicken breeds: Kaen Thong KKU50 (KT), Khai Mook Esarn KKU50 (KM), Soi Nin KKU50 (SN), and Soi Pet KKU50 (SP). Recently, TNC features have been retained in a Thai native crossbred chicken (TNC crossbred) known as KKU-ONE. This terminal cross between a TSC and a commercial broiler (BR) exhibits a native genetic fraction of 25%, with superior growth performance.

The improved growth and egg laying performance of these new breeds might alter the physical properties of their meat and could adversely affect meat tenderness. Generally, compared to slow-growing breeds, fast-growing chickens exhibit high muscle fiber diameters, and this difference exhibits a negative correlation with the chicken meat quality [9,10]. Therefore, it is crucial to identify the characteristics of chicken muscle fibers; however, the fibers are not the only important physical properties. In addition, the protein quality, and particularly the α-helixes of the protein secondary structure, exert a strong influence on the muscle fiber digestibility, as meat with a high percentage of α-helix proteins is more digestible [11,12]. Therefore, it is interesting to identify the key physical properties, i.e., characteristics of chicken muscle fibers and secondary protein structure of proteins, of TNC breeds and their crossbreds that have been improved under selection indices for revealing individual knowledge and assessing the suitability of the chicken breeds for use in breeding programs.

Typically, scanning electron microscopy (SEM) has been employed to assess properties or characteristics of chicken meat, which provides detailed surface information by the application of a directed electron beam in a raster pattern [13]. Previous studies have employed SEM to determine the microstructure, as well as the muscle fiber thickness and diameter, of chicken meat to assess chicken meat quality [14-16]. For example, Kang et al. [14] have employed SEM to examine the quality characteristics of chicken meat in salt at different temperatures. The secondary structure of proteins, such as the presence of α-helixes, is typically evaluated by synchrotron-based Fourier transform infrared (S-FTIR) microspectroscopy [11,12]. By this protocol, a synchrotron light beam, rather than a normal infrared beam, is used as the synchrotron exhibits a higher spatial resolution, and it can be applied to investigate the chemical composition of a tissue sample at the subcellular level. The brightness of the synchrotron light permits the examination of the chemical structure with better sensitivity and an improved signal-to-noise ratio. The beam size can be applied to measure samples with dimensions of 10×10 μm^2 compared to the conventional IR sample of about 50×50 μm^2 [11,12,17].

Previous studies have employed the use of S-FTIR microspectroscopy to determine protein secondary structures of flax seeds and during rumen degradation [11,12]. For example, Guo and Wang [18] have employed S-FTIR to confirm that the secondary structures of myofibrillar proteins are α-helixes and β turns. Therefore, in this study, SEM and S-FTIR microspectroscopy are employed to examine the chicken meat characteristics of TNC, TSC, and TNC crossbred chickens and compare these characteristics with those of meat obtained from BR. In this study, the aim was to identify the new breed that is most likely to have meat of a suitable texture, corresponding to a higher proportion of secondary α-helix structures, to aid in the future design of breeding programs for slowing-growth chicken breeds.

2. Materials and methods

2.1 Chicken breeds used in the study

Khon Kaen University's Institutional Animal Care and Use Committee (IACUC-KKU-58/62) provided approval for this experiment. The Network Center for Animal Breeding and Omics Research, Faculty of Agriculture, Khon Kaen University, Thailand, provided one-day-old chickens for two strains of TNCs (100% Thai native), referred to as PD and CH, respectively, TSC (50% Thai native), referred to as KM, while TNC crossbred (25% TNC), referred to as KKU-ONE. Except, 1-day-old BR (0% TNC) chickens were purchased from Arbor Acer, a division of the Charoen Pokphand Company.

2.2 Raising chickens and meat sample preparation

In the experiment, a totally randomized approach of four replicates per chicken breed was employed. Chickens from 1 to 3 weeks of age *ad libitum* with a starter diet, comprising 21% crude protein (CP) and 3,100 kcal of metabolizable energy (ME) per/kg, were used. Furthermore, these chickens were raised under the same conditions: (housing, vaccine program, and feeding). From 4 weeks of age until slaughter, the chickens were fed by a commercial broiler ration containing 19% CP and 3,200 kcal of ME per/kg with a growing diet. At the hatch date (0 day), 2, 4, 6, 8, 10, and 12 weeks, the live/body weights and feed intake were recorded. The chicken breast (*pectoralis major*) of the TNC crossbred, TSC, and TNC chickens was sampled at 8, 10, and 12 weeks of age, respectively, based on the weight of the retail cut market in Thai supermarkets of 2000-2200 g. The BR chickens were sampled at 6 weeks based on the weight of the retail cut market in Thai supermarkets of 2000-2200 g. The slaughter and collection of chicken meat have been described elsewhere [5,15]. The carcasses were plucked using a rotary-drum picker for 30 s after bleeding out, scalded at 60°C, and eviscerated. A 1 × 2 × 1 cm piece of the left *pectoralis major* was dissected from the middle region and fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for SEM analysis. Another 3 × 4 × 1 cm sample was dissected, placed into a vacuum-sealed bag, and stored overnight at 4 °C for S-FTIR analysis.

2.3 Physical properties

2.3.1 Identification of muscle fiber characteristics by scanning electron microscopy (SEM)

Chicken breast samples were washed with running water for 5 min and placed in an embedding cassette and cut into 0.5 × 0.5 × 0.5 cm^3 pieces. The specimens were subsequently dehydrated as previously described [15], with some adjustments in the graded ethanol series (25%, 50%, 70%, 95% and twice in absolute ethanol) for 30 min in each solution. Then, the water and ethanol were replaced with CO_2 by critical point drying (CPD) for 1 h. The sample was coated with gold for 3 min to increase the electron signal used for identifying the muscle fiber characteristics. SEM was set at an accelerating voltage of 10 kV for photography and under 500× magnification (10 kV) for recording video prints of 10 measurements per sample. Muscle images were recorded by SEM (Hitachi S-3400N, Japan). Numbers of muscle fibers and fiber diameters were recorded by Image J software [19].

2.3.2 Statistical analysis

The least-square means (LS means) of the body weight at the market weight as well as the muscle fibers numbers (MFNs) and fiber diameters across chicken breeds were analyzed by the analysis of variance (ANOVA) using the general linear model (GLM): $Y_{ijkl} = Breed_i + Sex_j + Breed_i * Sex_j + \varepsilon_{ijkl}$, where Y_{ijkl} represents the body weight, number of muscle fibers, or fiber diameter; $Breed_i$ represents KKU-ONE, KM, CH, PD, or BR; Sex_j represents male or female; and $Breed_i * Sex_j$ represents the interaction of breed and sex. The term ε_{ijkl} corresponds to the experimental error determined by the SAS statistical program.

2.3.3 Identification of the protein secondary structure by synchrotron-based FTIR microspectroscopy

2.3.3.1 Sample preparation

Approximately 0.5 × 1.0 × 0.5 cm^3 pieces were dissected from 60 chicken breasts and frozen in liquid nitrogen. The tissue pieces were embedded in an optimal cutting temperature (OCT) medium and stored at −80 °C until S-FTIR analysis. The frozen samples were cut into sections with a thickness of ~7 µm using a cryostat (Leica) at a temperature of −20 °C, and the sections were placed on barium fluoride (BaF_2) window slides (MirrIR slides, Tienta Sciences, OH, USA).

2.3.3.2 Raw spectra

Raw spectral data were recorded on an infrared microspectroscopy beamline (BL4.1 IR Spectroscopy and Imaging) at the Synchrotron Light Research Institute (SLRI), and computed the absorbance units of the average initial spectra were used to determine the secondary structure of the proteins. The spectra were recorded by using an IR microscope (Hyperion 2000, Bruker) and a Vertex 70 FTIR spectrometer (Bruker Optics, Ettlingen, Germany). The detector of the infrared microscope was a 100-µm mercury cadmium telluride (MCT-A) detector that was cooled by liquid nitrogen. FTIR measurements were recorded by using a 10 × 10 µm aperture and a spectral resolution of 6 cm^{-1}, with 64 scans co-added over a measurement spectrum from 4000 to 800 cm^{-1}. The OPUS 7.5 program (Bruker Optics Ltd, Ettlingen, Germany) was utilized for spectral acquisition and instrument monitoring.

2.3.3.3 Data analysis for S-FTIR spectra

The normalized spectra represented the normalized absorbance of the secondary structure analyzed with 300 The normalized spectra represented the normalized absorbance of the secondary structure analyzed with 300 good spectra obtained the absorbance 0.4 – 1.2 (25 spectra/sample). Next, these spectra were converted to the 2nd derivative spectra using 13 smoothing points, reduced the original spectra by averaging, and vector-normalized to consider the effects of differing sample thicknesses using the Savitzky-Golay method in the Unscrambler X software (version 10.1, Camo Analytics, Oslo, Norway).

3. Results and discussion

3.1 Body weight performance

The BRs (6 weeks) exhibited a significantly higher body weight at the market weight than those of the TNC crossbreds (8 weeks), TSCs (10 weeks), and TNCs (12 weeks) ($P < 0.0001$), similar to the results reported previously [20]. In addition, the CH utilization the TSCs and TNC crossbreds retained native genetic fractions of 50% and 25% known in the KM and KKU-ONE chickens, respectively. These breeds performed better in terms of body weight than TNCs, with the KKU-ONE crossbred exhibiting a higher growth rate intermediate between those of the TNCs and BRs. Notably, the body weight of the PD TNC breed was similar to that of the TSCs and greater than that of the CH breed (Table 1). Meanwhile, the interaction between breed and sex did not exert any effect on the body weight at the market weight ($P > 0.05$) (Table 1).

Table 1 Muscle fiber number (MFN) and fiber diameter (FD) of chicken breasts at marketing weight (LS means ± SE)

Effects	Breed/Age					SEM	p-Value
	BR (6wk)	KKU-ONE (8wk)	KM (10wk)	CH (12wk)	PD (12wk)		
Breeds							
Market weight (g)	2254.15±20.31^{A}	1634.73±21.73^{B}	1236.22±25.28^{C}	1142.40±25.50^{D}	1264.61±35.67^{C}	25.70	<.0001
MFN/0.05mm^2	16.26±1.21^{A}	23.29±1.12^{B}	32.59±1.78^{C}	31.23±1.22^{C}	31.2±1.23^{C}	1.19	<.0001
FD (μm)	64.42±2.52^{D}	40.49±0.46^{C}	31.52±0.54^{AB}	29.91±0.65^{A}	32.52±0.48^{B}	0.93	<.0001
Breed × sex							
Market weight (g)							0.2261
Male	2436.33±32.23^{AX}	1747.94±30.73^{BX}	1381.97±30.28^{CX}	1266.84±35.31^{DX}	1125.38±34.62^{CX}	32.63	
Female	2071.96±24.72^{AY}	1518.52±30.73^{BY}	1085.42±40.50^{CY}	1007.96±36.81^{DY}	1406.85±62.42^{CY}	39.04	
SEM	28.48	30.73	35.39	36.06	48.52		
p-value	<.0001	<.0001	<.0001	<.0001	0.0001		
MFN/0.05mm^2							<.0001
Male	16.55±1.75^{C}	20.95±1.75^{B}	39.95±1.66^{AX}	30.20±1.75^{A}	33.60±1.75^{A}	1.73	
Female	15.86±1.67^{C}	24.29±1.40^{B}	25.23±1.66^{AY}	32.14±1.71^{A}	28.80±1.75^{A}	1.64	
SEM	1.71	1.58	1.67	1.73	1.75		
p-Value	0.7765	0.1377	<.0001	0.4271	0.0535		
FD (μm)							<.0001
Male	69.12±3.03^{DY}	45.64±1.04^{CY}	33.90±0.48^{AB}	32.99±0.42^{A}	37.94±0.57^{BY}	1.26	
Female	59.71±2.08^{DX}	35.34±0.49^{CX}	29.13±0.95^{AB}	26.83±1.16^{A}	27.10±1.15^{BX}	1.17	
SEM	2.55	0.76	0.71	1.16	0.86		
p-Value	<0.0001	<0.0001	0.8470	0.9496	0.0004		

$^{A-D}$ within rows indicated a significant difference among the genotypes ($P<0.0001$). $^{X, Y}$ within columns indicated a significant difference among the sexes ($P<0.0001$). Commercial broiler (BR); Pradu Hang Dam Mor Kor 55 (PD); Chee KKU 12 (CH); Khai Mook E-san (KM), and KKU-ONE chickens. SEM = Standard error of means.

3.2 Identification of the muscle fiber and protein secondary structure in chicken breast

3.2.1 Muscle characteristic determined by SEM

In this study, meat texture characteristics of chicken breasts were determined by the numbers and diameters of the muscle fibers. The interaction between breed and sex exhibited a highly significant effect on the number and diameter of muscle fibers ($P < 0.0001$). The numbers of muscle fibers and fiber diameters of the TNC, TSC, and TNC crossbred chickens were significantly greater and significantly less than those of BR chickens, respectively ($P < 0.0001$) (Table 1 and Figure 1). The fiber diameters were significantly higher for the KKU-ONE (TNC crossbred), TNC PD, TSC KM, and TNC CH breed ($P < 0.0001$) (from the highest to the lowest), whereas the KM and CH fibers did not exhibit any difference in terms of the muscle fiber number or MFNs ($P > 0.05$), whereas the fiber diameter of the PD breed was significantly greater than that of the CH breed ($P < 0.0001$), possibly reflecting the more rapid growth of the PD breed than that of the CH breed.

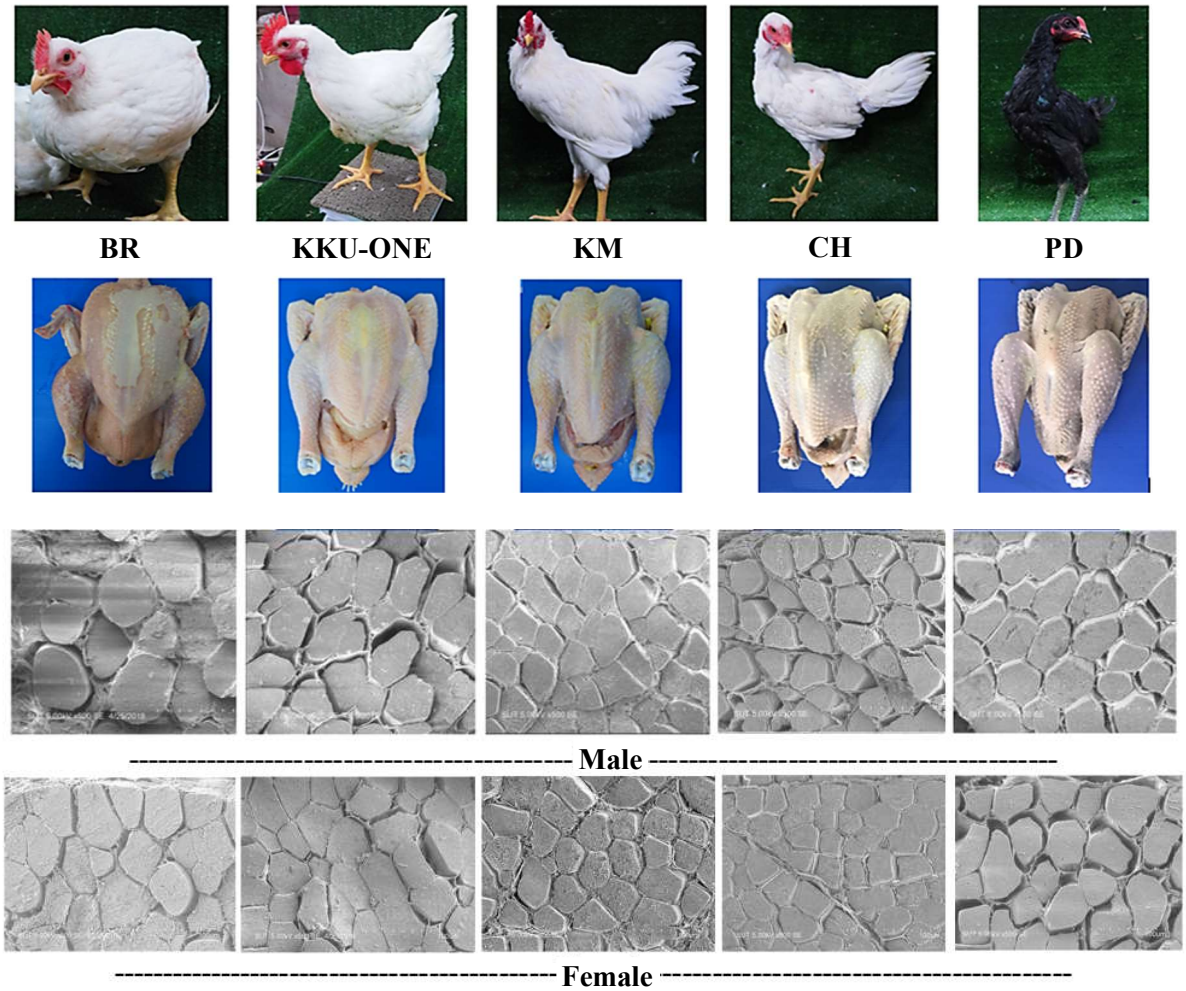

Figure 1 Scanning Eelectron microscope (SEM) image of muscle fibers of commercial broiler (BR); Pradu Hang Dam Mor Kor 55 (PD); Chee KKU 12 (CH); Khai Mook E-san (KM) and KKU-ONEchickens at marketing weight.

The body weight and muscle fiber characteristic results are interesting when the PD and KKU-ONE breeds are compared. The PD chicken, which was a Thai native purebred with 100% native blood fraction, was selected under the selection index due to its high growth rate, high egg production, and increased chest circumference. This study revealed some interesting features about the PD breed: Compared to the CH breed, the PD breed exhibited a better potential for growth performance, and it did not differ in growth characteristics in comparison to the synthetic KM breed, which exhibited a native blood fraction of 50%. Moreover, the MFNs for the KKU-ONE crossbred chicken was intermediate between those of the TNC and BR chickens, despite having a negative blood fraction level of only 25%. The KKU-ONE breeds also exhibited a lower diameter and higher muscle fiber

number than those of the BRs. Therefore, this crossbred may be a good selection for consumers who do not prefer a tough texture of TNC birds [6]. The majority of global consumers, including Thai consumers, prefer native chicken meat due to their more chewy, juicy, and tasty texture, all of the sensory properties that depend on the muscle fibers [4,5]. In addition, MFNs and fiber diameters affect the meat tenderness, as well as cooking loss, thawing loss, and sensory attributes [7,21]. According to Muth et al. [22], cooking loss of fast-growing chickens was significantly greater than that of slow-growing chickens. Chumngoen and Tan [7] have reported that the fiber texture characteristics are negatively correlated with the cooking loss, but positively correlated with attributes such as shear force, moisture release, and hardness. Waritthitham et al. [23] have demonstrated that the fiber cross-sectional area is positively correlated with the cooking and grilling loss. This result suggested that the PD and KKU-ONE breeds might sustainably increase the community incomes in comparison to the other breeds.

The interaction between breed and sex also significantly affected the MFN and fiber diameter ($P < 0.01$), for MFN as only the KM breed exhibited significant differences, where the males exhibited higher MFNs than those of the females. In terms of fiber diameter as the male of BR, KKU-ONE and PD breeds have larger of fiber diameter than those of the females (Table 1).

3.3 Secondary structure of proteins determined by synchrotron-based FTIR microspectroscopy

This fingerprint functional group for the chickens exhibited a unique molecular chemical structure (Table 2 and Figure 2). S-FTIR demonstrates the potential to identify differences in the lipid and protein secondary structures of TNCs, TSCs, TNC crossbreds, and BR chicken meat. Amide I exhibited an α-helix secondary structure of the protein, with an absorbance at ~1654 cm^{-1}, while the β-turn absorbance was observed at ~1685 cm^{-1}, which was in agreement with those reported previously [24,25]. In addition, differences among the TNC, TSC, TNC crossbred, and BR chickens were noticed in terms of the α-helixes and β-turn of the protein secondary structure. Moreover, compared to the other breeds, the two TNCs, viz. PD and CH, respectively, exhibited higher absorbance and lower absorbance for the α-helix protein structures and β-turns, respectively (Figure 3B).

Lipids of the considered chicken meat (Figure 2) were unique, with a CH_2 asymmetric stretching vibration at ~2927 cm^{-1} according to previous studies [24,25]. The result confirmed that S-FTIR can identify lipid differences among the TNC, TSC, TNC crossbred, and BR. The lipid profiles shown in Figure 3A, comprising average spectra and 2nd derivative spectra, indicated that the lipid content of the BR breed is greater than those of the TNC, TSC, and TNC crossbred chickens (Figure 3B).

The results obtained herein are in agreement with that reported previously [26]: Conventional techniques such as proximate analysis revealed that the fat content and protein content of TNC chickens are less than and greater than, respectively, those of commercial broilers. Similarity, Katemala et al. [27] have revealed that the α-helix structures of native chicken meat proteins are greater than those of commercial chickens. The α-helix secondary structure is known to affect digestibility, indicative of a better protein quality in TNC chickens [11,28]. By contrast, the β-turn secondary structure leads to decreased protein digestibility [29,30]. Therefore, compared to the other breeds, TNC chickens may provide more functional chicken meat. In addition, S-FTIR confirmed the lipid and protein differences of TNC, TSC, and TNC crossbred chickens compared with those of BR chickens.

The nucleic acid, carbohydrate, and glycogen contents among the chicken breeds were not clearly depicted by S-FTIR, possibly due to spectrum shifts and confounding of functional groups with similarity molecules. Another possibility is that the tissue preparation procedure used in this study might not have been suitable for nucleic acid measurements by S-FTIR (Figure 3C). Alternatively, some functional groups could have been small molecules that probably require extraction and further purification before analysis.

Table 2 Band assignment of functional groups of the chicken breasts at marketing weight

Band (cm^{-1})	Assignment: Functional groups	References
2927	Lipids: Asymmetric stretch CH_2	[24]
2871	Lipids: Symmetric stretch CH_3	[24]
2962	Lipids: Asymmetric stretch CH_3	[24]
1743	Phospholipids: CH_2–COOR	[24]
1730	Lipids: ν(C=O)	[24]
1685	Amide I (β-turn) protein: C=O stretching	[24, 25]
1654	Amide I (α-helix) protein: C=O stretching vibration (80%), C-N stretching	[24]
1546, 1513	Amide II: N–H bend (60%), C–N stretch (40%)	[24, 25]

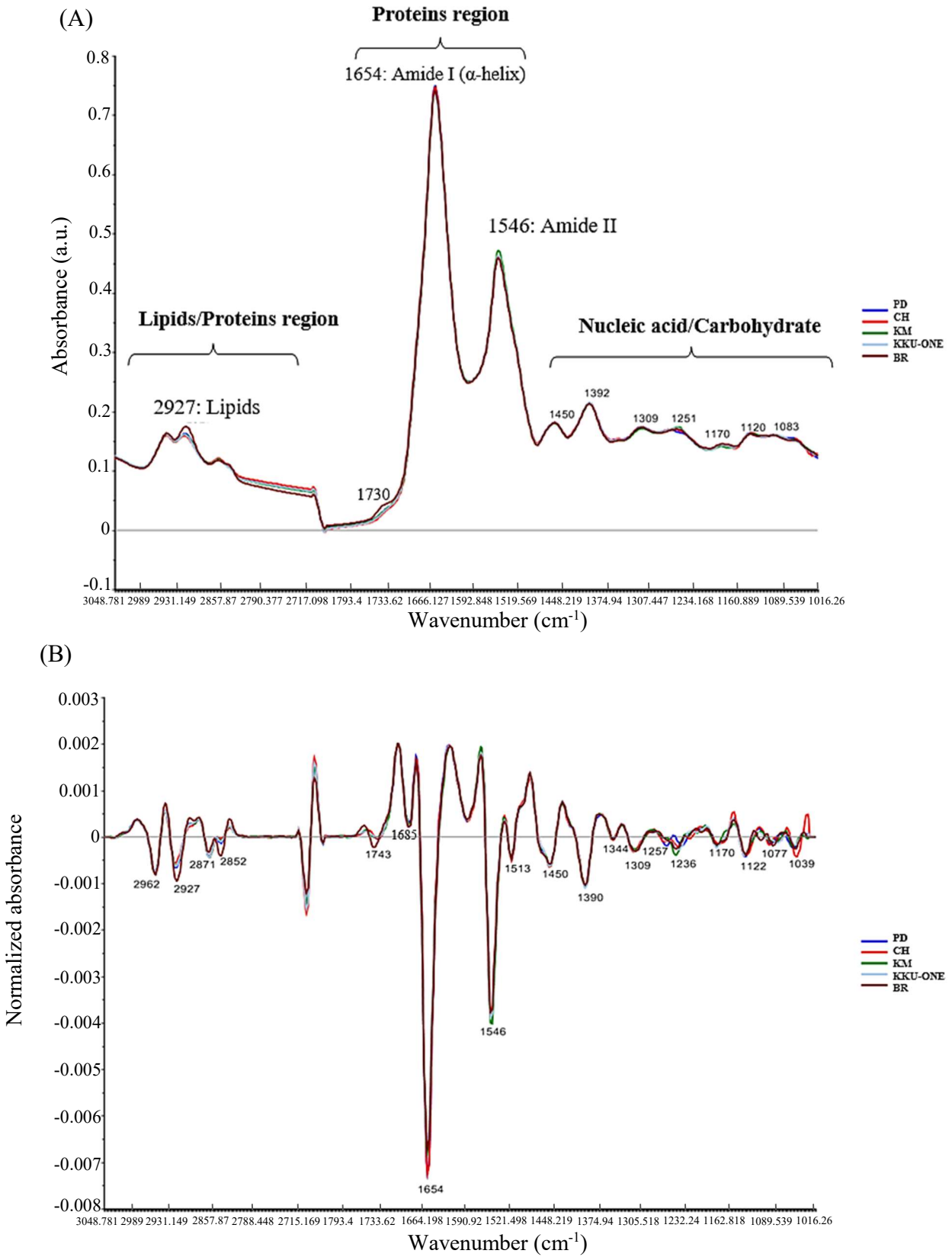

Figure 2 Comparison of the average original spectra (A) and second derivative spectra (B) from chicken breast of commercial broiler (BR); Pradu Hang Dam Mor Kor 55 (PD); Chee KKU 12 (CH); Khai Mook E-san (KM) and KKU-ONE chickens

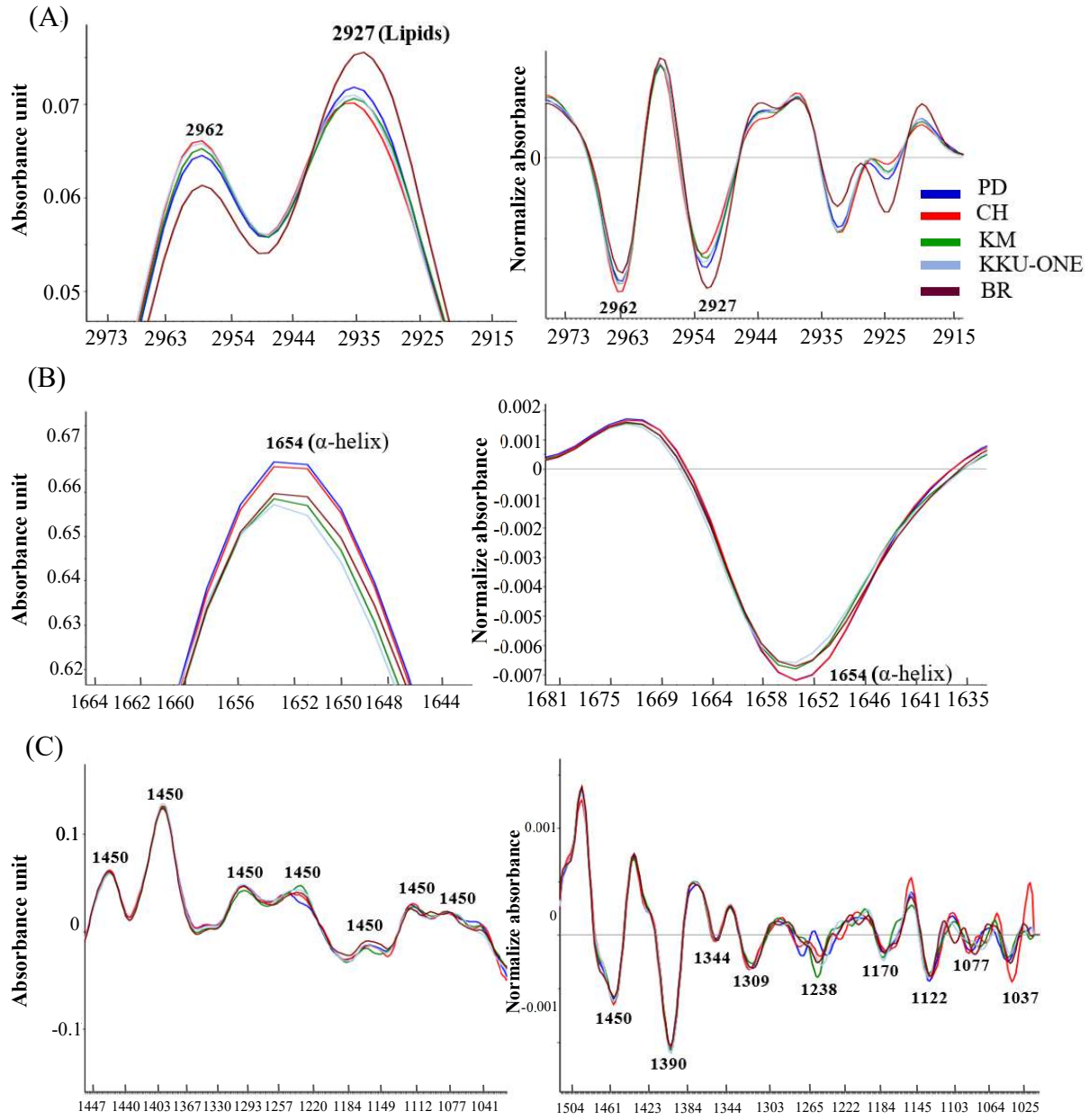

Figure 3 Average original and second derivative spectra of A) lipid spectral, B) protein spectral, C) nucleic acid spectral in chicken breast meat from commercial broiler (BR); Pradu Hang Dam Mor Kor 55 (PD); Chee KKU 12 (CH); Khai Mook E-san (KM) and KKU-ONE chicken breeds.

4. Conclusion

In this study, SEM and S-FTIR were employed to identify physical properties, such as characteristics of muscle fibers and protein secondary structures, in slow-growing TNC, TSC, and TNC crossbred chicken breeds and to compare these properties with those of fast-growing commercial broilers (BR). The goal was to identify the breeds that demonstrated the optimal potential to provide a good texture and suitable to be functional chicken meat, as well as for establishing breeding programs for slow-growing chickens. Compared to commercial BRs, TNC chickens exhibited higher muscle fiber numbers and smaller diameters. The TNC proteins contained a higher amount of α-helix secondary structures, and the lipid levels of the meat were less than those of the BR breed. The use of SEM and S-FTIR clearly identified differences in the physical properties, i.e., muscle fibers and secondary structures, of the meat proteins in chicken breast samples of slowing-growing TNC, TSC, and TNC crossbred chickens compared to a commercial faster-growing BR breed.

5. Ethical approval

The experiment was approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-58/62).

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