Proteolysis and changes in meat quality of chicken *pectoralis major* and *iliotibialis* muscles in relation to muscle fiber type distribution

Huilin Cheng,* Sumin Song,* Tae Sub Park,*,† and Gap-Don Kim ⓘ *,†,1

Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea; and †Institutes of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

ABSTRACT The proteolysis trends and meat quality of the chicken *pectoralis major* (**PM**) and *iliotibialis* (**IL**) muscles stored at 4°C for 7 d were investigated. After 7 d of storage, the purge loss was higher ($P <$ 0.05) in PM than in IL muscle. The difference in the composition of muscle fibers between PM (100% fast type) and IL (88.85% fast and 11.15% slow types) resulted in differences in proteolysis. Fructose-bisphosphate aldolase, troponin I, myosin heavy chain, and malate dehydrogenase exhibited the same tendencies, but pyruvate kinase, creatine kinase, L-lactate dehydrogenase, and triosephosphate isomerase exhibited different tendencies in the 2 muscles. The activity of cathepsin B was higher in PM than in IL during storage ($P < 0.05$). These results indicate that the proteolysis trend and changes in meat quality during cold storage are dependent on the different muscle fiber characteristics.

Key words: chicken, proteolysis, meat quality, muscle fiber characteristics, protease

INTRODUCTION

Globally, there is a constantly growing demand for meat and meat products, which are important for their protein content (Elahi et al., 2020; Alagawany et al., 2021; Zahari et al., 2021). Chicken meat is an abundant and readily available source of animal proteins (Chia et al., 2019). Undoubtedly, the pectoral muscle and the femoral muscle are considered to be 2 most important parts of the chicken carcass and are loved by consumers all over the world (Dogan et al., 2019). In addition, chicken breast muscle is recognized as the most valuable part of the chicken due to its distinct white meat (Mancinelli et al., 2017; Toomer et al., 2019). The proteins in chicken are also easily digestible, and it has a low proportion of saturated fats, making it one of the best choices for healthy meat.

The meat quality is determined by many factors that regulate the commodity value of meat, such as tenderness, color, juiciness, and flavor. These factors directly affect consumers' acceptance and purchase decisions. Many biological pathways are involved in a complex process that encompasses physiological, biochemical, and metabolic changes at the cellular level during the postmortem conversion of muscle to meat. For example, changes in myofibrillar protein degradation, metabolic enzymes, proteolytic enzyme activity, chemical state of myoglobin, production of heat shock protein, and apoptosis have important effects on final meat quality traits, especially tenderness, color, and water-holding capacity (Greaser, 1986; Matarneh et al., 2017; Huang et al., 2020). Aging is an important process in the conversion of muscle to meat, and it usually plays a vital role in improving the palatability of fresh meat and postmortem quality parameters (Mohan et al., 2020; Ramanathan et al., 2020). The ideal aging time can promote the degradation of the protein into small molecular peptide chains or amino acids via endogenous proteolytic systems through proteolysis in the internal and extracellular structure of the muscle fiber to maximize the palatability, stabilize the color, and improve the tenderness of the meat (Huff-Lonergan and Lonergan, 2005; Lana and Zolla, 2016; Barido and Lee, 2021). Although postmortem changes in tenderness are similar across species and time scales vary widely, chicken takes far less time than beef, lamb, and pork in tenderization (Lee et al., 2008; Li et al., 2012).

Although most of the research on proteolysis has so far focused on improving tenderness, the process of proteolysis improves not only the tenderness of meat but also other meat quality traits, such as meat color, pH,

and water-holding capacity. Kristensen and Purslow (2001) noticed that the water-holding capacity of meat reduced during the first 2 d post death but augmented during the subsequent storage period. They proposed a hypothesis that cytoskeleton degradation during aging increased meat water-holding capacity. According to Bond and Warner (2007), proteolysis of some proteins and variations in ion distribution can exacerbate the decline in the water-holding capacity of exercising meat. Endogenous protease systems, including calpain-2, proteasome, and lysosomal enzymes, have been observed to enhance the water-holding capacity of myofibrils through the proteolytic degradation of myofibrillar proteins (Zeng et al., 2017).

In previous study, we identified and quantified total proteolysis-induced peptides related to meat quality in beef during a 21-d cold storage period (Kim et al., 2021). However, chicken has not yet been fully studied to explore the postmortem proteolysis process at the peptide level. In addition, the proteolysis trends of different skeletal muscles are not the same, and the relationship between proteolysis trends and the characteristics of muscle fibers needs to be elucidated.

Therefore, this study was conducted to evaluate the quantitative changes in peptides derived from chicken skeletal muscles (M. *pectoralis major*, **PM** and M. *iliotibialis*, **IL**) by proteolysis during 7 d of cold storage, and the influences of these changes on meat quality characteristics were investigated to better understand the role of proteolysis in chicken meat during cold storage.

MATERIALS AND METHODS

Sample Preparation

Broiler chickens (Ross 308, male, 4 wk old, n = 10, 1.4 ± 0.05 kg of carcass weight) were purchased 6 h postmortem from a commercial slaughterhouse and transferred to the Laboratory of Meat Science at Seoul National University in Pyeongchang, Republic of Korea. Two major muscles (PM and IL) were removed from the chicken carcasses, and the connective tissue and visible fat were trimmed. Muscles were vacuum-packed (1.0 bar; MVAC 300, Maxima, The Netherlands) individually and stored in a cold room at 4°C. The storage period was fixed to 7 d based on the results of previous studies, which had reported that the proteolytic activity of chicken meat takes approximately 5 to 7 d to develop post-slaughter (Soglia et al., 2018; Barido and Lee, 2021). Among the PM (n = 20) and IL (n = 20) muscles from 10 chickens, the muscle from the left carcass was used for analysis on d 1 and that from the right carcass was used for analysis on d 7, respectively. The samples of both muscles for performing muscle fiber characteristics were sampled from d 1.

Proximate Composition

Moisture, crude protein, and crude ash were analyzed using the AOAC (2000) method. Crude fat content was determined using a modified version of the method by Folch et al. (1957). Five grams of samples were homogenized in 25 mL of Folch solution (chloroform: methanol, 2:1, v/v) at 8,000 rpm for 20 s (T18, IKA Works GmbH & Co., Staufen, Germany). The homogenates were filtered using Whatman No. 1 filter paper (Merck KGaA, Darmstadt, Germany). The filtrate was stirred with 5 mL of 0.88% NaCl and separated into 2 layers at room temperature for 2 h. After the upper layer was removed, the lower layer was transferred to a glass bottle, and the solvent was removed using nitrogen gas. Crude fat was expressed as a percentage of the weight of the sample. Proximate composition evaluation was conducted in triplicate for each sample.

Meat Quality Characteristics

pH To measure pH, three grams of sample were homogenized (T18, IKA Works GmbH & Co.) with 27 mL of deionized water and measured using a pH meter (MP230, Mettler-Toledo, Greifensee, Switzerland) calibrated with standard buffers (pH 9.21, 7.00, and 4.01).

Meat Color A Commission Internationale de l'Eclairage (CIE, 1978) system was used to determine the color values, including lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*). Chicken PM and IL muscles were removed from the packages and exposed to air for 20 min for myoglobin oxygenation. Meat color was measured on the muscle surface (both ventral and dorsal) using a colorimeter (CR-400, Minolta Co., Tokyo, Japan) calibrated with a white ceramic plate (Y = 93.5, x = 0.3132, y = 0.3198).

Water-Holding Capacity To determine the water-holding capacity, purge loss, and cooking loss were analyzed. After 7 d of storage, muscles were removed from the package and weighed. Purge loss was recorded as the difference in weight of the sample before and after storage as a percentage of the initial weight. To measure cooking loss, meat samples (approximately 20 g PM and 10 g IL) were packed with plastic bags and cooked in a water bath at 75°C. Samples were removed from the water bath when their internal temperatures reached 70°C. After being cooled at room temperature for 1 h, the samples were weighed, and the change in weight before and after cooking was recorded as the cooking loss (%).

Watner-Bratzler Shear Force To determine the tenderness of the meat, 3 cores (1.3 cm in diameter) were obtained from each cooked sample. The cores were prepared by cutting parallel to the muscle fiber direction. Shear force was measured by cutting the cores vertically using a texture analyzer (TA1, Ametek, Largo, FL) with a Warner-Bratzler shear blade. Watner-Bratzler shear force (**WBSF**) (kg$_f$/cm^2) value recorded for each sample was the average of the 3 cores.

Immunohistochemistry Immunohistochemistry was performed using a modified version of the method by Song et al. (2020). Muscle pieces (0.5 × 0.5 × 0.5 cm) were removed from each sample and immediately frozen

in 2-methylbutane cooled with liquid nitrogen. Transverse sections (10 μm in thickness) were obtained from the frozen samples using a cryostat microtome (CM 1860, Leica Biosystems, Nussloch, Germany) at −25 °C. For muscle fiber staining, 2 primary antibodies specific to each myosin heavy chain (MHC; slow, F59 and fast, S22) purchased from DSHB (Iowa, IA) were used. Fluorescent dye-conjugated anti-mouse IgG (Alexa Fluor 594, Thermo Fisher Scientific, Waltham, MA) and IgG$_{2a}$ (Alexa Fluor 488, Thermo Fisher Scientific) were used as secondary antibodies. All sections were visualized using a fluorescence microscope (EVOS M5000, Thermo Fisher Scientific). Muscle fibers were classified into 2 types (slow and fast) based on the distribution of slow or fast MHCs. Approximately 500 fibers in each section were analyzed using an Image Pro Plus Program (Media Cybernetics Inc., Rockville, MD) to determine muscle fiber characteristics: relative number (%), relative area (%), cross-sectional area (μm^2), and fiber density (number/mm^2).

Extraction of Peptides

Peptide extraction was conducted using a modified version of the method by Gallego et al. (2016). Ten grams of sample were homogenized with 50 mL of 0.01 N HCl for 3 min in a stomacher (BagMixer 400, Interscience, Saint Nom, France). The homogenate was centrifuged at 10,000 × g for 30 min at 4°C and filtered through glass wool. Three volumes of ethanol were mixed with the filtrate, and it was stored for 24 h at 4°C. The mixture was centrifuged at 10,000 × g for 30 min at 4°C, and the supernatant was lyophilized using a vacuum evaporator (SPD1010, Thermo Fisher Scientific Inc). Lyophilized samples were dissolved in 5 mL of 0.01 N HCl, neutralized to pH 7.0 using NaOH, and filtered using a 0.45-μm nylon membrane filter (Millipore Corp., Bedford, MA). The filtrates were centrifuged in centrifugal filter-containing tubes (Amicon Ultra-15 Centrifugal Filter Unit, Millipore) at 10,000 × g for 30 min.

LC−MS/MS Analysis and Label-Free Quantification

The LC-MS/MS analysis and the label-free quantification of peptides were done using a modified version of the method by Kim et al. (2021). To identify and quantify peptides, an LC device (Easy-n-LC, Thermo Fisher, San Jose, CA) equipped with a C18 nano bore column (150 mm × 0.1 mm, pore size of 3 μm, Agilent Technologies, Santa Clara, CA) and an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher) with a nano-electrospray source were used. The two mobile phases (A, 0.1% formic acid and 0.3% acetonitrile in deionized water; B, 0.1% formic acid in acetonitrile) were prepared for LC separation. The conditions for mobile phases were as follows: a linear increase from 0% B to 32% B for 23 min; 32% B to 60% B for 3 min; 95% B for 3 min; 100% A for

6 min; 1500 nL/min of the flow rate. Mass spectra were obtained using data-dependent acquisition with a full mass scan. The MS scan was followed by MS/MS scans of the top 5 precursor ions with dynamic exclusion (charge exclusion, 2−5 preferred and singly charged excluded; dynamic exclusion, 60 s). The orbitrap resolution was 15,000. The automatic gain control in the LTQ-Orbitrap XL mass spectrometer was 2 × 10^5 for MS and 1 × 10^4 for MS/MS.

Label-free quantification was performed using PEAKS Studio 10.0 (Bioinformatics Solutions Inc., Waterloo, ON, Canada). The MS/MS spectra were found using the database derived from UniProt (release March 2020 from http://www.uniprot.org, taxonomy *Gallus* 9031 [555,145 sequences]). Protein functions and interactions between them were evaluated through a String 11.5 database (http://string-db.org) search. Cellular components, molecular functions, biological processes, and KEGG pathways among gene ontology categories were examined for the proteins detected in chicken PM and IL muscles post the 7 storage days.

Proteolytic Enzymes

The analysis of the activities of cathepsin B and L was done using a modified version of the method by He et al. (2019). A total of 200 mg of sample was homogenized using an exaction buffer (150 mM NaCl, 25 mM Tris, 50 Mm EDTA, 1.0 mM DTT and 1.0% Triton-100, pH = 7.6) with a multipurpose mill (MM400, Retsch GmbH, Dusseldorf, Germany). The homogenates were centrifuged at 12,000 × g for 30 min at 4°C, and then the supernatant was collected. Protein concentration was measured using the method by Bradford (1976) and adjusted to 2.0 mg/mL. The extracted cathepsins B and L were mixed with the same volume of reaction buffer containing substrate (1.2 mM MgSO$_4$, 115 mM NaCl, 25 mM HEPES, 5.0 mM KCl, 2.0 mM CaCl$_2$, 1.0 mM KH$_2$PO$_4$, and 60 μm, pH = 7.4) at 37°C for 2 h. The substrates for cathepsin B and L were Z-RR-AMC (EMD, Merck, Darmstadt, Germany) and Z-FR-AMC (SC-3136, Santa Cruz Biotechnology, CA), respectively. The cathepsin inhibitor I (219415, Merck Millipore, Darmstadt, Germany) was used as a negative control. The absorbance value of the mixture was recorded at a wavelength of 380 nm/460 nm (excitation/emission) with a microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA). The enzyme activity was presented as the absorbance value relative to the control value. To extract calpains and proteasome 20S, 200 mg of sample was homogenized with an extraction buffer consisting of 100 mM Tris, 10 mM DTT, and 10 mM EDTA (pH 8.3). Protein concentration was analyzed using the Bradford (1976) method and adjusted to 2.0 mg/mL. The activities of calpains and proteasome 20S were measured using the Calpain Activity Fluorometric Assay Kit (MAK228, Sigma, St. Louis, MO) and the 20S Proteasome Activity Assay Kit (APT280, Merck) according to the manufacturers' instructions. The absorbance value was recorded

Table 1. Proximate composition and changes in meat quality characteristics of chicken M. *pectoralis major* and M. *iliotibialis* during 7 d of cold storage.

Measurements		M. *pectoralis major*		M. *iliotibialis*		Level of significance[3]		
		D 1	D 7	D 1	D 7	Muscle	Storage	M × S
Proximate composition[2] (%)	Moisture	74.10 ± 0.16[1]		75.36 ± 0.69		ns		
	Crude fat	1.56 ± 0.11		3.39 ± 0.25		**		
	Crude protein	22.82 ± 0.17		19.08 ± 0.13		***		
	Crude ash	1.78 ± 0.15		2.29 ± 0.12		ns		
pH		5.81 ± 0.04	5.82 ± 0.03	6.50 ± 0.04	6.61 ± 0.03	***	ns	ns
Meat color	CIE L*	52.17 ± 0.74	54.72 ± 3.54	$48.89 0 \pm 0.94$	52.48 ± 2.34	**	**	ns
	CIE a*	2.04 ± 0.24	3.16 ± 0.44	3.62 ± 0.33	3.58 ± 0.45	*	ns	ns
	CIE b*	6.04 ± 0.44	8.49 ± 0.48	4.57 ± 0.33	5.61 ± 0.50	***	***	ns
Purge loss (%)		-	1.17 ± 0.18	-	0.76 ± 0.07	*	ns	ns
Cooking loss (%)		20.99 ± 0.87	19.85 ± 0.99	21.25 ± 2.06	23.13 ± 2.12	ns	ns	ns
Warner-Bratzler shear force (kg_f/cm^2)		1.37 ± 0.08	1.14 ± 0.05	0.77 ± 0.07	0.68 ± 0.05	***	*	ns

[1]Data are means ± SE.

[2]Data for proximate composition were collected on d 1 and other data were collected on both d 1 and 7.

[3]Level of significance: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; Muscle, effect of muscle type; Storage, effect of storage time; M × S, combined effect of muscle and storage time.

at a wavelength of 380 nm/460 nm (excitation/emission) with a microplate reader (SpectraMax iD3, Molecular Devices). The enzyme activity was presented as the absorbance value relative to the control value. To extract caspase-3, 200 mg of the sample was homogenized with an extraction buffer consisting of 100 mM HEPES, 0.5 mM EDTA, 5 Mm DTT, 20% glycerol, and 0.2 % SDS (pH 7.5) using a multipurpose mill (MM400, Retsch GmbH, Dusseldorf, Germany). After centrifuging the homogenates, the supernatant was obtained, and its protein concentration was measured using the Bradford (1976) method. The activity of caspase-3 (2.0 mg/mL concentration) was measured using the EnzChek Caspase-3 Assay Kit [#]1 (E-13183, Molecular Probes, Inc., Eugene, OR) according to the manufacturer's instructions. The absorbance value of the mixture was recorded at a wavelength of 342 nm/441 nm (excitation/emission) with a microplate reader (SpectraMax iD3, Molecular Devices). The enzyme activity was presented as the absorbance value relative to the control value.

Statistical Analysis

Data on the approximate composition, pH, meat color, WBSF, and protease activity were collected from triplicate experiments of each sample, and data for purge and cooking loss were collected in one experiment of each sample. Muscle fiber characteristics were obtained based on 3 different areas of each section. All data were expressed as the mean and standard error of 10 muscles in each group (d 1 and d 7 of cold storage) of each muscle type (PM and IL). Data analysis was conducted using PROC MIXED with SAS 9.4 software (SAS Institute in Cary, NC) to assess muscle types and storage period effects. Two muscle types (PM and IL) and 2 storage time points (d 1 and 7) were set as fixed effects, and the effect each chicken was set as the random effect. Principal components analysis (**PCA**) was conducted to evaluate the relationship between proteolysis-induced peptides and meat quality traits. PCA was

performed based on the correlation matrix (PRINCOMP procedure). Significance was considered as $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

Approximate Composition

The approximate compositions of chicken PM and IL muscles, including moisture, crude fat, crude protein, and crude ash contents, are shown in Table 1. The moisture and ash content between PM and IL muscles were not different ($P > 0.05$). However, there were significant differences in the content of crude protein and crude fat between the 2 muscles ($P < 0.05$). The crude protein content in PM was significantly higher than that in IL muscle, while the crude fat content was significantly lower than that in IL muscle ($P < 0.05$).

Meat Quality Characteristics

Results on the postmortem changes in meat quality traits in PM and IL muscles during cold storage are presented in Table 1. The pH value was not impacted by storage time in either the PM or IL muscle ($P > 0.05$), and the IL muscle had a significantly higher pH value than the PM muscle at each stage ($P < 0.001$). There was no significant difference in the redness (CIE a*) value of each muscle between storage d 1 and 7 ($P > 0.05$); however, the redness value of the IL muscle was significantly higher than that of the PM muscle on storage d 1 and 7 ($P < 0.001$). Lightness (CIE L*) and yellowness (CIE b*) values of the PM muscle were higher than those of the IL muscle pertaining to the same storage period ($P < 0.01$). In addition, CIE L* ($P < 0.01$) and CIE b* ($P < 0.001$) values of both the muscles augmented significantly post the 7 storage days. The PM muscle had a higher purge loss than that of the IL muscle ($P < 0.05$); however, cooking loss of both the muscles was not influenced by the 7 storage days, and there was no difference in cooking loss ($P > 0.05$). Moreover, the WBSF values were significantly different between the
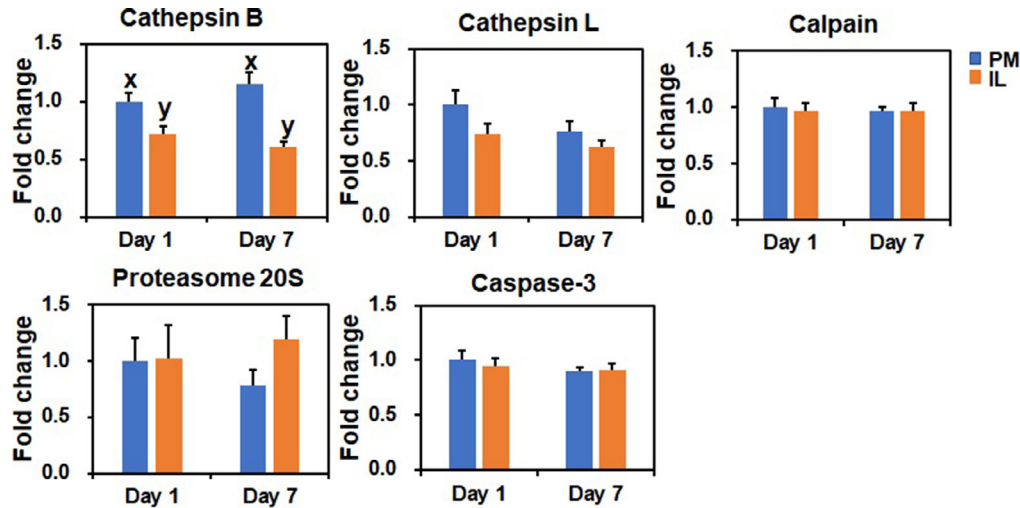
Figure 1. Changes in protease activity in chicken M. *pectoralis major* (PM) and M. *iliotibialis* (IL) during 7 d of cold storage. An effect of muscle type was observed on cathepsin B activity irrespective of the storage period ($P < 0.001$), whereas the effect of storage period and the combined effect of muscle type and storage period were not significant ($P > 0.05$). Cathepsin L, calpain, proteasome 20S, and caspase-3 activities were not significantly affected by muscle type, storage period, and the combination of muscle type and storage period ($P > 0.05$). Different letters (x, y) on the bar indicate significant differences between the PM and IL muscles on the same storage day at $P < 0.001$.

PM and IL muscles, irrespective of the storage day ($P < 0.001$). The WBSF values of the PM muscle significantly reduced ($P < 0.001$), whereas storage period did not influence the WBSF values of the IL muscle ($P < 0.05$). There was no combined effect of muscle type and storage period on the attributes of meat quality, including pH value, meat color, purge loss, cooking loss, and shear force ($P > 0.05$). The PM muscle had a higher purge loss than the IL muscle ($P < 0.05$); nevertheless, the cooking loss of both muscles was not affected by 7 days of cold storage, and there was no difference in cooking loss between the 2 muscles ($P > 0.05$). In addition, WBSF was significantly different between PM and IL muscles regardless of storage day ($P < 0.001$). The WBSF value was observed to significantly decline in the PM muscle ($P < 0.05$), whereas there was no effect of storage on WBSF of the IL muscle ($P > 0.05$).

Protease Activity

Changes in protease activity in chicken PM and IL muscles during 7 d of storage are shown in Figure 1. Both muscles showed no significant difference in activity of any proteases between d 1 and 7, respectively ($P > 0.05$). When comparing the protease activity between the 2 muscles, a higher activity of cathepsin B was observed in the PM muscle than in the IL muscle on the 2 storage days, respectively ($P < 0.05$). However, the other proteases did not show any significant difference between the 2 muscles during 7 d of storage ($P > 0.05$).
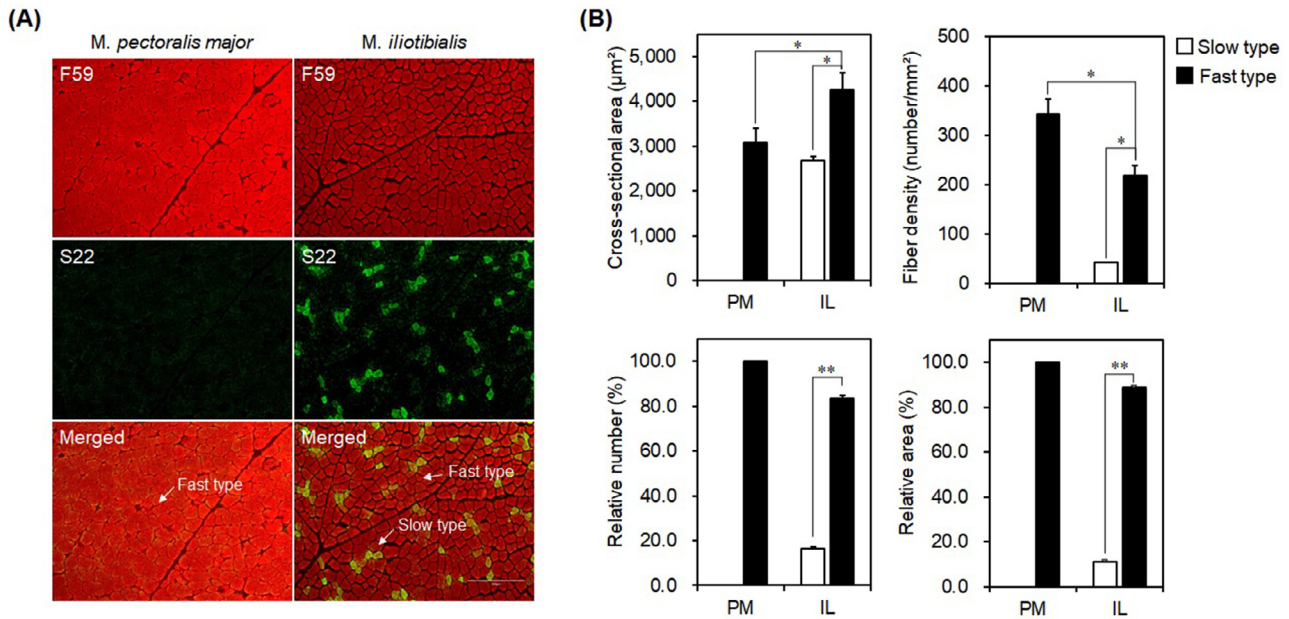
Muscle Fiber Characteristics

As shown in Figure 2A, the PM muscle was composed of only fast type of muscle fiber, whereas the IL muscle consists of both slow and fast types. When comparing muscle fiber size (**CSA**) between the 2 muscles within the same type, CSA of fast type was larger in IL muscle

than in PM muscle ($P < 0.05$; Figure 2B). Within the IL muscles, fast type was larger than slow type ($P < 0.05$). Contrary to the CSA of muscle fibers, the fiber density of the fast type was higher in the PM muscle than in the IL muscle, which consisted of a greater number of the fast type than in the slow type ($P < 0.05$). For IL muscle, muscle fiber composition, such as relative number and area, was significantly higher in the fast type (83.63% of relative number and 88.85% of relative area) than in the slow type (16.37% of relative number and 11.15% of relative area; $P < 0.01$).

Protein Degradation During Cold Storage

A total of 3,630 peptides were detected from 43 proteins of chicken PM and IL muscles during 7 d of cold storage (Table 2). Of these peptides, 451 and 431 peptides were identified as unique peptides in PM muscle on d 1 and 7, respectively, and 498 and 453 unique peptides were detected on d 1 and 7 in IL muscle, respectively. Most proteins discovered in the PM and IL muscles that degraded during the 7 storage days were identified as sarcoplasmic (cytosol) proteins. Most sarcoplasmic proteins are responsible for metabolic pathways, including glycolysis and gluconeogenesis (fructose-bisphosphate aldolase, pyruvate kinase, phosphoglycerate kinase, L-lactate dehydrogenase, creatine kinase M-type, adenylate kinase isoenzyme 1, malate dehydrogenase, triosephosphate isomerase, and glucose-6-phosphate isomerase; Table 2, Figure 3). Some myofibrillar proteins {myosin heavy chain, myosin-binding protein C [fast type], troponin I [fast skeletal muscle], PDZ domain-containing protein, myosin light chain 1, IF rod domain-containing protein, vinculin, and actin [alpha skeletal muscle]} were identified during seven storage days. Other proteins responsible for ECM-receptor interaction (collagen alpha-1(I) chain, collagen alpha-2(IX) chain, MFS domain-containing protein, and collagen alpha-(XVII) chain), structural molecule activity (c1q domain-

Figure 2. Representative stained sections (A) and muscle fiber characteristics (B) of chicken M. *pectoralis major* and M. *iliotibialis*. Sections were collected from chicken muscles on d 1. Antibodies: F59, anti-myosin heavy chain (fast type); S22, anti-myosin heavy chain (slow type). Bar = 300 μm.

containing protein and actin (cytoplasmic type 5), and oxygen binding (hemoglobin subunit alpha-A, hemoglobin subunit alpha-D, and hemoglobin subunit beta) were also identified. The proteolysis trends according to the spectral intensity of the unique peptides derived from each muscle are shown in Figure 4. We found many proteolysis-induced peptides to be affected by cold storage. Fructose-bisphosphate aldolase and hemoglobin subunit alpha-A showed the largest peptide spectral intensities on d 1 in PM and IL muscles, respectively. Several proteins exhibited similar tendencies in PM and IL muscles during cold storage. Intensities of peptides derived from myosin heavy chain and malate dehydrogenase were increased, while those of fructose-bisphosphate aldolase and troponin I were decreased after 7 d of storage. In contrast, pyruvate kinase, creatine kinase, L-lactate dehydrogenase, and triosephosphate isomerase exhibited different tendencies in the 2 muscles during storage. In PM, the peptide intensity of 2-phospho-D-glycerate hydrolyase, actin, alpha skeletal muscle, collagen alpha-1(I) chain, and the phosphorylase b kinase regulatory subunit decreased with increasing storage days increased. However, peptides from the myosin motor domain-containing protein, vinculin, aminotran-1-2 domain-containing protein, c1q domain-containing protein, and ribosomal protein lateral stalk subunit P2 were not detected on d 1, although they were detected a week later. This was also the case in IL muscle. Peptides derived from 5 proteins (Maillard deglycase, protein-glutamine gamma-glutamyltransferase 2, ribosomal protein lateral stalk subunit P2, IF rod domain-containing protein, and PPIase cyclophilin-type domain-containing protein) were produced over 7 d of storage. Conversely, the peptide intensity of 2-phospho-D-glycerate hydrolyase, circumsporozoite protein, MFS domain-containing protein, collagen alpha-1 chain, and phosphorylase b kinase regulatory subunit were entirely degraded from d 1 to d 7.

Relationship Between Proteins, Meat Quality Characteristics, and Proteolytic Enzyme Activity

As presented in PCA (Figure 5), the CIE a* and CIE b* in PM muscle were closely related to C1q domain-containing protein, and ribosomal protein lateral stalk subunit P2, whereas CIE L* was associated with hemoglobin subunit alpha-D and NAC-A/B domain-containing protein. WBSF and cooking loss in PM muscle were linked to L-lactate dehydrogenase and myosin motor domain-containing protein. However, pH did not show a similar tendency with any proteases and proteins. Among the proteases, cathepsin B was related to CIE a* and CIE b*; however, the other proteases were not associated with meat quality traits. Caspase 3 and calpain were linked to fructose-bisphosphate aldolase, adenylate kinase isoenzyme 1, myosin-binding protein C (fast type), and PDZ domain-containing protein. For IL muscle, CIE a* was associated with proteasome 20S, while pH was linked to calpain. However, caspase 3, cathepsins B and L did not show any relationships to meat quality traits. The relationships between L-lactate dehydrogenase and cooking loss and WBSF, and between C1q domain-containing protein and CIE a* were similar to those in PM muscle. CIE L* and CIE b* were associated with myosin heavy chain, whereas pH was closely related to NAC-A/B domain-containing protein. Adenylated kinase isoenzyme 1 and PDZ domain-containing protein were linked to cooking loss and WBSF in IL muscle, but these proteins did not show a relationship with any proteases, unlike those in PM muscle. Fructose-bisphosphate aldolase, pyruvate kinase, troponin I (fast type), collagen alpha-1 (XVII) chain, and phosphorylase b kinase regulatory subunit were linked to cathepsin L.

Table 2. Spectral count of peptides derived from chicken M. *pectoralis major* (PM) and M. *iliotibialis* (IL) during 7 days of cold storage.

Accession no.[a]	Protein name[a]	Cellular component / Molecular function[a]	Total spectral count	Unique peptides			
				PM		IL	
				Day 1	Day 7	Day 1	Day 7
P01994	Hemoglobin subunit alpha-A	Cytosol, oxygen binding	384	31	58	88	75
R4GM10	Fructose-bisphosphate aldolase	Glycolysis / gluconeogenesis, metabolic pathways, biosynthetic process	284	76	43	47	49
P00548	Pyruvate kinase	Glycolysis / gluconeogenesis, metabolic pathways	262	48	51	53	22
F1NU17	Phosphoglycerate kinase	Glycolysis / gluconeogenesis, metabolic pathways, biosynthetic process	207	38	29	34	23
E1BTT8	L-lactate dehydrogenase	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	188	60	54	28	31
P02457	Collagen alpha-1(I) chain	ECM-receptor interaction, structural molecule activity, biosynthetic process	52	2	*nd*	18	8
P00565	Creatine kinase M-type	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	206	8	10	28	32
P07322	Beta-enolase	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	145	18	11	22	15
P05081	Adenylate kinase isoenzyme 1	Cytosol, metabolic pathways, biosynthetic process	157	26	22	28	32
P13538	Myosin heavy chain, skeletal muscle	Myofibril	78	1	1	5	5
P02001	Hemoglobin subunit alpha-D	Cytosol, oxygen binding	118	4	9	24	25
P16419	Myosin-binding protein C, fast-type	Myofibril	194	17	16	24	21
P68246	Troponin I, fast skeletal muscle	Myofibril	77	2	5	21	2
P12108	Collagen alpha-2(IX) chain	ECM-receptor interaction, structural molecule activity	12	4	3	3	4
F1P194	C1q domain-containing protein	Structural molecule activity	15	*nd*	1	2	1
P53478	Actin, cytoplasmic type 5	Structural molecule activity	60	3	4	10	2
E1BVT3	Malate dehydrogenase	Metabolic pathways	84	4	6	7	8
P00504	Aspartate aminotransferase, cytoplasmic	Cytosol, metabolic pathways, biosynthetic process	47	8	8	6	10
A0A1D5P909	PDZ domain-containing protein	Myofibril	156	27	18	14	8
A0A1L1RRN7	2-phospho-D-glycerate hydro-lyase	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	23	2	*nd*	3	*nd*
E1BV54	SHSP domain-containing protein	Heat shock protein	8	*nd*	*nd*	3	1
A0A1L1RNL6	NAC-A/B domain-containing protein	Transcription regulation	368	16	14	9	14
P02112	Hemoglobin subunit beta	Cytosol, oxygen binding	19	*nd*	*nd*	1	1
F1N9H4	Elongation factor 1-alpha	Biosynthetic process	183	5	25	6	33
P00940	Triosephosphate isomerase	Cytosol, glycolysis / gluconeogenesis, metabolic pathways, biosynthetic process	46	29	19	4	7
A0A1D5P810	ATP synthase subunit O, mitochondrial	Metabolic pathways, biosynthetic process	8	*nd*	*nd*	1	1
P02604	Myosin light chain 1	Myofibril, structural molecule activity	69	*nd*	*nd*	3	9
A0A1D5PW37	MFS domain-containing protein	ECM-receptor interaction	9	*nd*	*nd*	2	*nd*
A0A1D5NVM0	Collagen alpha-1(XVII) chain	ECM-receptor interaction	7	*nd*	*nd*	1	*nd*
A0A1D5P1V6	Phosphorylase b kinase regulatory subunit	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	17	1	*nd*	2	*nd*
D5M8S2	Maillard deglycase	Cytosol, biosynthetic process	29	8	8	*nd*	8
Q01841	Protein-glutamine gamma-glutamyl-transferase 2	Cytosol	10	*nd*	*nd*	*nd*	1
A0A1D5PFB7	Myosin motor domain-containing protein	Myofibril	25	*nd*	1	*nd*	*nd*
A0A1D5PMT8	Ribosomal protein lateral stalk subunit P2	Cytosol, structural molecule activity, biosynthetic process	10	*nd*	1	*nd*	3
F1NJM8	IF rod domain-containing protein	Myofibril, structural molecule activity	12	*nd*	*nd*	*nd*	1
A0A1D5PRS4	Glucose-6-phosphate isomerase	Cytosol, glycolysis / gluconeogenesis, metabolic pathways	12	8	7	*nd*	*nd*
P12003	Vinculin	Myofibril, structural molecule activity	13	*nd*	2	*nd*	*nd*
A0A1D5PWY2	Aminotran_1_2 domain-containing protein	Biosynthetic process	5	*nd*	1	*nd*	*nd*
P68139	Actin, alpha skeletal muscle	Myofibril	4	1	*nd*	*nd*	*nd*
P07341	Fructose-bisphosphate aldolase B	Glycolysis / gluconeogenesis, metabolic pathways, biosynthetic process	3	3	3	*nd*	*nd*
Total			3,630	451	431	498	453

[a]Accession no., protein name, cellular component / molecular function were derived from the UniProt database, taxonomy *Gallus* 9031 (555,145 sequences). *nd*, not detected.

DISCUSSION

The degradation of major meat proteins, including sarcoplasmic and myofibrillar proteins, was observed at the initial storage time, as reported previously (Kim et al., 2021). The sarcoplasmic (cytosol) proteins, such as pyruvate kinase, L-lactate dehydrogenase, fructose-bisphosphate aldolase, beta-enolase, and creatine kinase
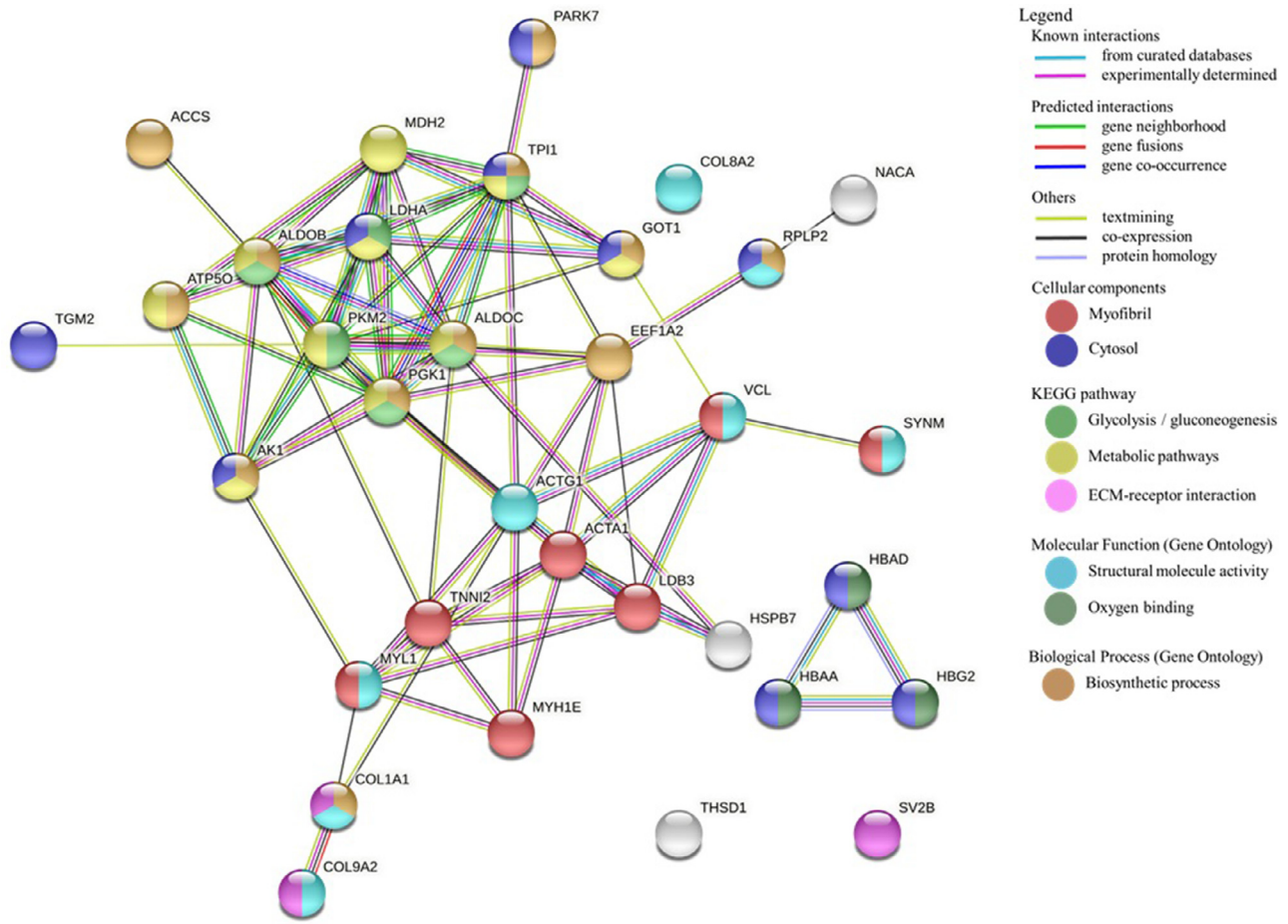
Figure 3. Interactions between the degraded proteins identified from chicken M. *pectoralis major* and M. *iliotibialis* during the seven storage days. Cellular components (myofibril and cytosol), KEGG pathway (glycolysis / gluconeogenesis, metabolic pathway, and ECM-receptor interaction), molecular function (structural molecule activity and oxygen binding), and biological process (biosynthetic process) are indicated by various colors. Proteins are represented by gene names as depicted in Table 1.

M-type, including hemoglobin subunit alpha-A, were degraded over 7 d regardless of muscle type (Figures 3 and 4). Peptides produced during storage were quantitatively identified as the majority of sarcoplasmic protein fragments. Among the myofibrillar proteins, actin and troponin I were especially degraded during storage (Figures 3 and 4). A previous study demonstrated that the degradation of sarcoplasmic and myofilament proteins as well as a decrease in μ-calpain activity were observed in the first hour after slaughter (Lee et al., 2008). This finding indicates that meat proteins, including sarcoplasmic proteins, can be affected by intrinsic proteases almost immediately after slaughter. In addition, most sarcoplasmic proteins do not play a direct role in meat tenderization, but the variability of sarcoplasmic proteins can affect other traits of meat quality, such as color and water-holding capacity (Marino et al., 2014; Kahraman and Gurbuz, 2016). In other studies, L-lactate dehydrogenase A chain isoform and fructose-bisphosphate aldolase A were negatively correlated with redness in beef (Wu et al., 2015; Nair et al., 2017; Gagaoua et al., 2018). These findings were consistent with the results of the present study, as evidenced by the degradation of L-lactate dehydrogenase and fructose-bisphosphate aldolase and the increase in redness during the storage of PM.

These results, unlike the results of previous studies, indicated that relatively few types of myofibrillar proteins were degraded. The results of previous studies (Lee et al., 2008; Li et al., 2012) have reported that many troponin T and desmin molecules were degraded. Herein, several myofibrillar proteins, including myosin heavy chain, actin, and troponin I, were observed; however, troponin T and desmin-derived peptides were not found. These results signified that the number and intensity of proteolysis-induced peptides acquired from sarcoplasmic proteins were relatively higher than those acquired from myofibrillar proteins, although both degraded during the storage period. Similar results were observed in our previous study on beef M. *longissimus lumborum* and M. *psoas major* and duck PM and IL muscles (Kim et al., 2021; Cheng et al., 2022). These studies observed greater degradation of sarcoplasmic proteins than myofibrillar proteins.

Previous studies have reported that endogenous proteolytic enzymes play a vital role in improving the meat tenderization process. Specifically, the activation of the calpain system results in the rapid development of
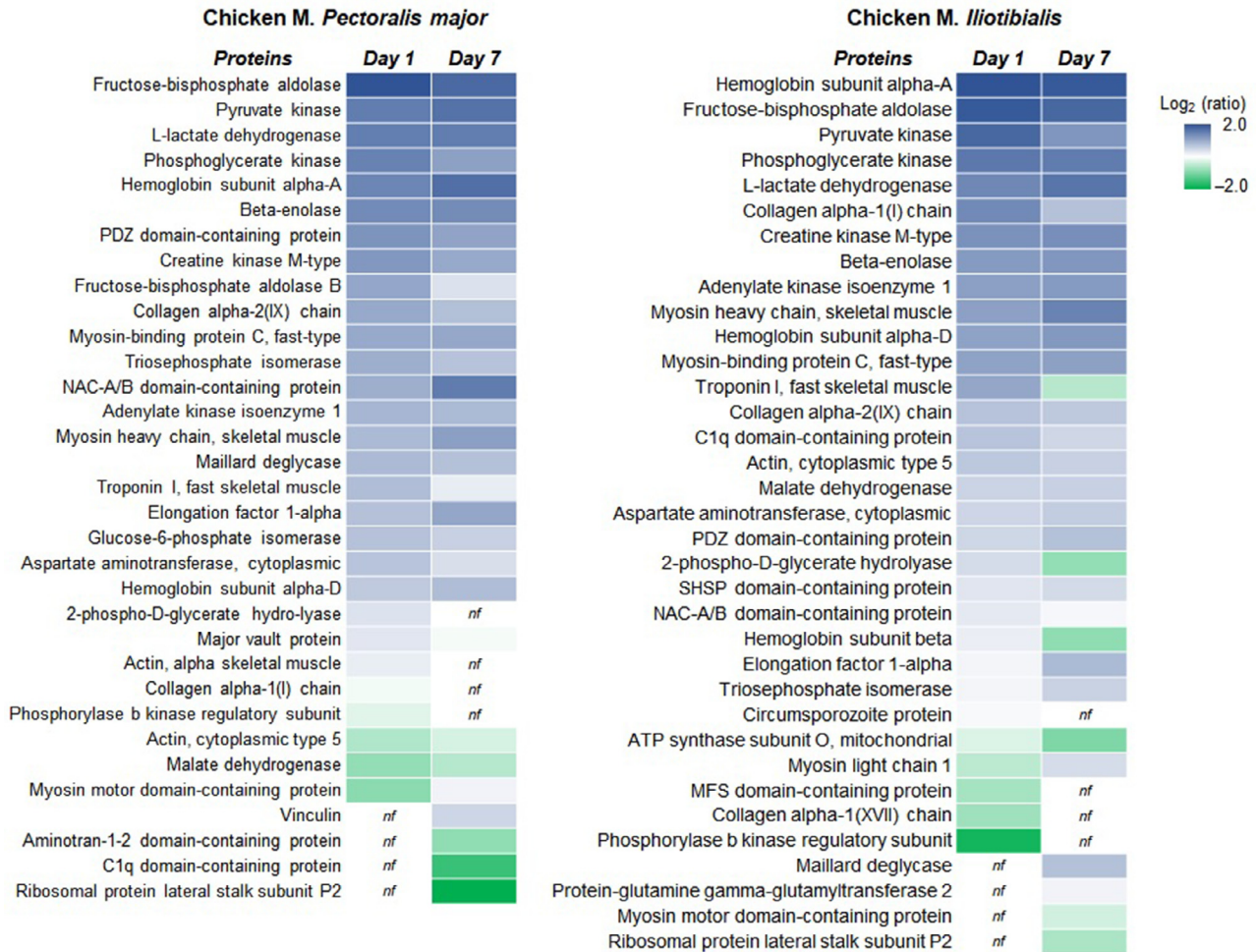
Figure 4. Quantitative changes in peptides derived from chicken M. *pectoralis major* and M. *iliotibialis* during 7 d of cold storage. Heat map: data expressed by the sum of the intensity of all peptides.

tenderness in meat via the destruction of the structure of myofibrils and the breakdown of connective tissue-containing protein (mainly collagen) (Lee et al., 2008). In the present study, there was no significant difference in the activities of proteolytic enzymes during 7 d of storage, although the tenderness of PM muscle was significantly improved. The results of previous studies have shown that the muscle tenderization process in chicken is faster than that in other species (Lee et al., 2008; Li et al., 2012; Biswas et al., 2016). That is, most proteolytic enzymes had strong activities during the early postmortem stage.

In terms of the meat quality characteristics of chicken, IL showed more stable characteristics in the aging process compared to PM muscle, and there were significant differences between both types of muscle, especially on d 1. The activity of cathepsin B was significantly different between the 2 muscles. The main reason for these differences is that PM and IL muscles have different muscle fiber compositions. Previous studies have classified poultry skeletal muscle fibers into 3 types: type I (slow-red-twitch fiber, slow oxidative), IIA (fast-red-twitch fiber, fast oxidative), and IIB (fast-white-twitch fiber, fast glycolytic) (Joo et al., 2013; Cong et al., 2017) based on the activity of enzymes. In this study, PM had solely fast

muscle fibers. It has been known that chicken breast is comprised of fast-twitch glycolytic muscle fibers (Smith and Fletcher, 1988; Roy et al., 2006; Petracci and Cavani, 2012; Verdiglione and Cassandro, 2013). IL muscle was mainly composed of 2 types of fast fibers (IIA and IIB; Ono et al., 1993), but in the present study, we found a small proportion of slow-type fibers. Berri et al. (2007) reported that cross-sectional area was positively correlated with tenderness in broilers, which was also confirmed in this study as PM had tougher meat than IL which had a bigger cross-sectional area. This is supported by Huo et al. (2021) who found that duck muscle fiber which more tender and has a smaller cross-sectional area, higher density, and can withstand lower shear force. In addition, increasing the percentage of type I fiber and decreasing the percentage of type IIB fiber can improve the tenderness of beef muscle (Hwang et al., 2010; Joo et al., 2013). In this study, PM showed a greater degree of lightness but a lower purge loss than IL, which can be explained by fast twitch glycolytic fibers (IIB), which was related to meat color and WHC. In previous studies, a higher composition of the fast twitch glycolytic IIB fiber in pork muscle was related to a greater degree of lightness and lower WHC (Kim et al., 2013). The PM muscle made up the largest part of
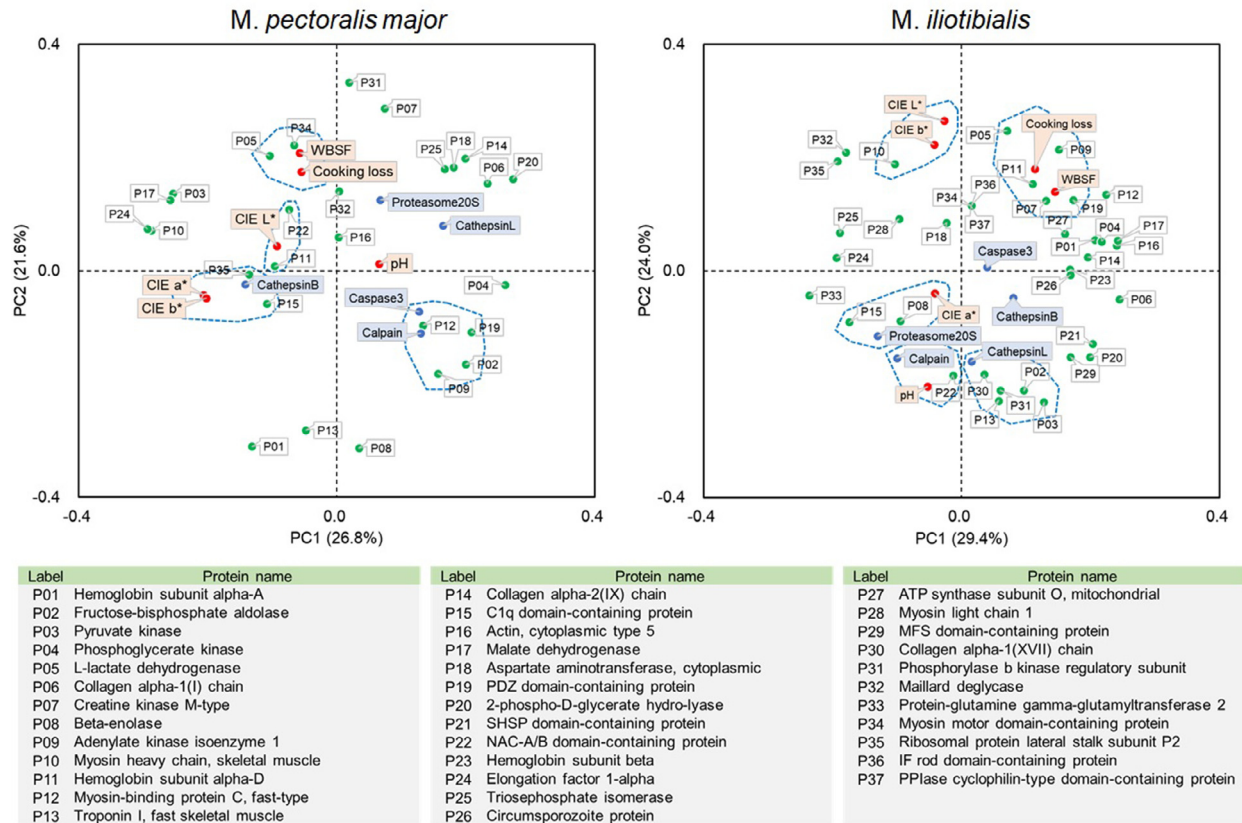
Figure 5. Principal components (PC) analysis between the proteins, proteases and meat quality traits of chicken M. *pectoralis major* (PM) and M. *iliotibialis* (IL). The first two PCs (higher than 2.0 of eigenvalues) were accepted: 26.8% and 21.6% for PM; 29.4% and 24.0% for IL. The blue dot lines indicate the similar tendencies between variables.

the muscles of the scapular belt, which lowers the wings. In contrast, IL forms the part of the thigh muscles that help with movement. The functional differences between the 2 regions in the chicken body result in the different muscle fiber compositions. Hence, the 2 muscles had different relationships among their peptides, proteases, and meat quality traits, as observed in the relationships of adenylated kinase isoenzyme 1 and PDZ domain-containing protein with proteases and meat quality traits: caspase 3 and calpain in PM muscle; cooking loss and WBSF in IL muscle. Previous studies have demonstrated a correlation between endogenous proteolytic activity and tenderness (Neath et al., 2007; Lana and Zolla, 2016; Barido and Lee, 2021), whereas our findings indicate that other meat quality characteristics, such as pH, meat color, and cooking loss, as well as the tenderness, are associated with proteolytic enzyme activity and degradation of proteins.

types (fast vs. slow and fast). Some proteins, such as pyruvate kinase, L-lactate dehydrogenase, fructose diphosphate aldolase beta-enolase, creatine kinase M-type, and hemoglobin subunits alpha-A, were observed to be closely related to the changes of chicken meat quality characteristics during cold storage. These results indicate that the proteolysis trend and changes of meat quality during cold storage are dependent on the different muscle fiber characteristics.

CONCLUSIONS

The study for chicken skeletal muscles revealed important new aspects of proteolysis-induced peptides to show the changes of postmortem chickens. The results showed there is a different tendency of chicken protein hydrolysis and meat quality characteristics between PM and IL muscles which are composed of different muscle fiber

DISCLOSURES

The authors declare that they have no conflicts of interest.

REFERENCES

Alagawany, M., S. S. Elnesr, M. R. Farag, R. Tiwari, M. I. Yatoo, K. Karthik, I. Michalak, and K. Dhama. 2021. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health − a comprehensive review. Vet. Q. 41:1–29.

AOAC. 2000. Official Methods of Analysis of AOAC USA. Association of Analytical Communities, Gaithersburg, MD.

Barido, F. H., and S. K. Lee. 2021. Changes in proteolytic enzyme activities, tenderness-related traits, and quality properties of spent hen meat affected by adenosine 5′-monophosphate during cold storage. Poult. Sci. 100:101056.

Berri, C., E. Le Bihan-Duval, M. Debut, V. Sante-Lhoutellier, E. Baeza, V. Gigaud, Y. Jego, and M. J. Duclos. 2007. Consequence of muscle hypertrophy on characteristics of Pectoralis major muscle and breast meat quality of broiler chickens. J. Anim. Sci. 85:2005–2011.

Biswas, A. K., S. Tandon, and C. K. Beura. 2016. Identification of different domains of calpain and calpastatin from chicken blood and their role in post-mortem aging of meat during holding at refrigeration temperatures. Food Chem 200:315–321.

Bond, J. J., and R. D. Warner. 2007. Ion distribution and protein proteolysis affect water holding capacity of *Longissimus thoracis et lumborum* in meat of lamb subjected to antemortem exercise. Meat Sci 75:406–414.

Cheng, H., S. Song, T. S. Park, and G. D. Kim. 2022. Comparison of meat quality characteristics and proteolysis trends associated with muscle fiber type distribution between duck pectoralis major and iliotibialis muscles. Food Sci. Anim. Resour. 42:266–279.

Chia, S. Y., C. M. Tanga, J. J. van Loon, and M. Dicke. 2019. Insects for sustainable animal feed: inclusive business models involving smallholder farmers. Curr. Opin. Environ. Sustain. 41:23–30.

CIE. 1978. Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms. de l'Eclairage, Commision Internationale, Paris.

Cong, J. H., L. Zhang, J. L. Li, S. H. Wang, F. Gao, and G. H. Zhou. 2017. Effects of dietary supplementation with carnosine on growth performance, meat quality, antioxidant capacity and muscle fiber characteristics in broiler chickens. J. Sci. Food Agr. 97:3733–3741.

Dogan, S. C., M. Baylan, A. Bulancak, and T. Ayasan. 2019. Differences in performance, carcass characteristics and meat quality between fast- and slow-growing broiler genotypes. Prog. Nutr. 21:558–565.

Elahi, U., Y.b. Ma, S.g. Wu, J. Wang, H.j. Zhang, and G.h. Qi. 2020. Growth performance, carcass characteristics, meat quality and serum profile of broiler chicks fed on housefly maggot meal as a replacement of soybean meal. J. Anim. Physiol. Anim. Nutr. 104:1075–1084.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

Gagaoua, M., M. Bonnet, L. De Koning, and B. Picard. 2018. Reverse phase protein array for the quantification and validation of protein biomarkers of beef qualities: the case of meat color from Charolais breed. Meat Sci 145:308–319.

Gallego, M., L. Mora, and F. Toldrá. 2016. Peptidomics as a tool for quality control in dry-cured ham processing. J. Proteom. 147:98–107.

Greaser, M. 1986. Conversion of muscle to meat. In: Muscle as foodAcademic Press, Inc, Orlando, FL, 37–102.

He, J., F. Deng, D. Pan, and X. Zeng. 2019. Investigation of the relationships between different enzymes and postmortem duck muscle tenderization. Poult. Sci. 98:6125–6130.

Huang, C., C. Hou, M. Ijaz, T. Yan, X. Li, Y. Li, and D. Zhang. 2020. Proteomics discovery of protein biomarkers linked to meat quality traits in post-mortem muscles: current trends and future prospects: a review. Trends Food Sci. Technol. 105:416–432.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Sci 71:194–204.

Huo, W., K. Weng, T. Gu, Y. Zhang, Y. Zhang, G. Chen, and Q. Xu. 2021. Effect of muscle fiber characteristics on meat quality in fast- and slow-growing ducks. Poult. Sci. 100:101264.

Hwang, Y. H., G. D. Kim, J. Y. Jeong, S. J. Hur, and S. T. Joo. 2010. The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. Meat Sci 86:456–461.

Joo, S. T., G. D. Kim, Y. H. Hwang, and Y. C. Ryu. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Sci 95:828–836.

Kahraman, H. A., and U. Gurbuz. 2016. Sarcoplasmic proteins and its effects on meat quality parameters in 5th Animal Health and Veterinary Medicine Congress.

Kim, G. D., S. Yun Lee, E.-Y. Jung, S. Song, and S. Jin Hur. 2021. Quantitative changes in peptides derived from proteins in beef tenderloin (psoas major muscle) and striploin (longissimus lumborum muscle) during cold storage. Food Chem 338:128029.

Kim, J., Y. Jeong, E. Y. Jung, H. S. Yang, H. T. Lim, and S. T. Joo. 2013. The influence of fiber size distribution of type IIB on carcass traits and meat quality in pigs. Meat Sci 94:267–273.

Kristensen, L., and P. P. Purslow. 2001. The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci 58:17–23.

Lana, A., and L. Zolla. 2016. Proteolysis in meat tenderization from the point of view of each single protein: a proteomic perspective. J. Proteom. 147:85–97.

Lee, H. L., V. Sante-Lhoutellier, S. Vigouroux, Y. Briand, and M. Briand. 2008. Role of calpains in postmortem proteolysis in chicken muscle. Poult. Sci. 87:2126–2132.

Li, S., X. Xu, and G. Zhou. 2012. The roles of the actin-myosin interaction and proteolysis in tenderization during the aging of chicken muscle. Poult. Sci. 91:150–160.

Mancinelli, A. C., M. G. Amato, D. Zillio, A. Dal Bosco, S. Mattioli, and C. Castellini. 2017. Chicken adaptability in alternative systems adaptive response of chicken strains to the organic and free range rearing systems. J. Dairy Vet. Sci. 4:555644.

Marino, R., M. Albenzio, A. della Malva, M. Caroprese, A. Santillo, and A. Sevi. 2014. Changes in meat quality traits and sarcoplasmic proteins during aging in three different cattle breeds. Meat Sci 98:178–186.

Matarneh, S. K., E. M. England, T. L. Scheffler, and D. E. Gerrard. 2020. The conversion of muscle to meat. Pages 159−185 in Lawrie's Meat Science: Woodhead Publishing Series in Food Science, Technology and Nutrition. F. Toldra, ed. Woodhead Publishing, Sawston, UK.

Mohan, K., R. Banerjee, and N. B. Maheswarappa. 2020. Recent developments in postmortem aging and evaluation methods. Pages 81−99 in Meat Quality Analysis. A. K. Biswas and P. K. Mandal, eds. Academic Press, UK.

Nair, M. N., B. R. C. Costa-Lima, M. Wes Schilling, and S. P. Suman. 2017. Proteomics of color in fresh muscle foods. Pages 163−175 in Proteomics in Food Science. M. L. Colgrave, ed. Academic Press, UK.

Neath, K. E., A. N. Del Barrio, R. M. Lapitan, J. R. V. Herrera, L. C. Cruz, T. Fujihara, S. Muroya, K. Chikuni, M. Hirabayashi, and Y. Kanai. 2007. Protease activity higher in postmortem water buffalo meat than Brahman beef. Meat Sci 77:389–396.

Ono, Y., H. Iwamoto, and H. Takahara. 1993. The relationship between muscle growth and the growth of different fiber types in the chicken. Poult. Sci. 72:568–576.

Petracci, M., and C. Cavani. 2012. Muscle growth and poultry meat quality issues. Nutrients 4:1–12.

Ramanathan, R., G. G. Mafi, L. Yoder, M. Perry, M. Pfeiffer, D. L. VanOverbeke, and N. B. Maheswarappa. 2020. Biochemical changes of postmortem meat during the aging process and strategies to improve the meat quality. Pages 67−80 in Meat Quality Analysis. A. K. Biswas and P. K. Mandal, eds. Academic Press, UK.

Roy, B. C., I. Oshima, H. Miyachi, N. Shiba, S. Nishimura, S. Tabata, and H. Iwamoto. 2006. Effects of nutritional level on muscle development, histochemical properties of myofibre and collagen architecture in the pectoralis muscle of male broilers. Br. Poult. Sci. 47:433–442.

Smith, D. P., and D. L. Fletcher. 1988. Chicken breast muscle-fiber type and diameter as influenced by age and intramuscular location. Poult. Sci. 67:908–913.

Soglia, F., Z. Zeng, J. Gao, E. Puolanne, C. Cavani, M. Petracci, and P. Ertbjerg. 2018. Evolution of proteolytic indicators during storage of broiler wooden breast meat. Poult. Sci 97:1448–1455.

Song, S., C. H. Ahn, and G. D. Kim. 2020. Muscle fiber typing in bovine and porcine skeletal muscles using immunofluorescence with monoclonal antibodies specific to myosin heavy chain isoforms. Food Sci. Anim. Resour. 40:132–144.

Toomer, O. T., M. L. Livingston, B. Wall, E. Sanders, T. C. Vu, R. D. Malheiros, K. A. Livingston, L. V. Carvalho, and P. R. Ferket. 2019. Meat quality and sensory attributes of meat produced from broiler chickens fed a high oleic peanut diet. Poult. Sci. 98:5188–5197.

Verdiglione, R., and M. Cassandro. 2013. Characterization of muscle fiber type in the pectoralis major muscle of slow-growing local and commercial chicken strains. Poult. Sci. 92:2433–2437.

Wu, W., X.-G. Gao, Y. Dai, Y. Fu, X.-M. Li, and R.-T. Dai. 2015. Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M. semitendinosus of Chinese Luxi yellow cattle. Int. Food Res. J. 72:98–105.

Zahari, S. M. S. N. S., N. S. M. Ali, A. R. Zabidi, D. Rosli, M. N. A. Manap, and N. Yahaya. 2021. Influence of neck slaughtering in broiler chicken meat on physicochemical analysis and metabolites 'fingerprinting'to enhance meat quality. Arab. J. Chem. 14:103042.

Zeng, Z., C. Li, and P. Ertbjerg. 2017. Relationship between proteolysis and water-holding of myofibrils. Meat Sci 131:48–55.