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ARTICLE INFORMATION	Fill in information in each box below
Article Title	Comparison of meat quality characteristics and proteolysis trends associated with muscle fiber type distribution between duck *pectoralis major* and *iliotibialis* muscles
Running Title (within 10 words)	Proteolysis and meat quality of duck skeletal muscles
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by the National Research Foundation of Korea (NRF) grant funded by Korea government (MSIT) (NRF-2019R1C1C1011056).
Author contributions (This field may be published.)	Conceptualization: Km GD, Cheng H; Data curation: Kim GD, Cheng H, Song S; Formal analysis: Cheng H, Song S; Methodology: Cheng H, Song SM, Kim GD, Park TS; Writing – original draft: Cheng H; Writing – review & editing: Kim GD, Park TS.
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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Comparison of meat quality characteristics and proteolysis trends associated with muscle fiber type distribution between duck *pectoralis major* and *iliotibialis* muscles

Abstract

This study was conducted to evaluate the proteolysis trends and change in meat quality during 10 days of cold storage in duck M. *pectoralis major* (PM) and M. *iliotibialis* (IL). Duck IL had a higher pH and greater degree of lightness but lower cooking loss than PM ($p<0.05$). During the 10-day cold storage, the pH value of PM declined significantly ($p<0.05$), while the meat quality traits of IL were not affected by cold storage ($p>0.05$). In PM, the redness increased from day 1 to day 5, while cooking loss was lower on day 10 compared to day 5 ($p<0.05$). There were no significant differences in the activities of cathepsin B and proteasome 20S during cold storage ($p>0.05$). The activity of calpains declined gradually during 10 days of storage ($p<0.05$), and the activity of calpains in PM was higher than that in IL ($p<0.05$). A total of 5,155 peptides were detected and derived from 34 proteins of duck PM muscle, whereas 4,222 peptides derived from 32 proteins were detected from duck IL muscle. Duck PM muscle was composed only of fast type of muscle fiber, whereas IL muscle was composed of both slow and fast types. The proteins responsible for glycolysis or myofibrillar proteins were closely related to changes in meat color or water-holding capacity during cold storage. These results suggest that changes in meat quality characteristics during cold storage are closely related to protein degradation, which is also related to the distribution of muscle fiber types.

Keywords: duck, proteolysis, meat quality, muscle fiber type

Introduction

Proteolysis is an enzymatic reaction involving multiple systems in which proteins are broken down into peptides or amino acids. Proteolysis plays an important role in controlling cell metabolic processes, such as cell division, apoptosis, transcription, signal transduction, protein degradation, and the regulation of multiple metabolic pathways (Ciechanover, 2005). Proteolysis also occurs in meat and is involved in vital activities of a series of enzymatic reactions (Barido & Lee, 2021; Lana & Zolla, 2016). Proteolysis mainly involves different endogenous proteolytic enzyme systems, such as calpains, cathepsins, proteasome, and caspases (Koohmaraie, 1996; Lana & Zolla, 2016; Sentandreu et al., 2002). The mechanism of proteolysis in beef and pork during storage has been widely elucidated (Lametsch & Bendixen, 2001; Lametsch et al., 2003; Lana & Zolla, 2016). However, this has not been fully studied in poultry meat, especially duck meat, due to the relatively little attention paid to poultry tenderization compared to that of beef or pork.

Recently, new insights into tenderness and meat color changes through proteomics and the importance of proteomics research in postmortem metabolic activities have been proposed (Purslow et al., 2021). Previous studies have demonstrated the proteome change and its relationship with beef or pork quality change postmortem (Gagaoua et al., 2020; Gagaoua et al., 2021; Jia et al., 2006; Lametsch & Bendixen, 2001; Lametsch et al., 2003). Duck meat, along with chicken and turkey meat, is classified as poultry meat, but its physicochemical properties and muscle fiber composition are different from those of other poultry meat (Kim et al., 2008). Thus, the differences in the trend of proteolysis between duck meat and chicken meat are expected due to the differences in the characteristics of muscle fiber and physicochemical properties between duck and chicken meat. However, until now, proteome change from a proteolysis perspective and its relation to meat quality

characteristics in duck meat has not been fully investigated.

Therefore, the purpose of this study was to investigate protein degradation by proteolysis during cold storage in two types of duck skeletal muscle (M. *pectoralis major*; PM and M. *iliotibialis*; IL) that have different physiological functions and different muscle fiber types. In addition, the relationship between proteolysis and meat quality characteristics was investigated to better understand the postmortem physicochemical changes in duck meat.

Materials and methods

Sample preparation

Fifteen ducks were slaughtered at a commercial slaughterhouse according to the standard commercial procedures of the Korean livestock production system. Duck PM (n = 30) and IL (n = 30) muscles were taken from both right and left sides of carcasses (6-week-old Cherry Valley ducks, 2.4 ± 0.3 kg) at 6 h postmortem. Muscles were weighed individually, vacuum-packed (1.0 bar; MVAC 300, Maxima, The Netherlands), and stored in a cold room at 4°C. Ten PM and IL muscles each were randomly selected at different storage times (1, 5, and 10 days), respectively. The meat quality characteristics, proteolytic enzyme activity, and proteolysis-induced peptides of each individual muscle were analyzed at different storage times. For analysis of proximate composition (moisture, crude protein, crude fat, and crude ash) and muscle fiber characteristics, samples were collected from both muscles on day 1.

Proximate composition

Moisture, crude protein, and crude ash contents were analyzed using the AOAC (2000) method. Crude fat content was determined using the Folch et al. (1957) method with

some modifications. Briefly, three grams of the sample were homogenized with 20 mL of Folch solution (chloroform: methanol, 2:1, v/v) at 8,000 rpm for 20 s using a homogenizer (T18, IKA Works GmbH & Co., Staufen, Germany). After filtrating the homogenate using filter paper (Whatman No. 1; Merck KGaA, Darmstadt, Germany), the filtrates were separated into two layers using 0.88% NaCl. The lower layer was collected, and the solvent was removed using nitrogen gas. Crude fat content was presented as a percentage of the weight of the sample. Proximate composition evaluation was conducted in triplicate for each sample.

Meat quality characteristics

Muscle pH was measured with homogenates of 3 g samples and 27 mL deionized water using a pH meter (MP230, Mettler-Toledo, Greifensee, Switzerland) calibrated with standard buffers (pH 4.01, 7.00, and 9.21). Meat color was measured using a colorimeter (CR-400, Minolta Co., Tokyo, Japan) calibrated with a white plate (Y = 93.5, x = 0.3132, y = 0.3198). A *Commission Internationale de l'Eclairage* (CIE, 1978) system was used to determine the color values (CIE L*, lightness; CIE a*, redness; CIE b*; yellowness). Purge loss and cooking loss were measured to determine water-holding capacity. To analyze the purge loss, each muscle was removed from the package and weighed at different storage times. The weight difference before and after storage was recorded as purge loss (%). To determine the cooking loss of the sample, muscles were cooked in a water bath at 75°C until their internal temperature reached 70°C. The weight difference before and after cooking was recorded as the cooking loss (%). The cooked samples were used to measure shear force. Three cores were removed from each sample by cutting parallel to the muscle fiber direction. Each core was cut vertically using a texture analyzer (TA1, Ametek, Largo, FL, USA)

103 equipped with a Warner-Bratzler shear blade. The Warner-Bratzler shear force (WBSF;
104 kg/cm^2) value was recorded for each sample with the average of the three cores.
105
106 *Immunohistochemistry*
107 To analyze muscle fiber characteristics, the muscle fibers of duck PM and IL muscles
108 were cut into small pieces (0.5 × 0.5 × 0.5 cm) and immediately frozen using 2-methylbutane
109 chilled with liquid nitrogen. Muscle fiber staining was performed using the Song et al. (2020)
110 method with some modifications. Briefly, transverse sections (10 μm in thickness) were
111 obtained from the frozen muscles using a cryostat microtome (CM 1860, Leica Biosystems,
112 Nussloch, Germany) at -21°C. The primary antibodies (F59 and S35; DSHB Iowa, IA, USA)
113 specific to each myosin heavy chain (MHC; slow and fast MHCs, respectively) were applied
114 to the section. To visualize the muscle fibers that reacted with primary antibodies, fluorescent
115 dye-conjugated anti-mouse IgG (Alexa Fluor 594, Thermo Fisher Scientific, Waltham, MA,
116 USA) and IgG_{2a} (Alexa Fluor 488, Thermo Fisher Scientific) were used. Stained sections
117 were captured using a fluorescence microscope (EVOS M5000, Thermo Fisher Scientific),
118 and approximately 800 muscle fibers were counted. The cross-sectional area (CSA, μm^2) of
119 each muscle fiber, relative area (%), relative number (%), and fiber density (number/mm^2)
120 were analyzed using an Image Pro Plus Program (Media Cybernetics Inc., Rockville, MD,
121 USA).
122
123 *Identification and quantification of peptides*
124 For peptide extraction, 5 g of the sample was homogenized with 30 mL of 0.01 N
125 HCl for 3 min in a stomacher (BagMixer®400, Interscience, Saint Nom, France). The
126 homogenate was centrifuged at 10,000×g for 30 min at 4°C. The supernatant was filtered

through glass wool, and filtrate was mixed with three volumes of ethanol. After being stored at 4℃ for 24 h, the mixture was centrifuged at 10,000×g for 30 min at 4℃, and the supernatant was lyophilized using a vacuum evaporator (SPD1010, Thermo Fisher Scientific Inc., MA, USA). The sample was dissolved with 5 mL of 0.01 N HCl, neutralized to pH 7.0 using NaOH, and filtered through a 0.45-μm nylon membrane filter (Millipore Corp., Bedford, MA, USA). The filtrate was centrifuged in centrifugal filter-containing tubes (Amicon® Ultra-15 Centrifugal Filter Unit, Millipore, MA, USA) at 10,000×g for 1 h.

The identification and quantification of peptides in duck muscles was conducted using the Kim et al. (2021) method with some modifications. Briefly, peptides were analyzed using an LC-MS/MS equipped with an LC device (Easy-n-LC, Thermo Fisher, San Jose, CA, USA) coupled with a C18 nano bore column (150 mm × 0.1 mm, pore size of 3 μm, Agilent Technologies, Santa Clara, CA, USA) and an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher, San Jose, CA, USA). The MS/MS spectra were identified and quantified using the PEAKS Studio 10.0 (Bioinformatics Solutions Inc., Waterloo, ON, Canada), and the database was derived from UniProt (release March 2020 from http://www.uniprot.org, taxonomy *Anas* 8835 [45,681 sequences]).

Activity of proteolytic enzymes

Three different extraction buffers were prepared to extract proteolytic enzymes from the samples: cathepsins B and L (150 mM NaCl, 25 mM Tris, 50 mM EDTA, 1.0 mM DTT and 1.0% Triton-100, pH = 7.6), calpains and proteasome 20S (100 mM Tris, 10 mM DTT, and 10 mM EDTA, pH to 8.3), and caspase-3 (100 mM HEPES, 0.5 mM EDTA, 5 mM DTT, 20% glycerol, and 0.2% SDS, pH 7.5). The extraction of enzymes was done using a modified version of the method by He et al. (2019). A total of 200 mg of the sample was homogenized

with 1.5 mL of extraction buffer using a multipurpose mill (MM400, Retsch GmbH, Dusseldorf, Germany) and centrifuged at 12,000×g for 30 min at 4 ℃. The supernatant was collected, and its concentration was measured using the method by Bradford (1976). Protein concentration was adjusted to 2.0 mg/mL. The activities of cathepsins B and L were measured using a reaction with the buffer containing the substrates (Z-RR-AMC; EMD, Merck, Darmstadt, Germany and Z-FR-AMC; SC-3136, Santa Cruz Biotechnology, CA, USA, respectively). The absorbance value was recorded at a wavelength of 380 nm/460 nm (excitation/emission) using a microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA, USA). Activities of other enzymes were analyzed using the Calpain Activity Fluorometric Assay Kit (MAK228, Sigma, St. Louis, MO, USA), 20S Proteasome Activity Assay Kit (APT280, Merck, Darmstadt, Gemany), and the EnzChek® Caspase-3 Assay Kit #1 (E-13183, Molecular Probes, Inc., Eugene, OR, USA) according to the manufacturers' instructions for calpains, proteasome 20S, and caspase-3, respectively. After reactions with substrates, the absorbance values were collected at a wavelength of 380 nm/460 nm (excitation/emission) for calpains and proteasome 20S and 342 nm/441 nm (excitation/emission) for caspase-3. The enzymes' activities were presented as the absorbance value relative to the control value.

Statistical analysis

All data were obtained from triplicate experiments per each sample and were expressed as the mean and standard error of 10 muscles in each group (day 1, day 5, and day 10 of cold storage) of each muscle type (PM and IL). Data analysis was performed using SAS 9.4 software (SAS Institute in Cary, North Carolina, USA). The effect of muscle type on proximate composition and muscle fiber characteristic was examined using the t-test, and the

effect of storage time and muscle type on meat quality characteristics and protease activity was examined using two-way ANOVA tests. Ducan's multiple range tests were used to determine statistical significance at $p<0.05$, $p<0.01$, and $p<0.001$. Principal components analysis (PCA) was conducted to evaluate the relationship between proteins, proteases, and meat quality traits. PCA was performed based on the correlation matrix (PRINCOMP procedure).

Results

Proximate composition and meat quality

The results of proximate composition and meat quality characteristics of duck PM and IL are shown in Table 1. There were no significant differences between the PM and IL muscles in the moisture, crude protein, and crude ash content ($p>0.05$), whereas the crude fat had a greater proportion in IL than in PM muscle ($p<0.05$). Duck IL had a higher pH and lightness (CIE L*) but lower cooking loss than PM ($p<0.05$). In addition, PM had a redder color (higher CIE a*) on day 1 and day 5 than that of IL ($p<0.05$), although they didn't show difference on day 10 ($p>0.05$). During the 10-day cold storage, the pH value of PM declined significantly ($p<0.05$), while the meat quality traits of IL were not affected by storage ($p>0.05$). In PM, the redness increased from day 1 to day 5, while cooking loss was less on day 10 than on day 5 ($p<0.05$). Regardless of muscle type, pH, yellowness (CIE b*), and purge loss were affected by 10 days of storage ($p<0.05$), but no combination effect with the muscle types and storage time was found ($p>0.05$).

Muscle fiber characteristics

Muscle fibers are classified into two types (slow and fast) based on the distribution of

MHCs, as shown in Fig. 1A. Duck PM muscle was composed only of fast type of muscle fiber, whereas IL muscle was composed of both slow and fast types. PM had a smaller cross-sectional area of fast-type fibers than IL muscle fibers ($p<0.001$; Fig. 1B). PM had a higher fast-type fiber density than IL muscle ($p<0.001$). There was no significant difference between fast- and slow-type muscle fibers within the IL muscle in terms of cross-sectional area and fiber density ($p>0.05$). However, muscle fiber compositions (relative fiber number and area) were significantly higher in the fast type than in the slow type within the IL muscle ($p<0.001$).

Proteolytic enzyme activities

The change in protease activities (calpains, cathepsins L and B, proteasome 20S, and caspase-3) was evaluated during 10 days of cold storage, as presented in Fig. 2. In both muscles, the activity of cathepsins B and L, and calpains decreased after 10 days of cold storage ($p<0.05$), whereas the activity of proteasome 20S and caspase-3 was decreased only in PM muscle after 5 or 10 days of storage ($p<0.05$). Duck PM muscle showed higher activity in cathepsin L and calpains than in IL muscle during storage ($p<0.05$), whereas the activity of cathepsin B and proteasome 20S was higher in PM than in IL only at 10 days of storage ($p<0.05$). However, the activity of caspase-3 did not show any difference between the two muscles during storage ($p>0.05$).

Protein degradation during cold storage

A total of 5,155 peptides were detected and derived from 34 proteins in duck PM, while 4,222 peptides derived from 32 proteins were detected in duck IL (Table 2). Peptides derived from actin, hemoglobin, fructose-bisphosphate aldolase, L-lactate dehydrogenase,

myosin heavy chain, pyruvate kinase, phosphagen kinase N-terminal domain-containing protein, grlyceraldehyde-3-phosphate dehydrogenase, and myoglobin accounted for a large fraction of the total peptides produced by proteolysis in both PM and IL muscle. The heat map (Fig. 3) showed quantitative changes in peptides derived from duck PM and IL muscles during 10 days of storage. As observed in spectral counts (Table 2), hemoglobin, actin, fructose-bisphosphate aldolase, L-lactate dehydrogenase, and creatine kinase showed strong intensities from day 1 regardless of muscle types. The strength of peptides derived from pyruvate kinase, triosephosphate isomerase, and cytochrome c oxidase polypeptide VIIc exhibited a similar tendency in both muscles and increased during 10 days of storage. However, degradation of glyceraldehyde-3-phosphate dehydrogenase, hemoglobin subunit beta, myoglobin, and glycerol-3-phosphate dehydrogenase showed different trends between PM and IL muscles: peptide intensities decreased in PM but increased in IL after 5 or 10 days of storage. For PM, alpha-galactosidase-derived peptides were not initially observed, but peptides were produced after 5 days of storage from alpha-galactosidase. In contrast, no peptides produced from glucose-6-phosphate isomerase after 10 days of storage. In IL muscle, peptides derived from adenylate kinase isoenzyme 1, ADP/ATP translocase, apolipoprotein A-1, and myosin binding protein C1 were not detected after 10 days of storage, whereas peptides derived from troponin T and glucose-6-phosphate isomerase were observed on day 10.

Relationship between proteins, meat quality characteristics, and proteolytic enzyme activity

PCA results are shown in Fig. 4 to evaluate the relationships between proteins, proteases, and meat quality characteristics in duck PM and IL muscles. For PM muscle, glucose-6-phosphate isomerase, phosphoglucomutase 1, and peptidylprolyl isomerase E were

247 closely related to lightness (CIE L*), while myosin heavy chain and glycerol-3-phosphate
248 dehydrogenase were associated with yellowness (CIE b*). Hemoglobin alpha a subunit was
249 linked to purge loss and caspase-3, whereas fructose-bisphosphate aldolase and phosphagen
250 kinase N-terminal domain-containing protein were linked to calpain, cathepsin B, proteasome
251 20S, and cooking loss. Alpha-glucosidase and ATP synthase subunit beta showed a
252 relationship with cathepsin L. However, these proteins, which showed a close relationship
253 with protease or meat quality traits in PM muscle, did not show a strong relationship with
254 protease or meat quality traits in IL muscle. For IL muscle, glceraldehyde-3-phosphate
255 dehydrogenase and ATP synthase subunit beta were linked to CIE L* and pH, while
256 cytochrome c oxidase polypeptide VIIc, glucose-6-phosphate isomerase, and myosin light
257 chain 1 were linked to CIE b*. In addition, L-lactate dehydrogenase, cytoplasmic actin,
258 ADP/ATP translocase, and myosin binding protein C1 were associated with cooking loss,
259 whereas myoglobin, peptidyl-prolyl cis-trans isomerase, myosin motor domain-containing
260 protein were closely related to calpain. Hemoglobin subunit beta, adenylate kinase isoenzyme
261 1, LIM domain binding 3, and uniquinol-cytochrome c reductase core protein 2 were linked
262 to caspase-3 and proteasome 20S, while actin alpha 1 and creatine kinase were linked to
263 cathepsin B.
264
265 **Discussion**
266 Meat quality during storage is influenced by external and internal factors. In
267 particular, intrinsic factors, such as muscle fiber type and proteolytic enzyme activity,
268 determine postmortem changes in meat quality characteristics. Poultry meat is generally
269 classified as white meat due to the high composition of white muscle fiber (type IIB) (Joo et
270 al., 2013). Similar to the findings of Huo et al. (2021), duck PM has only fast-type (type II)

271 fibers in the present study. However, another previous study (Kim et al., 2008), which
272 revealed that duck PM consisted of 73.3% type IIB and 26.7% type IIA muscle fibers, is
273 inconsistent with the present study. That is, duck PM muscle fibers can be classified into two
274 types (IIA and IIB) according to their contractile or metabolic properties, whereas muscle
275 fibers can be distinguished by the distribution of myosin isoforms (slow and fast) (Kim et al.,
276 2013). Duck IL muscle is composed of both slow and fast types of muscle fiber due to the
277 different morphological and physiological properties from those of PM. The different
278 distribution of muscle fiber types results in the differences in meat quality characteristics
279 between PM and IL muscles.

280 The relationships between protein hydrolysis of endogenous enzymes and meat
281 quality characteristics, especially meat tenderization, have been demonstrated (Bekhit et al.,
282 2014; Huang et al., 2011). In addition, Cheret et al. (2007) reported that cathepsin L plays a
283 role in the tenderization process of bovine and fish muscles, while cathepsin B plays a role
284 only in that of fish muscles. A similar role was found in the present study in which the
285 activity of cathepsin L changed after 10 days of storage, but cathepsin B showed no
286 difference in its activity. Calpains have been proven to be the main enzyme in the meat
287 tenderization process in most species (Lana & Zolla, 2016). In the present study, a significant
288 decrease in calpain activity was detected in PM and IL muscles of duck meat from the first
289 day to the tenth day. This was supported by the result of the degradation of myofibrillar
290 proteins such as actin, myosin heavy chain, and troponins. Robert et al. (1999) used
291 incubation experiments to demonstrate that proteasome 20S can effectively degrade bovine
292 myofibrils and interfere with the role of proteasome 20S in postmortem proteolysis.
293 However, in the present study, proteasome 20S activity was not different in IL muscle
294 throughout the storage period, while in PM muscle, its activity decreased after 10 days of

storage. Caspase-3 activity was decreased in PM muscle during cold storage. In the present study, the activities of proteases were maintained or gradually decreased from the initial storage time to the later storage stages, but tenderness was not affected by the proteases, as observed previously (He et al., 2019).

Several proteins showed the relationships between their degradation trends and protease activity or meat quality traits, especially meat color and water-holding capacity. In particular, meat color traits were majorly related with the degradation of enzymes responsible for glycolysis, such as glycerol-3-phosphate dehydrogenase, phosphoglucomutase 1, glyceraldehyde-3-phosphate dehydrogenase, and glucose-6-phosphate isomerase as reported in previous study which demonstrate the correlations among glycolytic dehydrogenase including glyceraldehyde-3-phosphate dehydrogenase, lactic dehydrogenase, and meat color stability were observed in ovine muscle (Xin et al., 2018). For water-holding capacity, Joo et al. (1999) demonstrated that the rate of drip loss declined according to the increasing of the sarcoplasmic protein solubility. In the present study, cooking loss in PM muscle was closely related to the degradation of proteins, such as fructose-bisphosphate aldolase, phosphagen kinase N-terminal domain-containing protein, and malate dehydrogenase, and these proteins were also associated with the activities of cathepsin B and calpain. However, IL muscle showed different trends in the relationship between protein and water-holding capacity, as observed in the relationship between cooking loss and myosin binding protein C1, cytoplasmic actin, ADT/ATP translocase, and L-lactate dehydrogenase. This was supported in part by a previous study (Hamm, 1986), which showed that myosin and actin had a significant effect on water-holding capacity of meat.

Conclusion

The differences in muscle fiber distribution between duck PM (fast type) and IL (slow and fast) resulted in different trends in proteolysis and changes in meat quality during cold storage. Large amounts of various proteolysis-induced peptides were observed in both muscles at the initial storage time (day 1), and this indicates that intrinsic proteolytic enzymes play a major role in protein degradation at the early postmortem stage. The proteins responsible for glycolysis or myofibrillar proteins were closely related to changes in meat color or water-holding capacity during cold storage. These results indicate that the proteolysis is affected by storage time and affects the change in duck meat quality during storage. This phenomenon also depends on the distribution of muscle fiber types, which is determined by physiological properties of the muscle.

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Figure Legends

Fig. 1: Cross-sections muscle fiber characteristics of duck M. *pectoralis major* and M. *iliotibialis*. (A) representative stained cross-sections. (B) Muscle fiber characteristics: cross-sectional area, fiber density, relative fiber number, and relative fiber area. F59, anti-myosin heavy chain (fast type); S35, anti-myosin heavy chain (slow type). Bar = 200 μm. ***, $p<0.001$.

Fig. 2: Changes in proteolytic enzymes' activities of duck M. *pectoralis major* (PM) and M. *iliotibialis* (IL) during 10 days of cold storage. Different superscripts indicate significant ($p<0.05$) differences between storage periods (a-c) within the same muscle or between muscle types (x, y) within the same storage period.

Fig. 3: Quantitative changes in peptides derived from duck M. *pectoralis major* and M. *iliotibialis* over 10 days of cold storage. Data are expressed by the sum of the intensity of all peptides for each protein from which the peptide originated. *nf*, not found.

Fig. 4: Principal components analysis between proteins, proteases, and meat quality traits in duck M. *pectoralis major* and M. *iliotibialis*.

Table 1. Proximate composition and changes in meat quality characteristics of duck M. *pectoralis major* and M. *iliotibialis* during 10 days of cold storage

Measurements		M. *pectoralis major*			M. *iliotibialis*			Level of significance[a)]		
		Day 1	Day 5	Day 10	Day 1	Day 5	Day 10	MT	ST	MT×ST
Proximate composition (%)	Moisture	74.84±0.73	-	-	74.32±1.58	-	-	ns	-	-
	Crude protein	20.43±0.86	-	-	19.70±0.88	-	-	ns	-	-
	Crude fat	2.80±0.23	-	-	4.06±0.32	-	-	***	-	-
	Crude ash	2.44±0.63	-	-	2.22±0.51	-	-	ns	-	-
pH		$6.03^{a,y}$±0.11	$5.95^{ab,y}$±0.06	$5.79^{b,y}$±0.22	6.66^{x}±0.14	6.62^{x}±0.15	6.55^{x}±0.15	***	**	ns
Meat color	CIE L*	40.58^{y}±2.99	39.55^{y}±1.11	38.25^{y}±3.03	43.29^{x}±2.47	43.00^{x}±1.33	43.06^{x}±1.12	***	ns	ns
	CIE a*	$15.59^{b,x}$±1.21	$17.26^{a,x}$±1.28	16.70^{ab}±0.43	13.60^{y}±1.96	14.73^{y}±1.45	14.38±2.44	**	ns	ns
	CIE b*	4.97±1.40	6.37±1.34	6.15±0.84	5.63±1.07	5.77±1.98	7.18±0.55	ns	*	ns
Purge loss (%)		-	1.29±0.18	1.82±0.85	-	0.87±0.38	1.42±0.24	ns	*	ns
Cooking loss (%)		$32.68^{ab,x}$±3.49	$33.25^{a,x}$±1.72	$29.05^{b,x}$±3.22	25.72^{y}±4.14	24.12^{y}±3.04	24.25^{y}±3.25	***	ns	ns
Warner-Bratzler shear force (kg/cm^2)		1.68±0.35	1.62±0.42	1.47±0.32	1.38±0.47	1.39±0.36	1.51±0.13	ns	ns	ns

Data are means±SE.

Different superscripts indicate significant ($p<0.05$) differences between storage days (a, b) within the same muscle or between muscle types (x, y) within the same storage day.

[a)]Level of significance: MT, muscle type; ST, storage time; MT×ST, combination effect of muscle type and storage time; ns, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table 2. Spectral count of peptides derived from duck M. *pectoralis major* and M. *iliotibialis* during 10 days of cold storage

Accession no.[a)]	Protein name[a)]	M. *pectoralis major*				M. *iliotibialis*			
		Total	Unique peptides			Total	Unique peptides		
			Day 1	Day 5	Day 10		Day 1	Day 5	Day 10
C7EK42	Hemoglobin alpha A subunit	631	264	91	80	359	53	33	30
U3I8T6	Actin alpha 1, skeletal muscle	562	190	58	65	510	100	39	56
R0KDK0	Fructose-bisphosphate aldolase	460	167	70	51	196	22	9	13
U3IE74	L-lactate dehydrogenase	220	95	26	21	328	37	13	18
U3ILF5	Phosphoglycerate kinase	143	62	23	24	78	1	3	4
R0LU47	Myosin heavy chain, skeletal muscle	419	173	60	50	129	24	15	11
U3I0F9	Pyruvate kinase	298	32	21	35	347	7	8	11
A0A493TED5	Phosphagen kinase N-terminal domain-containing protein	307	108	51	36	*nd*	*nd*	*nd*	*nd*
A0A493TFK1	Creatine kinase	133	71	15	19	158	41	23	18
A6ZIB9	Beta-actin	142	56	23	19	*nd*	*nd*	*nd*	*nd*
U3IA60	Malate dehydrogenase	156	25	12	12	128	29	19	12
U3I8D8	Triosephosphate isomerase	150	39	24	12	198	13	11	9
R9MH41	Alpha-galactosidase	10	*nd*	3	2	*nd*	*nd*	*nd*	*nd*
R0LER7	Myozenin-1	72	19	7	9	*nd*	*nd*	*nd*	*nd*
U3IHK4	ATP synthase peripheral stalk subunit	71	21	10	11	*nd*	*nd*	*nd*	*nd*
A0A493SVK7	Troponin T, fast skeletal type	54	8	*nd*	6	51	*nd*	*nd*	4
A0A493TQC9	Glyceraldehyde-3-phosphate dehydrogenase	316	120	12	27	258	15	8	9
P02114	Hemoglobin subunit beta	103	34	11	11	111	3	4	6
A0A493TX79	Adenylate kinase isoenzyme 1	50	15	6	10	49	2	*nd*	*nd*
A0A493SYZ4	Cytochrome c oxidase polypeptide VIIc	36	9	2	4	59	4	5	4
U3J9G0	NDUFA4 mitochondrial complex associated	94	21	6	8	*nd*	*nd*	*nd*	*nd*
Q7LZM2	Myoglobin	247	12	1	27	238	50	24	10
R0LLF5	Troponin I, fast skeletal type	37	3	4	6	*nd*	*nd*	*nd*	*nd*
U3IP65	ATP synthase subunit beta	64	4	4	2	113	14	16	8
U3I3Q8	Myosin binding protein H	26	21	9	4	16	1	1	*nd*
A0A493SU92	LIM domain binding 3	122	12	4	12	66	3	*nd*	1
A0A493T656	Glycerol-3-phosphate dehydrogenase	28	8	5	3	12	2	*nd*	1
A0A493TLM6	Aconitate hydratase, mitochondrial	41	10	2	6	36	3	5	1
U3IRP3	Glucose-6-phosphate isomerase	47	9	7	*nd*	45	*nd*	*nd*	2
U3J383	Phosphoglucomutase 1	13	5	2	2	*nd*	*nd*	*nd*	*nd*
R0LK98	Peptidyl-prolyl cis-trans isomerase	11	10	4	1	74	7	4	4
U3IQ96	Peptidylprolyl isomerase E	11	10	4	1	*nd*	*nd*	*nd*	*nd*
U3IMA7	Elongation factor 1-alpha	49	19	*nd*	4	*nd*	*nd*	*nd*	*nd*
A0A493TDJ5	Enoyl-CoA hydratase	32	1	2	4	120	5	2	2
R0LHA7	Actin, cytoplasmic 1	*nd*	*nd*	*nd*	*nd*	61	2	1	4
A0A493TU40	ADP/ATP translocase	*nd*	*nd*	*nd*	*nd*	94	1	1	*nd*
O42296	Apolipoprotein A-I	*nd*	*nd*	*nd*	*nd*	28	3	2	*nd*
A0A493T5E3	Ldh_1_N domain-containing protein	*nd*	*nd*	*nd*	*nd*	28	4	2	1
U3IA79	Myosin light chain 1	*nd*	*nd*	*nd*	*nd*	38	2	*nd*	1
U3J7T0	Myosin motor domain-containing protein	*nd*	*nd*	*nd*	*nd*	224	40	21	18
U3I342	Ubiquinol-cytochrome c reductase core protein 2	*nd*	*nd*	*nd*	*nd*	44	2	*nd*	1
A0A493T1G5	UTP—glucose-1-phosphate uridylyltransferase	*nd*	*nd*	*nd*	*nd*	26	1	3	1
Total		5155	1653	579	584	4222	491	272	260

[a)]Accession no. and protein name were derived from the UniProt database, taxonomy *Anas* 8839 (103,172 sequences). *nd*, not detected.

Fig. 1

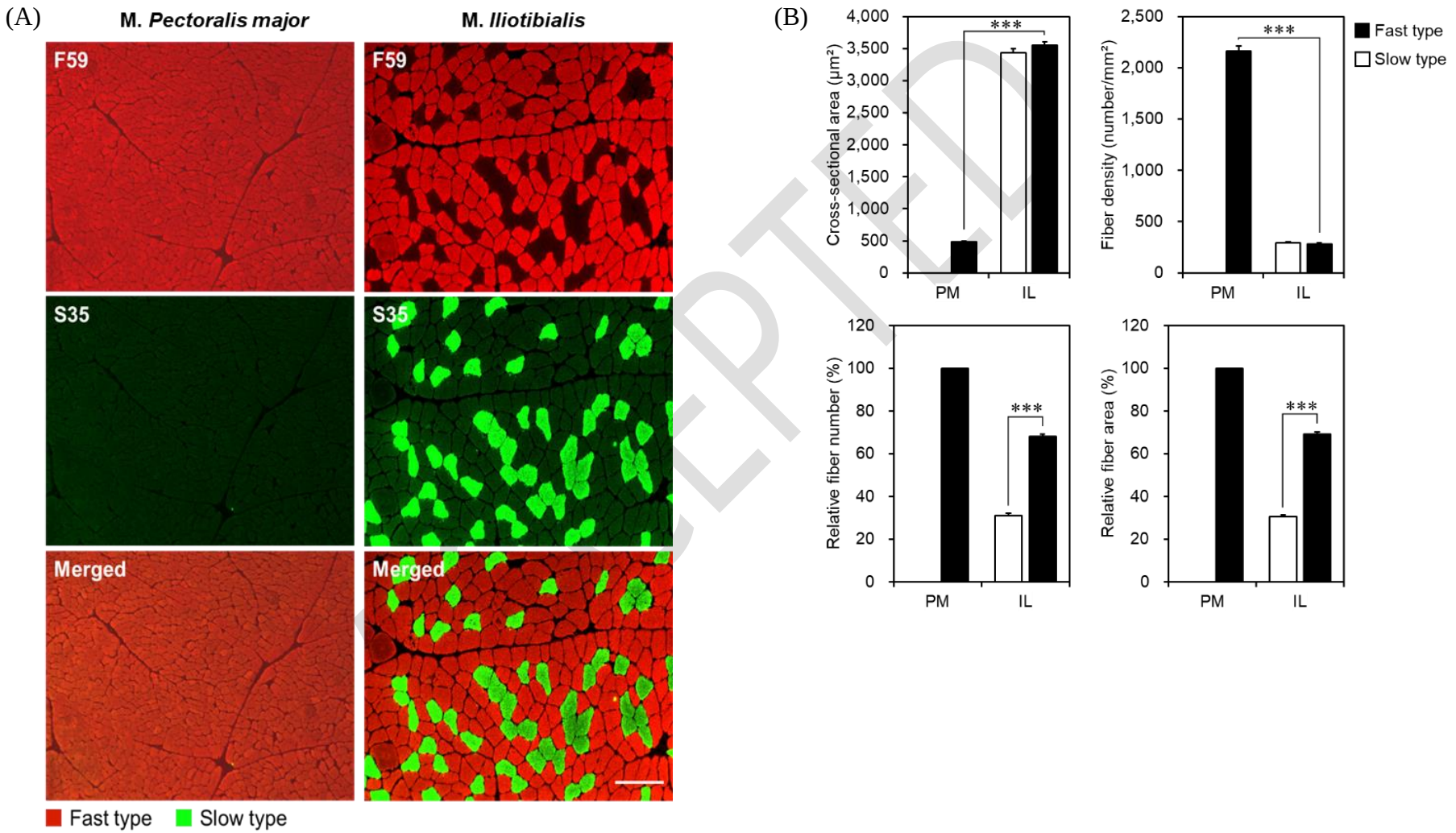


Fig. 2

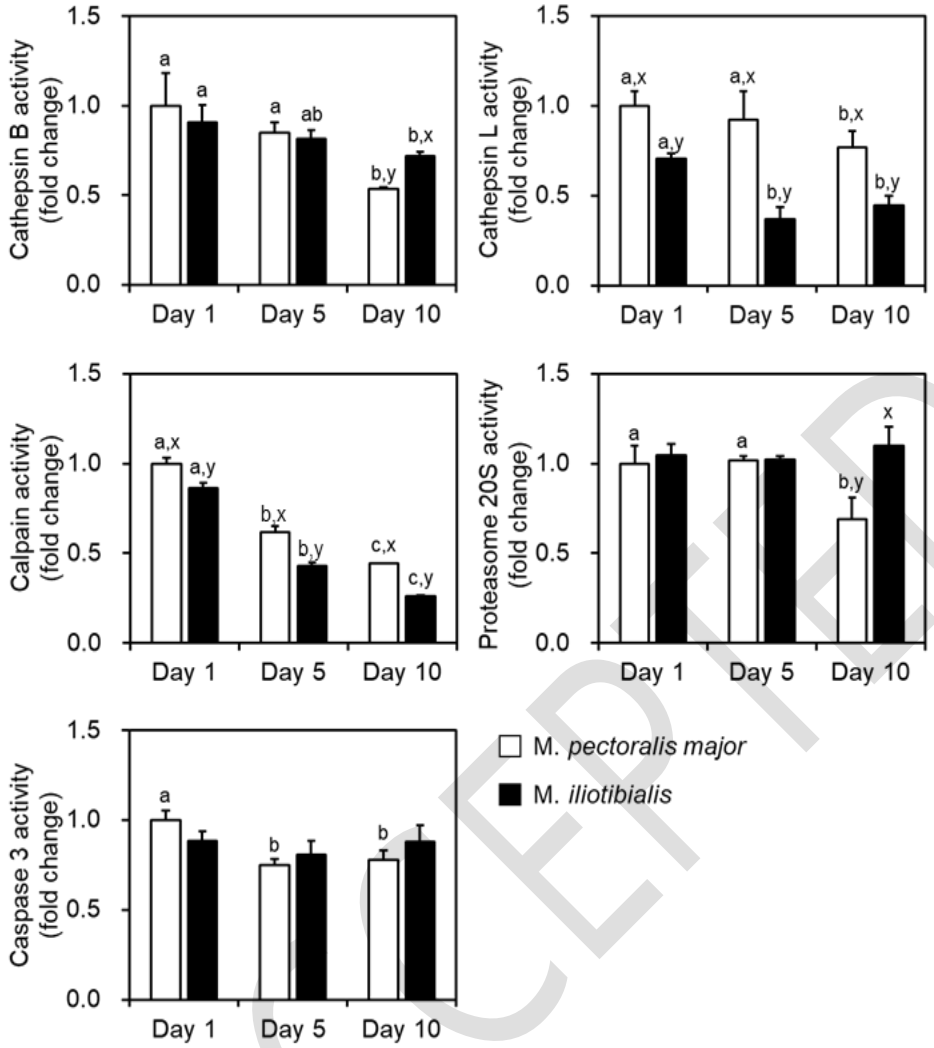


Fig. 3

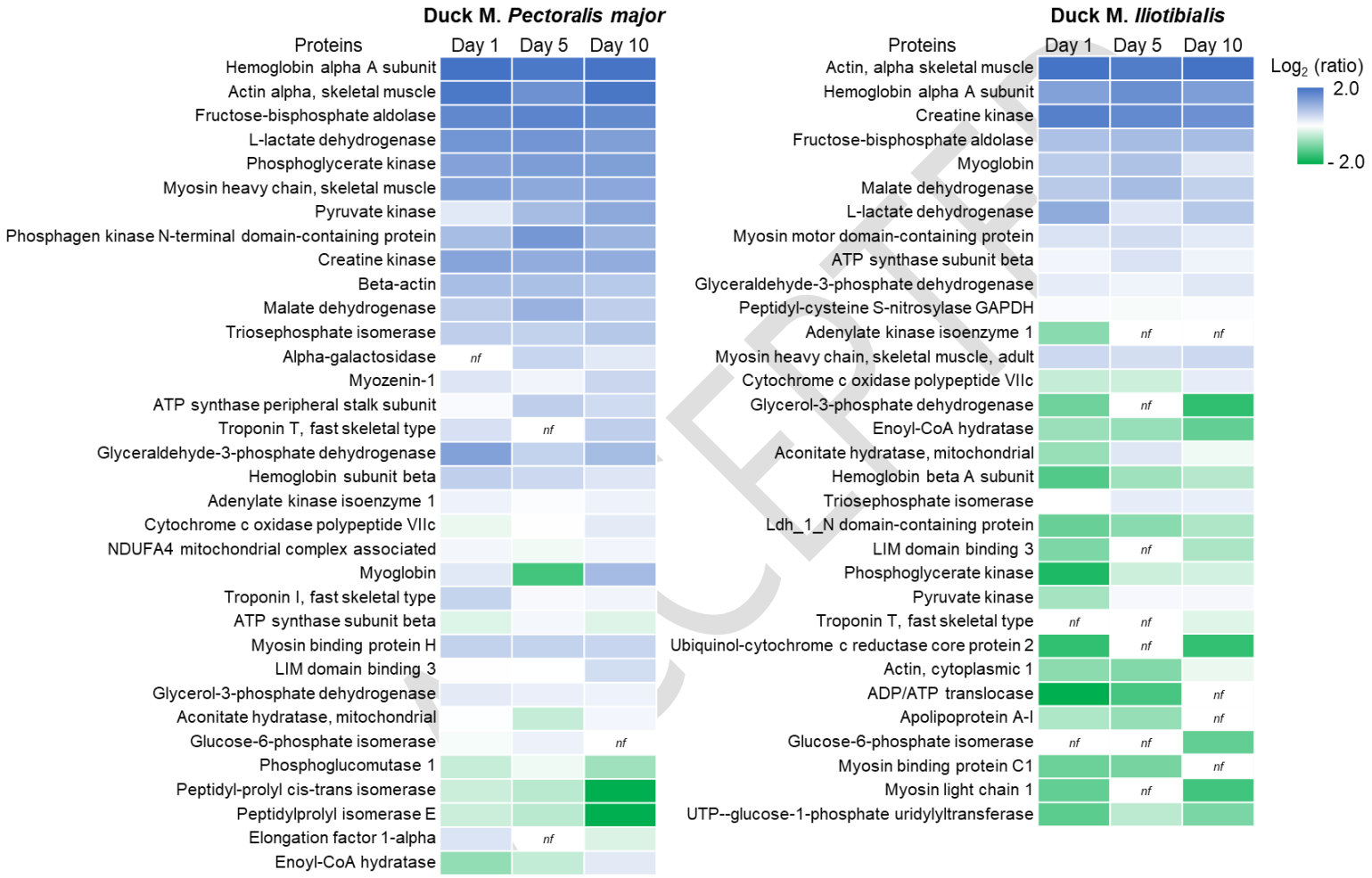
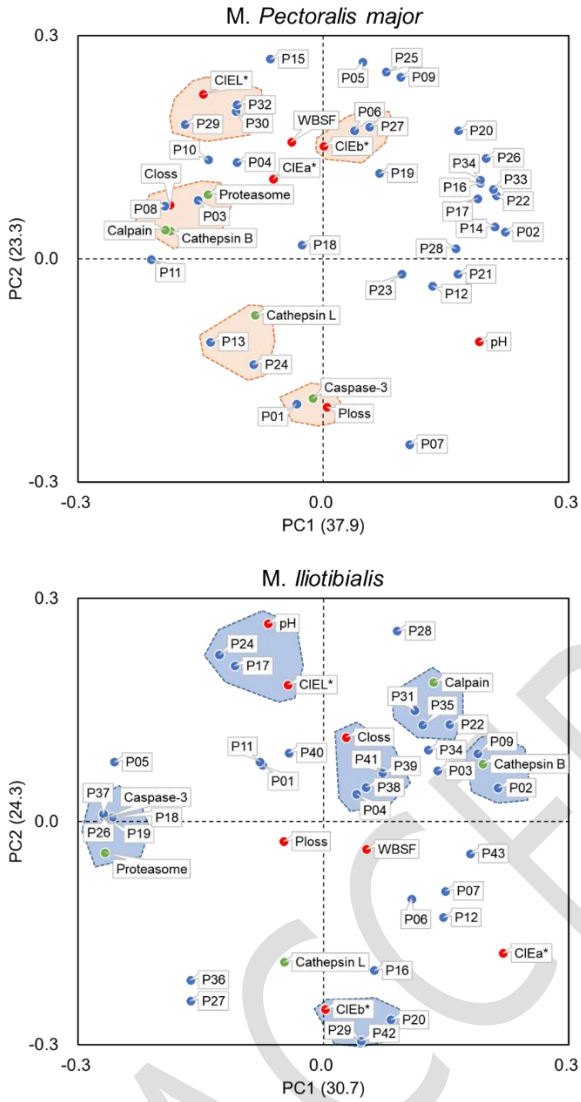


Fig. 4



Traits	Label	Full name
Meat quality	pH	pH
	Ploss	Purge loss
	Closs	Cooking loss
	WBSF	Warner-Bratzler shear force
	ClEL*	CIE L* (lightness)
	ClEa*	CIE a* (redness)
	ClEb*	CIE b* (yellowness)
Protease activity	Cathepsin B	Cathepsin B activity
	Cathepsin L	Cathepsin L activity
	Calpain	Calpain activity
	Proteasome	Proteasome 20S activity
	Caspase-3	Caspase 3 activity
Proteins	P01	Hemoglobin alpha A subunit
	P02	Actin alpha 1, skeletal muscle
	P03	Fructose-bisphosphate aldolase
	P04	L-lactate dehydrogenase
	P05	Phosphoglycerate kinase
	P06	Myosin heavy chain, skeletal muscle
	P07	Pyruvate kinase
	P08	Phosphagen kinase N-terminal domain-containing protein
	P09	Creatine kinase
	P10	Beta-actin
	P11	Malate dehydrogenase
	P12	Triosephosphate isomerase
	P13	Alpha-galactosidase
	P14	Myozenin-1
	P15	ATP synthase peripheral stalk subunit
	P16	Troponin T, fast skeletal type
	P17	Glyceraldehyde-3-phosphate dehydrogenase
	P18	Hemoglobin subunit beta
	P19	Adenylate kinase isoenzyme 1
	P20	Cytochrome c oxidase polypeptide VIIc
	P21	NDUFA4 mitochondrial complex associated
	P22	Myoglobin
	P23	Troponin I, fast skeletal type
	P24	ATP synthase subunit beta
	P25	Myosin binding protein H
	P26	LIM domain binding 3
	P27	Glycerol-3-phosphate dehydrogenase
	P28	Aconitate hydratase, mitochondrial
	P29	Glucose-6-phosphate isomerase
	P30	Phosphoglucomutase 1
	P31	Peptidyl-prolyl cis-trans isomerase
	P32	Peptidylprolyl isomerase E
	P33	Elongation factor 1-alpha
	P34	Enoyl-CoA hydratase
	P35	Myosin motor domain-containing protein
	P36	Ldh_1_N domain-containing protein
	P37	Ubiquinol-cytochrome c reductase core protein 2
	P38	Actin, cytoplasmic 1
	P39	ADP/ATP translocase
	P40	Apolipoprotein A-I
	P41	Myosin binding protein C1
	P42	Myosin light chain 1
	P43	UTP-glucose-1-phosphate uridylyltransferase