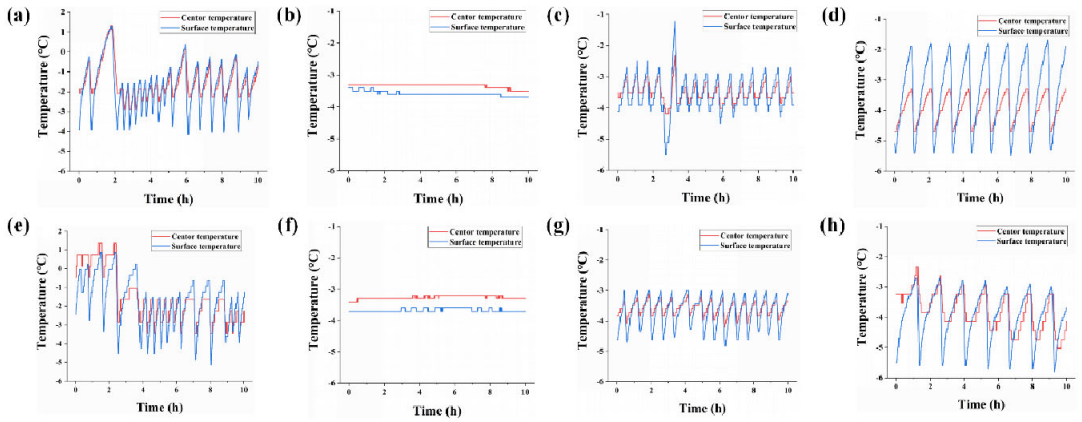

Fig. S1 Temperature changes inside the pork and salmon (center and surface) during the superchilling process. (a) The actual temperature changes (Control) of the superchilled area in pork (−3.5 ℃). (b) −3.5 ℃ constant temperature in pork. (c) Refrigerator temperature fluctuations of (−3.5±1.0) ℃ in pork. (d) Refrigerator temperature fluctuations of (−3.5±2.0) ℃ in pork. (e) The actual temperature changes (Control) of the superchilled area in salmon (−3.5 ℃). (f) −3.5 ℃ constant temperature in salmon. (g) Refrigerator temperature fluctuations of (−3.5±1.0) ℃ in salmon. (h) Refrigerator temperature fluctuations of (−3.5±2.0) ℃ in salmon.

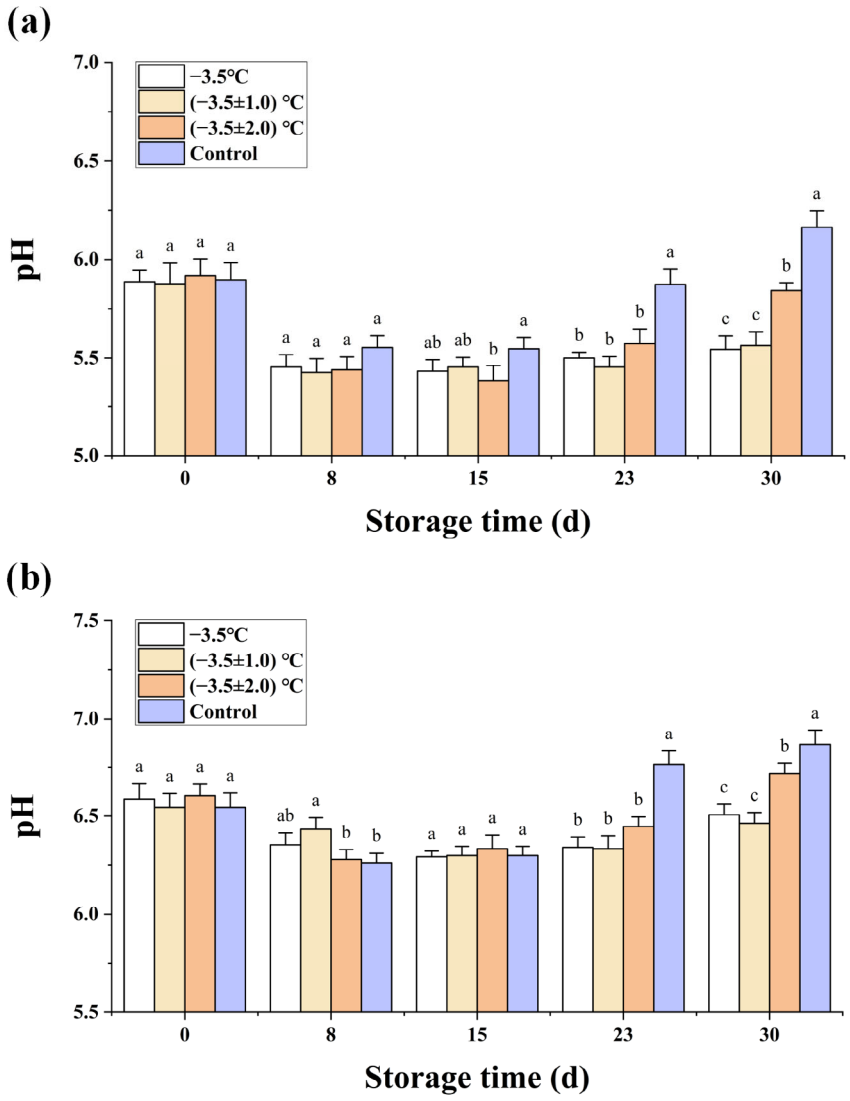

Fig. S2 Changes of pH during superchilling storage at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control. (a) Changes of pH in pork. (b) Changes of pH in salmon. The different lowercases indicate the significant differences among storage conditions for the same storage time (P<0.05). Data are presented as mean±standard deviation (n=3).

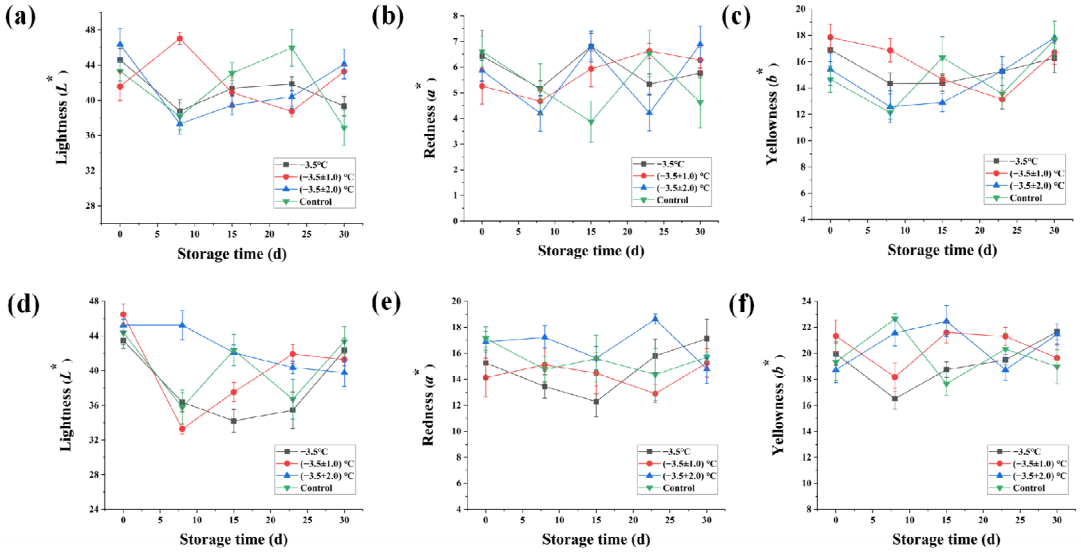

Fig. S3 Changes of meat color during superchilling storage at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control. (a) Changes of lightness (L^*) of pork. (b) Changes of redness (a^*) of pork. (c) Changes of yellowness (b^*) of pork. (d) Changes of lightness (L^*) of salmon. (e) Changes of redness (a^*) of salmon. (f) Changes of yellowness (b^*) of salmon. Data are presented as mean±standard deviation (n=5).

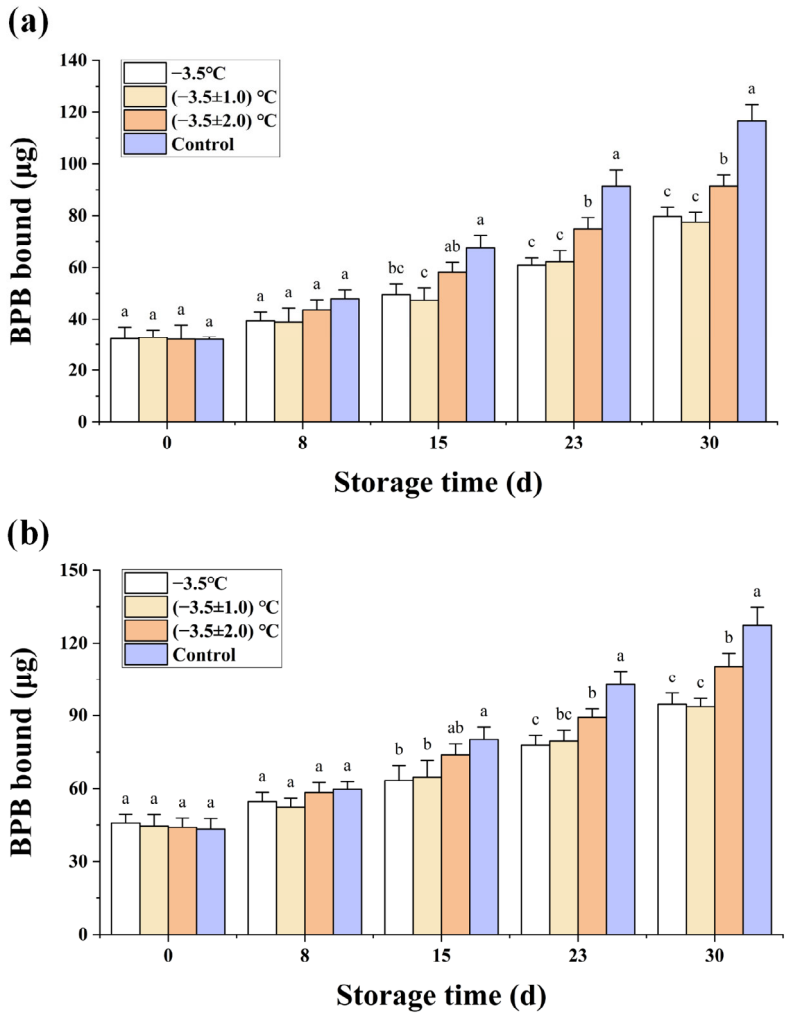

Fig. S4 Changes of bromophenol blue (BPB) bound during superchilling storage at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control. (a) Changes of BPB bound with pork proteins. (b) Changes of BPB bound with salmon proteins. The different lowercases indicate the significant differences among storage conditions for the same storage time (P<0.05). Data are presented as mean±standard deviation (n=3).

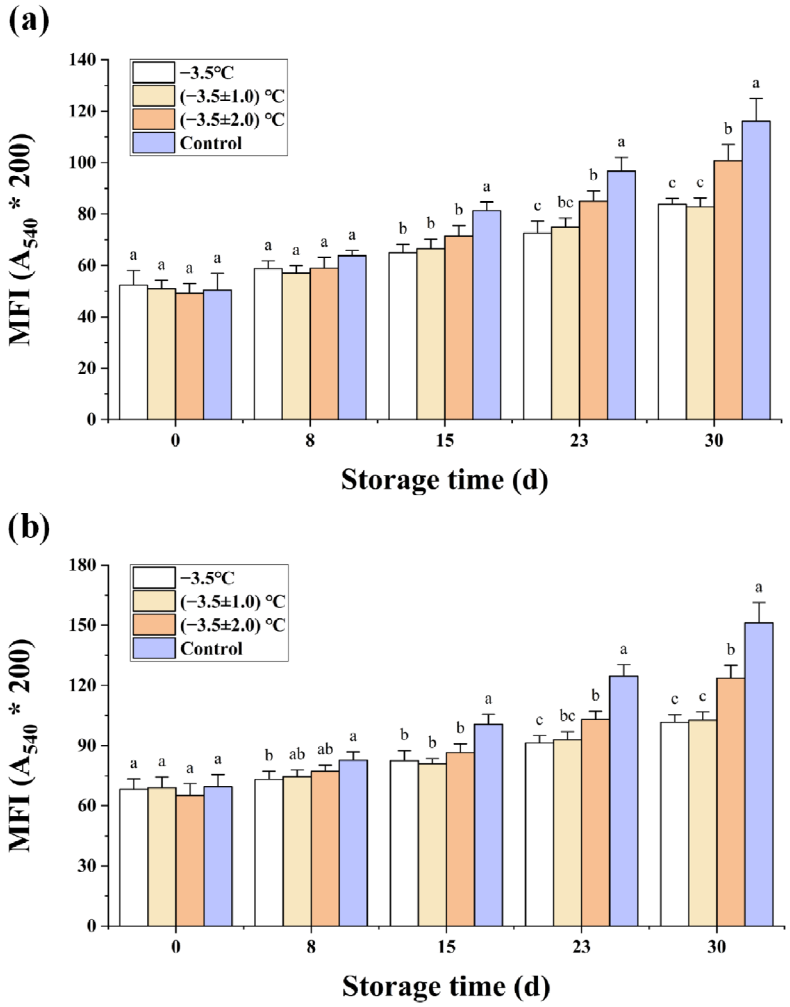

Fig. S5 Changes of myofibril fragmentation index (MFI) during superchilling storage at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control. (a) Changes of MFI in pork. (b) Changes of MFI in salmon. The different lowercases indicate the significant differences among storage conditions for the same storage time (P<0.05). Data are presented as mean±standard deviation (n=3).

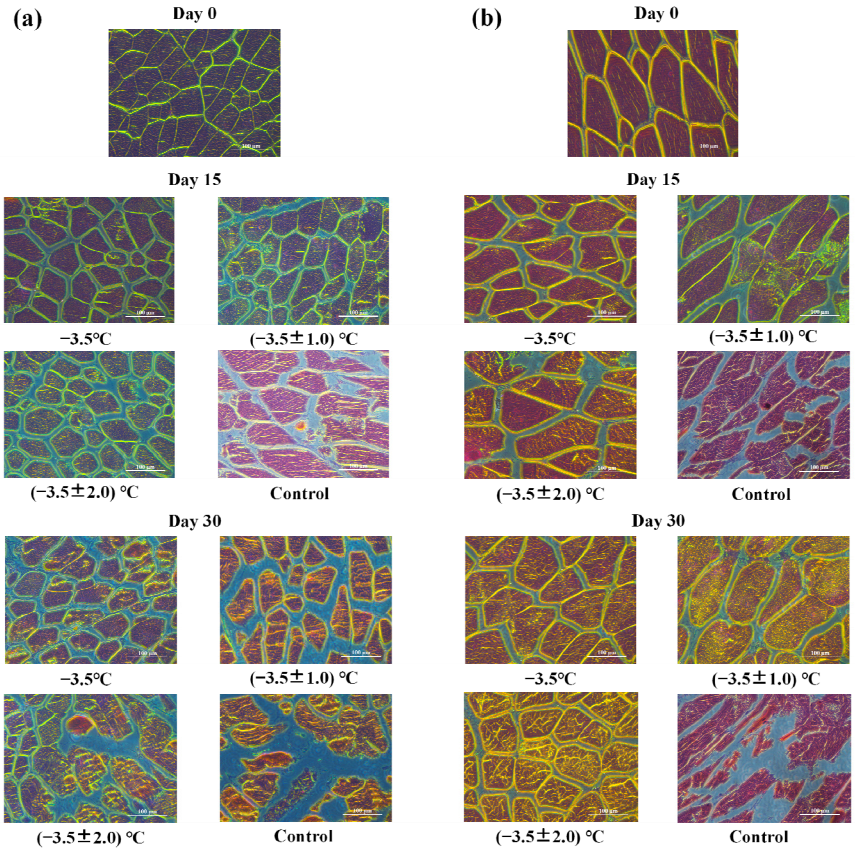

Fig. S6 Microstructures of meat samples stored at −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃, and the control after 0, 15 and 30 d observed using light microscopy (LM). (a) Microstructural changes in pork. (b) Microstructural changes in salmon.

Table S1 Effect of different superchilling temperatures, −3.5 ℃, (−3.5±1.0) ℃, (−3.5±2.0) ℃ and control on the drip loss of pork and salmon

		Drip loss (%)			
	Storage time (d)	−3.5℃	−3.5℃±1.0℃	−3.5℃±2.0℃	Control
Pork	0				
	8	6.11±1.00 [a]	6.53±0.99 [a]	6.34±0.85 [a]	7.12±0.88 [a]
	15	7.84±0.53 [b]	8.09±0.53 [b]	8.79±0.81 [ab]	9.98±1.03 [a]
	23	9.39±0.38 [a]	9.26±0.33 [a]	9.88±0.74 [a]	10.66±0.80 [a]
	30	10.45±0.62 [b]	10.19±0.60 [b]	11.06±0.73 [ab]	12.42±0.97 [a]
Salmon	0				
	8	1.87±0.29 [a]	2.20±0.19 [a]	2.03±0.43 [a]	2.17±0.24 [a]
	15	2.20±0.20 [a]	2.26±0.37 [a]	2.55±0.29 [a]	2.71±0.42 [a]
	23	2.89±0.41 [c]	3.08±0.48 [bc]	4.11±0.52 [ab]	4.49±0.50 [a]
	30	3.16±0.73 [b]	3.27±0.58 [b]	4.41±0.47 [ab]	5.42±0.32 [a]

Note: The different letters (a–c) indicate the significant differences among storage conditions at the same storage time (P<0.05). Data are presented as mean±standard deviation (n=3).

2 Materials and Methods

2.1 Materials and the superchilling process

The common chemicals used in this study were of analytical grade and supplied by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

The fresh pork and salmon samples were purchased from a local supermarket (Hangzhou, Zhejiang Province, China). The fresh raw materials were transported to the supermarket from a slaughterhouse early in the morning. Then, we collected the fish using a cryogenic storage box around 8.30am. Each piece of meat was cut into similar shaped rectangles as much as possible and divided into four groups based on similar weight ((250.0±25.0) g). The study groups were as follows: constant temperature group (−3.5 ℃), two temperature fluctuations groups ((−3.5±1.0) ℃, (−3.5±2.0) ℃), and actual temperature changes group (Control) in an ordinary refrigerator. The samples of all four groups (pork and salmon) were put into ziplock bags and placed in the designated space of the refrigerator according to the preset temperature fluctuations. Each group was sampled and measured 5 times within 30 days of storage at days 0, 8, 15, 23, and 30.

The following analyses were performed for each sample group at each time point.

2.2 Temperature control mode

The changes during the experiment were mainly temperature fluctuations. The refrigerators provided by Changhong Meiling Co., Ltd. were used to achieve constant temperature, temperature fluctuations, and actual temperature changes. Temperature recorders (L93-2L, HangZhou Loggertech Co., Ltd., Zhejiang, China) were used for

the measurement of temperature.

2.3 Evaluation of total volatile basic nitrogen (TVB-N)

The TVB-N was measured according to the Chinese standard GB 5009.228-2016 with some modifications. Briefly, 10.0 g of minced meat and 50 mL of trichloroacetic acid (20 g/L) was added to a beaker, shaken for 30 min, and then filtered. A mixture of 10 mL of this filtrate, 5 mL of MgO (10 g/L), and 10 mL of distilled water (DW) was added to a semi-micro nitrogen distillation apparatus (TianChang Kangpeng Experimental Equipment Co., Ltd., Anhui, China) for 5 min. For a control sample, 5 mL of DW was used instead of filtrate. The content of TVB-N was calculated using the formula below. The mean value was calculated from three replicates.

$$TVB-N\ (mg\ /\ 100g\ meat) = \frac{(V_1 - V_2) \times c \times 14}{m \times (10\ /\ 100)} \times 100$$

In the formula, V_1 denotes the titration volume (mL) of sample to be tested, V_2 denotes the titration volume (mL) of the blank sample, c denotes the actual concentration of HCl (0.01 mol/L), and m is the weight of minced meat sample (20.0 g).

2.4 Determination of total viable count (TVC)

The TVC was determined following the previously described method (Ojagh *et al.*, 2010) with some modifications. In brief, 5.0 g of minced meat sample was aseptically transferred to a sterile sampling bag (Changde Bikeman Biotechnology Co., Ltd., Hunan, China) and homogenized with 45 mL of sterile saline (0.86% NaCl) at room temperature. The mixture was diluted to the appropriate concentration using sterile saline. Then, 1 mL of diluent was spread on the Plate Counting Agar (PCA),

incubated at 37 ℃ for 48 hours, and then counted. The mean value was calculated from three replicates, and the result was expressed as the logarithm of meat colony-forming units (log CFU/g).

2.5 Quantification of thiobarbituric acid reactive substances (TBARS)

The TBARS value was measured following a previously described method (Castellini *et al.*, 2002) with some modifications. Firstly, 4.0 g of minced meat and 20 mL of 7.5% trichloroacetic acid (contained 0.1% EDTA) were mixed and shaken for 30 min. Next, 0.5 mL of filtrate was taken out and added with 0.5 mL of 0.02 mol/L thiobarbituric acid solution. The mixture was stored in a boiling water bath for 40 min, and shaken vigorously with 0.5 mL of chloroform after cooling. The absorbance of the supernatant was measured using a SPARK microplate reader (Tecan Austria GmbH, Salzburg, Austria) at the wavelengths of 532 and 600 nm. The TBARS was calculated using the below formula. The mean value was calculated from three replicates.

$$TBARS\ (mg\ /\ kg) = \frac{A_{532} - A_{600}}{155} \times 72.6 \times 100 \times 0.05 \times m$$

In the formula, m denotes the weight of the minced meat sample.

2.6 Measurement of drip loss

The drip loss determination was performed as per the method described previously (Zhang *et al.*, 2006) with some modifications. The meat was weighed before packaging and the original weight was recorded as W_1. After being stored for a certain number of days, the meat sample was removed from the ziplock bag. The liquid was removed from the meat surface with blotting paper, and the meat was weighed again as W_2. The drip loss was calculated using the below formula. The

mean value was calculated from three replicates.

$$Drip\ loss\ (\%) - \frac{W_1 - W_2}{W_1} \times 100$$

2.7 Measurement of pH

The determination of pH value was referred to the method described previously (Poleti *et al.*, 2018) with some modifications. At first, 5.0 g of ground meat sample was weighed, then added with 50 mL newly boiled and cooled DW, and vigorously shaken. After 30 min, the pH value of homogenate was measured with a pH meter (PB-10S, artorius, Göttingen, Germany). The mean value was calculated from three replicates.

2.8 Determination of meat color

Meat color was determined according to a previously established method (Castellini, Mugnai and Dal Bosco, 2002). After opening the package, the meat surface was wiped dry and left to stand exposed to the air for 30 minutes. Then, *L**, *a**, and *b** values of the sample were measured by a CR-400 chromatic aberration meter (Konica Minolta Inc., Japan). The color of each piece of meat was randomly measured at 5 locations. The mean value was calculated from five replicates.

2.9 Extraction of myofibrillar protein (MP)

The MP was prepared according to a previously described method (Amiri *et al.*, 2018) with some modifications. The meat was homogenized in 5 volumes (v/w) of a 10 mM phosphate buffer (pH 7.0). Then, the mixture was centrifuged at 8000*g*, 4 ℃ for 15 min. The above homogenization step was repeated with the obtained lower layer precipitate 3 times. The last obtained precipitate was mixed with 0.1 M NaCl

solution according to the mass-liquid ratio of 1:4 (w/v), and the same homogenous centrifugation operation step was repeated 3 times. The dispersion was filtered, dissolved, and homogenized in 10 mM PBS buffer after the last centrifugation step, which constituted a stable MP dispersion. After measuring the protein concentration by the biuret reaction, the concentration of MP was adjusted to 5 mg/mL with PBS buffer.

2.10 Evaluation of protein surface hydrophobicity

The protein surface hydrophobicity was determined according to a previously described method (Ilham *et al.*, 2006) with some modifications. Firstly, 1 mL of myofibril suspension (5 mg/mL) was reacted with 200 μL of bromophenol blue (BPB) solution (1 mg/mL) at 25 ℃ for 15 min. Subsequently, the sample was centrifuged to obtain the supernatant. For the control group, 1 mL of phosphate buffer (pH=7.5) was used instead of myofibril suspension. After 10 times diluting of supernatant, the absorbance value A was measured at 595 nm, and the combined BPB was used as the surface hydrophobicity index. The bound BPB was calculated using the below equation. The mean value was calculated from three replicates.

$$BPB\ bound\ (\mu g) = \frac{A_{control} - A_{sample}}{A_{control}} \times 200\ \mu g$$

2.11 Measurement of myofibril fragmentation index (MFI)

The myofibril suspension was extracted according to the method described in Section 2.9. After measuring the protein concentration by the biuret reaction, the concentration of myofibril suspension was adjusted to 0.5 mg/mL with PBS buffer. The absorbance was measured at a wavelength of 540 nm, and the MFI value was

calculated as 200×absorbance. The mean values were calculated from three replicates.

2.12 Histological study by light microscopy (LM)

A histological study was conducted according to a described protocol with some modifications (Awad *et al.*, 2009). The meat surface was removed from the meat sample and cut into cubes with a size of 4 mm×4 mm×5 mm. These were quickly put into the fixing solution and fixed at 4 ℃ for 24 h. The fixed samples were sequentially immersed in 70%, 80%, 95%, and 100% ethanol solutions for gradient dehydration. After soaking in wax, they were placed on a carton for embedding. The solidified wax block was sliced by a paraffin microtome, placed on glass slide at 45 ℃, and then baked for 24 h. Afterwards, the slides were successively stained with hematoxylin and eosin staining solution. The sections were taken out and dried naturally, covered with a coverslip, and then observed under a microscope. Images were taken using a Nikon Eclipse Ti microscope (Nikon Corp., Tokyo, Japan).

2.13 Histological study by scanning electron microscopy (SEM)

The microstructures in muscle samples were observed by using SEM according to the method described previously (Li *et al.*, 2014) with some modifications. The samples (4 mm×4 mm×5 mm, cut with a scalpel) were fixed with 2.5% glutaraldehyde overnight at 4 ℃, rinsed three times with 0.1 M phosphate buffer (pH=7.0), and then dehydrated with graded ethanol after being post-fixed in 1% osmium tetroxide at 4 ℃ for 2 h. The samples were dried in a Hitachi HCP-2 critical point dryer (Hitachi High-Tech Corp., Tokyo, Japan). Thereafter, the dried samples were mounted on a bronze stub and sputter-coated with gold in an MC1000 ion

sputter (Hitachi High-Tech Corp., Tokyo, Japan). The microstructures were observed under a Hitachi SU-8010 SEM (Hitachi High-Tech Corp., Tokyo, Japan) at a magnification of 250–500×.

2.14 Statistical analysis

The figures were drawn by Origin 2018. The results were analyzed by SPSS (Statistics 20, SPSS Inc., Chicago, USA). Each sample was detected at least 3 times in each index to confirm the results. The results were presented as means ± standard error of the mean (SEM). The Duncan's multiple range test was applied for multiple comparisons. $P<0.05$ was considered as statistical significance.

References

Amiri, A., Sharifian, P. and Soltanizadeh, N., 2018. Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins. *Int. J. Biol. Macromol.,* **111**:139-147. https://doi.org/10.1016/j.ijbiomac.2017.12.167

Awad, W.A., Ghareeb, K., Abdel, R.S., *et al*., 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poult. Sci.,* **88**(1):49-56. https://doi.org/10.3382/ps.2008-00244

Castellini, C., Mugnai, C. and Dal, B.A., 2002. Effect of organic production system on broiler carcass and meat quality. *Meat Sci.,* **60**(3):219-225. https://doi.org/10.1016/s0309-1740(01)00124-3

Ilham, C., Gatellier, P. and Sante, L.V., 2006. A simplified procedure for myofibril hydrophobicity determination. *Meat Sci.,* **74**(4):681-683. https://doi.org/10.1016/j.meatsci.2006.05.019

Li, Y., Li, X., Wang, J. Z., *et al.*, 2014. Effects of oxidation on water distribution and physicochemical properties of porcine myofibrillar protein gel. *Food Biophys*, **9**(2):169-178. https://doi.org/10.1007/s11483-013-9329-9

Ojagh, S.M., Rezaei, M., Razavi, S.H., *et al.*, 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chem.*, **120**(1):193-198.

https://doi.org/10.1016/j.foodchem.2009.10.006

Poleti, M.D., Moncau, C.T., Silva, V.B., *et al.*, 2018. Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. *Meat Sci.,* **145**:209-219. https://doi.org/10.1016/j.meatsci.2018.06.041

Zhang, W.G., Lonergan, S.M., Gardner, M.A., *et al.*, 2006. Contribution of postmortem changes of integrin, desmin and mu-calpain to variation in water holding capacity of pork. *Meat Sci.,* **74**(3):578-585. https://doi.org/10.1016/j.meatsci.2006.05.008