Quantity and functionality of protein fractions in chicken breast fillets affected by white striping

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ABSTRACT Recently, white striations parallel to muscle fibers direction have been observed on the surface of chicken breast, which could be ascribed to intensive growth selection. The aim of this study was to evaluate the effect of white striping on chemical composition with special emphasis on myofibrillar and sarcoplasmic protein fractions that are relevant to the processing features of chicken breast meat. During this study, a total of 12 pectoralis major muscles from both normal and white striped fillets were used to evaluate chemical composition, protein solubility (sarcoplasmic, myofibrillar, and total protein solubility), protein quantity (sarcoplasmic, myofibrillar, and stromal proteins), water holding capacity, and protein profile by SDS-PAGE analysis. White-striped fillets exhibited a higher percentage of moisture (75.4 vs. 73.8%; $P < 0.01$), intramuscular fat (2.15 vs. 0.98%; $P < 0.01$), and collagen (1.36 vs. 1.22%; $P < 0.01$), and lower content of protein (18.7 vs. 22.8%; $P < 0.01$) and ash (1.14 vs. 1.34%; $P < 0.01$), in comparison with normal fillets. There was a great decline in myofibrillar (14.0 vs. 8.7%; $P < 0.01$) and sarcoplasmic (3.2 vs. 2.6%; $P < 0.01$) content and solubility as well as an increase in cooking loss (33.7 vs. 27.4%; $P < 0.05$) due to white striping defects. Moreover, gel electrophoresis showed that the concentration of 3 myofibrillar proteins corresponding to actin (42 kDa); LC1, slow-twitch light chain myosin (27.5 kDa); and LC3, fast-twitch light chain myosin (16 kDa), and almost all sarcoplasmic proteins were lower than normal. In conclusion, the findings of this study revealed that chicken breast meat with white striping defect had different chemical composition (more fat and less protein) and protein quality and quantity (low content of myofibrillar proteins and high content of stromal proteins) with respect to normal meat. Furthermore, white striped fillets had lower protein functionality (higher cooking loss). All the former changes indicate that white striping has great impact on quality characteristics of chicken breast meat.

Key words: chicken breast meat, white striping, myofibrillar protein, sarcoplasmic protein, protein solubility

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INTRODUCTION

White striping defect is one of the recent emerging poultry quality issues, which can be attributed to selection for increased growth rate and breast yield. White striping is distinguished by the appearance of white striations parallel to muscle fibers on the surface of the pectoralis major muscle (Petracci and Cavani, 2012). The etiological causes of white striping are still poorly understood. What we know is that several factors can affect its incidence rate, which can be summarized as follows: genotype (high > standard breast yield; Petracci et al., 2013a), sex (males > females; Kuttappan et al., 2013a), growth rate (fast > low; Kuttappan et al., 2012a, 2013a), diet (high > low energy diet; Kuttappan et al., 2012a), and weight at slaughter (heavy > light; Kuttappan et al., 2013a).

The histological evaluations showed that white striping is normally associated with muscle degeneration and myopathic changes beneath the striation area with interstitial connective tissue (fibrosis) and fat (lipidosis) accumulation (Kuttappan et al., 2013b; Sihvo et al., 2014). A high incidence of similar histopathological anomalous features was also observed by Petracci et al. (2013b) in breast muscles from high-yield breast birds. Overall, fillets with severe white striping are characterized by a higher content of intramuscular fat and collagen and lower protein content (Kuttappan et al., 2012a; Petracci et al., 2014). White striping also lowers the acceptance of meat by consumers and affects purchase decisions (Kuttappan et al., 2012b). Petracci et al. (2013a) also found that white striping has a dramatic effect on water holding capacity (**WHC**; higher drip and cooking losses, and lower marinade uptake) and texture (lower shear force) of meat. On

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the contrary, Kuttappan et al. (2013a) did not found any major changes in cooked meat quality. All former changes showed that white striping defect can have different consequences on the quality and characteristics of chicken breast meat.

More attention should be given to the high incidence of white striping under commercial conditions (Petracci et al., 2013a) besides all former quality defects to evaluate the factors that affect the reduction of quality traits of white striped (**WS**) breast meat.

Protein composition of breast meat has a crucial impact on processing, sensorial, and nutritional quality traits (Smyth et al., 1999). Particularly, proteins are considered as the most important components of meat from a nutritional and processing viewpoint. Indeed, meat proteins contain all the amino acids essential to the human body, thus making them highly nutritious (Friedman, 1996). Moreover, meat proteins greatly contribute to processing abilities by imparting specific functionalities. The overall properties of meat and meat products, including appearance, texture, and mouth feel are dependent on protein functionality (Xiong, 2004). It is well known that myofibrillar proteins (i.e., myosin and actin) are mainly responsible for the WHC and textural properties of meat and meat products, whereas sarcoplasmic proteins (i.e., muscle enzymes) play a minor role (Smith, 2010; Sun and Holley, 2011; Petracci et al., 2013c). Solubility of myofibrillar and sarcoplasmic proteins is highly correlated with water retention (Li-Chan et al., 1987; Warner et al., 1997). Protein solubility also has a major role in the physical properties of the meat because lower protein solubility imparts poor functionality, as in the case of pale, soft, and exudative (**PSE**)-like meat (Van Laack et al., 2000; Bowker and Zhuang, 2013).

The aim of this study was to evaluate the effect of white striping on chemical composition with special emphasis on myofibrillar and sarcoplasmic protein fractions that are relevant to the processing features of chicken breast meat.

MATERIALS AND METHODS

Chemicals and solvents, unless specified, were of analytical grade and purchased from Carlo Erba Reagenti (Rodano, Italy), Merck (Darmstadt, Germany), and Sigma-Aldrich (St. Louis, MO).

Sample Selection and Preparation

Pectoralis major muscles (n = 12) were selected from normal and severe WS boneless breast fillets, which were collected from a single flock of 7-wk-old straight-run Ross 708 broilers (average live weight of 2.80 kg) after the deboning area in a commercial processing plant. Fillets were classified as normal and affected by severe white striping according to the criteria proposed by Kuttappan et al. (2012b). Fillets were bagged and transported under cold conditions (0–2°C) to the laboratory. The fillets were trimmed of excess fat and connective tissues and individually weighed. In addition, geometrical measurements were determined in centimeter with a caliper as described by Mehaffey et al. (2006), with slight modifications. Length was measured from the longest dimension of the fillet. Width was measured from the longest distance from side to side in the middle of fillet. Height was measured in 3 points: the first height (**H1**) was measured as vertical distance far from the end of caudal part by 1 cm toward dorsal direction; the second height (**H2**) was measured at the half distance of the breast length; the third height (**H3**) was measured at the highest point in the cranial part. Finally, the cranial part of each pectoralis major muscle was minced by small meat grinder with a 4 mm hole plate disk, mixed, and kept in freezer at −20°C for further analysis. The samples were used for determination of chemical composition (moisture, protein, lipid, ash, and collagen contents); total quantity of myofibrillar and sarcoplasmic proteins; solubility of sarcoplasmic, myofibrillar, and total proteins; and molecular protein profile by electrophoresis analysis.

Meat Quality Analysis

Chemical Composition. Proximate composition (moisture, protein, ash, and lipid contents) of breast meat was estimated in 3 replications for each sample using official methods of AOAC (1990). The moisture content was determined by accurately weighing 5 g of ground sample and then drying it in a conventional oven at 100 to 102°C for 16 h. Crude protein content was measured by Kjeldahl method, whereas intramuscular fat content was estimated by petroleum ether extraction using the Soxhlet method. Ash content was determined by incineration at 525°C. In addition, collagen content (hydroxyl proline as a measure) was determined using the colorimetric method proposed by Kolar (1990).

Total Content of Myofibrillar and Sarcoplasmic Proteins. Myofibrillar and sarcoplasmic proteins were extracted and separated following the method described for electrophoresis by Fritz et al. (1989). The total content of myofibrillar and sarcoplasmic proteins was measured by Bradford (1976) assay that involves the binding of proteins with Coomassie brilliant blue G-250 dye. The absorbance of the blue protein-dye complex was detected at 595 nm using a spectrophotometer UV-1601 from Shimadzu (Duisburg, Germany). The concentration was calculated using a calibration curve obtained with quick start BSA standard set from Bio-Rad (Segrate, Italy).

Protein Solubility. Protein solubility was estimated according to differences in extractability of proteins in different ionic strength solutions (Warner et al., 1997). Sarcoplasmic protein solubility was measured in 3 replications by weighing 1 g of breast meat sample. Ten milliliters of cold 25 m*M* potassium phosphate buffer (pH 7.2) were added to the samples and homogenized

by high-speed blender (Ultra-Turrax, T25 basic, New Brunswick, NJ) on the lowest speed (11,000 rpm/min). The homogenized samples were kept under refrigeration conditions (4°C) for 20 h and then centrifuged at 2,600 × *g* for 30 min at 4°C. The supernatant was decanted and protein concentration was measured using the Bradford assay (Bradford, 1976) with bovine serum albumin as a standard. Total protein solubility was similarly determined in a 1.1 *M* KI, 0.1 *M* potassium phosphate (pH 7.2) buffer. Myofibrillar protein solubility was calculated by the difference in the solubility of total and sarcoplasmic proteins.

WHC. Cooking loss was used as measure for WHC. About 6 g of minced breast meat were weighed into a 50-mL plastic test tube. After the addition of 10 mL 3.5% NaCl solution, the tubes were vigorously shaken for 15 s and then held for 30 min at room temperature. The supernatant was separated after centrifugation for 15 min at 3,000 × *g* at 4°C and the sediment was weighted (Van Laack et al., 2000). After cooking the tube content at 80°C for 20 min, cooking loss was determined as weight difference.

Electrophoresis Analysis. Normal and WS fillets were selected to separate the extracted proteins according to their molecular weights by SDS-PAGE analysis. The analysis was repeated twice for each sample. Minced meat sample (2 g) was added to 20 mL of rigor-buffer (**RB**) containing 75 m*M* KCl, 10 m*M* KH_2PO_4, 2 m*M* $MgCl_2$, and 2 m*M* ethylene glycol tetraacetic acid (pH 7.0) and homogenized with a high-speed blender (Ultra-Turrax, T 25 basic) on the lowest speed (11,000 rpm/min). The homogenate was centrifuged at 10,000 × *g*, keeping the temperature at 4°C for 10 min, and the supernatant (S1) decanted and saved. Twenty mL of fresh RB was added to the sediment and the homogenization repeated. A sample (0.5 mL) of this homogenate (P1) was saved and the centrifugation repeated. This process was repeated to obtain S1 up to S4 and P1 up to P4 (Fritz et al., 1989). The S1 was dedicated to sarcoplasmic protein evaluation, whereas P4 was used for myofibrillar protein evaluation. Moreover, a composite sample from S1 to S4 was used for sarcoplasmic fractions. Samples were mixed 1:1 with standard sample buffer that contained 8 *M* urea, 2 *M* thiourea, 3% (wt/vol) SDS, 75 m*M* DL-dithiothreitol, and 25 m*M* TrisHCl at pH 6.8 (Fritz et al., 1989), heated at 100°C for 5 min in a water bath, cooled, and applied to the gel. The concentration of extracted protein was measured using the Bradford assay (Bradford, 1976) before loading to gel. Fifteen microliters of myofibrillar protein extract was loaded on 12% Mini-Protean TGX Stain-Free Gel (Bio-Rad), and the same amount of sarcoplasmic extract was loaded on Mini-Protean TGX any kDa Stain-Free (Bio-Rad). The separated protein bands were identified by comparing their mobilities against those of a molecular weight marker (Precision plus Standard protein, all blue prestained, Bio-Rad) made of 10 purified proteins with different molecular weights (10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa). The point to point (semi-log) regression method was used to calculate the molecular weights. The reservoir buffer used in the Mini-protean II cell small electrophoresis unit (Bio-Rad) contained 50 m*M* Tris, 0.384 *M* glycine, and 0.1% (wt/vol) SDS. Small gels were run at a constant voltage of 80 and 120 V for stacking and running gel, respectively.

Sarcoplasmic and myofibrillar protein gel images were captured by a ChemiDoc MP tabletop scanner with Image Lab Rev 4.0 software (Bio-Rad). During the acquisition of images, Stain Free Gel application was used with 3.174 s (Auto-Intense Bands) as exposure time. Gels were activated by UV Trans illumination and subsequently managed by Image Lab Rev 4.0 software on a tabletop computer to determine protein concentration. Calibration curve was prepared by standard bovine serum proteins (BSA: 0.1, 0.25, 0.5, 0.75, 1.00, and 1.5 mg). Image area was X: 95.0, Y: 71.0 (mm) with a pixel size (μm) X: 68.2, Y: 68.2.

One dimensional SDS-PAGE analysis was used to evaluate the molecular weight profile of sarcoplasmic and myofibrillar proteins. The concentration of each band was expressed in 2 ways as absolute (mg/g of meat) and relative abundance (%). The latter was calculated based on the sum of protein concentration in all bands within the same lane to avoid the small differences due to protein loading among lanes. In each band, the dominant protein was determined based on molecular weight and relative abundance. Electrophoretic protein bands were assigned by comparison with data reported in literature using mass spectrometry (Huang et al., 2011; Zapata et al., 2012).

Statistical Analysis

The differences in quality traits between normal and WS fillets were determined by ANOVA using the GLM (SAS Institute Inc., 1988). The model tested the main effect for type of meat quality abnormality (normal vs. white striping) on meat quality traits. Each breast was considered as one experimental unit.

RESULTS

Breast Dimensions, Chemical Composition, and Gel Electrophoresis

The weights and dimensions of normal and WS fillets are presented in Table 1. The WS fillets exhibited higher weight (290.4 vs. 243.1 g, $P < 0.05$), length (19.9 vs. 18.4 cm; $P < 0.05$), and middle (H2, 3.1 vs. 2.4 cm; $P < 0.01$) and top (H3, 3.5 vs. 3.1 cm; $P < 0.01$) heights, whereas breast width and bottom height (H1) did not vary between groups.

The results of proximate composition of normal and WS fillets are reported in Table 2. All parameters were significantly modified by occurrence of white striping. The WS fillets showed significantly lower content of protein (18.7 vs. 22.8%; $P < 0.001$) and ash (1.14

Table 1. Weight and dimensions (means ± SEM) of normal and white striped (WS) chicken breast fillets (n = 6/group)

Parameter	Normal	WS	Probability
Weight (g)	243.1 ± 10.6	290.4 ± 15.8	*
Length (cm)	18.4 ± 0.1	19.9 ± 0.5	*
Width (cm)	8.9 ± 0.5	9.2 ± 0.6	NS
Bottom height (H1[1]; cm)	0.7 ± 0.1	0.8 ± 0.1	NS
Middle height (H2[2]; cm)	2.4 ± 0.1	3.1 ± 0.2	**
Top height (H3[3]; cm)	3.1 ± 0.2	3.5 ± 0.1	*

[1]H1 was measured far from the end of caudal part by 1 cm toward dorsal direction.

[2]H2 was measured at the half distance of the breast length.

[3]H3 was measured at the thickest point in the cranial part.

*$P < 0.05$; **$P < 0.01$.

vs. 1.34; $P < 0.001$), as well as higher percentage of moisture (75.4 vs. 73.8%; $P < 0.001$), intramuscular fat (2.15 vs. 0.98%; $P < 0.01$), and collagen (1.36 vs. 1.22%; $P < 0.01$).

Total sarcoplasmic and myofibrillar protein contents are shown in Figure 1. It was found that white striping determined a lower content of both sarcoplasmic (2.6 vs. 3.2 g/100 g of meat; $P < 0.01$) and myofibrillar (8.7 vs. 14.0 g/100 g of meat; $P < 0.05$) proteins.

The results of SDS-PAGE analysis for meat proteins from normal and WS fillets showed different patterns of both sarcoplasmic and myofibrillar proteins (Figure 2). Eight bands of myofibrillar proteins with molecular weights ranging from 16 to 80 kDa were quantified (Table 3). Instead, for sarcoplamic proteins, 12 bands were detected, but only 11, having molecular weight from 25 to 90 kDa, were quantified (Table 4). An unknown protein of 80 kDa, actin (42 kDa); LC1, slow-twitch light chain myosin (27.5 kDa); and LC3, fast-twitch light chain myosin (16 kDa) were significantly lower in the concentration in WS fillets than in normal ones. No significant difference was detected in protein concentration of troponin (70 kDa), desmin (53 kDa), partial hydrolysis of troponin (29 kDa), and LC2, slow-twitch light chain myosin (19 kDa) between normal and WS meat. The WS samples also exhibited lower relative abundance of 80 kDa band; LC1, slow twitch light chain myosin (27.5 kDa); LC3, fast-twitch light chain myosin; and partial hydrolysis of troponin. G-actin (42 kDa) had exceptionally higher value of relative abundance in WS meat. The remaining proteins did not show any significant difference in relative abundance. In general, all proteins showed the same trend between the concentration and relative abundance for both normal and WS fillets, except for partial hydrolysis of troponin (29 kDa). In this type of protein, there were no significant differences, but at the same time there was significant difference in relative abundance. Actin was the highest in concentration (156.2 and 136.5 mg/g) and relative abundance (81.2 and 86.1%) for both normal and WS fillets.

The concentration of sarcoplasmic proteins of band 1 (glycogen phosphorylase), 2 (pyruvate kinase), 3 (phosphoglucose isomerase), 4 (enolase), 6 (aldolase), 7 (glyceraldehyde dehydrogenase), 8 (lactate dehydrogenase), 9 (31.8 kDa), 10 (26.4 kDa), and 11 (phosphoglycerate mutase) were lower in WS meat samples (Table 4). On the contrary, there was no significant difference in the concentration of creatine kinase (43 kDa). The WS samples exhibited lower relative abundance of enolase (47 kDa), aldolase (39 kDa), and phosphoglycerate mutase (25 kDa) than normal ones. Glycogen phosphorylase (22.8 vs. 18.3%; $P < 0.01$) and lactate dehydrogenase (25.1 vs. 23.3%; $P < 0.05$) showed exceptionally higher relative abundance in WS fillets compared with normal ones. The remaining proteins did not exhibit any difference in relative abundance. The concentration and relative abundance of lactate dehydrogenase were the highest in both normal and WS samples compared with other types of proteins. In general, there was no increase in the concentration of any type of sarcoplasmic proteins.

WHC and Protein Solubility

Cooking loss is normally used to measure the loss of liquids as a result of protein denaturation and decomposition of cell membranes during cooking. In the current study, WS fillets exhibited higher values of cooking loss (33.7 vs. 27.4%; $P < 0.001$) in comparison with normal fillets (Figure 3).

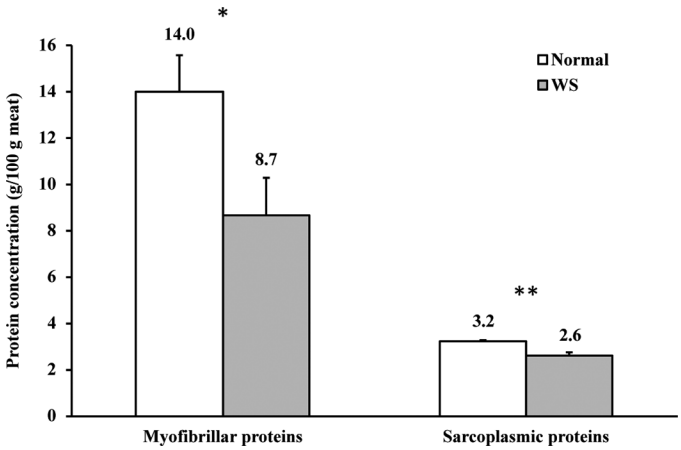

Figure 1. Total content (means ± SEM) of sarcoplasmic and myofibrillar proteins (g/100 g of meat) of normal and white striped (WS) chicken breast meat (n = 6/group; *$P < 0.05$; **$P < 0.01$).

Table 2. Chemical composition (means ± SEM) of normal and white striped (WS) chicken breast meat (n = 6/group)

Parameter	Normal	WS	Probability
Moisture (%)	73.8 ± 0.24	75.4 ± 0.31	**
Protein (%)	22.8 ± 0.63	18.7 ± 0.25	***
Intramuscular fat (%)	0.98 ± 0.23	2.15 ± 0.40	***
Ash (%)	1.34 ± 0.04	1.14 ± 0.02	***
Collagen (%)	1.22 ± 0.03	1.36 ± 0.04	**

**$P < 0.01$; ***$P < 0.001$.

The ranges of sarcoplasmic, myofibrillar, and total protein solubility for both severe and normal fillets were 44.8 to 52.0, 65.3 to 85.5, and 110.1 to 137.4 mg/g of meat, respectively. The solubilities of total, myofibrillar, and sarcoplasmic proteins are reported in Table 5. Protein solubility is frequently used to evaluate protein denaturation and its effect on WHC. Severe WS fillets showed lower protein solubility for sarcoplasmic, myofibrillar, and total protein fractions compared with normal fillets. The differences in protein solubility between normal and WS fillets were more tangible in total (137.4 vs. 110.1 mg/g; $P < 0.001$) and myofibrillar (85.5 vs. 65.3 mg/g; $P < 0.001$) proteins than in sarcoplasmic (52.0 vs. 44.8 mg/g; $P < 0.01$) proteins. On the other hand, when protein solubility based on total CP content was taken into account (Table 5), there were no significant differences between normal and WS meat samples.

DISCUSSION

The weight and dimension measurements were used to evaluate the growth pattern of breast fillets because it was found that high-yield breast hybrids were af-

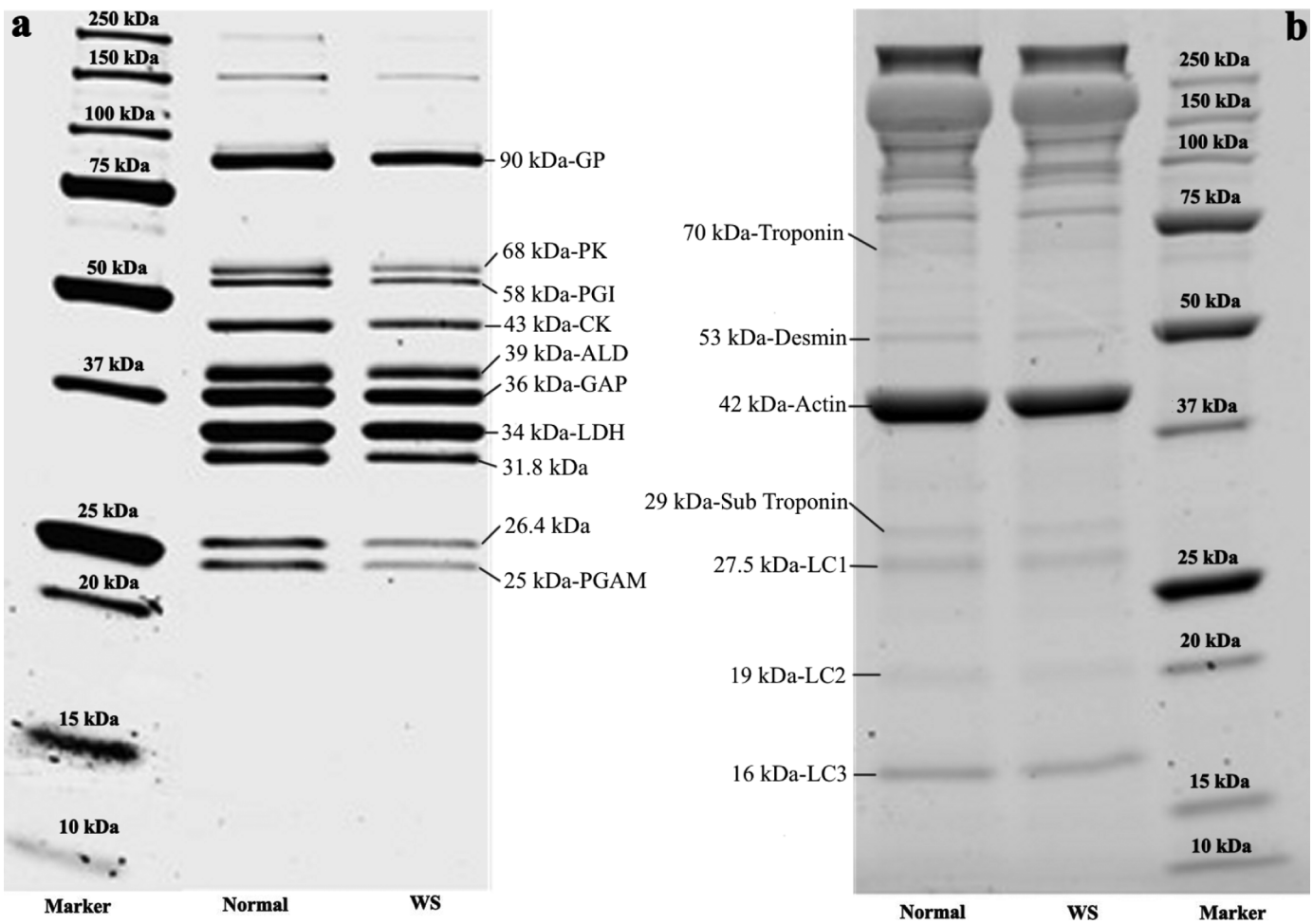

Figure 2. Stain-free SDS-PAGE of sarcoplasmic (a) and myofibrillar (b) proteins of normal and white striped (WS) samples. GP, glycogen phosphorylase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; ALD, aldolase; GAP, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglycerate mutase; LC1, slow-twitch light chain myosin; LC2, slow-twitch light chain myosin; LC3, fast-twitch light chain myosin.

Table 3. Concentration (mg/g)[1] and relative abundance (%)[2] of SDS-PAGE myofibrillar protein bands (means ± SEM) of normal and white striped (WS) chicken breast meat (n = 6/group)

Band No.	Protein name	Molecular weight (kDa)	Concentration (mg/g)			Relative abundance (%)		
			Normal	WS	Probability	Normal	WS	Probability
1	Unknown	80	7.6 ± 0.6	3.7 ± 0.7	***	3.9 ± 0.2	2.2 ± 0.3	***
2	Troponin	70	2.3 ± 0.6	1.2 ± 0.4	NS	1.1 ± 0.3	0.7 ± 0.2	NS
3	Desmin	53	2.3 ± 0.7	2.1 ± 0.9	NS	1.1 ± 0.3	1.2 ± 0.5	NS
4	Actin	42	156.3 ± 7.7	136.5 ± 6.3	*	81.2 ± 1.7	86.1 ± 1.5	**
5	Partial hydrolysis of troponin	29	2.0 ± 0.6	1.4 ± 0.4	0.06	0.9 ± 0.3	0.8 ± 0.2	*
6	LC1 slow-twitch light chain myosin	27.5	7.7 ± 1.1	4.0 ± 0.6	***	3.9 ± 0.3	2.4 ± 0.2	***
7	LC2 slow-twitch light chain myosin	19	1.5 ± 0.4	0.6 ± 0.4	NS	0.7 ± 0.2	0.3 ± 0.2	NS
8	LC3 fast-twitch light chain myosin	16	13.6 ± 1.2	9.6 ± 0.7	***	6.9 ± 0.2	6.0 ± 0.3	***

[1]The concentrations of proteins were expressed in milligrams per gram of raw chicken breast meat.

[2]The relative abundance was calculated by measuring the concentration of extracted protein on each band divided by the sum of the concentration for all bands in the same gel lane multiply by 100.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

fected by white striping and related histopathological anomalous features more than low-yield breast hybrids (Kuttappan et al., 2013b; Petracci et al., 2013b). In the current data, the WS fillets showed higher weight and dimensions than normal fillets. These results agree with Kuttappan et al. (2009), who used a similar approach and found that all of breast dimensions except the length were affected by white striping. In a more recent study, the same authors showed that fillet weights and yields increased as severity of striping increased (Kuttappan et al., 2012a). Even if a small number of samples was considered in the present study, it was confirmed that occurrence of white striping was mainly associated with thicker or heavier fillets as suggested by Kuttappan et al. (2013a), and as a consequence, birds within the same flock showing higher breast sizes are more prone to develop white striping abnormality.

Overall, the results confirmed that white striping defect had a dramatic effect on chemical composition. Severe WS breast meat had higher fat and moisture content and lower levels of total protein and ash. Moreover, even if total protein content was dramatically reduced in fillets showing white striping, there was an increase in collagen. These results were consistent with Petracci et al. (2014) who found that WS fillets showed higher fat and collagen content and lower protein content. Kuttappan et al. (2012a, 2013a) also found that severe WS fillets had higher fat content and lower protein content compared with normal fillets, although they found no effects on moisture and ash content. It should not be underestimated that overall these changes may determine a strong reduction of nutritional value of chicken breast meat as previously reported by Kuttappan et al. (2012a) and Petracci et al. (2014).

Table 4. Concentration (mg/g)[1] and relative abundance (%)[2] of SDS-PAGE sarcoplasmic protein bands (means ± SEM) of normal and white striped (WS) chicken breast meat (n = 6/group)

Band No.	Protein name	Molecular weight (kDa)	Concentration (mg/g)			Relative abundance (%)		
			Normal	WS	Probability	Normal	WS	Probability
1	Glycogen phosphorylase	90	12.1 ± 0.6	9.2 ± 0.6	**	18.3 ± 0.4	22.8 ± 1.1	**
2	Pyruvate kinase	60	2.8 ± 0.3	1.4 ± 0.2	**	4.2 ± 0.2	3.3 ± 0.3	*
3	Phosphoglucose isomerase	58	2.0 ± 0.3	1.0 ± 0.2	**	2.9 ± 0.2	2.4 ± 0.3	NS
4	Enolase	47	4.0 ± 0.3	2.0 ± 0.2	***	6.0 ± 0.2	4.9 ± 0.2	**
5	Creatine kinase	43	1.6 ± 0.2	1.0 ± 0.2	NS	2.4 ± 0.3	2.4 ± 0.3	NS
6	Aldolase	39	2.8 ± 0.2	1.4 ± 0.1	***	4.1 ± 0.1	3.4 ± 0.1	***
7	Glyceraldehyde phosphate dehydrogenase	36	9.4 ± 0.5	5.5 ± 0.6	***	14.3 ± 0.4	13.2 ± 0.6	NS
8	Lactate dehydrogenase	34	15.3 ± 0.7	10.3 ± 0.9	***	23.3 ± 0.5	25.1 ± 0.5	*
9	Unknown	31.8	6.6 ± 0.3	4.0 ± 0.4	***	10.0 ± 0.3	9.6 ± 0.2	NS
10	Unknown	26.4	4.5 ± 0.6	2.6 ± 0.3	**	6.6 ± 0.4	6.3 ± 0.3	NS
11	Phosphoglycerate mutase	25	4.9 ± 0.4	2.7 ± 0.30	***	7.4 ± 0.1	6.5 ± 0.2	**

[1]The concentrations of proteins were expressed in milligrams per gram of raw chicken breast meat.

[2]The relative abundance was calculated by measuring the concentration of extracted protein on each band divided by the sum of the concentration for all bands in the same gel lane multiplied by 100.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

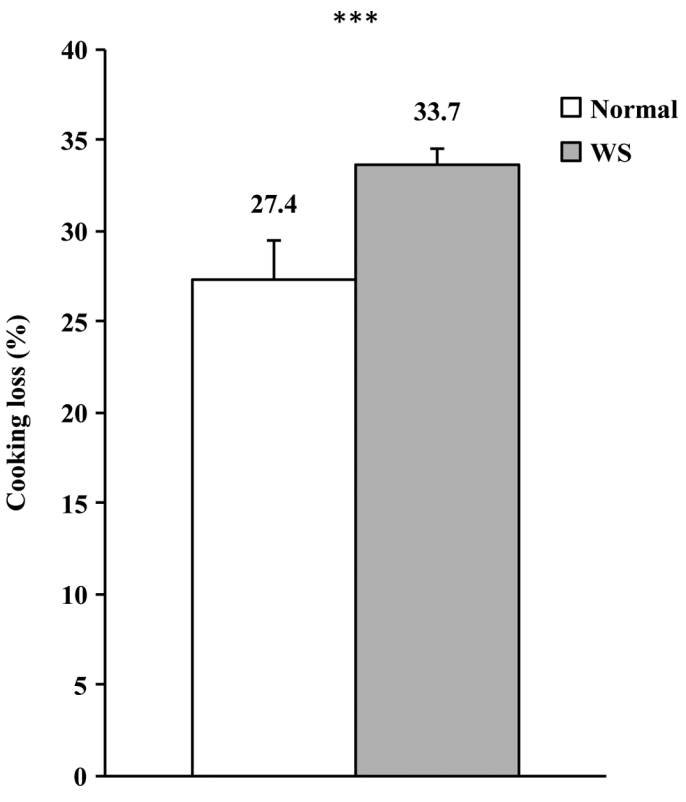

Figure 3. Cooking loss (means ± SEM) of normal and white striped (WS) chicken breast meat (n = 6/group; $^{***}P < 0.001$).

These dramatic differences in proximate composition can be likely due to muscular degeneration previously observed in WS breast muscles (Kuttappan et al., 2013b) that can explain the reduction in protein content. In addition, increase of fat accumulation due to lipidosis can explain the higher intramuscular lipid content, whereas higher content of collagen can be explained by fibrosis (Kuttappan et al., 2013b; Sihvo et al., 2014). Thereupon, lower total protein level may be an indirect effect of fiber degeneration and atrophy, coupled with increased fat accumulation. This hypothesis is reinforced by the remarkable reduction of both myofibrillar and sarcoplasmic proteins observed in the present study. It is well known that degeneration of fibers in muscular dystrophy is characterized by an extensive loss of sarcoplasmic and contractile protein with replacement of fat and connective tissue (Stracher et al., 1979). Reduction of myofibrillar proteins can be mainly due to increased myofibrillar catabolism (Hillgartner et al., 1981), whereas sarcoplasmic protein decline can be a consequence of leakage due to sarcolemma damage and alteration of muscular enzymes (Patnode et al., 1976). On the other hand, similar degenerative processes and histopathological lesions have been also described for some of the major poultry myopathies, which have been associated with selection for growth rate in chickens (MacRae et al., 2006).

The SDS-PAGE revealed that the absolute concentrations of myofilament proteins such as actin, LC1 slow-twitch light chain myosin, and LC3 fast-twitch light chain myosin, which are components of contractile fibers, were decreased. Furthermore, the decrease of concentration of specific myofibrillar proteins (actin, LC1, and LC3) may indicate that the degeneration process could be selective in some sites of myofilament. However, it was possible to observe a reduction of both absolute and relative concentrations of LC1 slow-twitch and LC3 fast-twitch light chain myosins. Previously, Stracher et al. (1979) reported that myosin from dystrophic chickens contained less LC3 myosin than normal birds and suggested that dystrophic myosin might be embryonic in nature and more susceptible to proteolysis. In general, SDS-PAGE analysis revealed to a certain extent that WS meat had a different quantitative distribution for myofibrillar proteins.

On the other hand, all identified sarcoplasmic proteins (glycogen phosphorylase, pyruvate kinase, phosphoglucose isomerase, enolase, aldolase, glyceraldehyde phosphate dehydrogenase, lactate dehydrogenase, and phosphoglycerate mutase) exhibited lower absolute concentrations with the exception of creatine kinase. Previous microscopic examinations on WS muscle fibers showed that a part of myofibrils had poor functionality of sarcolemma and there was a loss of sarcoplasmic fluids, which contain sarcoplasmic proteins (Stracher et al., 1979; Sihvo et al., 2014). However, when concentrations were expressed as relative abundance, sarcoplasmic bands did not show the same trend. Relative abundance for some bands (glycogen phosphorylase and lactate dehydrogenase) increased, whereas others

Table 5. Total, myofibrillar, and sarcoplasmic protein solubility (means ± SEM) of normal and white striped (WS) chicken breast meat (n = 6/group)

Solubility	Normal	WS	Probability
Total proteins			
mg/g of meat	137.4 ± 5.1	110.1 ± 3.5	***
mg/g of protein[1]	604.3 ± 25.7	587.7 ± 19.8	NS
Myofibrillar proteins			
mg/g of meat	85.5 ± 5.8	65.3 ± 4.3	***
mg/g of protein[1]	375.2 ± 27.3	348.6 ± 28.5	NS
Sarcoplasmic proteins			
mg/g of meat	52.0 ± 1.1	44.8 ± 1.7	**
mg/g of protein[1]	229.0 ± 9.4	239.1 ± 11.1	NS

[1]Based on total CP content.

$^{**}P < 0.01$; $^{***}P < 0.001$.

decreased or did not change in WS samples. This behavior can be explained assuming that protein turnover has not had the same rate during muscle degeneration and regeneration for all proteins, as suggested by previous histological studies that showed polyphasic degeneration (Kuttappan et al., 2013b; Petracci et al., 2013b; Sihvo et al., 2014).

As a result of quantitative changes in myofibrillar and sarcoplasmic protein contents, both protein solubility and WHC were measured to evaluate if there were also changes in protein functionality. The results of protein solubility were in agreement with Warner et al. (1997) who found very wide changes in sarcoplasmic (50–70 mg/g), myofibrillar (55–130 mg/g), and total protein (100–200 mg/g) solubility for meats having different quality defects such as PSE-like and dark, firm, and dry abnormalities. Several studies showed that myofibrillar and sarcoplasmic protein solubility was highly correlated with some processing properties such as WHC (drip loss, moisture uptake, cooking loss; Van Laack et al., 1994; Warner et al., 1997) and texture and gel characteristics (Li-Chan et al., 1987; Sun and Holley, 2010). Even in the current study, WS breast meat showed lower protein solubility (sarcoplasmic, myofibrillar, and total proteins) when based on the weight of fresh meat in comparison with normal meat as usually expressed in the literature, but when the solubility was based on protein content, there were no significant differences. The WS fillets also exhibited a lower WHC, which was in agreement with previous findings of Petracci et al. (2013a) who found lower marinade uptake and increased cook losses in fillets with severe white striping. Hence, the reduction in protein solubility and cooking loss for WS fillets can be explained by reduction of total protein content and in particular of myofibrillar and sarcoplasmic fractions and to a lesser extent by collagen increase, and not to actual differences in protein solubility. These results can also be supported by the previous studies that showed that the decrease of protein solubility was attributed to protein denaturation as a result of low pH, which formed insoluble aggregates (Fischer et al., 1979; Bowker and Zhuang, 2013). Van Laack et al. (2000) found that pale chicken fillets characterized by low pH and WHC had decreased solubility for both sarcoplasmic and total protein. By contrast, WS fillets were characterized by higher pH values than normal meat (Petracci et al., 2013a). Therefore, the reduction of myofibrillar and sarcoplasmic protein contents could be the main reason for reduction of WHC in WS fillets and this contributes to excluding any similarities between PSE-like and white striping abnormalities.

This study concluded that appearance of white striping is associated with a dramatic change in the chemical composition and nutritional value of chicken breast represented by an increase of intramuscular fat, moisture, and ash, and a consequent reduction of the protein content. In this regard, there is a relevant decrease in protein fractions with higher nutritional value and processing features (e.g., myofibrillar rather than sarcoplasmic), whereas collagen is increased. Overall, these changes likely play a major role in the reduction of the processing properties of meat affected by white striping. Moreover, the disparities in molecular weight profile patterns for myofibrillar and sarcoplasmic proteins that were observed by SDS-PAGE analysis showed different availability of some protein subfractions.

REFERENCES

AOAC. 1990. Meat and meat products. Pages 931–948 in Official Methods of Analysis. 15th ed. Vol. 2. Assoc. Off. Anal. Chem., Washington, DC.

Bowker, B. C., and H. Zhuang. 2013. Relationship between muscle exudate protein composition and broiler breast meat quality. Poult. Sci. 92:1385–1392.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Fischer, C., R. Hamm, and K. O. Honikel. 1979. Changes in solubility and enzymatic activity of muscle glycogen phosphorylase in PSE-muscle. Meat Sci. 3:11–19.

Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem. 44:6–29.

Fritz, J. D., D. R. Swartz, and M. L. Greaser. 1989. Factors affecting polyacrylamide gel electrophoresis and electro blotting of high-molecular-weight myofibrillar proteins. Anal. Biochem. 180:205–210.

Hillgartner, F. B., A. S. Williams, J. A. Flanders, M. Dexter, and R. J. Hansen. 1981. Myofibrillar protein degradation in the chicken. 3-Methylhistidine release in vivo and in vitro in normal and genetically muscular-dystrophic chickens. Biochem. J. 196:591–601.

Huang, H., M. R. Larsen, A. H. Karlsson, L. Pomponio, L. Nanni Costa, and R. Lametsch. 2011. Gel-based phosphoproteomics analysis of sarcoplasmic proteins in postmortem porcine muscle with pH decline rate and time differences. Proteomics 11:4063–4076.

Kolar, K. 1990. Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. J. Assoc. Off. Anal. Chem. 73:54–57.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012a. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677–2685.

Kuttappan, V. A., V. B. Brewer, F. D. Clark, S. R. McKee, J. F. Meullenet, J. L. Emmert, and C. M. Owens. 2009. Effect of white striping on the histological and meat quality characteristics of broiler fillets. Poult. Sci. 88 (E-Suppl. 1):136–137. (Abstr.)

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2013a. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult. Sci. 92:811–819.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J. F. Meullenet, S. R. McKee, and C. M. Owens. 2012b. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240–1247.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013b. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.

Li-Chan, E., S. Nakai, and D. F. Wood. 1987. Muscle protein structure—Functionality relationships and discrimination of functionality by multivariate analysis. J. Food Sci. 52:31–36.

MacRae, V. E., M. Mahon, S. Gilpin, D. A. Sandercook, and M. A. Mitchell. 2006. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (*Gallus domesticus*). Br. Poult. Sci. 47:264–272.

Mehaffey, J. M., S. P. Pradhan, J. F. Meullenet, J. L. Emmert, S. R. McKee, and C. M. Owens. 2006. Meat quality evaluation of

minimally aged broiler breast fillets from five commercial genetic strains. Poult. Sci. 85:902–908.
Patnode, R., E. Bartle, E. J. Hill, V. LeQuire, and J. H. Park. 1976. Enzymological studies on hereditary avian muscular dystrophy. J. Biol. Chem. 251:4468–4475.
Petracci, M., M. Bianchi, S. Mudalal, and C. Cavani. 2013c. Functional ingredients for poultry meat products. Trends Food Sci. Technol. 33:27–39.
Petracci, M., and C. Cavani. 2012. Muscle growth and poultry meat quality issues. Nutrients 4:1–12.
Petracci, M., S. Mudalal, E. Babini, and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:179–183.
Petracci, M., S. Mudalal, A. Bonfiglio, and C. Cavani. 2013a. Occurrence of white striping and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670–1675.
Petracci, M., F. Sirri, M. Mazzoni, and A. Meluzzi. 2013b. Comparison of breast muscle traits and meat quality characteristics in 2 commercial chicken hybrids. Poult. Sci. 92:2438–2447.
SAS Institute Inc. 1988. SAS/STAT Guide for Personal Computers. Version 6.03 ed. SAS Inst. Inc., Cary, NC.
Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623. http://dx.doi.org/10.1177/0300985813497488.
Smith, D. M. 2010. Functional properties of muscle proteins in processed poultry products. Pages 181–195 in Poultry Meat Processing, 2nd ed. C. M. Owens, C. Alvarado, and A. R. Sams, ed. CRC Press, Boca Raton, FL.
Smyth, A. B., E. O'Neill, and D. M. Smith. 1999. Functional properties of muscle proteins in processed poultry products. Pages 377–396 in Poult. Meat Sci. Poult. Sci. Symp. Series Vol. 25. R. I. Richardson and G. C. Mead, ed. CABI Publishing, Wallingford, UK.
Stracher, A., E. B. McGowan, L. Siemankowski, V. Molak, and S. A. Shafiq. 1979. Relationship between myosin structure and muscle degeneration. Ann. N. Y. Acad. Sci. 317:349–355.
Sun, X. D., and R. A. Holley. 2010. High hydrostatic pressure effects on the texture of meat and meat products. J. Food Sci. 75:R17–R23.
Sun, X. D., and R. A. Holley. 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. Compr. Rev. Food Sci. Food Saf. 10:33–51.
Van Laack, R. L. J. M., R. G. Kauffman, W. Sybesma, F. J. M. Smulders, G. Eikelenboom, and J. C. Pinheiro. 1994. Is colour brightness (L-value) a reliable indicator of water-holding capacity in porcine muscle? Meat Sci. 38:193–201.
Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. Poult. Sci. 79:1057–1061.
Warner, R. D., R. G. Kauffman, and M. L. Greaser. 1997. Muscle protein changes post mortem quality traits relation to pork quality traits. Meat Sci. 45:339–352.
Xiong, Y. L. 2004. Muscle protein. Pages 100–122 in Proteins in Food Processing. R. Y. Yada, ed. Woodhead Publishing Limited, Oxford, UK.
Zapata, I., J. M. Reddish, M. A. Miller, M. S. Lilburn, and M. Wick. 2012. Comparative proteomic characterization of the sarcoplasmic proteins in the pectoralis major and supracoracoideus breast muscles in 2 chicken genotypes. Poult. Sci. 91:1654–1659.