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Biochemical and Ultramicroscopic Evaluation of Myofibril Proteins and Collagen during Ageing in Broiler Chicken PSE (Pale, Soft, Exudative) Meat

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HIGHLIGHTS

- Cathepsins B + L and L activities have a prolonged action in PSE fillets.
- PSE meat presents accelerated protein degradation in relation to the control fillets.

Abstract: The aim of this work was to study the myofibril proteins and collagen fraction changes in broiler chickens PSE (pale, soft, exudative) meat during ageing and their relationship to meat quality. The results presented an increase of myofibril proteins and collagen solubility promoted by the enhanced proteases activities during storage. Ultramicroscopically, the PSE meat samples revealed intracellularly a sarcomere super contraction and lacunas within the A and I bands while Z-lines appeared very dense and fragmented in comparison to normal samples. This observation was noticed already at 4h storage while extracellularly collagen fibrils decreased visually within the endomysium only after 24h of conditioning. These results influenced the quality as the PSE meat presented better functional properties at the first hours of conditioning before further proteins degradation by proteases. Thereafter, at the later ageing stage a further disintegration of the abnormal meat structure would affect the meat functional properties.

Keywords: Cooking loss; Tenderness; Collagen content; Myofibrillar Fragmentation Index.

INTRODUCTION

Meat tenderness is one of the most important factors to consumers. The development of this tenderness during ageing is dependent on the meat architecture, the integrity of the skeletal muscle cell, the activity of endogenous proteases such as calpains and cathepsins within the cell and the extracellular matrix proteins [1,2]. The development of tenderness in poultry is dependent also on the fast decrease in pH *post mortem* under warm carcass temperature developing PSE meat (pale, soft, exudative) leading to the denaturation of myofibril proteins impairing their functional properties [3]. There are controversies over the tenderness of PSE meat and its relationship to the proteases activity. We have previously reported that chicken PSE meat had lower shear force and higher myofibrillar fragmentation index attributing these results to the calpains enzymatic activities as the consequence of the surplus of Ca^{2+} within the muscle cell milieu promoted by PSE developmental conditions [3]. According to Claeys *et al.* [4], a fast decrease in pH values followed by an increase in the concentration of Ca^{2+} can result in a premature activation of calpains and subsequently this activity decreased by the calpains autolysis during *post mortem* conditions. In skeletal muscle, the calpain system consists of several proteases, and two types of them, μ - and m-calpain and their inhibitor calpastatin, are thought to be involved in the proteolytic meat tenderization. Both μ - and m-calpains are calcium dependent and are composed of 80 and 28 kDa subunits, and both suffer autolysis in the presence of Ca^{2+} [5]. Furthermore, several reports have shown that collagen properties such as fiber sizes, genetic types, total content and the presence of crosslinking mediated by lysyl oxidase are important for determining the contribution of this protein for meat texture [2,6]. There is evidence that *post mortem* changes occur within the connective tissue due to the action of lysosomal proteases (cathepsins), which act on the degradation of collagen during ageing [7]. Cathepsins are a group of enzymes comprised of both exo- and endo-peptidases [8]. Reports have indicated that cathepsins B and L activities at 8 h *post mortem* have been found positively correlated with tenderness in beef and can act on collagen molecules [9,10]. Cathepsins are located within the lysosomes and must be released in order to have access to myofibril and connective tissue proteins, thus providing meat tenderness [11]. However, low pH values and high carcass temperature can enhance the disruption of the lysosome membrane¹⁰, as it seems to happen in PSE meat [12-14]. Several studies have indicated that proteases activity may be associated with fast decrease in pH *post mortem* [3,15,16], but the extent of these changes, as well as specifically the changes in PSE meat quality during ageing is unknown. Therefore, the aim of this present work was to study both myofibril proteins and collagen fraction biochemical and ultrastructural changes during ageing of broiler chicken PSE meat and its relationship to quality.

MATERIAL AND METHODS

Samples Preparation

Breast meat (*Pectoralis major* m.) samples were obtained from 42-day old broilers from a commercial plant located in the South of Brazil. The animals were slaughtered according to the standard industrial practice and essentially consisted of electrical stunning, bleeding, defeathering, evisceration, water-cooling the carcass, deboning and refrigeration [3]. The length of time from slaughtering to samples collection was about 3 hours. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee.

Biochemical and Physicochemical Parameters

pH was measured 3 and 24 hours *post mortem* by inserting electrodes into the meat samples using a contact pH meter system (Testo 205), as reported in [17]. Thirty-six samples were classified as PSE meat ($\text{pH}_{3\text{h}} \leq 5.8$) and thirty-six samples as control ($\text{pH}_{3\text{h}} > 5.9$). A Minolta CR400 colorimeter was used to evaluate the color (L^* , a^* , b^*) of the posterior surface of intact skinless breast muscle at 24 h *post mortem*. The L^* , a^* and b^* values were measured at three different sites on the same sample: the proximal extremity of muscle, the distal extremity of the muscle and the medial side half-way between the proximal and distal extremities [12]. Samples were vacuum packed in plastic bags and aged for 0 ± 1 °C for 24, 72 and 120 h *post mortem* (twelve fillets meat for ageing time), totaling 36 samples from each group (PSE and control meat). Samples were taken for Myofibrillar Fragmentation Index (MFI) determination, Cooking Loss, Shear Force Measurement, Total and Soluble Collagen content at 24, 72 and 120 h *post mortem*.

Myofibrillar Fragmentation Index (MFI)

MFI was determined in triplicate as an indirect measurement of calpain activity, according to Culler *et al.* [18] as described in Wilhem *et al.* [3]. Two grams of muscle samples, free of external fat and visible connective tissue, were homogenized for 30 s in 10 mL of MFI buffer (100 mM KCl, 20 mM potassium phosphate, 1 mM EDTA, 1 mM MgCl_2 and 1 mM NaN_3 at pH 7.0). The homogenate was centrifuged at 10,000 rpm for 15 min at 2 °C, the supernatant discarded and the pellet resuspended in 10 mL of the MFI buffer and centrifuged at 10,000 rpm for 15 min at 2 °C. The supernatant was discarded and the pellet suspended in 5 mL of the same MFI buffer. The myofibril suspension was poured through a filter paper to remove connective tissue, and then assayed for protein concentration using the Biuret method. Aliquots of the suspensions were diluted in the MFI buffer to a final protein concentration of 0.5 mg/mL. The diluted protein suspension was poured into a cuvette and the absorbance at 540 nm was immediately measured with a spectrophotometer. The MFI was expressed as $A_{540\text{nm}} \times 200$.

Cooking Loss (CL)

CL was measured according to Honikel [19]. Samples were packed in plastic bags and submitted to cooking in a water bath for 30 min until internal temperature reached 75 °C. CL was expressed as the weight difference before and after cooking.

Shear Force (SF) Measurement

The samples were the same as the CL analysis. Samples were cut into 1x1x2 cm ($n = 08$ from each sample), and analyzed on a texturometer TATX-2i. The results were expressed in newton (N).

Cathepsin Activities

Cathepsins B + L, B, and H activities were determined in triplicate, according to Toldrá and Etherington [20]. Samples ($n = 10$ per group) were collected at 4, 24, and 72 h *post mortem*, totaling 30 samples per group. Protease activity was measured using a spectrofluorometer at an excitation wavelength of 355 nm and an emission wavelength of

460 nm. Results were expressed in fluorescence units (FU); 1 FU corresponds to the amount of enzyme capable of hydrolyzing 1 μmol of substrate in 1 h at 37 °C.

Collagen Content

The total collagen content in samples was determined in triplicate after 15 h of hydrolysis of 1.0 g of meat with 15 mL 6 M HCl at 105 °C, as reported by Woessner [20]. The hydrolysate was filtered and its pH adjusted between 6.0 and 7.0 with 33 % (w/v) NaOH, and diluted with distilled water to 250 mL. An aliquot of 2 mL of hydrolyzed sample and 1 mL of cloramine T solution were mixed in a test tube and left for 20 min at room temperature and 1 mL of 3.15 M HClO₄ was added and left for 5 min at room temperature. Then, 1 mL of 4-dimethyl-aminobenzaldehyde was added and solutions were shaken and heated at 60 °C for 20 min. The samples were cooled down for 5 min in water tap and the absorbance measured at 560 nm. The amount of hydroxyproline was determined from a standard curve. The Total Collagen content (TC) was calculated from hydroxyproline content using the coefficient 8.0 [20].

Soluble collagen (SC) was extracted in triplicate according to the modified method of Oliveira *et al.* [21]. Samples (2.5 g) were mixed for 1 min with 20 mL of deionized water and it was heated for 60 min at 80 °C. Samples were homogenized at 22,000 rpm in Ultra Turrax and centrifuged for 15 min at 4,000 rpm. Supernatant was filtered and 30 mL of 6 M HCl added for hydrolysis as described previously. Soluble collagen was evaluated as described for total collagen content.

Histological Evaluation

Histological evaluation by electron microscopy was performed as in Guarnieri *et al.* [22]. Samples (n = 6 from ageing time) after 3, 24, 72, and 120 hours *post mortem* were fixed in 2 % glutaraldehyde in a 0.14 M sodium cacodylate buffer at pH 7.4, containing 0.18 M sucrose. After washing in phosphate buffer, the samples were post-fixed in 1 % osmium tetroxide in phosphate buffer for 2 h, followed by dehydration in acetone, and embedding in Araldite resin. Ultrathin sections (50 nm) were stained with saturated uranyl acetate in 50 % ethanol and lead citrate for 1 h. The sample ultrastructure was observed with a JEOL JEM-1010 scanning electron microscope.

Statistical Analysis

Statistical analysis was carried out using Statistic software, version 7.0. Student t-test was used to determine significant difference among two samples of PSE and control meat at the same storage time. Tukey's test was used to determine significant difference among storage periods of time for PSE or control meat samples. Pearson correlations coefficients were used for testing correlations between pH_{3h}, pH_{24h}, L* values, MFI, CL, SF, TC and SC.

RESULTS

Physicochemical Parameters

pH and L* values were significantly different typical for control and PSE meats, as previously reported [3,12,17,23]. The pH values decreased gradually throughout the *post mortem* periods evaluated in the control group while in PSE meat samples there was a faster decrease (p = 0.27), due to the rapid glycolysis, consequently the meat was paler, showing higher L* and b* (yellowness) values typical of PSE meat (Table 1). The a* value was higher for the control samples, showing higher red value (Table 1).

Table 1 Means (\pm SD) Comparison of physicochemical parameters between control and PSE (Pale, Soft, Exudative) broiler breast meat (*Pectoralis major* m.) samples

Groups	PSE (n = 36)	Control (n = 36)
pH _{3h}	5.64 ^{b,B} \pm 0.051	6.05 ^{a,A} \pm 0.053
pH _{24h}	5.67 ^{b,A} \pm 0.086	5.98 ^{a,B} \pm 0.063
L*	60.21 ^a \pm 1.279	52.10 ^b \pm 3.776
a*	0.19 ^b \pm 0.485	2.27 ^a \pm 0.619
b*	6.46 ^x \pm 2.195	4.97 ^y \pm 0.990

pH_{3h}: pH 3 hours *post mortem*; pH_{24h}: pH 24 hours *post mortem*; L*: lightness; a*: green-red component; b*: yellow-blue component.

^{a-b} Means within each line with different superscripts are different ($p \leq 0.01$).

^{x-y} Means within each line with different superscripts are significantly different ($p \leq 0.05$).

^{A-B} Means within each column with different superscripts are different ($p \leq 0.01$).

\pm Standard Deviation

Cooking Loss (CL)

There was no difference ($p \geq 0.05$) between CL 24 h and CL 72 h in both groups (Table 2). However the CL 120 h *post mortem* value increased significantly ($p \leq 0.05$) approximately 9.2 % for PSE.

The CL was higher for PSE fillets throughout the experiment. The highest CL value as observed in the PSE fillets samples was the consequence of rapid *post mortem* glycolysis leading to the denaturation of myofibrillar proteins that reduces their ability to retain water [17]. Therefore the CL amount was inversely proportional to the water holding capacity (WHC) [3]. The use of chicken PSE meat generates a product with low WHC and poorer cut when compared to the normal meat [24,25]. Thus, these biochemical events were expected to increase during ageing, especially in PSE meat due to the higher CL 120 h *post mortem*.

Table 2 Cooking loss of control and PSE (Pale, Soft, Exudative) meat samples of broiler breast stored at 0 ± 1 °C for 24, 72 and 120 hours *post mortem*.

Groups	Cooking Loss (%)		
	24 h (n = 12)	72 h (n = 12)	120 h (n = 12)
PSE meat	28.31 ^{b,A} \pm 2.32	28.62 ^{b,A} \pm 1.16	30.91 ^{a,A} \pm 2.62
Control meat	23.85 ^{b,B} \pm 1.28	25.46 ^{ab,B} \pm 1.43	24.90 ^{a,B} \pm 1.19

^{a-b}: significantly different in the same group ($p \leq 0.05$).

^{A-B}: significantly different between groups ($p \leq 0.05$).

\pm Standard Deviation

Breast Meat Tenderness Measurement

Table 3 shows the Shear Force (SF) values for PSE and control meat samples. It was observed that at 24 h *post mortem*, the SF showed lower values for PSE samples (20.50 N) comparing to control samples (24.99 N) ($p \leq 0.05$) corroborating the results observed previously by Wilhelm *et al.* [3] stating that the lower SF value was due to the earlier activation of calpain in PSE meat samples in a pre rigor conditions.

Table 3 Shear force of control and PSE (Pale, Soft, Exudative) meat samples of broiler breast stored at 0 ± 1 °C for 24, 72 and 120 hours *post mortem*.

Groups	Shear Force (N)		
	24 h (n = 12)	72 h (n = 12)	120 h (n = 12)
PSE meat	20.50 ^{b,B} ± 3.31	28.72 ^{b,A} ± 11.54	48.61 ^{a,A} ± 14.07
Control meat	24.99 ^{a,A} ± 5.69	24.31 ^{a,A} ± 5.45	27.38 ^{a,B} ± 6.82

^{a-b}: significantly different in the same group ($p \leq 0.05$).

^{A-B}: significantly different between groups ($p \leq 0.05$).

± Standard Deviation

After 72 h of the broiler chicken slaughter time it was found that SF values of PSE meat samples were higher than the control samples ($p \leq 0.05$) while in the control samples, SF was not affected during storage although significant changes were observed in PSE fillets meat samples ($p \leq 0.05$). In PSE meat samples, there was a gradual increase of shear force values during storage occurring 2.5-fold after 120 h *post mortem* in relation to 24 h and approximately 2-fold than the control samples ($p \leq 0.01$) (Table 3). After 120 h, the texture was not affected during storage under refrigeration. One explanation for these results lies on the excessive water loss during storage leading to the protein aggregation as observed in cooking loss of PSE meat at 120 h *post mortem* (Table 2). In addition, some authors have reported that inactivation of calpains was due to its early autolysis [3,5], which probably contributed to the increase of shear force 120 h *post mortem* value (Table 3).

Myofibrillar Fragmentation Index (MFI)

Table 4 shows the results of MFI evaluation for control and PSE broiler breast meat samples stored at 0 ± 1 °C for 120 h. There was no significant difference ($p \geq 0.05$) for MFI in sample kept for 24 h between both samples. PSE meat samples did not change significantly in the MFI values during storage, however from 72 h *post mortem* onwards the MFI of PSE meat was higher ($p \leq 0.05$) than control group probably due to earlier activation of calpain. Increased protease activity in PSE samples was due to the higher concentration of intracellular Ca^{2+} . This higher ion concentration should primarily enhance the μ -calpain activity when, according to Lee *et al.* [1], the activity of this enzyme ceases due to autolysis.

Table 4 Myofibrillar Fragmentation Index (MFI) of control and PSE (Pale, Soft, Exudative) samples of broiler breast stored at 0 ± 1 °C for 24, 72 and 120 hours *post mortem*.

Groups	Myofibrillar Fragmentation Index		
	24 h (n = 12)	72 h (n = 12)	120 h (n = 12)
PSE meat	81.31 ^{a,A} ± 8.06	76.79 ^{a,A} ± 3.32	77.06 ^{a,A} ± 3.56
Control meat	79.19 ^{a,A} ± 3.43	73.78 ^{b,B} ± 2.43	73.18 ^{b,B} ± 1.82

^{a-b}: significantly different in the same group ($p \leq 0.05$).

^{A-B}: significantly different between groups ($p \leq 0.05$).

± Standard Deviation

Wilhelm *et al.* [3] found a higher MFI values in PSE meat samples. These authors observed that after 72 h *post mortem* the ultrastructure of the fillets from PSE meat was more fragmented than the control samples, indicating higher proteolytic activities, similar to the results reported herein. Santos *et al.* [15] noted that in acute stressed chicken, MFI changed, although these results were not enough to influence SF values.

Cathepsin Activities

At 4 h *post mortem*, only cathepsins L and H activities were higher in the control group (Table 4). However, at 24 h *post mortem*, control samples had a marked decline in cathepsins B + L and L activities, whereas PSE meat samples had no significant changes in

cathepsin activity, which demonstrates that these proteases have a prolonged action in PSE fillets. In the 24 h *post mortem* period, cathepsins B + L and L activities in PSE fillets were 28 % and 30 % higher than those of control fillets, respectively. These isoforms remain active in PSE meat probably because of its low pH (5.64) compared to that of control fillets (5.98). As cathepsins B and L exhibit optimal activities at approximately pH 5.5 [9,27], PSE samples offered better conditions for the prolonged action of these enzymes.

Table 5 Cathepsin activities of control and PSE (Pale, Soft, Exudative) samples of broiler breast stored at 0 ± 1 °C for 4, 24 and 72 hours *post mortem*.

	PSE meat	Control meat
<i>post mortem</i>		
	B + L Cathepsins (FU)*	
4 h (n = 10)	869.29 ^{a,A}	935.31 ^{a,A}
24 h (n = 10)	770.03 ^{a,A}	551.21 ^{b,B}
72 h (n = 10)	582.67 ^{a,B}	585.68 ^{a,B}
	B Cathepsins (FU)*	
4 h (n = 10)	132.03 ^{a,A}	137.17 ^{a,A}
24 h (n = 10)	115.07 ^{a,A}	115.56 ^{a,A}
72 h (n = 10)	133.59 ^{a,A}	140.05 ^{a,A}
	L Cathepsins (FU)*	
4 h (n = 10)	732.26 ^{b,A}	798.14 ^{a,A}
24 h (n = 10)	654.96 ^{a,A}	435.65 ^{b,B}
72 h (n = 10)	449.08 ^{a,B}	445.62 ^{aB}
	H Cathepsins (FU)*	
4 h (n = 10)	113.19 ^{b,A}	147.03 ^{a,A}
24 h (n = 10)	140.27 ^{a,A}	136.74 ^{a,A}
72 h (n = 10)	125.57 ^{a,B}	141.14 ^{a,A}

*FU: Fluorescence unit.

^{a-b}: significantly different between groups ($p \leq 0.05$).

^{A-B}: significantly different in the same groups ($p \leq 0.05$).

Collagen Content

Collagen content in PSE meat was 14.6 % lower ($p \leq 0.05$) than in control group at 24 h *post mortem* (Table 6). This result was probably related to the accelerated decline in pH values while the carcass temperature was still warm and under these conditions would lead to lysosomes rupture thus liberating cathepsins, which in turn acted enzymatically on the collagen molecules [26]. B and L were probably the cathepsin components as they present an activity of pH optimum about 5.5 [9,27], as observed in our study (Table 5). Other reports have shown that these isoforms enzymes may affect the collagen molecules in its native form inducing the fibers cross-linking depolymerization [9,27]. Considering that pH_{3h} value of the PSE meat samples was 5.64 and this fact might have contributed to the these enzymes digestion on collagen fibers. Although the mechanism of cathepsins over collagen is as yet not well understood. Kirschke *et al.* [27] suggested that these proteases act mainly on the collagen telopeptides digesting the crosslinkages regions. Based on studies with differential scanning calorimetry, Beltrán *et al.* [28] concluded that bacterial collagenase and cathepsin B have similar mechanisms acting on the insoluble collagen.

Table 6 Total collagen content of control and PSE (Pale, Soft, Exudative) samples of broiler breast stored at 0 ± 1 °C for 24, 72 and 120 hours *post mortem*.

Groups	Total Collagen (g/100g)		
	24 h	72 h	120 h
PSE meat	0.35 ^{b,B} ± 0.04	0.38 ^{ab,A} ± 0.04	0.40 ^{a,A} ± 0.02
Control meat	0.41 ^{a,A} ± 0.03	0.36 ^{a,A} ± 0.05	0.36 ^{a,A} ± 0.04

^{a-b}: significantly different in the same group ($p \leq 0.05$).
^{A-B}: significantly different between groups ($p \leq 0.05$).
± Standard Deviation

In addition to the probable role of calpain, the lower collagen content in PSE meat probably contributes to the lowest shear force observed in this 24 h *post mortem* meat samples. Furthermore, the soluble collagen content of PSE meat was about 25 % higher ($p \leq 0.05$) than the control samples 24 h *post mortem* (Table 7), collaborating with the tenderness of PSE meat. This fact suggested that cathepsins may also act earlier in PSE meat improving meat tenderness.

Table 7 Soluble collagen content of control and PSE (Pale, Soft, Exudative) samples of broiler breast stored at 0 ± 1 °C for 24, 72 and 120 hours *post mortem*.

Groups	Soluble Collagen (g/100g)		
	24 h	72 h	120 h
PSE meat	0.12 ^{a,A} ± 0.02	0.13 ^{a,A} ± 0.02	0.14 ^{a,A} ± 0.02
Control meat	0.09 ^{b,B} ± 0.02	0.14 ^{a,A} ± 0.04	0.12 ^{a,A} ± 0.01

^{a-b}: significantly different in the same group ($p \leq 0.05$).
^{A-B}: significantly different between groups ($p \leq 0.05$).
± Standard Deviation

At 72 and 120 h *post mortem*, the collagen content of fillets was similar ($p \geq 0.05$) in both control and PSE meat samples (Table 7). The soluble collagen (SC) content increased in the control samples, similarly to the PSE meat kept for 72 h *post mortem* (Table 6). Histochemical studies in beef samples have shown that in refrigerated stored meat a progressive rupture of lysosomes happened and after 14 days of storage, its breakdown was almost completed [29]. Therefore, in our experiment, probably in the control meat sample, it may undergo a gradual rupture of lysosomes throughout the storage period thus releasing cathepsins. As the pH values of the fillets already were at in the ideal range of the enzymes activity thus there were suitable conditions for breaking down the collagen molecules and consequently promoting their degradation.

Pearson Correlation

Correlations between the different measurements, such as pH_{3h}, pH_{24h}, L*, SF, CL, TC, MFI and SC were evaluated. A significant negative Pearson correlation is observed between pH_{3h} and CL (-0.76, $p \leq 0.01$) and L* (-0.83, $p \leq 0.01$) indicating that, the lower the pH_{3h}, the higher was the impairment of the functional properties of the meat proteins. Similar behavior was also observed for L* values in relation to CL (0.79, $p \leq 0.01$), suggesting that, as meat becomes paler, the CL increases. MFI values correlated positively with L* (0.53, $p \leq 0.05$) and CL (0.56, $p \leq 0.01$), indicating that a higher proteolysis was correlated with higher impairment of the functional properties of meat. TC content correlated with pH_{3h} (0.64, $p \leq 0.01$) and pH_{24h} indicating that PSE meat influenced the collagen content reduction.

Histological Evaluation

Longitudinal and transverse electron micrographs confirmed this premature accelerated degradation of PSE meat myofibril and connective tissue proteins, as fewer collagen fibres were observed in these sections at 24 h *post mortem* (Figs 1A and 1B). This higher protease activity was noted by transmission micrograph observations (Figs 1A-a, -b, -c and -d for the normal samples and Figs 1A-e, -f, -g and -h for the PSE meat samples, taken at 3, 24, 72 and 120 h *post mortem* and refrigerated). As shown in Fig. 1A, striking differences were already apparent in the transmission micrographs at 3 h *post mortem*. In the normal samples (Fig. 1A-a), the sarcomere components possessed intact bands from I and A (zone H) and lines Z and M; the same finding was not observed for the PSE samples (Fig. 1A-e). The myofilament structure of the PSE meat was partially shrunken, and the Z lines were beginning to be enzymatically digested (Fig. 1A-e, arrow). It appears that the calpain systems initiated their activity in the PSE samples significantly earlier than in the normal samples [3]. This observation can be explained by the increase in the concentration of Ca^{2+} in the PSE samples, which likely occurred even before slaughtering. This hypothesis was supported by consistently lower pH values with respect to the normal samples. In fact, the excess Ca^{2+} within the tissue at this stage activates the μ -calpain activity prematurely in birds when compared with mammals [5]. In mammals, a certain amount of time is necessary to initiate the maturation assembly, presumably because the Ca^{2+} concentration is not sufficiently high to induce muscle catabolism [5]. There is a specific relationship between the initiation of the enzymatic activity and the animal species. After 24 h of ageing, the normal samples presented with some depolymerised myofilaments, and the Z lines were starting to be enzymatically digested at some locations (Figs 1A-c and -d). In the PSE meat samples, the sarcomere was more disorganized at 4 h *post mortem*, and some lacunas were evident within the bands, indicating protein fragmentation. In the PSE samples, the typical dark and light pattern was not evident, and the Z lines appeared more pronounced and were followed by lacunas (Fig. 1A-e). Myosin filaments were predominant within the sarcomeres and even touched the Z lines, as shown in Fig. 1A-e; actin filaments were seldom observed. Open spaces were also visible, and a super muscle contraction was evident. One of the most affected muscle cell regions was at the triad, which is where the T tubules join the sarcoplasm reticulum at the transition between bands I and A. This is the region where Ca^{2+} is released and promotes super contraction, which draws some of the sarcomere components towards the Z-lines and makes them comparatively very dense. From 72 to 120 h, it was clear there was Z-line digestion within the normal samples because some of the components at the sarcomere began to fragment (Figs 1A-c and -d as indicated by the arrowhead). In the PSE meat samples, a more dramatic enzymatic reaction occurred, which was observed at the 72 and 120 h storage periods, when the meat structure was likely to be collapsed (Figs 1A-g and -h). This enzymatic activity provided a lower availability of the myofibrillar protein, with the minimum quantities observed at the 72 to 120 h storage periods; approximately 18.0 % of the protein was lost in the PSE meat samples (Table 4). A difference in MFI between the PSE and normal meats was also apparent; the PSE meat had significantly greater MFI (approximately 5 % more), indicating higher protease activity (Table 4).

The transverse electron micrographs presented in Fig. 1B corroborated the previous results observed in longitudinal studies. A larger space was observed at the endomysial region, and fewer collagen fibres were distributed throughout the extracellular spaces (Figs 1B-d, -e and -f). This reduction was the result of lysozyme activity, as calpains are unlikely to display any activity towards connective tissue proteins [1]. Therefore, the candidates are cathepsin enzymes. In fact, under bird pre-slaughter management conditions, the meat suffers dramatic stress. Furthermore, the cathepsin system can be activated when lysosomes are degraded under a higher temperature and lower pH, which liberates these enzymes from the organelles [26,28], presenting prolonged activity in PSE meat, as observed in Table 5. This occurrence was corroborated by the results shown in Table 7; the total collagen fractions were 14.6 % lower in the PSE meat samples and, conversely, 25.0 %

higher in the 24 h *post mortem* soluble fractions. In fact, cathepsins B and L are optimally active towards collagen molecules between a pH of 5.5 to 6.0, which favours cathepsinic activity [26]. Moreover, at the age of 42 days, a broiler chicken contains more soluble than insoluble collagen fibres because the mature collagen crosslinks and pyridinolines are beginning to be synthesized [2]. This finding is shown in Table 6, in which the total collagen was more concentrated in the polymerized form in the normal samples. Conversely, there was more soluble collagen in the PSE meat samples regardless of the ageing period (Table 7).

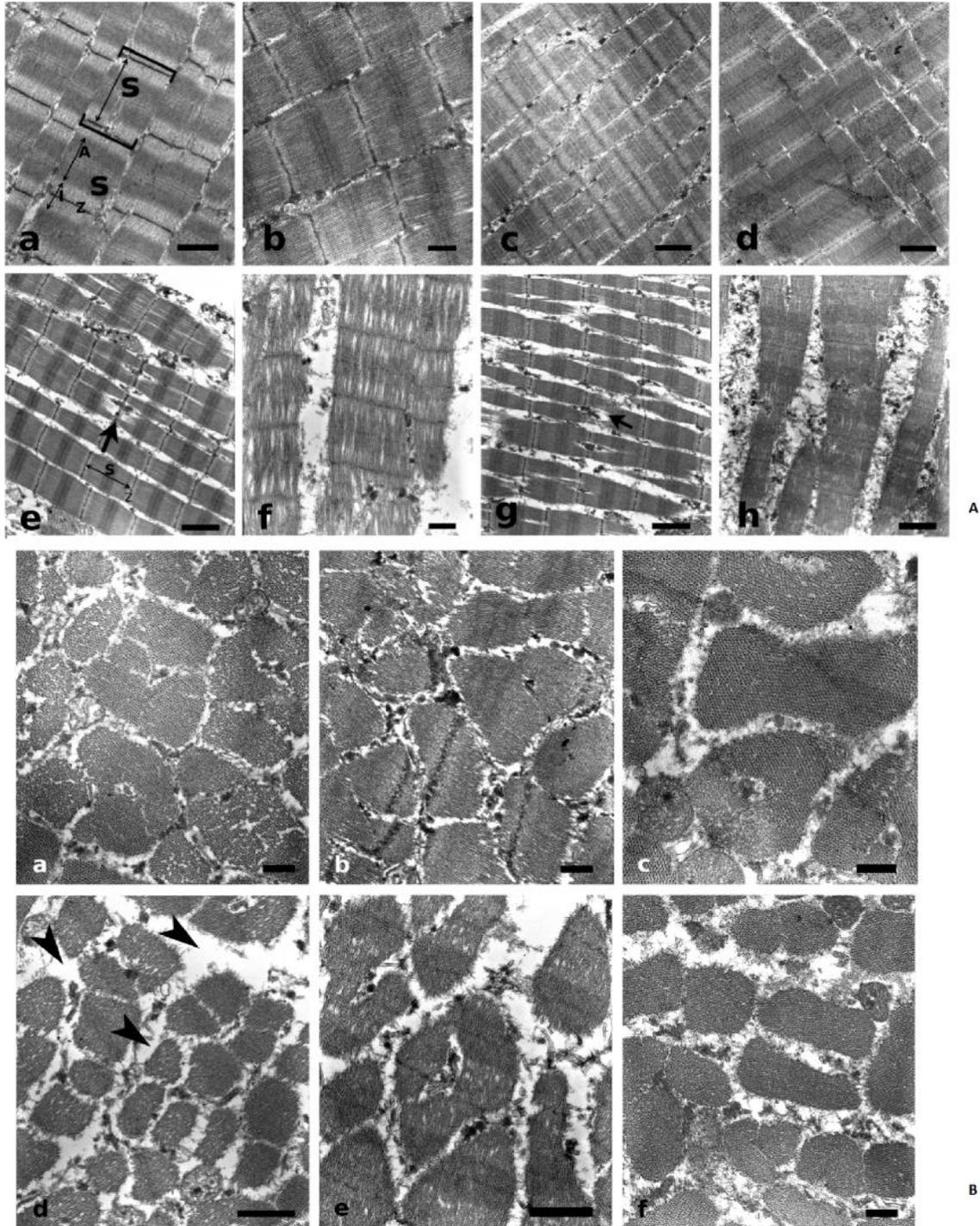


Figure 1 (A) Longitudinal electron micrograph of *pectoralis major* m. from control group stored at 0 ± 1 °C for different times: **a, b, c, d**: control 4, 24, 72 e 120 h *post mortem*, respectively; **e, f, g, h**: PSE (Pale, Soft, Exudative) 4, 24, 72 e 120 h *post mortem*, respectively. **s**: sarcomere; **a**: a band; **i**: i band; **h**: h zone; **z**: z line. (bar = 1 µm). **(b)** electron micrograph of a transverse section of broiler *pectoralis*

major m. stored at 0 ± 1 °C for different times: **a, b, c**: control 4, 24 e 120 h *post mortem*, respectively (Bar = 400 nm); **d, e, f**: PSE 4, 24 E 120 h *post mortem*, respectively. [Bar = 600 nm (d), 600 nm (e), 400 nm (f)].

CONCLUSION

PSE meat samples presented accelerated protein degradation in relation to the control samples due to the premature and prolonged proteases activity thus contributing initially to their relative higher tenderness. However, during ageing PSE meat showed higher Cooking Loss and Shear Force compromising its quality.

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