Changes in collagen fractions during PSE meat (*Pale, Soft, Exudative*) chicken installation

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Abstract – **The aim of this work was to investigate the postmortem changes in collagen fractions during broiler PSE meat development. *Pectoralis major* m samples were characterized by measuring pH and lightness values (L*). Samples with pH>5.9 and L*<53.0 were classified as control and PSE meat with pH≤5.8 and L*≥53.0. Analysis of cooking loss (CL), shear force (SF), total (TC) and soluble collagen (SC) were performed in fillets stored at 0C for 1 day and 5 days postmortem. Results were compared by Student t-test in the STATISTICA 7.0. TC from 1 day control samples (0.35g/100g) was 14.6% lower in relation to PSE meat (0.41g/100g) whereas on 5th day no difference was observed (p≥0.05). SC was 25.6% higher in PSE meat in comparison to control samples from 1 day storage whereas on 5th day no significant difference was detected (p≥0.05). CL of 1 day stored PSE samples was 15.7% higher (p≤0.01) and on 5th day an increase by 17.6% (p≤0.01) was observed in relation to control samples. SF of 1 day samples was 18% lower (p≤0.05) in PSE samples however on 5th day this value doubled in relation to control samples. PSE meat presented an accelerated degradation of collagen molecules on 1 day samples and lower SF in relation to control samples. On 5th day of storage, the TC and SC content did not differ significantly, but there was an increase of both SF and CL and these values seemed to be related to collagen content alteration. We concluded that changes in collagen fraction have implication on the broiler PSE meat qualities.**

Keywords – **pH, shear force, cooking loss**

I. INTRODUCTION

PSE meat originates from a rapid decline in pH while the carcass of the animal is still warm, leading to the denaturation of myofibril proteins, which jeopardizes their functional properties [1, 2]. The consequence is low-quality meat with their functional properties and its tenderness compromised [3, 4]. Meat tenderness is a highly valued consumer trait and thus definition of the multiple processes that influence meat tenderness will provide clues toward improving meat quality and value [5]. Tenderness is dependent on the architecture and the integrity of the skeletal muscle cell and on events that modify those proteins and their interactions [6]. Some reports emphasized that properties of collagen, such as fiber sizes, genetic types, total content and solubility are important for determining the contribution of this protein for meat texture [7].

There is evidence that postmortem changes occur in the connective tissue due to the action of lysosomal proteases (cathepsins), which act on the degradation of collagen during maturation [8]. Cathepsins are a group of enzymes comprised of both exo- and endo-peptidases [9]. There are indications that cathepsins B and L activities at 8 h postmortem have been found positively correlate with tenderness in beef and can act on collagen molecules [10, 11]. Cathepsins are located within the lysossomes and must therefore be released from them in order to have access to myofibril and connective tissue proteins thus influencing the meat tenderness [12]. Moreover, low pH levels and high carcass temperature can enhance the disruption of the lysossomal membrane [11], as it seems to happen in PSE meat [13]. Therefore, the aim of this work was to investigate the postmortem changes in collagen fractions during broiler PSE meat development.

II. MATERIALS AND METHODS

A. Sample preparation

Breast fillet meat (*Pectoralis major* m.) samples were obtained from 42-day old broilers of either sex from a commercial plant located in the south of Brazil. The animals were slaughtered according to the standard industry practices [4]. The time from bird slaughtering to sample collection was about 3h.

B. Biochemical and physicochemical parameters

pH was measured by inserting electrodes into the meat samples [14] using a contact pH meter system (Testo 205), as reported by Olivo et al. [2]. Twenty-four samples were classified as PSE (pH_{3h} ≤5.8) and

twenty-four samples as control (pH_{3h} >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 postmortem. The L*, a* and b* values were measured at three different sites on the same sample [13]. Vacuum packed in plastic bags, PSE and control fillet meats before storing at 0 ± 1 ºC were divided into two groups of 12 samples and thus kept for 1 day and 5 days postmortem for further analysis. Samples were taken for Cooking Loss (CL), Shear Force Measurement (SF), Total (TC) and (SC) Collagen content at 1 day and 5 days postmortem storage.

CL was measured according to Honikel [15] and essentially samples were packed in plastic bags and submitted for cooking in a water bath for 30 min. until internal temperature reached 75 ºC. CL was expressed as the weight difference before and after cooking.

SF was measured in the same samples used for CL analysis. Samples were cut into 1x1x2 cm, and analyzed on a texturometer TATX-2i. The results were expressed in Newtons (N).

TC content in samples was determined after 15h hydrolysis of 1.0 g of meat with 15 mL 6 M HCl at 105 ºC, as reported by Woessner [16]. The collagen content was calculated from hydroxyproline content using the coefficient 8.0 (Woessner, 1961). SC was extracted according to the modified method of Oliveira et al. [17]. Samples (2.5 g) were mixed for 1 min with 20 mL of deionized water and it was heated for 60 min at 80 ºC. 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. SC was quantitatively assayed as described for TC.

Statistical analysis was carried out using Statistic software, version 7.0. Student t-test was used to determine significant difference among the same storage periods of storage of PSE or control meat samples.

III. RESULTS

Table 1 shows the differences values in CL and SF between the control and PSE broiler breast meat samples stored at 0ºC for 1 day and 5 days. CL of 1 day stored PSE samples was 15.7 % higher and on 5th day an increase by 17.6 % was observed in relation to control samples. During storage of 5 days period, PSE meat presented CL_{5d} 9.2 % higher that CL_{1d} and control samples showed CL_{5d} 6.5 % higher that CL_{1d} within the same group. In 1 day stored samples, SF_{1d} of control samples was by 20.0 % higher than PSE meat samples. During storage of 5 days period, SF_{5d} of PSE samples increased by 140.0 % that SF_{1d} of PSE and almost 80.0 % that SF_{5d} of control samples.

Table 1 Cooking Loss (CL) and Shear Force (SF) measurements of control and PSE broiler breast meat samples stored at 0 ºC for 1 day and 5days.

Parameters	Control	PSE
*CL_{1d} (%)	23.85^{bB} ±1.28	28.31^{aB} ±2.32
*CL_{5d} (%)	25.46^{bA} ±1.19	30.91^{aA} ±2.62
SF_{1d} (N)	24.99^{aA} ±5.69	20.50^{bB} ±3.31
*SF_{5d} (N)	27.38^{bA} ±6.82	48.61^{aA} ±14.06

$^{a-b}$ Means within each line with different superscripts are different ($p \leq 0.05$). * ($p \leq 0.01$).
$^{A-B}$ Means within each column with different superscripts are different ($p \leq 0.05$).

Table 2 shows difference values in SC and TC in control and PSE broiler breast meat samples kept at 0 ºC for 1day and 5 days storage. TC_{1d} from 1 day storage samples was 14.6 % lower in PSE meat (0.35 g/100 g) in relation to control (0.41 g/100 g) whereas on 5th day no difference was observed ($p \geq 0.05$). SC_{1d} was 25.6 % higher in PSE meat in comparison to control samples whereas on 5th day no significant difference was observed ($p \geq 0.05$).

Table 2 Soluble Collagen (SC) Total Collagen (TC) contents of control and PSE broiler breast meat samples stored at 0 ºC for 1 day and 5 days.

Parameters	Control	PSE
*TC_{1d}	0.41^{aB} ± 0.02	0.35^{bA} ± 0.03
*TC_{5d}	0.36^{aA} ± 0.05	0.39^{aA} ± 0.02
*SC_{1d}	0.090^{bB} ± 0.02	0.121^{aB} ± 0.03
*SC_{5d}	0.118^{aA} ± 0.05	0.140^{aA} ± 0.02

*g/100g of sample
$^{a-b}$ Means within each line with different superscripts are different ($p \leq 0.05$).
$^{A-B}$ Means within each column with different superscripts are different ($p \leq 0.05$).

IV. DISCUSSION

A. Cooking Loss and Shear Force measurements

CL was higher for PSE fillets throughout the experiments (Table 1). The CL is the consequence of meat soluble and insoluble matters within the drip during cooking and the main component lost is water [18]. The highest CL value observed in the PSE meat samples are assigned to rapid postmortem glycolysis leading to the denaturation of myofibrillar proteins,

reducing their ability to retain water [2, 19]. The CL is inversely proportional to the Water Holding Capacity (WHC) (4). The use of chicken PSE meat within the industry for commercial purpose generates a product with low WHC and poor cut when compared to normal meat [2, 3, 20]. Thus during storage these problems tend to increase as the highest CL observed was from PSE meat samples stored for 5 days postmortem.

It was observed that in 1 day postmortem samples, the SF was lower ($p \leq 0.05$) for PSE fillets samples (20.50 N) compared to control samples (24.99 N) (Table 1). Similar results were observed by Wilhelm et al. [4], which attributed the lower SF for earlier activation of calpain during installation of PSE meat probably before the initiation of *rigor mortis*. The same authors suggested that the increased protease activity in PSE meat samples might be due to the high concentration of intracellular Ca^{2+}. Furthermore, in PSE meat samples there was a gradual increase of SF during storage. PSE meat samples showed at the 5th day postmortem values of SF 2.5-fold ($p \leq 0.01$) than 1 day postmortem and almost twice as much higher ($p \leq 0.01$) than the control samples at the same time of storage probably due to excessive water loss leading to myofibril proteins aggregation.

B. Collagen Contents

TC_{1d} was 14.6 % lower in PSE meat. This result is probably related to the accelerated decline in pH while the carcass temperature is warm leading probably to the rupture of lysosomes, which contain cathepsin enzymes, which in turn is able to degrade collagen molecules [21]. Cathepsins B and L are the main components to digest these molecules and have a pH optimum of activity between 5.5 and 6.0. Research has shown that these isoforms enzymes may affect the collagen in its native form inducing the depolymerization of its fibers [10, 22]. Because the fillet PSE samples pH_{3h} was 5.64, this value may have contributed to enhance the activity of these enzymes over collagen molecules. Although the cathepsin mechanism of action over collagen is not well understood, Kirschke et al. [22] suggested that these proteases act mainly on the collagen telopeptides. Based on the studies with differential scanning calorimetry, Beltran et al. [23] concluded that bacterial collagenase and cathepsin B have similar mechanisms acting on the insoluble collagen. Furthermore, the soluble collagen content of PSE meat was about 25 % higher ($p \leq 0.05$) than in the control samples in 1 day postmortem samples (Table 2), collaborating with the tenderness of PSE meat. This demonstrates that these cathepsins may show their activity earlier before the slaughtering the birds as discussed in Wilhem et al. [4] thus improving the meat tenderness.

After 5 days postmortem, the collagen content of fillets was similar ($p \geq 0.05$) in both control and PSE meat samples (Table 2). The SC content was higher in the control group in a similar amount in relation to PSE meat stored for 5 days postmortem (Table 1). Histochemical studies in beef have shown that a progressive rupture of lysosomes took place and after 14 days of storage its breakdown was almost completed [24]. Therefore, it is reasonable to speculate that in our experiment in the control group a gradual rupture of lysosomes may have occurred during the 5 days of storage thus releasing cathepsins. Finally, as the pH of the fillets already was in the ideal range of activity of these enzymes, possibly occurred conditions for the cathepsins to hydrolyze the collagen molecules and consequently promoting their degradation.

V. CONCLUSION

Changes in collagen fraction during meat ageing may have implication on the broiler PSE meat qualities, in particular at 1 day *postmortem* samples contributing to its tenderness.

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