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Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat

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ABSTRACT

The connections between the redox imbalance in post-mortem muscle, early protein oxidation and the onset of pale, soft and exudative (PSE) condition in chicken breast are studied. PSE was induced by incubation of post-mortem chicken carcasses at 37 °C for 200 min. PSE-induced muscle consistently had faster pH decline and lower pH at 200 min (5.84 vs. 6.59) and 24 h (5.69 vs. 5.96), higher L^* (54.4 vs. 57.3), and lower texture and water holding capacity (WHC) than normal meat. The activities of catalase, glutathione peroxidase and superoxide dismutase were significantly lower in PSE-induced samples than in the normal counterparts. PSE was more susceptible to proteolysis and protein oxidation than normal meat during succeeding chilled storage with more intense tryptophan and thiols depletion, higher protein carbonylation and more intense formation of protein cross-links. We provide plausible explanations to support the role of protein oxidation in the impaired quality PSE chicken.

1. Introduction

The poultry industry is confronting a major challenge due to an increasing occurrence of pale, soft, and exudative (PSE)-like meat (Barbut et al., 2008). PSE meat occurs in swine by two causes, (i) a genetic mutation on the ryanodine receptor (Ca^{++} metabolism) in muscle tissue, and (ii) severe stress conditions prior to slaughter (Fujii et al., 1991). In chicken, however, only the latter has been confirmed as a driving cause for PSE occurrence (Carvalho et al., 2015; Wilhelm, Maganhini, Hernández-Blazquez, Ida, & Shimokomaki, 2010). Several factors influence the onset of PSE-like chicken meat, such as season (Langer et al., 2010), environment (Carvalho et al., 2015), heat stress (Spurio et al., 2016), and pre- and post-slaughter handling practices (Guarnieri et al., 2004). The consequences of broiler breast PSE meat have recently been the subject of experimental studies by several research groups (Barbut et al., 2008; Carvalho et al., 2014).

PSE meat is characterized by low water holding capacity (WHC), soft texture, and light appearance. This functional defect is believed to take place as a result of protein denaturation caused by rapid post-mortem muscle glycolysis, which lowers pH while the carcass is still warm (Barbut et al., 2008; Lesiow & Xiong, 2013). Elevated post-mortem temperatures around 40 °C facilitates protein denaturation and the onset of PSE meat characteristics in turkey (McKee & Sams, 1998) and swine (Lesiow & Xiong, 2013) by accelerating the development of rigor mortis and a fast pH decline. Along with denaturation and proteolysis, muscle proteins also suffer oxidative damage after slaughter and during the subsequent meat aging (Estevez, 2015). Pre-slaughter stress is linked to an increased susceptibility to lipid and protein oxidation in broiler muscles (Estevez, 2015) and protein oxidation has been recurrently associated to an impaired functionality of meat proteins (Utrera & Estevez, 2012; Xiong, 2000). It is hence, reasonable to hypothesize that protein oxidation may also play a role in the onset of PSE-like meat, but this extent has never been confirmed.

Muscle proteins undergo oxidative damage in the presence of reactive-oxygen species (ROS), transition metals and reducing sugars (Estevez, 2015). Among the assorted chemical manifestations of

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this damage, the depletion of tryptophan residues and free thiols and the formation of carbonyl residues and crosslinks have been already reported in raw and processed meat and poultry (Estévez, 2011; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In post-mortem muscle, the collapse of the endogenous antioxidant system and the biochemical changes occurred during the conversion of muscle to meat promote the occurrence of post-mortem oxidative stress in poultry muscles (Estevez, 2015). The pH-decline, in particular, is responsible of the alteration of cellular compartmentalization and the release of pro-oxidant enzymes. Enzymes such as glutathione peroxidase, superoxide dismutase and catalase are known for contributing to counteract pro-oxidant factors and protect against lipid and protein oxidation in post-rigor meat (Liu et al., 2014). The connection between the redox imbalance in post-mortem muscle, the occurrence of protein oxidation and the onset of PSE-like meat is waiting to be unveiled. A better understanding of the biochemical mechanisms behind the role of oxidative stress in PSE occurrence was the objective of this study.

2. Material and methods

2.1. Materials and experimental design

A total of 16 female Ross broilers (308 lineage, 34 days of age and 1.8 kg) were obtained from a commercial processing plant (Veravic S.L., Cáceres Spain). Feed was removed 10 h before slaughter and water was provided *ad libitum*. Typical commercial corn-soybean meal diet was utilized and the birds were transported (155 km) to the slaughterhouse. The animals were slaughtered according to standard industry practices, which consisted of hanging, carbon dioxide stunning, bleeding, scalding, defeathering and evisceration. At that point (5 min after slaughter), sixteen carcasses ($T^a \sim 37.5$ °C) were collected and randomly divided into two groups. One group of carcasses (n = 8) was subjected to induction of PSE as described by Lesiow and Xiong (2013), with minor modifications. The carcasses were placed in separate plastic bags (Ziploc bags), and stored at 37 °C in an oven (JP Selecta-2001244, Barcelona, Spain) for 200 min to generate PSE-like meat. The pH changes were monitored over time. After that, the carcasses were placed in separate plastic bags and stored at 4 °C for up to 24 h for further analyses. The other group of samples (normal; n = 8) were held by the hocks on shackles in the cold room for approximately 60 min. After cooling, the carcasses (4 °C) were placed in separate plastic bags and stored at 4 °C for up to 24 h for further analyses. The pH and temperatures values of PSE and normal breast samples were measured at 20, 80, 200 min and 24 h post-mortem. Thereafter, breast muscles were dissected from carcasses and immediately subjected to color, texture and WHC measurements as described below. A slice (1 cm thickness) from the middle of each breast was placed in falcon test tubes, kept under refrigeration (4 °C) for 7 days, and analyzed for lipid and protein oxidation and WHC as described below. Lipid and protein oxidation measurements were made at days 1, 4 and 7 of chilled storage. The remaining samples were placed in plastic bags and kept in a −80 °C freezer for enzymes activities and free amino acids (FAA) determination.

2.2. Temperature and pH determination

The pH and temperature of carcasses were measured (in duplicate in each sample) using portable pH-meter (Crison PH25, Barcelona, Spain) and thermometer (Testo 735, Lenzkirch, Germany) by inserting electrodes into the *pectoralis major* as described in Carvalho et al. (2014).

2.3. Color determination

This evaluation was performed using a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ) with illuminant D65 and 0° standard observer. Before each measurement, the chromameter was calibrated on the CIE color space system using a white tile. Measurements were made at three different random reading points on the surface of the breast muscle and in the core after slicing as previously described by Carvalho et al. (2014).

2.4. Texture

Texture profile analysis (TPA) was performed at room temperature (21 °C) with a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK). Seven cube samples (1 × 1 × 1 cm) were taken from the middle of the raw breast and subjected to a two-cycle compression test. The samples were compressed to 40% of their original height with a cylindrical probe of 5 cm diameter and a cross-head speed of 5 mm/s. Texture profile parameters were determined following descriptions by Bourne (1978) and the SMS manual (Stable Micro Systems, Surrey, UK). All analyses were performed in heptaplicate in each breast sample.

2.5. Water holding capacity (WHC)

2.5.1. Press method (WHC_p)

This measurement was carried out based on the technique of Hamm (1960), as described in Carvalho et al. (2014), with minor modifications. After 24 h postmortem, samples were collected from the cranial side of the breast filets and cut into cubes 1.0 ± 0.01 g. A total of 16 samples were analyzed in duplicate. Initially they were carefully placed between 2 pieces of filter paper and then left under a 1-kg weight for 2 min. The samples were weighed and WHC was determined using the following equation: 100 − [(Wi − Wf/Wi) × 100], where Wi and Wf are the initial and final sample weight, respectively.

2.5.2. Cooking loss (CL)

CL was measured according to Honikel (1998), with minor modifications. The samples were weighed (5 ± 0.1 g) before and after 30 min of cooking. The CL was determined by equation: 100 − [(Wi − Wf/Wi) × 100], where Wi and Wf are the initial and final sample weight, respectively.

2.5.3. Centrifugation method (WHC_c)

A total of 16 samples were analyzed in duplicate as described by Honikel (1998), with minor modifications. The piece of breast meat (5 g) was placed in plastic bag (Ziploc bags) and heated at 70 °C for 30 min in a water bath. After heating, samples were left at room temperature (21 °C for 45 min). The WHC was determined by a centrifugal method. Briefly, samples were centrifuged at 500*g* for 15 min at 4 °C. WHC (%) was calculated as aforementioned.

2.5.4. Centrifugation method with salt addition (WHC_s)

A piece of breast meat (5 g) was placed in falcon tubes and added 8 mL NaCl 0.6 M (Barbut, 1993). Samples were then heated at 70 °C for 30 min in a water bath. After heating, samples were left at room temperature (21 °C for 45 min). WHC was determined by the centrifugal method previously described.

2.6. Free amino acids (FAA) analysis

Ten grams of broiler breast muscle samples were homogenized twice with 50 mL deionized water and centrifuged at 5000*g*, 4 °C for 10 min. Combined supernatants, containing water-soluble