Oxidative changes in cooled and cooked pale, soft, exudative (PSE) chicken meat

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Abstract

The mechanisms involved in the development of oxidative changes in pale, soft, exudative (PSE) chicken meat during storage in the dark at 4°C for 5 days and after cooking at 80°C for 30 min, light exposure and reheating were explored in this study. The results indicate that myoglobin, lipid and protein oxidation occurred concomitantly during both treatments in PSE chicken meat during storage, and each process seemed to promote the others. Transition metals and metmyoglobin played pivotal roles in the generation of free radicals that triggered lipid and protein oxidation in cooled and cooked PSE, respectively. In contrast, light played a secondary role as an oxidative inducer of these processes. Different pathways triggered the production of compounds from the interactions between oxidative reactions in cooled and cooked PSE chicken meat. The impact of these reactions on the functionality of PSE chicken meat requires further study.

Keywords: PSE meat, carbonyls, lipid oxidation, metmyoglobin, heme iron, thiol groups.

1. Introduction

Global poultry meat production and consumption have increased rapidly over the past few decades. Compared to other forms of poultry, chicken meat constitutes more than 90% of the demand in the chicken market and is influenced by, among other factors, its nutritional value, such as high protein quality and the presence of vitamins and minerals that can meet human needs. However, the development of PSE chicken meat results in a series of problems related to quality and revenue loss for the industry (Zhao., *et al*, 2016; Xu., *et al*, 2018).

PSE meats are characterized by their pale color, soft texture and low water holding capacity (WHC). Extensive studies have shown that the occurrence of PSE properties in chicken meat is linked to genetic and metabolic factors (as a consequence of accelerated postmortem glycolysis, which promotes a sharp decrease in pH while the muscle is still in the rigor state) and to the overall stressful preslaughter environmental conditions (temperature, relative humidity, transport) (Langer., *et al*, 2010; Ziober., *et al*, 2010). The rapid decrease in the pH of the meat causes denaturation of myofibrillar and sarcoplasmic proteins, as well as excessive loss of exudate, thus compromising the functional, technological and sensory properties of the meat (Yang., *et al* 2021).

In animals raised under harsh conditions, a genetically induced imbalance in ryanodine receptors leads to an excessive release of calcium from the sarcoplasmic reticulum, which leads to hypermetabolism and a consequent increase in the activity of proteases and lipases. The latter enzymes hydrolyze membrane phospholipids that are converted into hydroperoxides, whose instability results in transformation into reactive compounds. Concomitantly, the excess hydrogen ions in the intracellular environment due to the high concentration of lactic acid facilitate reactions that lead to protein carbonylation and lipid oxidation. On the other hand, storage and processing conditions also influence the development of oxidative changes in lipids and meat proteins (Soares., *et al*, 2009; Ziober., *et al*, 2010; Al-Dalali., *et al*, 2021; Qian., *et al*, 2022).

In refrigerated meats, changes in cell compartmentalization, iron release from sarcoplasmic proteins and increased oxidizing enzyme activity lead to the propagation of lipid oxidation (Wang, He, Gan & Li, 2018).

During the cooking process, the formation and cleavage of lipid radicals, the release of nonheme iron from meat and the oxidation of myoglobin intensify oxidative reactions. In addition, membrane rupture during cooking leads to the exposure of phospholipids to oxygen and/or a reduction in size (thus increasing the surface contact), thus accelerating the development of oxidative rancidity during storage. Reheating meat increases the levels of thiobarbituric acid-reactive substances

(TBARS) and volatile compounds in meat (Byrne, Bredie, Moltram & Martens, 2002; Estevez & Cava, 2004; Xiong., *et al*, 2020).

Displaying meat under lights on a market shelf is important for consumer acceptability, as the specific color of meat is an important factor in quick visual assessments of the quality and freshness of the product. However, light in combination with oxygen may induce photooxidation in meat due to the presence of naturally occurring photosensitizers, such as tryptophan and tyrosine residues, as well as riboflavin. These photosensitizers are excited upon absorbing light energy and may either react directly with lipids and proteins or attack molecular oxygen to produce superoxide anions ($O_2^{\cdot -}$) or singlet oxygen ($^1O_2$), inducing oxidative damage (Baptista., *et al*, 2017; Zainudim, Poojary, Jongberg, & Lund, 2019).

Oxidative reactions in PSE chicken meat promote extensive changes in the major meat components, affecting properties that are already altered, such as water retention capacity, loss by dripping, loss by cooking, and color, and reducing the quality and industrial applicability of the meat. Recently, the influence of oxidation on the quality of PSE chicken meat was discussed in our previous studies (Carvalho., *et al*, 2017); however, the pathways related to triggering oxidative reactions in cooled and thermally treated PSE chicken meat need further discussion. Thus, the mechanisms involved in the development of oxidative changes in PSE chicken meat during storage in the dark at 4°C for 5 days and after cooking at 80°C for 30 min, exposure to light and reheating (oxidative induction) were explored in this study.

2. Materials and methods

2.1 Chemicals

1,1,1,3-Tetraetoxypropane (TEP), 2,4-dinitrophenylhydrazine (DNPH), bovine serum albumin (BSA), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), L-cysteine and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). $Na_4P_2O_7$, Tris-maleate, KCl, $MgCl_2$, and ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) were purchased from Merck (Merck, Darmstadt, Germany). All aqueous solutions were prepared using a Milli-Q system. All other chemicals were of analytical or better grade.

2.2 Meat samples

Ross lineage broiler fillet meat (*Pectoralis major m.*) samples were obtained at a commercial plant located in southern Brazil from 42-day-old male and female broilers slaughtered in the summer according to standard industrial practices. The slaughtering process consisted of a sequence of electrical stunning, bleeding, defeathering, evisceration, carcass water cooling, deboning and

refrigeration, as described in Wilhelm, Maganhini, Hernandez-Blazquez, Ida & Shimokomaki (2010). After the analyses in a commercial plant, the chicken meat samples were packed in transparent, flexible, oxygen-permeable polyethylene bags that were layered in thermal boxes with ice and cooling gels around the meat, and the samples were then sent to a university for further analysis. The breast fillets previously classified as control and PSE meat were stored at 4°C in the dark under low oxygen pressure for 5 days in a cool chamber inside a scientific laboratory at the university. On Days 1, 3 and 5, these samples were analyzed under cooled and cooked conditions. The cooled PSE meat remained in the cold chamber, while some of the samples were separated on each day of analysis for thermal treatment. For thermal treatment, the samples were vacuum packed and kept in a water bath at 80°C for 30 min. Then, the vacuum packaging was removed from the cooked samples, and the samples were repacked with the same packaging used for the cooled samples and stored at 4°C in an isolated section within the cool chamber, where they were exposed to fluorescent lighting for 4 h. The samples were then reheated in an 800-Watt microwave for 4 min. Last, the samples were cooled to room temperature as described by Byrne, Bredie, Moltram & Martens (2002) with modifications.

2.3 pH and color analysis

pH and color determination was performed as described in Wilhelm, Maganhini, Hernandez-Blazquez, Ida & Shimokomaki (2010). Briefly, the pH was measured by inserting electrodes into the breast muscle using a contact pH meter system previously calibrated with buffer solutions (pH= 4 and pH= 7). The pH analyses were performed in triplicate at 4 h and 24 h post-mortem. The pH of the control and PSE samples was measured at 4 h. The pH at 24 h post-mortem was measured as described by Olivo, Soares, Ida & Shimokomaki (2001). A Minolta CR400 colorimeter (BYK - Gardner Gmbh; color-guide 45°/0°, 11 mm; light source: D65) was used to evaluate the color, lightness (L*), redness (a^*), and yellowness (b^*) of the posterior surface of the intact skinless breast muscles at 24 h post-mortem. The color parameters were measured at three different points on the same sample. The final color values are expressed as the means of triplicate values. Seventy-six samples were tested and identified by coding. Fourteen samples with values of L*24 h < 53.0 and pH > 5.80 were classified as controls, and fourteen other samples with values of L*24 h ≥ 53.0 and pH ≤5.80 were classified as PSE meat according to (Barbut, 1993; Soares, Lara, Ida, Guarnieri & Shimomkomaki, 2002).

2.3 Protein denaturation

The degree of protein denaturation in the control and PSE samples was measured 48 h postmortem as described by Swatland (1995). Five grams of sample (in triplicate) was homogenized with 15 mL of distilled water using a Turrax (1000 g) for 15 s. The homogenate was centrifuged at 3500g at 2°C for 10 min and filtered through filter paper. One milliliter of the filtrate was dispensed in a test tube, and 5 mL of citrate/phosphate buffer (0.2 M) was added (pH=4.6). The mixture was incubated in a thermostatted bath at 20°C for 30 min along with a blank sample. A blank was prepared for each treatment and consisted of 1 mL of filtrate and 5 mL of distilled water. The transmittance of the samples was measured spectrophotometrically at 600 nm. The results are expressed as the measure of transmittance by subtracting the value of the blank.

2.4 Water holding capacity (WHC)

The WHC was measured 24 h post-mortem as described by Ham (1964). Samples were collected from the cranial side of the breast fillets and cut into 2 g cubes (in triplicate). These samples were placed between two filter papers. The whole sample was placed between two acrylic plates, and a 10 kg mass was applied for 5 min. The WHC is expressed as a percentage and calculated according to the following equation:

1. 100 - [(Pi -Pf/Pi) x100], where Pi and Pf are the initial and final sample weights, respectively.

2.5 Lipid oxidation

Lipid oxidation in breast fillets was determined by the TBARS assay using the method adapted by Soares, Olivo, Shimokomaki & Ida (2004). The results are expressed in mg TBARS kg^{-1} of sample according to Tarladgis, Pearson & Dugan (1964). Ten grams of sample (in triplicate) was homogenized in 98 mL of distilled water, 2.5 mL of 4 N HCl and 5 drops of Span 80 + Tween 20. This solution was distilled for 10 min, and 50 mL was collected. An aliquot of 5 mL of distillate was transferred to a test tube, and 5 mL of 0.02 M thiobarbituric acid (TBA) reagent was added. The mixture was placed in a boiling water bath for 35 min along with a blank containing 5 mL of TBA plus 5 mL of distilled water. After cooling to room temperature, the absorbance was measured spectrophotometrically at 530 nm. A standard curve was generated with known concentrations of TEP and was used to calculate the TBARS content in the samples.

2.6 Protein carbonyl analysis

The protein carbonyls in the breast fillets were monitored by derivatization with 2,4-dinitrophenylhydrazine (DNPH) in a manner similar to that reported by Fagan, Slezka & Sohar

(1999). Three grams of sample (in quadruplicate) was solubilized in 25 mL of buffer (pH=7.4) containing $Na_4P_2O_7$ (2.0 mM), Tris-maleate (10 mM), KCl (100 mM), $MgCl_2$ (2.0 mM) and EGTA (2.0 mM). The mixture was homogenized with a Turrax (twice at 8000 × g and 9500 × g for 15 s). The homogenate was centrifuged at 8000 × g for 20 min at 4°C. The supernatant was clarified by centrifugation at 38720 × g for 30 min at 4°C. An aliquot of 1 mL of the filtrate was precipitated with 1 mL of HCl/acetone (3:1). The mixture was centrifuged at 8000 × g for 20 min at 4°C. The resulting pellet was treated with 1 mL of 10% TCA and centrifuged at 8000 × g for 20 min at 4°C. The pellet was resuspended in 1 mL of buffer and 1 mL of DNPH solution. A blank was prepared with 1 mL of buffer and 1 mL of 2 N HCl. The mixture (or blank) was incubated with agitation (in the dark) for 30 min at 4°C. One milliliter of 30% TCA was added, and the pellet was incubated in an ice bath for 10 min. This mixture was centrifuged at 8000 × g for 20 min at 4°C. The resulting pellet was washed twice with 1 mL of ethanol/ethyl acetate (1:1) in 10 mM HCl and centrifuged at 8000 × g for 20 min at 4°C for the removal of DNPH. The pellet was then solubilized in 1 mL of 6 M guanidine solution in monobasic sodium phosphate buffer. The resulting mixture was centrifuged at 8000 × g for 2 min at 4°C. The carbonyl content was determined by analyzing the supernatant spectrophotometrically at 370 nm using a molar absorbance coefficient of 22.000 $M^{-1}cm^{-1}$. The protein concentration was determined spectrophotometrically at 280 nm using bovine serum albumin (BSA) in 6 M guanidine as the standard. The results are expressed in nmol carbonyls/mg protein.

2.7 Metmyoglobin analysis

The percentage of metmyoglobin in the samples on Days 1, 3 and 5 was determined according to Krzwwicki (1982). Five grams of sample (in triplicate) was solubilized in 25 mL of phosphate buffer (40 mM) at pH 6.8. The mixture was homogenized with a Turrax at 13500 × g for 10 s and stored for 1 h at 4°C. The homogenate was centrifuged at 5000 × g for 30 min at 4°C. The supernatant was filtered through Whatman No. 1 filter paper. The absorbance of the filtrate was measured spectrophotometrically at 525, 572 and 700 nm, and myoglobin values were obtained according to the following equation:

2. $MetMb = \{1.395 - [(Abs_{572} - Abs_{700})/(Abs_{525} - Abs_{700})]\} x\ 100$

where Abs_{525} is the absorbance of the supernatant at 525 nm, Abs_{572} is the absorbance of the supernatant at 572 nm, and Abs_{700} is the absorbance of the supernatant at 700 nm.

2.8 Heme iron analysis

Heme iron analysis of the samples was performed using the method adapted by Souza, Arthur & Canniati-Brazaca (2007). Five grams of sample (in triplicate) was dispensed in 50 mL Eppendorf tubes, and 20 mL of acetone and 0.5 mL of HCl were added. Deionized water was added until the total weight of the sample was 4.5 g. The amount of water to be added was calculated by moisture analysis of the material. The samples were centrifuged at 2000 × g for 15 min and filtered through a qualitative filter paper. The absorbance of the filtrate was measured at 640 nm using the extractive solution as a blank, and the heme iron content was calculated using the formula below:

3. $\text{Hematin} = Abs_{640} * 680 * P(\text{sample})$

4. $$\text{Heme iron} = \frac{\text{Hematin} * 88.2}{P(\text{sample}) * 10000}$$

Abs = absorbance (nm)

P = weight (mg).

2.9 Free thiol group analysis

For the determination of free thiol groups in proteins, DTNB was used as described by Ellman (1959). Two grams of sample (in triplicate) was homogenized with 50 mL of 5% sodium dodecyl sulfate (SDS) in 0.10 M Tris buffer (pH=8.0) in a Turrax at 13500 × g for 30 s. The homogenate was incubated in a water bath at 80°C for 30 min and then cooled and centrifuged at 1200 × g for 20 min. The supernatant was filtered with Whatman No. 1 filter paper. Two milliliters of Tris buffer (pH=8.0) and 0.5 mL of 10 mM DTNB in 0.10 M Tris buffer (pH=8.0) were added to 0.5 mL of the filtrate. The absorbance at 412 nm was determined spectrophotometrically after 30 min against a blank containing 0.5 mL of 5% SDS, 2 mL of Tris buffer (pH=8.0) and 0.5 mL of 10 mM DTNB in 0.10 M Tris buffer (pH=8.0). The data obtained were plotted on a standard curve of L-cysteine according to the method of Stapelfeldt, Bjerrum & Skibsted (1997). The protein concentration was determined spectrophotometrically at 280 nm using BSA as a standard. The results are expressed in nmol/mg of protein.

2.10 Fatty acid analysis

On the third day of storage, the fatty acid (FA) profiles of both cooled PSE and control samples were determined. The FA profiles were determined in duplicate by preparing methyl esters by transesterification according to the method of ISO 5509 (1978) using 2 M KOH in methanol and

n-heptane. The FA methyl esters (FAMEs) were quantified with a Shimadzu 14A gas chromatograph (Japan) equipped with a flame ionization detector and a fused silica capillary column (100 m × 0.25 mm × 0.25 µm film thickness, CP-7420 Varian). The column temperature was increased at 10°C min^{-1} from 80 to 240°C. The temperatures of the injector and detector were maintained at 200 and 220°C, respectively. The carrier gas was hydrogen (1.2 mL min^{-1}), and nitrogen was the make-up gas (30 mL min^{-1}) and flame gas (300 mL.min^{-1}). The sample split mode was 1/100. The identification of FAs was based on authentic standards (Product 18919-1AMP, C4-C24 FAME Mix, Sigma–Aldrich). The peak areas were determined by means of a CG-300 integrator (CG Instruments, Brazil). The results are expressed as the relative percentages of the identified FAs according to Soares *et al.* (2009).

2.11 Statistical analysis

All results were analyzed using STATISTICA for Windows version 5.0 STATSOFT (1995). Tukey's test (5%) was used to evaluate the color, pH, WHC, protein denaturation, fatty acids and metmyoglobin. Student’s t test (1% and/or 5%) was used to evaluate the protein carbonyls, TBARS, heme iron and thiol groups. Pearson's correlation coefficient test was used to assess the correlation between pH and L*, TBARS and carbonyl compounds.

3. Results and discussion

3.1 pH, color, WHC, and protein denaturation

Table 1 shows the pH, color, WHC and protein denaturation values obtained in this study. A consistent difference in pH between the PSE and control samples at 4 h post-mortem was found in this study, similar to the results of Lesiow & Xiong (2013). The pH continued to decrease with time and reached a minimum of 5.77 after 24 h. In contrast, the pH values of the control samples were 6.54 and 5.99 after 4 h and 24 h, respectively. After slaughter, because of the bleeding and the interruption of oxygen supply, muscles begin using anaerobic metabolism to produce energy and maintain function as long as ATP is available. With the depletion of ATP reserves, muscles cannot relax, and permanent cross-links are formed between the myosin and actin filaments, leading to rigor mortis. In normal chicken meat, rigor mortis sets in after 1 h post-mortem, and the rate of pH drop varies between strains and individuals. In pos-tmortem PSE poultry, quantitative alteration in the isoforms of ryanodine receptors alters muscle calcium homeostasis. The increased calcium concentration in the intracellular medium increases the activity of calcium-dependent enzymes and accelerates glycolysis, which promotes a fast decline in pH while the muscle is still in rigor mortis and the carcass temperature remains high. Metabolic heat and the accumulation of lactic acid in the

muscles change the properties of the meat (Estevez, 2015). In this study, the faster decline in pH in the first hours post-mortem in PSE meat compared to controls led to the lower WHC of the meat, as well as, the higher light scatter, resulting in the pale color, as verified by the high L value (Table 1).

There was a significant difference ($p<0.05$) between the a/b ratios of the PSE samples and control samples (Table 1). In the CIELAB system, the a* coordinate represents redness, and b* represents yellowness. The a/b ratio provides a quantitative measure of the balance between oxymyoglobin and metmyoglobin formation in meat, so the higher the a/b ratio is, the redder the meat will appear. In the PSE chicken meat, the lower a/b ratio in relation to that of the control indicates that the reducing environment promoted by the low post-mortem pH facilitated the formation of metmyoglobin.

As shown in Table 1, PSE chicken meat showed a lower WHC than the control. Water retention capacity is the ability of meat to retain water under the application of external mechanical or thermal forces. This property influences the appearance and palatability of meat and is directly related to water loss before and during cooking. The water loss in PSE meat is higher, corresponding to a lower WHC. Nondenatured proteins maintain their connection with water during the conversion of muscle into meat, but when denatured, the proteins modify the behavior of the free water present in the muscle. Free water is retained by weak bonding forces; that is, it is maintained solely by surface forces. The bound water present in muscles at a proportion of 4 to 5% is strongly bound to the protein hydrophilic groups to the point of resisting intense mechanical forces, and the immobilized water cannot easily be removed from the muscles due to this strong bond with the proteins. The changes observed in the water retention capacity of muscle proteins are due to the changes experienced by free water and structural alterations in the myofibrils. Myosin denaturation and the exposure of hydrophobic groups result in large clusters that are accelerated by myosin unfolding and aggregation owing to head-to-head interactions of myosin. Furthermore, changes in myosin conformation are sensitive to low pH because of electrostatic repulsion, and the similarity between positive and negative charges allows the best approximation between the thin and thick filaments, preventing these molecules from binding with water. However, the high activity of proteases in PSE chicken meat facilitates the movement of water from the intracellular compartment to the endomysium and finally to the surface, thus increasing the loss and impairing the quality of the meat (Yang *et al*, 2021). Currently, processors are attempting to identify suitable methods to improve the WHC and textural properties of PSE-like chicken breast meat, such as high-intensity ultrasound treatment (Li., *et al* 2014) and isoelectric solubilization/precipitation processing (Zhao *et al*., 2016). Glycosylation, one type of posttranslational protein modification, is a prevailing

approach for improving the functional properties of proteins (Xu., *et al* 2018). These authors grafted glucosamine onto the peptide backbone in the PSE-like chicken breast using microbial transglutaminase as a biocatalyst. The results of secondary structure analysis suggested that the glycosylated myofibrils had decreased α-helix contents and increased β-sheet, β-turn and random coil contents. After glycosylation with glucosamine, the surface hydrophobicity was significantly reduced, and the solubility of the myofibrils at the isoelectric point was markedly improved. Glycosylation could be used in future studies to measure the possible differences and their effects on other functional properties of PSE and PSE meat under in vitro-induced oxidation used in food products.

There was a significant difference between the L* values of the PSE and control samples. A high L* value characterizes pale PSE meat (Barbut *et al*, 2008). A negative correlation ($r = -0.20978$ $p < 0.001$) between pH_{24h} and L* was found in this study. These results confirm the inverse relationship between pH and the L* value in PSE chicken meat. The color of the meat surface results from the selective absorption of myoglobin, caused by the distribution of light that reflects off the meat, as well as important components, such as muscle fibers and their proteins, and is also influenced by the amount of free liquid present in the meat. In PSE meat, a low pH increases the birefringence, resulting in less light being transmitted and more light being reflected. Lateral refraction through myofibrils is a dominant mechanism of light scattering, and this can increase in meat with a low ultimate pH compared to normal meat because the closer packing of myofilaments at lower pH values increases the difference in the reflective index between the myofibril and its surroundings (Purslow., Warner., Clarke., & Hughes, 2020).

There was a significant difference ($p<0.05$) in the degree of protein denaturation between the PSE and control samples (Table 1). The superior loss of native structure and further hydrolysis in PSE samples facilitated protein unfolding, leading to the exposure of nonpolar residues that were previously buried in the hydrophobic core of the protein structure (Estevez, 2011), thus increasing the susceptibility of meat proteins to oxidation. The native structure (size, composition, sequence and distribution of the lateral chains of amino acids) of myofibrillar proteins and their ability to bind with water plays an essential role in their functionality within meat systems, determining the quality of fresh meat, and the success of numerous processes applied during the manufacture of meat products includes the optimization of myofibrillar protein preservation (Souza, 2015). Oxidation causes changes in the secondary and tertiary structures of proteins, leading to an increase in protein hydrophobicity and protein aggregation (Estevez, 2011). In PSE meat, changes in surface hydrophobicity reflect changes in the chemical and physical characteristics of the protein structure, leading to aggregation and polymerization through dityrosine and disulfide bonds that likely

combine to reduce protein solubility (Chen., *et al* 2016). In addition, the high content of intracellular calcium and denaturation promotes the intense activity of calpains in the myofibrillar proteins of PSE chickens, which propagate through the Z-line, resulting in collapse of the sarcomere structure (Wilhelm, Maganhini, Hernandez-Blazquez, Ida & Shimokomaki, 2010). The combination of these ultrastructural alterations could unfortunately interfere with properties such as gelation and emulsification, thus decreasing the functional quality of the meat.

3.2 Metmyoglobin and TBARS (under cooked conditions)

In Figure 1, the percentage of metmyoglobin in the cooled and cooked PSE samples and their respective controls are shown.

There was a higher production of metmyoglobin in the PSE samples cooled until the third day of storage (Fig. 1). From the third day to the fifth day, there was no change in the levels of metmyoglobin in the PSE and control groups, but the significant difference between treatments was maintained. In all the analyses performed on the cooled samples, with the exception of pH, color, WHC and protein denaturation, there was no significant difference within the same treatment from the third day to the fifth day of storage. However, as low temperature is a factor that delays the development of oxidative reactions, it is believed that perhaps with a slightly longer storage time between the analyses, significant differences within the same sample could be detected.

Three forms of myoglobin, oxymyoglobin, deoxymyoglobin and metmyoglobin, coexist in meat systems. The predominance of each form depends on the redox status of iron. Deoxy(II)Mb and Oxy(II)Mb can be converted into the ferric state (Met(III)Mb) in meat. This process is known as myoglobin autooxidation. Formation of Met(III)Mb from Oxy(II)Mb is a proton-mediated reaction; protons enter the heme pocket and protonate ligated O_2. The positive charge on O_2, which results from protonation, causes single-electron removal from the iron atom of ferroprotoporphyrin IX to be energetically favorable. The neutral superoxide radical then dissociates, resulting in Met(III)Mb formation (Oxy(II)Mb + $H^+ \rightarrow {}^+$HOO-Mb(II)$\rightarrow$ Met(III) Mb+$HO_2^{\bullet}$) (Wang, He, Emara, Gan, & Li, 2019).

In general, Met(III)Mb can be reduced back to a ferrous myoglobin state in fresh meat because of the activity of the Met(III)Mb-reducing enzyme (MRE) system (Wang *et al*., 2018). However, MRE activity can decrease when meat is subjected to regular aging or freezing. In cooled PSE meat, the relatively low pH may reduce the activity of this system, increasing the content of metmyoglobin in the meat. In addition, early acid denaturation of sarcoplasmic proteins facilitates the oxidative change from oxymyoglobin into metmyoglobin.

Higher levels of metmyoglobin were observed in PSE meat than in control meat after cooking at 80°C for 30 min, exposure to light and reheating on the first day of storage (Fig. 1). The

stabilization of the levels of metmyoglobin in the cooked PSE meat and the control between Days 3 and 5 was consistent with the lack of change in heme iron levels.

The greater oxidation of myoglobin in cooked PSE meat than in cooked control meat was influenced by physicochemical changes induced by temperature during cooking, such as disruption of cell membranes and denaturation, which increased the release of heme iron (Fig 2) and probably increased the nonheme iron content in meat, and by the catalytic effect of light, which generates reactive substances such as superoxide anions that may undergo nonenzymatic disproportionation through the conjugate acid ($HO_2^{\bullet}$), producing hydrogen peroxide (H_2O_2). Both nonheme iron and metmyoglobin in the presence of hydrogen peroxide produced free radicals that enhanced oxidative reactions during refrigerated storage (Estevez & Cava, 2004; Carlsen, Moller, & Skibsted, 2005).

However, the high levels of metmyoglobin (Fig. 1) and TBARS (Table 2) observed in the PSE chicken meat after cooking indicate that lipid radicals likely oxidized myoglobin, and this oxidation of myoglobin produced free radicals that attacked unsaturated lipids. There is evidence that the interaction of metmyoglobin with hydrogen peroxide (metmyoglobin-mediated oxidation systems – (MetMb/H_2O_2)) or lipid hydroperoxides (LOOH) results in the formation of hypervalent species such as ferrylmyoglobin (Mb(IV)=O) (Estevez, 2011). Hypervalent myoglobin species have been highlighted as powerful contributors to lipid and protein oxidation in meat. Mb(IV)=O is able to abstract a hydrogen atom from lipid and protein molecules (Mb(IV)=O + LH→ Mb(IV)= O + $L^{\bullet}$ + H^{+}), initiating radical chain reactions of these components. Furthermore, ferrylmyoglobin and metmyoglobin can degrade LOOH into free radicals such as peroxyl and alkoxyl radicals, which can initiate or catalyze a series of propagation and termination steps of lipid and protein oxidation (Met (III)Mb + LOOH → Mb(IV)=O + $LO^{\bullet}$ + H^{+}; Mb(IV)=O + LOOH + H^{+} → Met (III)Mb + $LOO^{\bullet}$ + H_2O; $LOO^{\bullet}$ + PH → $P^{\bullet}$ + LOOH; $P^{\bullet}$ + $P^{\bullet}$; P-P) (Carlsen, Jensen, & Skibsted, 2005; Wang, He, Emara, Gan, & Li, 2019). The reaction between peroxyl radicals and proteins promotes the formation of protein radicals that react other protein radicals, resulting in cross-linking and decreasing protein solubility (Xiong., *et al*, 2020).

3.3 Heme iron

In Figure 2, the heme iron contents of the cooled and cooked PSE samples and their respective controls are shown. There was a greater depletion of heme iron in both cooked and cooled PSE samples until the third day of storage.

During refrigerated storage or cooking of meat, the porphyrin ring of myoglobin may break down, releasing iron from the globin structure. However, the greater denaturation and oxidative

induction by heat in cooked PSE meat led to unfolding of the protein and exposure of heme iron, thereby affecting the redox stability of myoglobin (Estevez & Cava, 2004).

Transition metals can react with H_2O_2 to generate a reactive intermediate (hydroxyl radical; $HO^{\bullet}$) via the Fenton reaction (Estevez, 2011). Hydroxyl radicals are the most reactive oxygen radicals and are very reactive toward lipids and proteins in meat. The oxidation-promoting effect of transition metals in meat muscle can be termed a metal-catalyzed oxidation system. In general, the role of transition metals in generating free radicals *in vivo* is insignificant since the metals are bound to proteins and have limited availability for participation in the Fenton reaction (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Thus, it is plausible that the release of nonheme iron resulting from myoglobin degradation promotes the Fenton reaction (Wang, He, Emara, Gan, & Li, 2019).

Heme iron can promote oxidation via different mechanisms, involving Fenton-like iron(III), iron(IV) and iron(II), or iron(IV) species (Carlsen, Moller, & Skibsted, 2005). Under such conditions, the side chains of the susceptible amino acids can be oxidatively deaminated by heme iron. The reactive species attack the amino group from the amino acid side chain by abstracting a hydrogen atom from the neighboring carbon, leading to the formation of a carbon-centered protein radical. In a further step, oxidized forms of heme iron accept the lone electron of the carbon radical to form an imino group, which is spontaneously hydrolyzed to yield the corresponding aldehyde moiety (Stadman, 1990; Carlsen, Jens & Skibsted, 2005; Estevez, 2011; Soladoye, Juárez, Aalhus, Shand & Estévez, 2015). Utrera, Parra, & Estevez (2014) found a significant positive correlation between heme iron and TBARS contents ($r=0.79$; $p<0.01$), indicating that heme iron in conjunction with FAs may play a greater role in meat instability to lipid oxidation. The combination of these endogenous factors in PSE chicken meat enhanced the susceptibility of the meat to lipid oxidation. On the other hand, the microstructural differences in the muscle membranes of PSE chicken meat, such as extracellular enlargement of endomysium and perimysium sheaths, could facilitate the accessibility of phospholipids for myoglobin, which may be more important than unsaturation in the role of myoglobin as a catalyst for lipid oxidation (Guarnieri., *et al*, 2004; Wu, Xiao, Yin, Zhang & Richards, 2021).

3.4 TBARS, carbonyls and FA profile (under cooled conditions)

Table 2 and Table 3 show the TBARS content and the FA profiles, respectively, found in cooled and cooked PSE meat and the control samples at 4°C for 5 days.

As shown in Table 2, there was a greater increase in TBARS levels in the cooled PSE samples than in the control samples. These results indicate the oxidative degradation of unsaturated

lipids during storage. However, the relatively low TBARS values found during the whole storage period together with the loss of free thiol groups suggest that malondialdehyde (MDA, one of the most abundant secondary products of lipid oxidation found in meat) (Estevez, 2015) reacted with these amino acids, reducing the detection threshold of reactive compounds and making it difficult to determine the level of oxidation by the method employed. MDA is an electrophile that can react easily with protein nucleophiles. The cysteine side chain in proteins is the most reactive nucleophile due to the presence of a thiol moiety (Wang, He, Emara, Gan, & Li, 2019). The TBARS results for the PSE samples after cooking at 80°C for 30 min, exposure to fluorescent light and reheating are discussed in section 3.2 and section 3.5.

As detailed in Table 3, there was a significant difference ($p<0.05$) in total n-6 FAs and a higher arachidonic acid production in cooled PSE samples than in the control samples. The higher arachidonic acid content found in PSE samples than in control samples is in agreement with observations reported in our previous studies (Soares., *et al* 2009). In PSE chicken meat, the production of n-6 FAs suggests that the enhanced action of free radicals and lipid peroxidation contributed to the imbalance in calcium homeostasis, increased activity of phospholipase A_2, metabolism and/or oxidation of arachidonic acid. In fact, our studies confirm the pivotal role of arachidonic acid as a substrate for triggering lipid oxidation in PSE chicken meat (Soares., *et al*, 2003; Soares., *et al,* 2009).

As shown in Table 4, there was a greater increase in protein carbonyl levels during storage in the cooled PSE samples than in the cooled control samples. These results were influenced by several pro-oxidant agents, such as pH, protein denaturation, and myoglobin, and by the reduced activity of endogenous antioxidant enzymes. Among these factors, the low pH played an essential role in protein oxidation in the cooled PSE through different pathways: the low pH provided a reducing environment that facilitated the oxidation of myoglobin and the production of free radicals through Fenton reactions; promoted denaturation with consequent unfolding and exposure of highly reactive amino acids on the surface of proteins such as thiol, basic and aromatic groups that may react quickly with free radicals or reactive substances being converted into carbonyl compounds; and decreased endogenous antioxidant enzyme (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px)) activity, which increased the susceptibility of the PSE muscle to postmortem oxidation (Carvalho., *et al*, 2017).

Furthermore, the high activity of proteolytic enzymes (calpains, cathepsins) in PSE chicken meat contributed to the breakdown of the protein structure into smaller and more reactive compounds, such as peptides and free amino acids, as well as the production of new $-NH_2$ groups

(Wilhelm, Maganhini, Hernandez-Blazquez, Ida, & Shimokomaki, 2010; Chen., *et al*, 2016). The combination of these physical, chemical and structural changes promoted reactions that led to protein carbonylation in the cooled PSE meat.

Based on the results obtained in this study, we proposed an interrelationship between myoglobin, lipids and protein oxidation in cooled PSE meat and hypothesized that these processes may follow a sequence of reactions: a) in post-mortem meat, the synergism between the low pH and denaturation of sarcoplasmic proteins enhanced the change in redox status of myoglobin in cooled PSE chicken meat. Thus, the Fe^{+2} ion was oxidized to its more reactive Fe^{+3} form (Oxy(II)Mb oxidation to Met(III)Mb); b) the release of heme iron from the myoglobin molecule and the increase in nonheme iron in the intracellular environment led to the reaction of the latter with endogenous pro-oxidants (metal-catalyzed oxidation systems), producing free radicals such as hydroxyl radicals (non heme-Fe(III) + $H_2O_2 \rightarrow OH^{\bullet}$ + heme-Fe(II) + OH^-); c) the hydroxyl radical reacted with arachidonic acid (as a result of the relatively low energy load and fast oxidation of unsaturated lipids) released by the enzyme phospholipase A_2 (Soares *et al.*, 2009). Arachidonic acid, under the action of lipoxygenases and cyclooxygenases, was converted to hydroperoxide (primary product of lipid oxidation). This hydroperoxide reacted with hydroxyl radicals, producing lipid radicals (such as peroxyl radicals) that propagated lipid oxidation ($C_{20}H_{32}O_2 \xrightarrow{\text{lipoxygenase}} LOOH$; $LOOH + OH^{\bullet} \rightarrow ROO^{\bullet} + H_2$; $ROO^{\bullet} + RH \rightarrow R^{\bullet} + ROOH$; $R^{\bullet} + R^{\bullet} \rightarrow R\text{-}R$); d) the peroxides formed during the propagation of lipid oxidation underwent cleavage, producing lower-molecular-weight volatile and nonvolatile compounds (secondary products) such as aldehydes (hexanal, MDA and 4-hydroxy-2-trans-nonenal (HNE)) (Estevez, 2015); e) $LOOH \xrightarrow{\text{scission}} C_3H_4O_2$; and f) carbonylation is an irreversible and nonenzymatic modification of proteins that can occur through different pathways, such as the oxidative deamination of alkaline amino acids (mainly lysine, arginine and proline) via Fenton reactions, oxidative cleavage of the peptide backbone via the α-amidation or oxidation of glutamyl side chains, covalent binding to nonprotein carbonyl compounds such as HNE or MDA, and dicarbonyl formation from Maillard degradation of sugars such as glucose and ribose that can induce the oxidative deamination of amino group residues in proteins, leading to the formation of α-aminoadipic semialdehyde (AAS) and γ-glutamic semialdehyde (GGS) (Estevez, 2011; Utrera; Parra & Estevez, 2014). Our results indicate that thiol groups react with MDA with the loss of SH groups and undergo further hydrolysis to form a protein carbonyl compound. Both lipid and protein processes terminate when the formed radicals react with each other ($C_3H_4O_2 + C_3H_7NO_2S \xrightarrow{(-H_2O)}$ protein carbonyl).

3.5 TBARS and carbonyl levels (under cooked conditions)

Table 2 and Table 4 show the TBARS and carbonyl levels in the PSE and control samples after cooking at 80°C, exposure to fluorescent light for 4 h and reheating in a microwave for 4 min.

In Table 4, higher levels of protein carbonyls were found in PSE meat than in control meat after cooking at 80°C for 30 min, exposure to light and reheating on the first day of storage.

Cooking accelerates free radical generation, thus increasing the oxidation of proteins in meat. Several changes resulting from protein oxidation during cooking include increased surface hydrophobicity and protein aggregation, thiol group loss, increased carbonylation, Schiff base formation, aromatic group oxidation, carboxylation, bityrosine formation, disulfide bond formation, cross-linking, impaired digestibility and hardness modifications in meat (Ferreira, Morcuende, Madruga, Silva & Estevez, 2018).

The increase in protein carbonylation levels in both cooked PSE and control samples was also influenced by the different actions of oxidative inducers (heat + light + reheating) in meat. With cooking, there was an enhancement in the exposure of reactive groups of proteins, increasing the accessibility between amino acids and pro-oxidants, mainly heme iron (Utrera, Parra, & Estevez, 2014), whereas during refrigeration, photooxidation promoted by light may have accelerated the formation of peroxides that propagated lipid and protein oxidation when reacting with free radicals; the catalytic effect of light along with dehydration caused by cooking probably increased the collision between reactants, leading to an increase in TBARS and carbonyl levels during refrigerated storage. However, the cooked PSE samples showed the highest protein and lipid oxidation.

The high levels of TBARS and carbonyls found in cooked PSE meat suggest the existence of an interrelationship between these processes. In fact, a positive correlation ($r = 0.96369$, $p < 0.001$) between TBARS content (Table 2) and carbonyl compound content (Table 4) was found in the cooked PSE samples.

In meat systems, oxidation can be considered a cascade reaction, and there may be a transfer of oxidative processes between the myoglobin, lipid and protein fractions. In a principal component analysis, Wang, He, Gan & Li (2018) observed good correlation between TBARS, carbonyl protein and metmyoglobin contents in rabbit meat during refrigerated and super chilled storage. The authors showed that myoglobin, lipid and protein oxidation occurred concurrently in rabbit meat during both storage treatments, and each oxidation process seemed to promote the others. Thus, in the cooked PSE samples, as a result of the reactions between metmyoglobin and its hypervalent forms

with hydroperoxides (from heat cleavage and the catalytic effect of light), lipid radicals were produced, which promoted lipid and protein oxidation. The resulting peroxyl radicals could react with unsaturated lipids, generating peroxides and lipid radicals. The latter can be converted back to react with oxygen, thus promoting lipid oxidation. Hydroperoxides can undergo cleavage into various secondary compounds, such as aldehydes, alcohols, furans, and hydrocarbons, increasing the TBARS levels in cooked PSE meat. Similar to the metal-catalyzed oxidation system, lipid radicals can abstract a hydrogen atom from (C-H or S-H) bonds, thereby resulting in the formation of carbon-centered radicals (Davies, 2016). Carbon-centered radicals generally react with O_2 to yield peroxyl radicals ($H_3C^{\bullet} + O_2 \rightarrow POO^{\bullet}$) (Soladoye, Juarez, Aalhus, Shand, & Estevez, 2015). Peroxyl radicals that contain α-heteroatoms (α-hydroxyl or α-amino groups) can undergo rapid unimolecular elimination of $HOO^{\bullet}/O_2^{\bullet -}$ to generate protein carbonyl compounds (Davies, 2016), thus increasing the occurrence of carbonylation in cooked PSE. However, on Days 3 and 5 of storage, there was a decrease in TBARS and carbonyl levels. These results indicate that oxidative induction by light played a secondary role in triggering oxidative reactions in cooked PSE meat because even with induction on Days 3 and 5, the TBARS and carbonyl levels did not differ significantly from those in the control. This result agrees with that of Zainudin, Poojary, Jonberg, & Lund (2019). The authors showed that light influenced protein carbonylation under high-oxygen conditions. In contrast, the stabilization during the formation of metmyoglobin along with the decrease in TBARS and carbonyl levels provided evidence of the essential role of metmyoglobin-mediated oxidation systems, such as a catalyst of lipid and protein oxidation in cooked PSE meat. On the other hand, the products of lipid and protein oxidation may react with each other, MDA may interact with amino groups and thiol, or the aldehyde moiety from specific protein carbonyls may be involved in several reactions, including oxidative degradation by which an aldehyde moiety is oxidized to form carboxylic acid and reacts with an aldehyde moiety from another protein-bound carbonyl residue to form an aldol condensation product or with an α-amino group from a free amino acid to form a Strecker aldehyde via Strecker-type degradation (oxidative deamination and decarboxylation of the amino acid in the presence of a carbonyl compound), thus decreasing the TBARS and carbonyl levels in cooked PSE meat (Estevez, 2011).

In addition, under intense oxidative conditions, the ongoing oxidation of AAS leads to the formation of the stable end-product α-aminoadipic acid (AAA) (Ferreira, Morcuende, Madruga, Silva, & Estevez, 2018).

3.6 Thiol groups

The free thiol group contents of the cooled and cooked PSE samples and their respective controls are shown in Figure 3.

There was a greater loss of thiol groups in the cooled PSE until the third day of storage. In the cooled PSE samples, the exposure of sulfhydryls that are normally buried inside of protein due to the relatively high surface hydrophobicity (Chen *et al*, 2016) facilitated reactions with free radicals and the formation of disulfide bonds (Carvalho *et al*, 2017) and with other sulfur derivatives such as sulfones and sulfoxides, as well as the interactions with MDA, by decreasing the content of free thiol groups during storage.

In the cooked PSE samples, reheating promoted the breakdown of disulfide bonds, thus increasing the content of free thiol groups that were oxidized by free radicals such as peroxyl radicals and hydroxyl radicals and increasing the levels of carbonyl compounds in the meat. At the same time, the formation of disulfide bridges and the reactions of thiol groups with other compounds, such as MDA, influenced the compromising of free thiol groups, and no increase was observed on Days 3 and 5 of storage. (Ferreira, Morcuende, Madruga, Silva, & Estevez, 2018).

The greater loss of thiol groups in the cooked PSE samples than in the control samples and the consistent formation of TBARS support our hypothesis that the thiol groups reacted with aldehydes resulting from lipid oxidation, such as MDA.

Protein thiols, in addition to being essential to the human diet, are also involved as cofactors in several metabolic reactions (Estevez, Geraert, Liu, Delgado, Mercier & Zhang, 2020), so their decreased content in the PSE samples reduced the nutritional value of the meat.

4. Conclusion

The results of this study indicate that myoglobin, lipid and protein oxidation occurred concomitantly in both cooked and cooled PSE chicken meat during storage, and each type of oxidation seemed to promote the others.

The metal-catalyzed oxidation systems and metmyoglobin-mediated oxidation systems played a pivotal role in the generation of free radicals that triggered the propagation and final termination steps of lipid and protein oxidation in cooled and cooked PSE meat, respectively.

In the cooked PSE samples, the decrease in TBARS and carbonyl levels on Days 3 and 5 during storage provided evidence of the secondary role of light as an oxidative inducer of these processes. Moreover, other compounds may have been generated by the reaction between the products of lipid and protein oxidation with themselves and the formation of specific carbonyls.

Different pathways triggered the production of compounds resulting from the interactions between myoglobin, lipid and protein oxidation in the cooled and cooked PSE samples. The impact of these compounds on the functionality of PSE chicken meat needs to be further studied.

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Table 1. pH, L, a/b, WHC and DP values in the control, cooled PSE and cooked PSE chicken breast

	C1	Cooled PSE	C2	Cooked PSE
$pH_{4\,h}$	6.54^{a} ± 0.02	5.79^{b} ± 0.015		
$pH_{24\,h}$	5.99^{a} ± 0.05	5.77^{b} ± 0.017		
L	54.67^{a} ± 2.52	58.50^{b} ± 2.16		
a/b	0.19^{a} ± 0.014	0.16^{b} ± 0.009		
WHC	68.54^{a} ± 2.25	63.75^{b} ± 1.78		
DP	36.27^{a} ± 2.74	43.62^{b} ± 2.51	51.57^{c} ± 2.62	60.75^{d} ± 2.69

Values with different letters in the same row differ significantly ($p<0.05$).

Values are expressed as the mean ± standard deviation of results obtained in triplicate.

C1: cooled control;

C2: cooked control. WHC (%): water holding capacity. DP (%): protein denaturation .

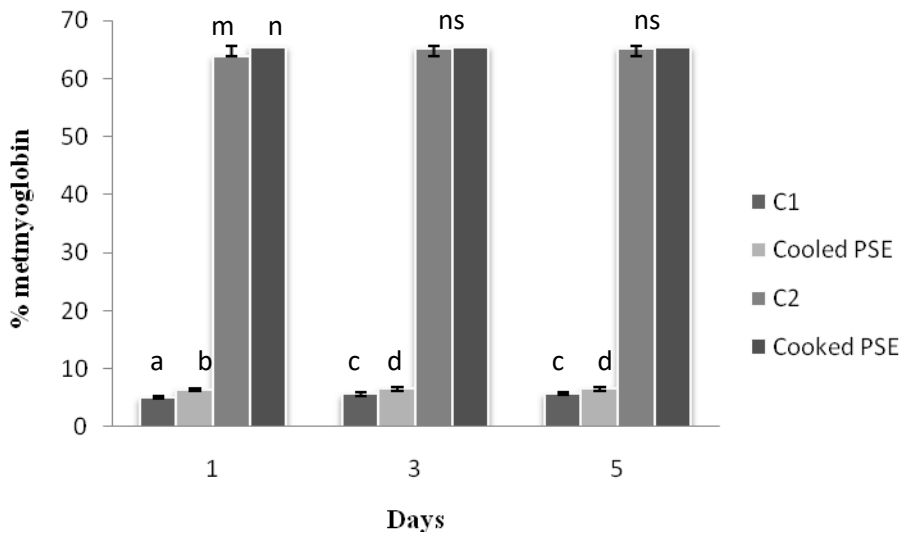

Fig. 1 Metmyoglobin (%) for PSE and their respective controls.

Bars with different letters are significantly different ($p < 0.05$).

Values are expressed as the mean of results obtained in triplicate.

ns: no significant difference

C1: cooled control; C2: cooked control.

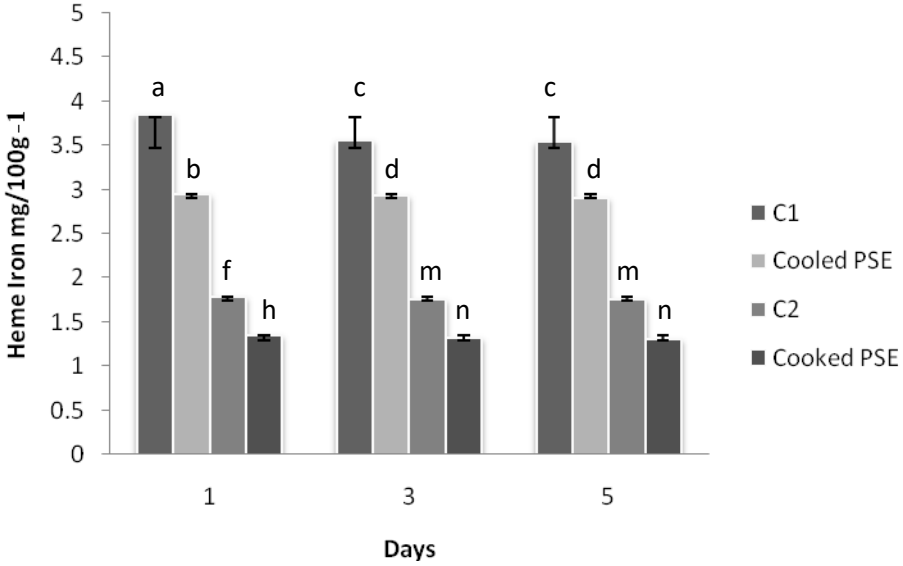

Fig. 2 Heme iron (mg/100 g^{-1}) for PSE meat and the respective controls.

Bars with different letters are significantly different ($p < 0.05$).

Values are expressed as the mean of results obtained in triplicate.

C1: cooled control; C2: cooked control.

Table 2. TBARS (mg/kg) for PSE meat and the respective controls

Days	C1	Cooled PSE	C2	Cooked PSE
1	*$0.042^{B,a}$ ± 0.0025	*$0.057^{A,a}$ ± 0.012	**$6.54^{M,n}$ ± 0.125	**$6.75^{N,n}$ ± 0.119
3	**$0.045^{B,b}$ ± 0.0043	**$0.065^{A,b}$ ± 0.023	$2.75^{M,m}$ ± 0.107	$2.77^{M,m}$ ± 0.110
5	**$0.045^{B,b}$ ± 0.0046	**$0.065^{A,b}$ ± 0.025	$2.67^{M,m}$ ± 0.129	$2.72^{M,m}$ ± 0.134

(a, b, m, n) Within the same columns, means marked with different superscript letters are significantly different. (A, B, M, N) Within the same row, means marked with different superscript letters are significantly different. *($p<0.01$). **($p<0.05$). Values are the means of results obtained in triplicate.

C1: cooled control; C2: cooked control.

Table 3. FA profile of control and cooled PSE samples on the third day of storage in the dark at 4°C

	Control	Cooled PSE
Myristic acid (14:0)	$0.44^{a} \pm 0.031$	$0.36^{a} \pm 0.044$
Palmitic acid (16:0)	$20.91^{a} \pm 0.121$	$20.62^{a} \pm 0.109$
Palmitoleic acid (16:1n-7)	$4.43^{a} \pm 0.340$	$3.12^{b} \pm 0.127$
Stearic acid (18:0)	$5.68^{a} \pm 0.157$	$8.82^{b} \pm 0.186$
Oleic acid (18:1n-9)	$34.96^{a} \pm 0.101$	$30.44^{b} \pm 0.094$
Vaccenic acid (18:1n-7)	$1.91^{a} \pm 0.133$	$2.04^{a} \pm 0.064$
Linoleic acid (18:2n-6)	$27.47^{a} \pm 0.574$	$26.90^{a} \pm 0.506$
Alpha-linolenic acid (18:3n-3)	$1.89^{a} \pm 0.083$	$1.47^{b} \pm 0.030$
Gamma-linolenic acid (18:3n-6)	$0.16^{a} \pm 0.11$	$0.16^{a} \pm 0.12$
Arachidic acid (20:0)	$0.28^{a} \pm 0.034$	$0.45^{a} \pm 0.021$
Arachidonic acid (20:4n-6)	$1.15^{a} \pm 0.154$	$2.57^{b} \pm 0.252$
Behenic acid (22:0)	$0.26^{a} \pm 0.026$	$0.40^{a} \pm 0.056$
Lignoceric acid (24:0)	$0.30^{a} \pm 0.017$	$1.33^{b} \pm 0.050$
Nervonic acid (24:1n-9)	$0.32^{a} \pm 0.015$	$0.46^{a} \pm 0.021$
*SFAs	$27.87^{a} \pm 2.45$	$31.98^{a} \pm 2.77$
*MUFAs	$41.62^{a} \pm 1.57$	$36.06^{b} \pm 1.09$
*PUFAs	$30.51^{a} \pm 0.42$	$31.96^{b} \pm 0.63$

Means within rows marked by different superscript letters are significantly different ($p<0.05$). The values are expressed as the mean ± standard deviation of results obtained in duplicate.

*SFAs - Saturated fatty acids = (14:0 +16:0 +18:0 + 20:0 + 22:0 + 24:0)

*MUFAs - Monounsaturated fatty acids = (16:1n-7 + 18:1n-9 + 18:1n-7 + 24:1n-9)

*PUFAs – Polyunsaturated fatty acids (18:2n-6 + 18:3n-3 + 18:3n-6 + 20:4n-6)

Table 4. Carbonyl (nmol/mg) for PSE meat and the respective controls

Days	C1	Cooled PSE	C2	Cooked PSE
1	*$0.36^{B,a}$ ± 0.034	*$0.54^{A,a}$ ± 0.037	*$6.48^{M,n}$ ± 0.021	*$7.55^{N,n}$ ± 0.043
3	**$0.40^{B,b}$ ± 0.042	**$0.60^{A,b}$ ± 0.072	$3.80^{M,m}$ ± 0.045	$4.02^{M,m}$ ± 0.052
5	**$0.40^{B,b}$ ± 0.045	**$0.60^{A,b}$ ± 0.069	$2.58^{M,m}$ ± 0.037	$2.65^{M,m}$ ±0.041

(a, b, m, n) Within the same columns, means marked with different superscript letters are significantly different. (A, B, M, N) Within the same row, means marked with different superscript letters are significantly different. *($p<0.01$). **($p<0.05$). Values are the means of results obtained in quadruplicates.

C1: cooled control; C2: cooked control.

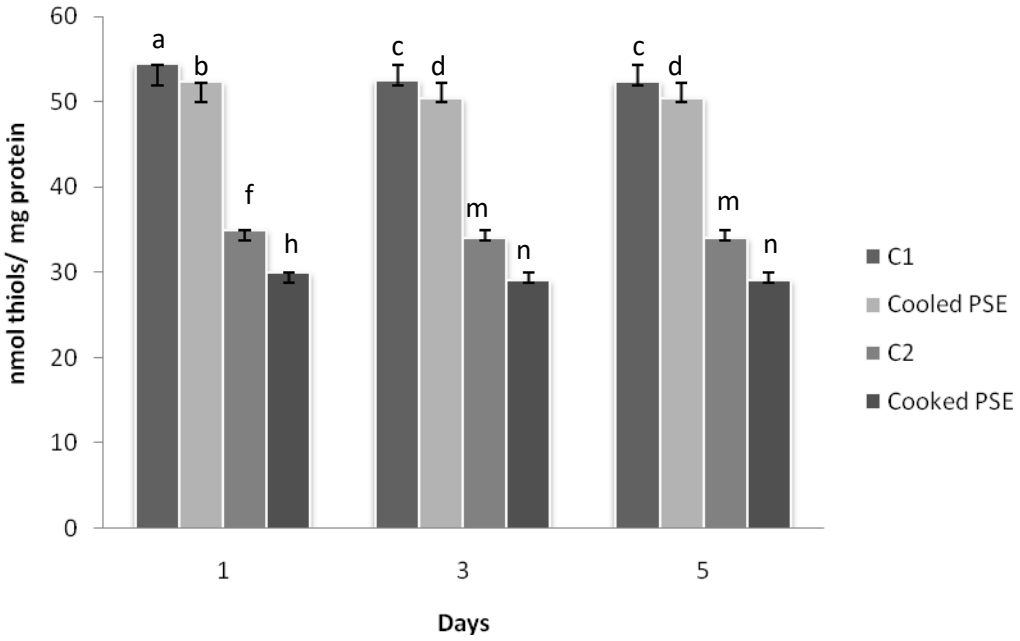

Fig. 3 Thiol groups (nmol thiols/mg protein) for PSE meat and the respective controls.

Bars with different letters are significantly different ($p < 0.05$).

Values are expressed as the means of results obtained in triplicate. C1: cooled control; C2: cooked control.