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

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## 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.

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34

35 1. Introduction

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

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

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

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

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

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

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

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

86

## 87 2. Materials and methods

### 88 2.1 Chemicals

89 1,1,1,3-Tetraethoxypropane (TEP), 2,4-dinitrophenylhydrazine (DNPH), bovine serum albumin  
90 (BSA), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), L-cysteine and sodium dodecyl sulfate (SDS)  
91 were purchased from Sigma Chemical Co. (St. Louis, MO, USA).  $Na_4P_2O_7$ , Tris-maleate, KCl,  
92  $MgCl_2$ , and ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA) were  
93 purchased from Merck (Merck, Darmstadt, Germany). All aqueous solutions were prepared using a  
94 Milli-Q system. All other chemicals were of analytical or better grade.

95

### 96 2.2 Meat samples

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

101 refrigeration, as described in Wilhelm, Maganhini, Hernandez-Blazquez, Ida & Shimokomaki  
102 (2010). After the analyses in a commercial plant, the chicken meat samples were packed in  
103 transparent, flexible, oxygen-permeable polyethylene bags that were layered in thermal boxes with  
104 ice and cooling gels around the meat, and the samples were then sent to a university for further  
105 analysis. The breast fillets previously classified as control and PSE meat were stored at 4°C in the  
106 dark under low oxygen pressure for 5 days in a cool chamber inside a scientific laboratory at the  
107 university. On Days 1, 3 and 5, these samples were analyzed under cooled and cooked conditions.  
108 The cooled PSE meat remained in the cold chamber, while some of the samples were separated on  
109 each day of analysis for thermal treatment. For thermal treatment, the samples were vacuum packed  
110 and kept in a water bath at 80°C for 30 min. Then, the vacuum packaging was removed from the  
111 cooked samples, and the samples were repacked with the same packaging used for the cooled  
112 samples and stored at 4°C in an isolated section within the cool chamber, where they were exposed  
113 to fluorescent lighting for 4 h. The samples were then reheated in an 800-Watt microwave for 4  
114 min. Last, the samples were cooled to room temperature as described by Byrne, Bredie, Moltram &  
115 Martens (2002) with modifications.

116

### 117 2.3 pH and color analysis

118 pH and color determination was performed as described in Wilhelm, Maganhini, Hernandez-  
119 Blazquez, Ida & Shimokomaki (2010). Briefly, the pH was measured by inserting electrodes into  
120 the breast muscle using a contact pH meter system previously calibrated with buffer solutions (pH=  
121 4 and pH= 7). The pH analyses were performed in triplicate at 4 h and 24 h post-mortem. The pH of  
122 the control and PSE samples was measured at 4 h. The pH at 24 h post-mortem was measured as  
123 described by Olivo, Soares, Ida & Shimokomaki (2001). A Minolta CR400 colorimeter (BYK -  
124 Gardner GmbH; color-guide 45°/0°, 11 mm; light source: D65) was used to evaluate the color,  
125 lightness (L\*), redness (a\*), and yellowness (b\*) of the posterior surface of the intact skinless breast  
126 muscles at 24 h post-mortem. The color parameters were measured at three different points on the  
127 same sample. The final color values are expressed as the means of triplicate values. Seventy-six  
128 samples were tested and identified by coding. Fourteen samples with values of L\*<sub>24 h</sub> < 53.0 and  
129 pH > 5.80 were classified as controls, and fourteen other samples with values of L\*<sub>24 h</sub> ≥ 53.0 and  
130 pH ≤ 5.80 were classified as PSE meat according to (Barbut, 1993; Soares, Lara, Ida, Guarnieri &  
131 Shimomkomaki, 2002).

132

133

134

### 135 2.3 Protein denaturation

136 The degree of protein denaturation in the control and PSE samples was measured 48 h  
137 postmortem as described by Swatland (1995). Five grams of sample (in triplicate) was homogenized  
138 with 15 mL of distilled water using a Turrax (1000 g) for 15 s. The homogenate was centrifuged at  
139 3500g at 2°C for 10 min and filtered through filter paper. One milliliter of the filtrate was  
140 dispensed in a test tube, and 5 mL of citrate/phosphate buffer (0.2 M) was added (pH=4.6). The  
141 mixture was incubated in a thermostatted bath at 20°C for 30 min along with a blank sample. A  
142 blank was prepared for each treatment and consisted of 1 mL of filtrate and 5 mL of distilled water.  
143 The transmittance of the samples was measured spectrophotometrically at 600 nm. The results are  
144 expressed as the measure of transmittance by subtracting the value of the blank.

### 145 2.4 Water holding capacity (WHC)

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

- 151 1.  $100 - [(P_i - P_f/P_i) \times 100]$ , where  $P_i$  and  $P_f$  are the initial and final sample weights,  
152 respectively.

### 154 2.5 Lipid oxidation

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

### 166 2.6 Protein carbonyl analysis

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

169 (1999). Three grams of sample (in quadruplicate) was solubilized in 25 mL of buffer (pH=7.4)  
170 containing  $\text{Na}_4\text{P}_2\text{O}_7$  (2.0 mM), Tris-maleate (10 mM), KCl (100 mM),  $\text{MgCl}_2$  (2.0 mM) and EGTA  
171 (2.0 mM). The mixture was homogenized with a Turrax (twice at  $8000 \times g$  and  $9500 \times g$  for 15 s).  
172 The homogenate was centrifuged at  $8000 \times g$  for 20 min at  $4^\circ\text{C}$ . The supernatant was clarified by  
173 centrifugation at  $38720 \times g$  for 30 min at  $4^\circ\text{C}$ . An aliquot of 1 mL of the filtrate was precipitated  
174 with 1 mL of HCl/acetone (3:1). The mixture was centrifuged at  $8000 \times g$  for 20 min at  $4^\circ\text{C}$ . The  
175 resulting pellet was treated with 1 mL of 10% TCA and centrifuged at  $8000 \times g$  for 20 min at  $4^\circ\text{C}$ .  
176 The pellet was resuspended in 1 mL of buffer and 1 mL of DNPH solution. A blank was prepared  
177 with 1 mL of buffer and 1 mL of 2 N HCl. The mixture (or blank) was incubated with agitation (in  
178 the dark) for 30 min at  $4^\circ\text{C}$ . One milliliter of 30% TCA was added, and the pellet was incubated in  
179 an ice bath for 10 min. This mixture was centrifuged at  $8000 \times g$  for 20 min at  $4^\circ\text{C}$ . The resulting  
180 pellet was washed twice with 1 mL of ethanol/ethyl acetate (1:1) in 10 mM HCl and centrifuged at  
181  $8000 \times g$  for 20 min at  $4^\circ\text{C}$  for the removal of DNPH. The pellet was then solubilized in 1 mL of 6  
182 M guanidine solution in monobasic sodium phosphate buffer. The resulting mixture was centrifuged  
183 at  $8000 \times g$  for 2 min at  $4^\circ\text{C}$ . The carbonyl content was determined by analyzing the supernatant  
184 spectrophotometrically at 370 nm using a molar absorbance coefficient of  $22.000 \text{ M}^{-1}\text{cm}^{-1}$ . The  
185 protein concentration was determined spectrophotometrically at 280 nm using bovine serum  
186 albumin (BSA) in 6 M guanidine as the standard. The results are expressed in nmol carbonyls/mg  
187 protein.

## 188 2.7 Metmyoglobin analysis

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

$$196$$
$$197 \quad 2. \text{ MetMb} = \{ 1.395 - [(\text{Abs}_{572} - \text{Abs}_{700})/(\text{Abs}_{525} - \text{Abs}_{700})] \} \times 100$$

198 where  $\text{Abs}_{525}$  is the absorbance of the supernatant at 525 nm,  $\text{Abs}_{572}$  is the absorbance of the  
199 supernatant at 572 nm, and  $\text{Abs}_{700}$  is the absorbance of the supernatant at 700 nm.

200

201



## 202 2.8 Heme iron analysis

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

$$210 \quad 3. \text{ Hematin} = \text{Abs}_{640} * 680 * P(\text{sample})$$

211

$$212 \quad 4. \text{ Heme iron} = \frac{\text{Hematin} * 88.2}{P(\text{sample}) * 10000}$$

213

214  
215 Abs = absorbance (nm)

216 P = weight (mg).

217

## 218 2.9 Free thiol group analysis

219 For the determination of free thiol groups in proteins, DTNB was used as described by  
220 Ellman (1959). Two grams of sample (in triplicate) was homogenized with 50 mL of 5% sodium  
221 dodecyl sulfate (SDS) in 0.10 M Tris buffer (pH=8.0) in a Turrax at  $13500 \times g$  for 30 s. The  
222 homogenate was incubated in a water bath at 80°C for 30 min and then cooled and centrifuged at  
223  $1200 \times g$  for 20 min. The supernatant was filtered with Whatman No. 1 filter paper. Two milliliters  
224 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  
225 0.5 mL of the filtrate. The absorbance at 412 nm was determined spectrophotometrically after 30  
226 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  
227 mM DTNB in 0.10 M Tris buffer (pH=8.0). The data obtained were plotted on a standard curve of  
228 L-cysteine according to the method of Stapelfeldt, Bjerrum & Skibsted (1997). The protein  
229 concentration was determined spectrophotometrically at 280 nm using BSA as a standard. The  
230 results are expressed in nmol/mg of protein.

231

## 232 2.10 Fatty acid analysis

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

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

246

## 247 2.11 Statistical analysis

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

253

## 254 3. Results and discussion

### 255 3.1 pH, color, WHC, and protein denaturation

256 Table 1 shows the pH, color, WHC and protein denaturation values obtained in this study. A  
257 consistent difference in pH between the PSE and control samples at 4 h post-mortem was found in  
258 this study, similar to the results of Lesiow & Xiong (2013). The pH continued to decrease with time  
259 and reached a minimum of 5.77 after 24 h. In contrast, the pH values of the control samples were  
260 6.54 and 5.99 after 4 h and 24 h, respectively. After slaughter, because of the bleeding and the  
261 interruption of oxygen supply, muscles begin using anaerobic metabolism to produce energy and  
262 maintain function as long as ATP is available. With the depletion of ATP reserves, muscles cannot  
263 relax, and permanent cross-links are formed between the myosin and actin filaments, leading to  
264 rigor mortis. In normal chicken meat, rigor mortis sets in after 1 h post-mortem, and the rate of pH  
265 drop varies between strains and individuals. In pos-tmortem PSE poultry, quantitative alteration in  
266 the isoforms of ryanodine receptors alters muscle calcium homeostasis. The increased calcium  
267 concentration in the intracellular medium increases the activity of calcium-dependent enzymes and  
268 accelerates glycolysis, which promotes a fast decline in pH while the muscle is still in rigor mortis  
269 and the carcass temperature remains high. Metabolic heat and the accumulation of lactic acid in the

270 muscles change the properties of the meat (Estevez, 2015). In this study, the faster decline in pH in  
271 the first hours post-mortem in PSE meat compared to controls led to the lower WHC of the meat,  
272 as well as, the higher light scatter, resulting in the pale color, as verified by the high L value (Table  
273 1).

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

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

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

313 There was a significant difference between the  $L^*$  values of the PSE and control samples. A  
314 high  $L^*$  value characterizes pale PSE meat (Barbut *et al*, 2008). A negative correlation ( $r = -$   
315  $0.20978$   $p < 0.001$ ) between  $pH_{24h}$  and  $L^*$  was found in this study. These results confirm the  
316 inverse relationship between pH and the  $L^*$  value in PSE chicken meat. The color of the meat  
317 surface results from the selective absorption of myoglobin, caused by the distribution of light that  
318 reflects off the meat, as well as important components, such as muscle fibers and their proteins, and  
319 is also influenced by the amount of free liquid present in the meat. In PSE meat, a low pH increases  
320 the birefringence, resulting in less light being transmitted and more light being reflected. Lateral  
321 refraction through myofibrils is a dominant mechanism of light scattering, and this can increase in  
322 meat with a low ultimate pH compared to normal meat because the closer packing of myofilaments  
323 at lower pH values increases the difference in the reflective index between the myofibril and its  
324 surroundings (Purslow., Warner., Clarke., & Hughes, 2020).

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

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

### 344 3.2 Metmyoglobin and TBARS (under cooked conditions)

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

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

355 Three forms of myoglobin, oxymyoglobin, deoxymyoglobin and metmyoglobin, coexist in meat  
356 systems. The predominance of each form depends on the redox status of iron. Deoxy(II)Mb and  
357 Oxy(II)Mb can be converted into the ferric state (Met(III)Mb) in meat. This process is known as  
358 myoglobin autooxidation. Formation of Met(III)Mb from Oxy(II)Mb is a proton-mediated reaction;  
359 protons enter the heme pocket and protonate ligated O<sub>2</sub>. The positive charge on O<sub>2</sub>, which results  
360 from protonation, causes single-electron removal from the iron atom of ferroprotoporphyrin IX to  
361 be energetically favorable. The neutral superoxide radical then dissociates, resulting in Met(III)Mb  
362 formation (Oxy(II)Mb + H<sup>+</sup> → <sup>+</sup>HOO-Mb(II) → Met(III) Mb + HO<sub>2</sub><sup>•</sup>) (Wang, He, Emara, Gan, & Li,  
363 2019).

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

370 Higher levels of metmyoglobin were observed in PSE meat than in control meat after cooking at  
371 80°C for 30 min, exposure to light and reheating on the first day of storage (Fig. 1). The

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

374 The greater oxidation of myoglobin in cooked PSE meat than in cooked control meat was  
375 influenced by physicochemical changes induced by temperature during cooking, such as disruption  
376 of cell membranes and denaturation, which increased the release of heme iron (Fig 2) and probably  
377 increased the nonheme iron content in meat, and by the catalytic effect of light, which generates  
378 reactive substances such as superoxide anions that may undergo nonenzymatic disproportionation  
379 through the conjugate acid ( $\text{HO}_2^*$ ), producing hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Both nonheme iron and  
380 metmyoglobin in the presence of hydrogen peroxide produced free radicals that enhanced oxidative  
381 reactions during refrigerated storage (Estevez & Cava, 2004; Carlsen, Moller, & Skibsted, 2005).

382 However, the high levels of metmyoglobin (Fig. 1) and TBARS (Table 2) observed in the  
383 PSE chicken meat after cooking indicate that lipid radicals likely oxidized myoglobin, and this  
384 oxidation of myoglobin produced free radicals that attacked unsaturated lipids. There is evidence  
385 that the interaction of metmyoglobin with hydrogen peroxide (metmyoglobin-mediated oxidation  
386 systems – ( $\text{MetMb}/\text{H}_2\text{O}_2$ )) or lipid hydroperoxides (LOOH) results in the formation of hypervalent  
387 species such as ferrylmyoglobin ( $\text{Mb(IV)=O}$ ) (Estevez, 2011). Hypervalent myoglobin species have  
388 been highlighted as powerful contributors to lipid and protein oxidation in meat.  $\text{Mb(IV)=O}$  is able  
389 to abstract a hydrogen atom from lipid and protein molecules ( $\text{Mb(IV)=O} + \text{LH} \rightarrow \text{Mb(IV)=O} + \text{L}^\bullet + \text{H}^+$ ),  
390 initiating radical chain reactions of these components. Furthermore, ferrylmyoglobin and  
391 metmyoglobin can degrade LOOH into free radicals such as peroxy and alkoxy radicals, which  
392 can initiate or catalyze a series of propagation and termination steps of lipid and protein oxidation  
393 ( $\text{Met(III)Mb} + \text{LOOH} \rightarrow \text{Mb(IV)=O} + \text{LO}^\bullet + \text{H}^+$ ;  $\text{Mb(IV)=O} + \text{LOOH} + \text{H}^+ \rightarrow \text{Met(III)Mb} +$   
394  $\text{LOO}^\bullet + \text{H}_2\text{O}$ ;  $\text{LOO}^\bullet + \text{PH} \rightarrow \text{P}^\bullet + \text{LOOH}$ ;  $\text{P}^\bullet + \text{P}^\bullet \rightarrow \text{P-P}$ ) (Carlsen, Jensen, & Skibsted, 2005; Wang,  
395 He, Emara, Gan, & Li, 2019). The reaction between peroxy radicals and proteins promotes the  
396 formation of protein radicals that react other protein radicals, resulting in cross-linking and  
397 decreasing protein solubility (Xiong., *et al.*, 2020).

### 398 3.3 Heme iron

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

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

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

406 Transition metals can react with H<sub>2</sub>O<sub>2</sub> to generate a reactive intermediate (hydroxyl radical;  
407 HO•) via the Fenton reaction (Estevez, 2011). Hydroxyl radicals are the most reactive oxygen  
408 radicals and are very reactive toward lipids and proteins in meat. The oxidation-promoting effect of  
409 transition metals in meat muscle can be termed a metal-catalyzed oxidation system. In general, the  
410 role of transition metals in generating free radicals *in vivo* is insignificant since the metals are bound  
411 to proteins and have limited availability for participation in the Fenton reaction (Bekhit, Hopkins,  
412 Fahri, & Ponnampalam, 2013). Thus, it is plausible that the release of nonheme iron resulting from  
413 myoglobin degradation promotes the Fenton reaction (Wang, He, Emará, Gan, & Li, 2019).

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

431  
432 3.4 TBARS, carbonyls and FA profile (under cooled conditions)

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

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

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

446 As detailed in Table 3, there was a significant difference ( $p < 0.05$ ) in total n-6 FAs and a  
447 higher arachidonic acid production in cooled PSE samples than in the control samples. The higher  
448 arachidonic acid content found in PSE samples than in control samples is in agreement with  
449 observations reported in our previous studies (Soares., *et al* 2009). In PSE chicken meat, the  
450 production of n-6 FAs suggests that the enhanced action of free radicals and lipid peroxidation  
451 contributed to the imbalance in calcium homeostasis, increased activity of phospholipase A<sub>2</sub>,  
452 metabolism and/or oxidation of arachidonic acid. In fact, our studies confirm the pivotal role of  
453 arachidonic acid as a substrate for triggering lipid oxidation in PSE chicken meat (Soares., *et al*,  
454 2003; Soares., *et al*, 2009).

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

467 Furthermore, the high activity of proteolytic enzymes (calpains, cathepsins) in PSE chicken  
468 meat contributed to the breakdown of the protein structure into smaller and more reactive  
469 compounds, such as peptides and free amino acids, as well as the production of new -NH<sub>2</sub> groups



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

473 Based on the results obtained in this study, we proposed an interrelationship between  
474 myoglobin, lipids and protein oxidation in cooled PSE meat and hypothesized that these processes  
475 may follow a sequence of reactions: a) in post-mortem meat, the synergism between the low pH and  
476 denaturation of sarcoplasmic proteins enhanced the change in redox status of myoglobin in cooled  
477 PSE chicken meat. Thus, the  $\text{Fe}^{+2}$  ion was oxidized to its more reactive  $\text{Fe}^{+3}$  form (Oxy(II)Mb  
478 oxidation to Met(III)Mb); b) the release of heme iron from the myoglobin molecule and the increase  
479 in nonheme iron in the intracellular environment led to the reaction of the latter with endogenous  
480 pro-oxidants (metal-catalyzed oxidation systems), producing free radicals such as hydroxyl radicals  
481 (non heme-Fe(III) +  $\text{H}_2\text{O}_2 \rightarrow \text{OH}^\bullet + \text{heme-Fe(II)} + \text{OH}^-$ ); c) the hydroxyl radical reacted with  
482 arachidonic acid (as a result of the relatively low energy load and fast oxidation of unsaturated  
483 lipids) released by the enzyme phospholipase  $A_2$  (Soares *et al.*, 2009). Arachidonic acid, under the  
484 action of lipoxygenases and cyclooxygenases, was converted to hydroperoxide (primary product of  
485 lipid oxidation). This hydroperoxide reacted with hydroxyl radicals, producing lipid radicals (such  
486 as peroxy radicals) that propagated lipid oxidation ( $\text{C}_{20}\text{H}_{32}\text{O}_2$  lipoxygenase LOOH;  $\text{LOOH} + \text{OH}^\bullet \rightarrow$   
487  $\text{ROO}^\bullet + \text{H}_2$ ;  $\text{ROO}^\bullet + \text{RH} \rightarrow \text{R}^\bullet + \text{ROOH}$ ;  $\text{R}^\bullet + \text{R}^\bullet \rightarrow \text{R-R}$ ); d) the peroxides formed during the  
488 propagation of lipid oxidation underwent cleavage, producing lower-molecular-weight volatile and  
489 nonvolatile compounds (secondary products) such as aldehydes (hexanal, MDA and 4-hydroxy-2-  
490 trans-nonenal (HNE)) (Estevez, 2015); e) LOOH scission  $\text{C}_3\text{H}_4\text{O}_2$ ; and f) carbonylation is an  
491 irreversible and nonenzymatic modification of proteins that can occur through different pathways,  
492 such as the oxidative deamination of alkaline amino acids (mainly lysine, arginine and proline) via  
493 Fenton reactions, oxidative cleavage of the peptide backbone via the  $\alpha$ -amidation or oxidation of  
494 glutamyl side chains, covalent binding to nonprotein carbonyl compounds such as HNE or MDA,  
495 and dicarbonyl formation from Maillard degradation of sugars such as glucose and ribose that can  
496 induce the oxidative deamination of amino group residues in proteins, leading to the formation of  $\alpha$ -  
497 aminoadipic semialdehyde (AAS) and  $\gamma$ -glutamic semialdehyde (GGS) (Estevez, 2011; Utrera;  
498 Parra & Estevez, 2014). Our results indicate that thiol groups react with MDA with the loss of SH  
499 groups and undergo further hydrolysis to form a protein carbonyl compound. Both lipid and protein  
500 processes terminate when the formed radicals react with each other ( $\text{C}_3\text{H}_4\text{O}_2 + \text{C}_3\text{H}_7\text{NO}_2\text{S} (-\text{H}_2\text{O})$   
501 protein carbonyl).

502

503

504 3.5 TBARS and carbonyl levels (under cooked conditions)

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

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

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

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

525 The high levels of TBARS and carbonyls found in cooked PSE meat suggest the existence  
526 of an interrelationship between these processes. In fact, a positive correlation ( $r = 0.96369$ ,  $p <$   
527  $0.001$ ) between TBARS content (Table 2) and carbonyl compound content (Table 4) was found in  
528 the cooked PSE samples.

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

536 with hydroperoxides (from heat cleavage and the catalytic effect of light), lipid radicals were  
537 produced, which promoted lipid and protein oxidation. The resulting peroxy radicals could react  
538 with unsaturated lipids, generating peroxides and lipid radicals. The latter can be converted back to  
539 react with oxygen, thus promoting lipid oxidation. Hydroperoxides can undergo cleavage into  
540 various secondary compounds, such as aldehydes, alcohols, furans, and hydrocarbons, increasing  
541 the TBARS levels in cooked PSE meat. Similar to the metal-catalyzed oxidation system, lipid  
542 radicals can abstract a hydrogen atom from (C-H or S-H) bonds, thereby resulting in the formation  
543 of carbon-centered radicals (Davies, 2016). Carbon-centered radicals generally react with O<sub>2</sub> to  
544 yield peroxy radicals (H<sub>3</sub>C• + O<sub>2</sub>→POO•) (Soladoye, Juarez, Aalhus, Shand, & Estevez, 2015).  
545 Peroxy radicals that contain α-heteroatoms (α-hydroxyl or α-amino groups) can undergo rapid  
546 unimolecular elimination of HOO•/O<sub>2</sub>•<sup>-</sup> to generate protein carbonyl compounds (Davies, 2016),  
547 thus increasing the occurrence of carbonylation in cooked PSE. However, on Days 3 and 5 of  
548 storage, there was a decrease in TBARS and carbonyl levels. These results indicate that oxidative  
549 induction by light played a secondary role in triggering oxidative reactions in cooked PSE meat  
550 because even with induction on Days 3 and 5, the TBARS and carbonyl levels did not differ  
551 significantly from those in the control. This result agrees with that of Zainudin, Poojary, Jonberg, &  
552 Lund (2019). The authors showed that light influenced protein carbonylation under high-oxygen  
553 conditions. In contrast, the stabilization during the formation of metmyoglobin along with the  
554 decrease in TBARS and carbonyl levels provided evidence of the essential role of metmyoglobin-  
555 mediated oxidation systems, such as a catalyst of lipid and protein oxidation in cooked PSE meat.  
556 On the other hand, the products of lipid and protein oxidation may react with each other, MDA may  
557 interact with amino groups and thiol, or the aldehyde moiety from specific protein carbonyls may be  
558 involved in several reactions, including oxidative degradation by which an aldehyde moiety is  
559 oxidized to form carboxylic acid and reacts with an aldehyde moiety from another protein-bound  
560 carbonyl residue to form an aldol condensation product or with an α-amino group from a free amino  
561 acid to form a Strecker aldehyde via Strecker-type degradation (oxidative deamination and  
562 decarboxylation of the amino acid in the presence of a carbonyl compound), thus decreasing the  
563 TBARS and carbonyl levels in cooked PSE meat (Estevez, 2011).

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

567  
568  
569

### 570 3.6 Thiol groups

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

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

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

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

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

### 591 4. Conclusion

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

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

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

602 Different pathways triggered the production of compounds resulting from the interactions  
603 between myoglobin, lipid and protein oxidation in the cooled and cooked PSE samples. The impact  
604 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 <sub>4h</sub>	6.54 <sup>a</sup> ± 0.02	5.79 <sup>b</sup> ± 0.015		
pH <sub>24h</sub>	5.99 <sup>a</sup> ± 0.05	5.77 <sup>b</sup> ± 0.017		
L	54.67 <sup>a</sup> ± 2.52	58.50 <sup>b</sup> ± 2.16		
a/b	0.19 <sup>a</sup> ± 0.014	0.16 <sup>b</sup> ± 0.009		
WHC	68.54 <sup>a</sup> ± 2.25	63.75 <sup>b</sup> ± 1.78		
DP	36.27 <sup>a</sup> ± 2.74	43.62 <sup>b</sup> ± 2.51	51.57 <sup>c</sup> ± 2.62	60.75 <sup>d</sup> ± 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 .

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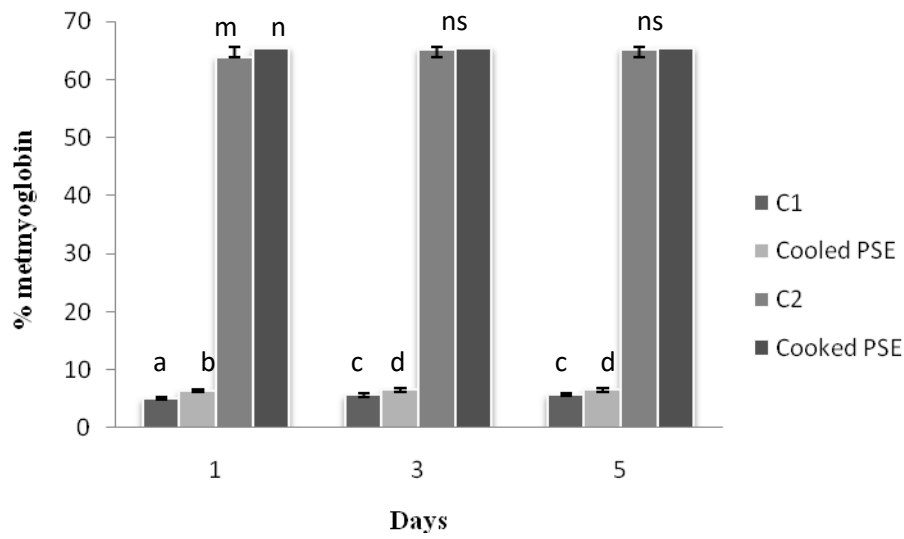
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812 Fig. 1 Metmyoglobin (%) for PSE and their respective controls.

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

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

815 ns: no significant difference

816 C1: cooled control; C2: cooked control.

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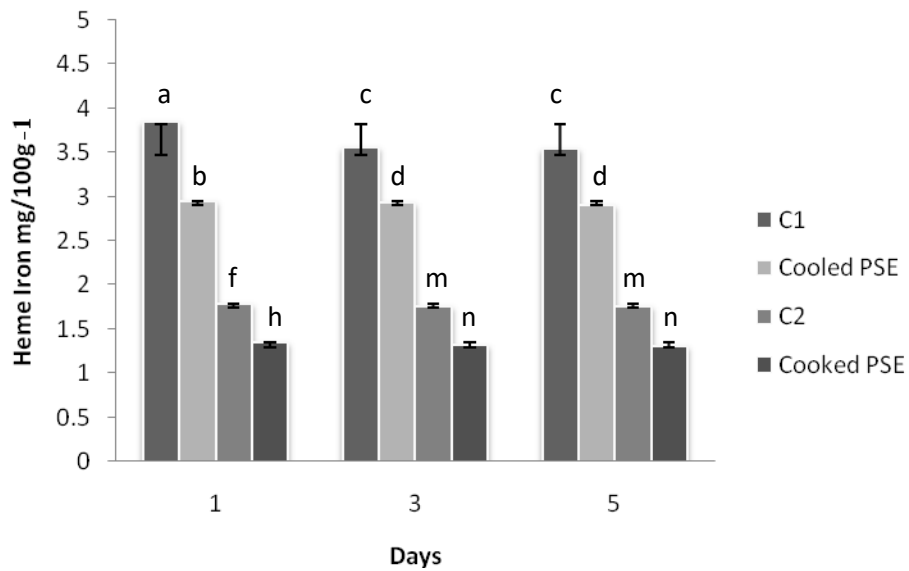
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832 Fig. 2 Heme iron (mg/100 g<sup>-1</sup>) for PSE meat and the respective controls.

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

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

835 C1: cooled control; C2: cooked control.

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

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

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(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.

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

880		Control	Cooled PSE
881	Myristic acid (14:0)	0.44 <sup>a</sup> ± 0.031	0.36 <sup>a</sup> ± 0.044
882	Palmitic acid (16:0)	20.91 <sup>a</sup> ± 0.121	20.62 <sup>a</sup> ± 0.109
883	Palmitoleic acid (16:1n-7)	4.43 <sup>a</sup> ± 0.340	3.12 <sup>b</sup> ± 0.127
884	Stearic acid (18:0)	5.68 <sup>a</sup> ± 0.157	8.82 <sup>b</sup> ± 0.186
885	Oleic acid (18:1n-9)	34.96 <sup>a</sup> ± 0.101	30.44 <sup>b</sup> ± 0.094
886	Vaccenic acid (18:1n-7)	1.91 <sup>a</sup> ± 0.133	2.04 <sup>a</sup> ± 0.064
887	Linoleic acid (18:2n-6)	27.47 <sup>a</sup> ± 0.574	26.90 <sup>a</sup> ± 0.506
888	Alpha-linolenic acid (18:3n-3)	1.89 <sup>a</sup> ± 0.083	1.47 <sup>b</sup> ± 0.030
889	Gamma-linolenic acid (18:3n-6)	0.16 <sup>a</sup> ± 0.11	0.16 <sup>a</sup> ± 0.12
890	Arachidic acid (20:0)	0.28 <sup>a</sup> ± 0.034	0.45 <sup>a</sup> ± 0.021
891	Arachidonic acid (20:4n-6)	1.15 <sup>a</sup> ± 0.154	2.57 <sup>b</sup> ± 0.252
892	Behenic acid (22:0)	0.26 <sup>a</sup> ± 0.026	0.40 <sup>a</sup> ± 0.056
893	Lignoceric acid (24:0)	0.30 <sup>a</sup> ± 0.017	1.33 <sup>b</sup> ± 0.050
894	Nervonic acid (24:1n-9)	0.32 <sup>a</sup> ± 0.015	0.46 <sup>a</sup> ± 0.021
895	*SFAs	27.87 <sup>a</sup> ± 2.45	31.98 <sup>a</sup> ± 2.77
896	*MUFAs	41.62 <sup>a</sup> ± 1.57	36.06 <sup>b</sup> ± 1.09
897	*PUFAs	30.51 <sup>a</sup> ± 0.42	31.96 <sup>b</sup> ± 0.63

898 Means within rows marked by different superscript letters are significantly different (p<0.05).

899 The values are expressed as the mean ± standard deviation of results obtained in duplicate.

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

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

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

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

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

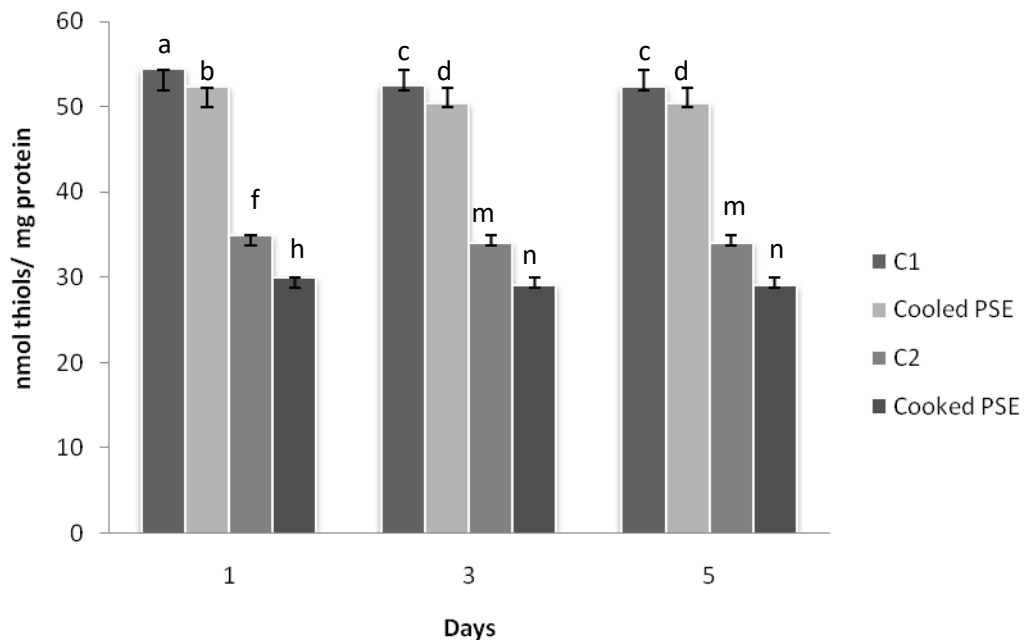
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(a, b, m, n) Within the same columns, means marked with different superscript letters are significantly different. (A, B, M, N) Within the same row, means marked with different superscript letters are significantly different. \*(p<0.01). \*\*(p<0.05).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

C1: cooled control; C2: cooked control.



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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.