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Abstract

8

9

10 The effects of presalting conditions (storage temperature and duration) with/without
11 sodium tripolyphosphate (STPP) on the color and pigment characteristics of cooked ground
12 chicken breast were investigated. Meat mixtures containing 2% NaCl (control) or 2% NaCl
13 and 0.5% STPP (STPP treatment) were stored for 0, 3, 5, 7, and 10 d at 2 or 7°C, followed
14 by cooking to 75°C, and cooling and storage at 2–3°C until further analysis. The treatment
15 was the most effective on the pink color defect of all independent variables. The effect of
16 storage temperature was only observed on CIE L* values and percentage myoglobin
17 denaturation (PMD). The control was redder than the STPP treated samples and the CIE a*
18 values increased ($p < 0.05$) from 0 to 5 d in the control and STPP treated samples. Compared
19 to the STPP treatment, the control exhibited increased reducing conditions (more negative
20 oxidation reduction potential), lower undenatured myoglobin, and greater PMD. No
21 differences in the cooking yields of the control and STPP-treated samples were observed for
22 various storage durations. Products with STPP showed higher ($p < 0.05$) pH values than those
23 without STPP, but no differences ($p > 0.05$) in PMD were observed over the storage period in
24 the control and STPP treated samples, except for day 0. Thus, STPP is effective at reducing
25 the pink color in cooked chicken breasts. In addition, presalting for longer than 5 d resulted
26 in increased pink color of the cooked chicken breasts.

27 **Key words:** ground chicken breast, NaCl, sodium tripolyphosphate, pink color, pigment
28 properties

Introduction

29

30

31 Pink color defect is a condition where well-cooked white meat retains a pink color or
32 develops pinkness with storage after cooking (King and Whyte, 2006). This is a major
33 problem widely reported and studied in poultry and can affect the purchasing behavior of
34 consumers because the appearance of uncooked pink color in fully cooked, uncured poultry
35 products is not unexpected (Holownia et al., 2003; Suman et al., 2016).

36 The causes of the pink defect have been proposed to be related to various types of
37 pigments, preslaughter factors, stunning techniques, incidental nitrate/nitrite contamination
38 through the water supply, diet, processing equipment, and nonmeat ingredients, cooking
39 method, and irradiation of the precooked products (Cornforth et al, 1998; Froning, 1995;
40 Maga, 1994; Holownia et al., 2003). Because the factors associated with pink color
41 development in poultry meat are complex, previous studies have attempted to explain the
42 mechanism and identify novel methods to control this defect. Several studies have reduced
43 or prevented the development of pink color in cooked meat products by incorporating pink
44 inhibiting ingredients such as whey protein concentrate (Sammel and Claus, 2003b; Sammel
45 et al., 2007; Slesinski et al., 2000), non-fat dry milk (Dobson and Cornforth, 1992; Slesinski
46 et al., 2000), sodium citrate (Sammel and Claus, 2003a; Sammel and Claus, 2006; Sammel
47 et al., 2006), citric acid (Kieffer et al., 2000; Sammel and Claus, 2006), and calcium chloride
48 (Sammel and Claus, 2007; Claus et al., 2010). Most of these studies successfully reduced
49 pink color defects, but the products tested with pink inhibiting ingredients contained
50 intentionally added pink-color-generating ligands such as nitrite and/or nicotinamide to
51 develop a cured pink color (Kieffer et al., 2000; Sammel et al., 2007; Sammel and Claus,
52 2006; Sammel and Claus, 2007). Therefore, some studies have attempted to create a natural

53 pink color defect without pink generating ligands by presalting before cooking over a few
54 days of storage (Claus and Jeong 2018; Jeong, 2017; Jeong and Claus, 2010). Jeong (2017)
55 reported that salt addition to a level of more than 2% to ground chicken breasts may reduce
56 the redness of cooked products and presalting storage longer than 3 d showed a natural pink
57 color of the products with less than 1% salt. Claus and Jeong (2018) found that 2% NaCl
58 added to ground turkey breasts and stored for 7 d resulted in the most reducing condition
59 (lowest oxidation reduction potential) and the most red coloration in cooked products.

60 NaCl (sodium chloride) and phosphate are the most commonly used ingredients in the
61 meat processing industry and can affect meat color. Sodium chloride addition to meat may
62 solubilize myofibrillar proteins, providing amino acid chains to form more heme complexes
63 and decrease the redox potential, resulting in a pink color defect in cooked products (Ahn
64 and Maurer, 1989a; Cornforth et al., 1986). Sodium chloride increases the stability of
65 hemoglobin and myoglobin due to the presence of chloride ions. The addition of sodium
66 tripolyphosphate results in increased heat stability of myoglobin due to increased pH (Ahn
67 and Maurer, 1989c). Thus, changes in the pH by NaCl and phosphate addition may affect
68 the state of myoglobin and redox potential of the meat, which are critical for forming heme
69 complexes and developing the pink color of cooked products. Additionally, prolonged
70 storage of presalted meat before cooking promotes pink defect formation after cooking
71 (Cornforth et al., 1991).

72 Although early studies have reproduced the natural pink color defect without adding pink
73 color generating ligands and revealed the effects of salt and salt addition timing of 0 d or 7
74 d on pink color defects in cooked chicken and turkey products (Jeong, 2017; Jeong and Claus,
75 2018), the effect of temperature and presalting duration in the absence and presence of
76 sodium polyphosphate on pigment characteristics of cooked meats has not yet been

77 established.

78 Therefore, this study was performed to examine the effects of presalting conditions
79 (storage temperature and duration) with and without sodium tripolyphosphate (STPP) on the
80 color and pigment characteristics of cooked ground chicken breasts.

81

82

Materials and Methods

83

84 Processing and preparation

85 Fresh, skinless, and boneless chicken breasts (1 d postmortem) were obtained from a local
86 processor (Kwangsung Food, Korea). The raw material was shipped in an insulated cooler
87 and refrigerated at 2-3°C until use. Three replicates of ground chicken breast were received
88 and used immediately after arrival in this study. A total of 50 kg of raw chicken trimmings
89 were used for each replicate and ground with a 0.3 cm plate using a chopper (TC-22 elegant
90 plus, Tre Spade, Italy). The ground meat was separated into two portions for addition of
91 sodium tripolyphosphate (STPP). For the first portion, the ground meat was mixed with only
92 2% NaCl of meat weight basis using a mixer (5K5SS, Whirlpool Inc., USA) for 10 min
93 (control). In the second portion, the ground meat was mixed with 2% NaCl and 0.5% STPP
94 for 10 min (STPP treatment group), as was tested by previous studies (Jeong and Claus,
95 2010; Sammel and Claus, 2007) for turkey breasts. Based on USDA-FSIA (2019)
96 recommendation, industrial practice was considered and two temperature were selected (2
97 or 7°C). Therefore, all salted ground meat was divided into two sets depending on the storage
98 temperature (2 or 7°C) and individually vacuum-packaged (2 kg each) in polyethylene/nylon
99 bags (oxygen transmission rate = 0.7 cm³/m²/24 at 23°C and 90% relative humidity; SNF-
100 100, SamYoung Chemical, Korea) using a vacuum packaging machine (M6-TM, Leepack

101 Co., Ltd., Korea), which were stored for 0, 3, 5, 7, and 10 d under refrigeration (2 or 7°C)
102 prior to being remixed and stuffed. At the designated day, the packages were opened, the
103 salted ground meat was remixed using a mixer (5K5SS, Whirlpool Inc., USA) for 5 min,
104 and subsequently stuffed into conical centrifuge tubes (50 g each). These tubes were
105 centrifuged at $2,000 \times g$ for 10 min (FELTA5, Hanil Science Corp., Korea) to remove any
106 air pockets. All stuffed samples were cooked to an internal endpoint temperature of 75°C in
107 a 90°C water bath (CB60L, Dongwon Scientific Machinery Corp., Korea). The temperature
108 was monitored by placing four thermocouples attached to a 4-channel digital thermometer
109 (Tes-1384, Ketch Scientific Instrument Co., Ltd., Taiwan) in the center of the extra samples
110 throughout the water bath. After cooking, the samples were immediately cooled on ice for
111 20 min and stored at 2-3°C overnight in the dark before further analysis. Experiments were
112 performed in triplicate.

113

114 **Instrumental color determination**

115 A colorimeter (CR-400, 8 mm aperture, illuminant C, 2° standard observer; Konica
116 Minolta Corp., Japan) calibrated with a white plate ($L^* 94.90$, $a^* -0.39$, $b^* 3.88$) was used
117 for determination of the CIE $L^*a^*b^*$ values and measured freshly cut surfaces of each
118 cooked sample following immediately cutting.

119

120 **Cooking yield, pH, and oxidation-reduction potential (ORP) determination**

121 Stuffed ground chicken meat samples were weighed prior to cooking to determine raw
122 sample weights. Cooked weights were also measured to determine cooking yields. Cooking
123 yield was calculated as: $[\text{cooked sample weight}/\text{raw sample weight}] \times 100$. The sample (5 g)
124 was homogenized in 25 mL of distilled water and pH values were measured with a pH

125 electrode attached to a pH meter (Accumet AB50, Thermo Fisher Scientific Inc., Singapore).
126 Oxidation-reduction potential (ORP) was measured for cooked chicken breasts following
127 the method of Cornforth et al. (1986) and John et al. (2005) with slight modifications.

128

129 **Myoglobin content, percentage myoglobin denaturation (PMD), pigment** 130 **determination**

131 Myoglobin (Mb) was extracted from both uncooked and cooked chicken products using a
132 procedure of Warriss (1979) and Trout (1989). The absorbance (A) of the filtrate was
133 subsequently determined at 525, 572, and 700 nm (Krzywicki, 1979) using a UV/VIS
134 spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan). The total myoglobin (Mb)
135 content and PMD were calculated using the following formulas (Trout, 1989): Mb (mg/g) =
136 $(A_{525} - A_{700}) \times 2.303 \times \text{dilution factor}$; PMD = $[1 - (\text{Mb concentration after heating} / \text{Mb}$
137 $\text{concentration before heating})] \times 100$. To obtain the percentage reflectance, the absorbance
138 data on the filtrate from 400 to 700 nm were converted to percentage reflectance using the
139 equation described by Stewart et al. (1965). Nitrosyl hemochrome (rNIT) was estimated
140 using the percent reflectance ratio, %R650 nm/%R570 nm (AMSA, 1991). Nicotinamide
141 hemochrome (rNIC) was estimated by the percent reflectance ratio of %R537 nm/%R553
142 nm (Schwarz et al., 1998).

143

144 **Statistical Analysis**

145 All experiments were performed in triplicate and were analyzed in a completely
146 randomized $2 \times 2 \times 5$ factorial design (absence and presence of STPP by storage temperature
147 by storage duration before cooking). The main effects and their interactions were analyzed
148 using a Proc Mixed procedure in the SAS 9.4 software (SAS, 2013). When significance

149 (p<0.05) was observed in the models, the means were separated by pairwise comparisons
150 using the PDIFF (P-values for DIFFerences of the Least Square means) option in the
151 software.

152

153

Results and Discussion

154 Instrumental color

155 The CIE L* values of the cooked ground chicken breasts were affected by the treatment
156 (T, P<0.0001), storage temperature (S, P<0.05), and storage duration (D, P<0.05; Table 1).
157 However, no three-way interactions between the main effects were observed (P>0.05) for
158 the CIE L* values. The samples treated with 0.5% STPP exhibited lower (P<0.05) CIE L*
159 values compared to the control without STPP (Table 2). These results are similar to those of
160 Lopez et al. (2012), showing that the addition of 1.5% NaCl and 0.35% STPP in the marinade
161 solution decreased lightness (CIE L* values) for marinated broiler breasts compared to those
162 with 0% NaCl and 0.35% STPP. Overall, when the samples were stored at the higher
163 temperature (7°C), the CIE L* values were increased compared to those obtained at the
164 lower temperature (2°C). After 5 d storage, the CIE L* values of the cooked chicken
165 products increased (P<0.05) compared to those stored for 0 or 3 d. However, no differences
166 in the CIE L* values of the cooked chicken breast were observed when stored from 5 to 10
167 d. These findings may be related to light scattering effect during presalting duration of
168 chicken meat at higher temperature rather than lower temperature (Nam and Ahn, 2002;
169 O'Keeffe and Hood, 1980-1981), which resulted in increased lightness.

170 Treatment (T, P<0.0001) had significant effects on CIE a* in the cooked ground chicken
171 breasts (Table 1). Higher CIE a* values were observed in the control (CIE a* 4.18) compared
172 to the STPP treatment (CIE a* 3.86; Table 2). Holownia et al. (2003) showed that the pink

173 discoloration could be visually detected when the a^* value was >3.8 in chicken breast meat.
174 Therefore, when ground chicken breasts were presalted, the pink color in the cooked ground
175 chicken products developed regardless of the presence of STPP. Herein, the CIE a^* values
176 of cooked chicken breasts were similar those reported by Sammel et al. (2006), ranging from
177 3.4 to 4.5 for cooked ground turkey meat. The storage duration (D, $P<0.001$) affected CIE
178 a^* in the cooked ground chicken breasts (Table 1). Presalting storage of >5 d before cooking
179 resulted in higher ($P<0.05$) CIE a^* values compared to chicken products stored for 0 or 3 d
180 (Table 2). Similarly, Jeong and Claus (2010) reported that cooked ground turkey breast
181 showed pink discoloration when 2% NaCl was added without pink color generating agents
182 and stored for 6 d prior to cooking. However, no differences ($P>0.05$) in the CIE a^* values
183 were observed in cooked ground chicken breasts stored for 5 to 10 d. Recently, Claus and
184 Jeong (2018) showed that ground turkey breasts with adding 2% salt and storing for 7 d were
185 redder (higher CIE a^* values) than those without salt stored the same duration or with added
186 salt and immediately cooked after 1 d storage. They speculated that there may be a
187 synergistic effect between the salt and storage for pink color defect development in cooked
188 ground turkey breasts. However, the storage temperature (S) did not affect ($P>0.05$) the CIE
189 a^* values of cooked ground chicken breasts and no two-way or three-way interactions were
190 observed between the main effects ($P>0.05$; Tables 1 and 2).

191 The CIE b^* values of cooked ground chicken breast were affected by the treatment (T,
192 $P<0.0001$) and storage duration (D, $P<0.0001$; Table 1). The control without STPP showed
193 higher ($P<0.05$) CIE b^* values (yellowness) compared to the samples with added STPP
194 (Table 2). Sammel and Claus (2007) reported that STPP decreased the yellowness of cooked
195 ground turkey formulated with pink-color inducing ligands, such as 10 ppm sodium nitrite
196 or 1% nicotinamide. As the storage duration was prolonged from 0 to 10 d, the CIE b^* values

197 of the cooked chicken breast decreased ($P < 0.05$). The CIE b^* values were the highest
198 ($P < 0.05$) in samples stored for 0 d and decreased for samples stored for up to 5 d, but no
199 differences in CIE b^* values ($P > 0.05$) were observed among the samples stored for 5, 7, or
200 10 d. Jeong (2017) reported that a short-term presalting period of 3 d did not significantly
201 affect CIE b^* values, except that chicken products with 1% NaCl showed lower CIE b^*
202 values after 3 d storage compared to those cooked immediately, which is partially consistent
203 with our results in the current study. However, the storage temperature (S) did not affect
204 ($P > 0.05$) CIE b^* values and no two-way or three-way interactions were observed ($P > 0.05$)
205 for the CIE b^* values (Tables 1 and 2).

206

207 **Reflectance estimator of nitrosyl hemochrome (rNIT) and nicotinamide hemochrome** 208 **(rNIC)**

209 Nitrosyl hemochrome is the stable pink pigment of cured meat formed by the reaction
210 between myoglobin and nitrates/nitrites after the cooking (Cassens, 1997; Suman and Joseph,
211 2013), leading to the pink color of cooked meat products. Even though nitrite is not
212 intentionally added to meat, the inherent nitrite present in the meat itself has been observed
213 in poultry meat (Ahn and Maurer, 1987; Claus and Jeong, 2018). In this study, the rNIT ratio,
214 a reflectance estimator of nitrosyl hemochrome, was affected by treatment (T, $P < 0.0001$) of
215 the cooked ground chicken breasts (Table 1). The samples with STPP (STPP treatment)
216 showed higher ($P < 0.05$) rNIT ratios than the control without STPP (Table 2). These results
217 were likely due to the increased pH by STPP addition, which promoted hemochrome
218 formation (Sammel and Claus, 2007; Trout, 1989). However, the storage temperature (S)
219 and storage duration (D) showed no effect ($P > 0.05$) on the rNIT ratio in the cooked ground
220 chicken products. Significant interaction effects between the treatment (T) and storage

221 temperature (S) were found for rNIT ($P<0.05$) in cooked ground chicken breasts (Tables 1
222 and 4). The storage temperature had no effect ($P>0.05$) on the rNIT ratio of the control
223 without STPP. However, STPP treatment at 7°C had higher ($P<0.05$) rNIT ratios compared
224 to those stored at 2°C. STPP treatment had higher rNIT ratio ($P<0.05$) than the control at
225 both storage temperatures.

226 The rNIC ratio, a reflectance estimator of nicotinamide hemochrome, was not influenced
227 by the treatment (T), storage temperature (S), or storage duration (D) of the cooked ground
228 chicken products ($P>0.05$; Table 1). No two-way or three-way interactions significantly
229 affected the rNIC ratio. Nicotinamide is a pyridine derivative with a relatively high
230 concentration in poultry meat and has been reported as a potential pigments involved in the
231 pink defect of cooked, uncured turkey (Ahn and Maurer, 1990; Schwarz et al., 1998). Claus
232 and Jeong (2018) indicated that the storage of ground turkey and salt addition promote
233 conditions associated with the formation of reduced nicotinamide-denatured globin
234 hemochrome, leading to pink color in the cooked products. However, this discrepancy from
235 our results is probable affected by nicotinamide content for different types of poultry meat
236 (Schwarz et al., 1997).

237

238 **Cooking yield, pH values, and oxidation-reduction potential (ORP)**

239 The treatment (T, $P<0.0001$) affected cooking yield and pH values in the cooked ground
240 chicken breast, but storage temperate (S) and storage duration (D) did not affect ($P>0.05$)
241 cooking yields or pH values (Tables 1 and 3). No significant interactions were found among
242 the main effects ($P>0.05$; Table 1). The samples with STPP (STPP treatment) had higher
243 ($P<0.05$) cooking yield than the control (Table 3). As expected, the pH values of cooked
244 ground chicken breast was increased by 0.5% STPP addition and was higher ($P<0.05$) than

245 the control without STPP. These results are similar to those reported by Holownia et al.
246 (2004), showing that the combination of sodium chloride and tripolyphosphate significantly
247 increased the pH of cooked chicken meat. It is well-known that alkaline phosphate such as
248 tripolyphosphate can increase the pH of meat and improve the water holding capacity,
249 resulting in reduced cooking loss of meat products (Lamkey, 1998; Long et al., 2011;
250 Sebranek, 2015). In terms of meat pigments, increased pH values can increase the heat
251 stability of myoglobin, allowing it to retain a native state after cooking (Ahn and Maurer,
252 1989c), in agreement with the results of the myoglobin content and PMD reported in this
253 study (Table 3).

254 The ORP is important for pink color formation and is affected by processing ingredients
255 such as salt and phosphate (Holownia et al., 2003). Lower ORP values (more reducing
256 conditions) is favorable for the development of pink discoloration in cooked meat because
257 it encourages the complexation of denatured protein with heme pigments (Dobson and
258 Cornforth, 1992). In the current study, ORP was affected by the treatment (T, $P < 0.05$; Table
259 1). The addition of 2% NaCl alone to ground chicken breasts (control) promoted reducing
260 conditions (more negative ORP values) to a larger extent compared to the STPP treatment
261 (Table 3). Ahn and Maurer (1989b) suggested that the decreased ORP by salt addition may
262 influence the formation of heme complexes in turkey breast products. Cornforth et al. (1986)
263 also showed that reducing conditions (more negative ORP values) can promote pink color
264 formation in cooked turkey rolls. These previous reports support the CIE a^* values reported
265 in this study. However, the storage temperature (S, $P > 0.05$) and storage duration (D, $P > 0.05$;
266 Table 1) did not affect ORP. These results are similar to those reported by Jeong (2017),
267 showing that no significant differences in the ORP of cooked chicken breasts occur when
268 samples are presalted and stored for 0 to 3 d prior to cooking.

269

270 **Myoglobin content and percentage myoglobin denaturation (PMD)**

271 The treatment (T, $P < 0.0001$) affected the myoglobin content and PMD of cooked ground
272 chicken breasts (Table 1). The STPP treatment resulted in higher ($P < 0.05$) myoglobin
273 content compared to the control (Table 3). Trout (1989) reported that the addition of
274 phosphate increased the stability of myoglobin in meat system. Ahn and Maurer (1989c)
275 reported that sodium chloride significantly decreased myoglobin heat stability, whereas
276 sodium tripolyphosphate increased the stability of myoglobin. It is also possible that water
277 retention ability by STPP addition could reduce water release containing water soluble
278 components such as myoglobin in meat (Ghorpade et al., 1992). However, storage
279 temperature (S) and duration (D) did not affect ($P > 0.05$) the myoglobin content of the
280 cooked ground chicken products. Interaction effects between the treatment (T) and storage
281 temperature (S) were significant ($P < 0.05$) for myoglobin content in the cooked ground
282 chicken breasts (Tables 1 and 4). The control samples were not different ($P > 0.05$) in
283 myoglobin content at both storage temperatures. However, the chicken products with STPP
284 at 7°C showed greater myoglobin content ($P < 0.05$) than those stored at 2°C or the control at
285 7°C. It could be speculated that an increase of storage temperature may reduce the oxygen
286 tension (Seideman et al., 1984) and the redox form of myoglobin can be changed into be
287 more deoxymyoglobin, which may result in greater thermal stability at higher pH (Hunt et
288 al., 1999).

289 In the case of PMD, STPP treatment resulted in lower ($P < 0.05$) PMD values compared to
290 the control and samples stored at 2°C showed higher ($P < 0.05$) PMD than those stored at 7°C
291 (Table 3). Holownia et al. (2004) found that the presence of STPP with sodium chloride
292 decreased the PMD in cooked chicken breast meat, which is similar to our result. In addition,

293 presalting periods of >3 d prior to cooking resulted in higher ($P<0.05$) PMD values compared
294 to the initial storage (0 d; Table 3). The increased PMD is probably due to the synergistic
295 effect between the salt and storage (Rhee and Ziprin, 2001). It is likely that salt addition
296 destabilized myoglobin in the meat (Ahn and Maurer 1989c; Min et al., 2010) and the anion
297 or cation promoted alteration in the denaturation behavior of myoglobin (Trout, 1989),
298 resulting in increased PMD. The treatment (T) and storage temperature (S) interaction was
299 found ($P<0.05$) for PMD in cooked ground chicken breasts (Tables 1 and 4). The addition of
300 STPP to samples did not result in differences ($P>0.05$) in PMD compared to the control at
301 2°C (Table 4). When the samples were stored at 7°C, PMD was decreased ($P<0.05$) by
302 addition of STPP to the ground chicken breast. PMD was not changed ($P>0.05$) with varying
303 storage temperature in the control, but decreased ($P<0.05$) when stored at 7°C compared to
304 the 2°C storage in the STPP treatment.

305 306 **Conclusions**

307 Presalting of ground chicken meat in the absence of STPP rather than the presence of
308 STPP facilitated the development of a natural pink color in the final products without the
309 addition of nitrogenous compounds such as nitrate and nitrite. However, the storage
310 temperature prior to cooking did not affect the redness and pigments associated with the pink
311 color in cooked ground chicken breasts. Storage periods of >5 d may contribute to
312 spontaneous pink color development in cooked ground chicken products. The results
313 indicate that poultry meat processors can control the natural pink color defects by limiting
314 the storage periods to <5 d when chicken meat is presalted without the addition of any pink-
315 inhibiting ligands. Further researches are necessary to investigate the effectiveness of pink
316 inhibiting ligands to reduce the development of pink color defects in cooked meat products.

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320
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448

449 **Table 1. Significance of main and interaction effects on presalting condition with/without sodium tripolyphosphate on color and**
 450 **pigments properties of cooked ground chicken breasts**

Main and interaction effects ¹	Dependent variables ²									
	CIE L*	CIE a*	CIE b*	rNIT	rNIC	Cooking Yield	pH	ORP	Myoglobin	PMD
Treatment (T) ³	**	**	**	**	NS	**	**	*	**	**
Storage temperature (S) ⁴	*	NS	NS	NS	NS	NS	NS	NS	NS	*
Storage day (D) ⁵	*	**	**	NS	NS	NS	NS	NS	NS	*
T × S	NS	NS	NS	*	NS	NS	NS	NS	*	*
T × D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S × D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T × S × D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

451 ¹ Main and interaction effects: * = P<0.05, ** = P<0.0001, NS = not significant.

452 ² Dependent variables: CIE L* (lightness), CIE a* (redness), CIE b* (yellowness), rNIT (reflectance estimator of nitrosyl
 453 hemochrome, %R650nm/%R570nm, higher ratio more), rNIC (reflectance estimator of nicotinamide hemochrome, %R537nm/%R553nm, higher ratio
 454 more), ORP (oxidation-reduction potential), Myoglobin (amount of undenatured myoglobin), PMD (percentage myoglobin denaturation).

455 ³ Treatment: Ground meat were mixed with 2% NaCl alone (Control) or 2% NaCl and 0.5% sodium tripolyphosphate (STPP treatment).

456 ^{4,5} Storage temperature and day: Treatments were periodically stored for 0, 3, 5, 7, or 10 d at different temperatures (2°C or 7°C) before being cooked.

457

458 **Table 2. Least square means and standard errors for presalting condition effects on CIE L*, a*, b* values, reflectance estimator of**
 459 **nitrosyl hemochrome, and reflectance estimator of nicotinamide hemochrome in cooked ground chicken breasts formulated without**
 460 **adding pink generating ligands**

Main effects	Dependent Variables ¹				
	CIE L*	CIE a*	CIE b*	rNIT	rNIC
Treatment (T) ²					
Control	76.35 ^a	4.18 ^a	8.29 ^a	1.0137 ^b	1.0093
STPP	74.46 ^b	3.86 ^b	8.03 ^b	1.0167 ^a	1.0091
(SEM)	(0.17)	(0.05)	(0.07)	(0.0006)	(0.0005)
Storage temperature (S) ³					
2°C	75.27 ^b	4.00	8.12	1.0147	1.0092
7°C	75.54 ^a	4.04	8.19	1.0157	1.0092
(SEM)	(0.17)	(0.05)	(0.07)	(0.0006)	(0.0005)
Storage day (D) ⁴					
Day 0	75.12 ^b	3.59 ^c	8.72 ^a	1.0160	1.0087
Day 3	75.18 ^b	3.89 ^b	8.26 ^b	1.0157	1.0090
Day 5	75.62 ^a	4.19 ^a	8.04 ^c	1.0146	1.0091
Day 7	75.61 ^a	4.18 ^a	8.01 ^c	1.0151	1.0094
Day 10	75.48 ^a	4.25 ^a	7.76 ^c	1.0146	1.0098
(SEM)	(0.19)	(0.07)	(0.09)	(0.0008)	(0.0006)

461 ^{a-c} Means within a column with unlike superscripts are different (P<0.05). Two way interaction found for rNIT(T ×S; P<0.05).

462 ¹ Dependent variables: CIE L* (lightness), CIE a* (redness), CIE b* (yellowness), rNIT (reflectance estimator of nitrosyl
 463 hemochrome, %R650nm/%R570nm, higher ratio more), and rNIC (reflectance estimator of nicotinamide hemochrome, %R537nm/%R553nm, higher
 464 ratio more).

465 ² Treatment: Ground meat were mixed with 2% NaCl alone (Control) or 2% NaCl and 0.5% sodium tripolyphosphate (STPP treatment).

466 ^{3,4} Storage temperature and day: Treatments were periodically stored for 0, 3, 5, 7, or 10 d at different temperatures (2°C or 7°C) before being cooked.

467 **Table 3. Least square means and standard errors for presalting condition effects on cooking yield, pH values, oxidation-reduction**
 468 **potential, myoglobin content, and percentage myoglobin denaturation in cooked ground chicken breasts formulated without adding**
 469 **pink generating ligands**

Main effects	Dependent Variables ¹				
	Cooking yield (%)	pH	ORP (mV)	Myoglobin (mg/g)	PMD (%)
Treatment (T) ²					
Control	97.94 ^b	6.19 ^b	-109.75 ^a	0.18 ^b	85.46 ^a
STPP	98.96 ^a	6.39 ^a	-97.50 ^b	0.21 ^a	82.95 ^b
(SEM)	(0.18)	(0.01)	(3.64)	(0.005)	(0.85)
Storage temperature (S) ³					
2°C	98.52	6.30	-102.12	0.19	84.79 ^a
7°C	98.38	6.29	-105.13	0.20	83.62 ^b
(SEM)	(0.19)	(0.01)	(3.64)	(0.005)	(0.85)
Storage day (D) ⁴					
Day 0	98.59	6.33	-113.50	0.19	81.46 ^b
Day 3	98.27	6.28	-108.37	0.20	84.86 ^a
Day 5	98.62	6.26	-103.79	0.19	85.33 ^a
Day 7	98.31	6.28	-90.53	0.19	84.31 ^a
Day 10	98.47	6.30	-101.93	0.19	85.06 ^a
(SEM)	(0.25)	(0.01)	(5.60)	(0.006)	(0.95)

470 ^{a,b} Means within a column with unlike superscripts are different (P<0.05). Two way interaction found for myoglobin contents (T × S; P<0.05) and PMD
 471 (T × S; P<0.05).

472 ¹ Dependent variables: ORP (oxidation-reduction potential), Myoglobin (amount of undenatured myoglobin), PMD (percentage myoglobin denaturation).

473 ² Treatment: Ground meat were mixed with 2% NaCl alone (Control) or 2% NaCl and 0.5% sodium tripolyphosphate (STPP treatment).

474 ^{3,4} Storage temperature and day: Treatments were periodically stored for 0, 3, 5, 7, or 10 d at different temperatures (2°C or 7°C) before being cooked.

475 **Table 4. Interaction effects of presalting condition on estimator of nitrosyl hemochrome (rNIT), myoglobin contents, and percentage**
 476 **myoglobin denaturation (PMD) in cooked ground chicken breasts formulated without adding pink generating ligands**

477

Treatment ¹	rNIT			Myoglobin (mg/g)			PMD (%)		
	Storage temperature ²			Storage temperature			Storage temperature		
	2°C	7°C	(SEM)	2°C	7°C	(SEM)	2°C	7°C	(SEM)
Control	1.0138 ^b	1.0136 ^b	(0.0006)	0.18	0.18 ^b	(0.005)	85.22	85.71 ^a	(0.85)
STPP	1.0157 ^{ay}	1.0178 ^{ax}	(0.0006)	0.19 ^y	0.22 ^{ax}	(0.005)	84.36 ^x	81.53 ^{by}	(0.85)
(SEM)	(0.0006)	(0.0006)		(0.005)	(0.005)		(0.85)	(0.85)	

478 ^{a,b} Means within a column with unlike superscripts are different (P<0.05). Standard error of interaction effects was 0.0008 for rNIT, 0.006 for myoglobin
 479 contents, and 0.92 for PMD.

480 ^{x,y} Means within a row with unlike superscripts are different (P<0.05). Standard error of interaction effects was 0.0008 for rNIT, 0.006 for myoglobin
 481 contents, and 0.92 for PMD.

482 ¹ Treatment: Ground meat were mixed with 2% NaCl alone (Control) or 2% NaCl and 0.5% sodium tripolyphosphate (STPP treatment).

483 ² Storage temperature: Treatments were periodically stored at different temperatures (2°C or 7°C) before being cooked.

484