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ARTICLE

Effects of Short-Term Presalting and Salt Level on the Development of Pink Color in Cooked Chicken Breasts

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Abstract

The objective of this study was to determine the effects of short-term presalting on pink color and pigment characteristics in ground chicken breasts after cooking. Four salt levels (0%, 1%, 2%, and 3%) were presalted and stored for 0 and 3 d prior to cooking. Cooking yield was increased as salt level was increased. However, no significant differences in pH values or oxidation reduction potential (ORP) of cooked chicken breasts were observed. Cooked products with more than 2% of salt level had less redder (lower CIE a* value) on day 3 than on those on day 0. As salt level was increased to 2%, myoglobin was denatured greatly. Myoglobin denaturation was leveled off when samples had 3% of salt. With increasing salt levels, residual nitrite contents were increased while nitrosyl hemochrome contents were decreased. These results demonstrate that salt addition to a level of more than 2% to ground meat may reduce the redness of cooked products and that presalting storage longer than 3 d should be employed to develop a natural pink color of ground chicken products when less than 1% salt is added to ground chicken meat.

Keywords ground chicken breast, NaCl, pink color, pigment properties

Introduction

For fully cooked poultry items, the presence of a pink or undercooked appearance is a major concern. This pink color defect is not a food safety issue, but a quality issue. It is of economic concern to the processor (Friesen and Marcy, 2000). The causes of undesirable pink color include high pH, high reducing conditions, the state and level of meat pigments, incidental nitrate/nitrite contamination, and processing ingredients (Holownia *et al*., 2003; Maga, 1994). Many researchers investigated solutions to reduce pink color development by food ingredients (Sammel and Claus, 2003; Sammel and Claus, 2006; Sammel and Claus, 2007; Schwarz *et al*., 1999; Slesinski *et al*., 2000). Slesinski *et al*. (2000) observed that dairy proteins can reduce pink color in ground turkey breasts. They found that nonfat dry milk (NFDM) and specific whey protein concentrates (WPC) can reduce the pink color defect in samples containing 10 ppm of sodium nitrite (Slesinski *et al*., 2000). Sammel and Claus (2003) reported that citric acid and sodium citrate can consistently reduce natural or induced pink color in ground turkey rolls but have no effect on pink color of intact turkey breast. Recently, Kim *et al*. (2015) found that dipping in citric acid solution in sous vide processed chicken breasts can reduce the pink color.

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Sodium chloride is one of the fundamental and common ingredients used in the production of meat and poultry products. In cured meat, chloride ion from salt has been found to be able to promote cured color formation by accelerating the formation of nitric oxide from nitrite (Sebranek and Fox, 1991). Regarding meat pigments, sodium chloride can significantly decrease the heat stability of myoglobin and hemoglobin but increase the heat stability of cytochrome c (Ahn and Maurer, 1989c). Trout (1989) reported that the percentage myoglobin denaturation is increased linearly with increasing sodium chloride concentration in cooked meat products and the pinkness of products is decreased with increasing levels of sodium chloride. However, Ahn and Mauer (1989a) reported that sodium chloride added at 2% to turkey breast can significantly increase the redness values of oven roasted products and concluded that sodium chloride might be the most important added ingredient affecting meat color.

With respect to presalting condition, processors reported that turkey rolls hold for several hours before cooking exhibits pink color in cooked products probably due to microbial growth and reduced oxidation-reduction potential (Cornforth *et al*., 1991). Ahn and Mauer (1989a) speculated that the increasing redness values of turkey breast added with salt could be the reaction of solubilized myofibrillar proteins with heme pigments. Jeong and Claus (2010) attempted to create a natural pink color defect without the addition of pink generating ligands such as nitrite or nicotinamide in ground turkey breast products at different processing conditions during storage before cooking. They found that prolonged storage for 7 d of presalted ground turkey meat under anaerobic conditions might have contributed to the undesirable development of a pink color defect in cooked ground turkey. However, the interaction between presalting period and salt level in ground chicken before cooking in relation to the development of pink color in cooked products has not been elicited.

Therefore, the objective of this study was to investigate the effects of short-term presalting period and salt level on the development of pink color and identify the pigment properties in cooked ground chicken breasts.

Materials and Methods

Processing and preparation

Fresh, skinless, and boneless chicken breasts (1 d postmortem) were obtained from a local processor. Raw material was shipped in an insulated cooler and refrigerated (2-3°C) until used. Three separate replications of ground chicken breast were received and used in this study. From each replication, a representative sample of raw ground chicken was used for pH determination and residual nitrite content analysis. A total of 20 kg of raw chicken trimmings were used for each replication and ground with a 0.3 cm plate using a chopper (TC-22 elegant plus, Tre Spade, Italy). The ground meat was randomly separated into four individual batches (4 kg each) depending on NaCl level as follows: 0%, 1%, 2%, and 3% NaCl. In each treatment, salt was incorporated into the ground meat and mixed (5K5SS, Whirlpool Inc., USA) for 5 min followed by division into two sets for two different lengths of storage (day 0 and day 3). All salted ground meat was vacuum-packaged into polyethylene/nylon bags using a vacuum packaging machine (M6-TM, Leepack Co., Ltd., Korea). Samples for day 3 were further stored under refrigeration (2-3°C) prior to being remixed and stuffed. At designated day (day 0 or day 3), the ground meat was stuffed into conical centrifuge tubes (50 g each). These tubes were centrifuged at 2,000 × g for 10 min (FELTA5, Hanil Science Corp., Korea) to remove air pockets. All samples were then cooked to an internal endpoint temperature of 75°C in a 90°C water bath (CB60L, Dongwon Scientific Machinery Corp., Korea). Temperature was monitored by randomly placing four thermocouples attached to a 4-channel digital thermometer (Tes-1384, Ketech Scientific Instrument Co., Ltd., Taiwan) in the center of extra samples throughout the water bath. After cooking, samples were immediately cooled on ice for 20 min and stored at 2-3°C overnight in the dark until further analysis. Experiments were replicated three times.

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

Stuffed ground chicken meat samples were weighed prior to cooking to determine raw sample weights. Cooked weights were also measured to determine cooking yields using the following equation: Cooking yield = [cooked sample weight/raw sample weight] × 100. A pH meter (Accumet AB50, Thermo Fisher Scientific Inc., Singapore) was used to measure pH values of 5 g raw meat or cooked chicken sample homogenized in 25 mL of distilled, deionized water. Oxidation-reduction potential (ORP) was measured for cooked turkey products following the method of Cornforth *et al*. (1986) and John *et al*. (2005) with slight modifications. Briefly, a sample (10 g) of each

chicken product was homogenized (DI 25 basic, IKA-Werke Gmbh & Co., KG, Germany) in 20 mL 0.1 M sodium carbonate for 15 s at 13,000 rpm. Then 50 μL of butylated hydroxytoluene (BHT, 7.2% in ethanol) was added to minimize sample oxidation during blending. The ORP values were determined after 3 min of stabilization using a platinum Ag/AgCl combination electrode (Model 13-620-631, Thermo Fisher Scientific Inc., Singapore) attached to the pH meter set at millivolt scale.

Instrumental color determination

CIE $L^*a^*b^*$ values were measured for freshly cut surfaces of each cooked sample using a chroma meter (CR-400, 8 mm aperture, illuminant C; Konica Minolta Corp., Japan) calibrated with a white plate (L^* 94.90, a^* -0.39, b^* 3.88). Two slices were cut parallel to the longitudinal axis of each cooked ground chicken breast sample and three measurements per slice (six readings per sample) were taken immediately following cutting.

Myoglobin content and percentage myoglobin denaturation (PMD) determination

Myoglobin (Mb) was extracted from both uncooked and cooked turkey breast products using a procedure of Warriss (1979) and Trout (1989). The extracted supernatants were further clarified by filtration using Whatman No. 1 filter paper. The absorbance (A) value of the filtrate was then determined at 525, 572, and 700 nm (Krzywicki, 1979) using a UV/VIS spectrophotometer (UV-1800, Shimadzu Corp., Japan). Total myoglobin (Mb) content and percentage myoglobin denaturation (PMD) were calculated using the following formulas (Trout, 1989): Mb (mg/g) = (A525 – A700) × 2.303 × dilution factor; PMD = [1 – (Mb concentration after heating/Mb concentration before heating)] × 100.

Nitrite, nitrosyl hemochrome, and total pigment analysis

Representative fresh ground chicken samples from each replication and cooked products were analyzed for residual nitrite contents using the method of AOAC (2007). Nitrosyl hemochrome and total pigments were measured for cooked chicken samples after extraction with 80% acetone and acidified acetone (Hornsey, 1956). For nitrosyl hemochrome determination, 10 g of cooked chicken product were blended with 40 mL acetone and 3 mL of distilled, deionized water using a homogenizer (Polytron Model PT10-35, Kinematica AG, Switzerland). The homogenized samples were kept in the dark for 15 min before absorbance measurement. The homogenate was filtered through a Whatman No. 1 filter paper and then the absorbance of the filtrate was measured at wavelength of 540 nm (A540) using a spectrophotometer (Model UV mini 1240, Shimadzu Corporation, Japan). Nitrosyl hemochrome concentration (ppm) = A540 × 290. For total pigment measurement, 10 g of cooked samples were blended with 40 mL acetone, 1 mL HCl, and 2 mL distilled, deionized water and kept in the dark at cold temperature (2-3°C) for 1 h, and then filtered through a Whatman No. 1 filter paper. Absorbance value of the filtrate was measured at wavelength of 640 nm (A640). Total pigment (ppm) = A640 × 680.

Statistical analysis

All experiments were replicated three times. Data were statistically analyzed with two-way analysis of variance (ANOVA) to determine the effect of presalting period before cooking and NaCl level using statistical analysis system (SAS, 2012). Duncan's multiple range test was used to determine differences between mean values. Statistical significance was considered when p value was less than 0.05.

Results and Discussion

pH values and residual nitrite contents of raw ground chicken breast

The pH values and residual nitrite contents of representative raw ground chicken breast samples were 5.98 (standard deviation, 0.14) and 0.18 ppm (standard deviation, 0.04), respectively. Ahn and Maurer (1987) reported that the residual nitrite contents of 0.2 ppm were naturally present in raw turkey breasts, which is similar to our result.

Cooking yield, pH values, and ORP

The effects of presalting period before cooking and salt levels on cooking yield, pH values, and ORP of cooked ground chicken breast are shown in Table 1. As expected, products containing salt had greater (p<0.05) cooking yields compared to those without salt regardless of the presalting period (Table 1). On day 0, samples with 2% or 3% NaCl had higher (p<0.05) cooking yields than those with 0% or 1% NaCl. These results were similar to those reported by Jeong and Claus (2010). The cooking yields of products with 0% and 1% NaCl were higher (p<0.05) on day 3 than those on day 0. However, the 2% and 3%

Table 1. Effects of presalting period and salt level on cooking yield, pH, and oxidation reduction potential (ORP) in cooked ground chicken breasts[1)]

Traits	Presalting	NaCl level			
		0%	1%	2%	3%
Cooking yield (%)	Day 0	89.45±0.24^{Cy2}	96.05±0.11^{By}	97.82±0.26^{A}	97.45±0.21^{A}
	Day 3	90.40±0.33^{Bx}	98.03±0.42^{Ax}	98.61±0.31^{A}	97.97±0.50^{A}
pH	Day 0	6.16±0.03	6.13±0.04	6.12±0.03	6.12±0.03
	Day 3	6.11±0.03	6.10±0.03	6.10±0.02	6.12±0.01
ORP (mV)	Day 0	-115.53±2.26	-114.62±2.37	-112.88±2.56	-114.55±2.06
	Day 3	-113.12±2.64	-112.70±1.75	-109.33±2.35	-108.25±2.29

All values are presented as means ± standard error of triplicates.
[1)]Raw ground meat was salted (0, 1, 2, and 3% NaCl, respectively), stored for 0 or 3 d, and then cooked to 75°C in a 90°C water bath.
[A-C]Means within a row with different superscript letters are significantly different (p<0.05).
[x,y]Means within a column with different superscript letters are significantly different (p<0.05).

NaCl products had similar (p>0.05) cooking yields on day 0 and day 3. The pH values were not affected (p>0.05) by presalting period or salt level (Table 1). These results were in agreement with those of a previous study (Medyński *et al*., 2000) demonstrating that sodium chloride added to pork did not have statistically significant influence on the pH values of the meat after cooking. The oxidation reduction potential (ORP) is an important factor in pink color formation and determines the ability of pigments to bind to molecules in meat system (Holownia *et al*., 2003). Cornforth *et al*. (1986) found that the pink color of commercial and laboratory prepared turkey meat samples is due to more reduced condition (lower ORP), thereby promoting hemochrome formation. Ahn and Maurer (1989b) reported that the redox potential is decreased by 2.5% NaCl in turkey breast meat, which might have resulted in heme complex formation and more undenatured pigment in the cooked products. However, in this study, neither presalting period nor salt level had significant effect (p> 0.05) on ORP values (Table 1). This might be due to different pH values among treatments because reducing condition is pH dependent (Atonini and Brunori, 1971; Holownia *et al*., 2003).

Instrumental color, myoglobin (Mb) contents, and percentage myoglobin denaturation (PMD)

On day 0, 2% NaCl samples had more red color (higher CIE a* values, p<0.05) than 0% or 3% NaCl products. The redness values of 2% NaCl samples were not different (p>0.05) from those of 1% NaCl products (Table 2). On day 3, the CIE a* values were decreased with increasing salt levels, except for products with 1% NaCl. Products with 3% NaCl had less red color (lower CIE a* values, p<0.05) compared to those with 0% or 1% NaCl. Presalting period did not significantly (p>0.05) affect the CIE a* values of products containing 0% or 1% NaCl. However, on day 3, samples treated with 2% or 3% NaCl showed decreased (p<0.05) CIE a* values compared to those on day 0 (Table 2). Ahn and Mauer (1989a) reported that the addition of 2.0% salt to turkey breast significantly increased the redness values compared to turkey products without the addition of salt. They have indicated that adding sodium chloride might solubilize myofibrillar proteins so that they could have more chances to react with heme pigments, thereby resulting in more pink color formation (Ahn and Mauer, 1989a). However, Trout (1989) reported that sodium chloride up to 3% decreased redness in ground turkey products, probably due to increasing degradation of myoglobin during the cooking process. Therefore, our results suggest that salt addition to chicken breast meat might be able to increase CIE a* value in cooked products on day 0 when compared to products without the addition of salt, on the other hand, excessive amounts of sodium chloride (at more than 2%) coupled with 3 days of presalting period might be responsible for the reduction of redness in cooked samples which might be due to more myoglobin denaturation and oxidative conditions (Min *et al*., 2010; Rhee and Ziprin, 2001; Trout, 1989). In this study, NaCl level affected CIE L* and b* values (Table 2). CIE L* values were decreased (p<0.05) with increasing salt level on both presalting days. However, there was no significant (p>0.05) difference between the two presalting periods (day 0 and day 3) at the same salt level. Cooked chicken products with 2% and 3% salt levels had lower CIE b* (p<0.05) values than those with 0% or 1% salt level on day 0. However, on day 3, the addition of salt resulted in either lower (1% and 2% NaCl) or similar (3% NaCl) CIE b* values compared to those of 0% NaCl

products. Presalting period had no significant (p>0.05) effect on CIE b* values except that the 1% NaCl products had lower CIE b* values on day 3 than those on day 0. Myoglobin contents were reduced (p<0.05) with increasing salt levels on both presalting days except for products with 3% NaCl (Table 2). It appears that at salt level of less than 2%, myoglobin content is destabilized by salt addition (Jeong and Claus, 2010; Min *et al*., 2010; Trout, 1989). When samples were treated with 0%, 1%, and 2% of NaCl and stored for 3 d prior to cooking process, myoglobin contents were lower (p<0.05) compared to 0 d samples. A similar finding has been reported by Min *et al*. (2010) showing that the addition of NaCl can significantly decrease myoglobin content in raw beef loin during storage. However, no differences (p>0.05) in myoglobin contents of 3% NaCl samples were found between day 0 and day 3 (Table 2). It is generally believed that sodium chloride will decrease the heat stability of myoglobin and hemoglobin in meat system due to the chloride ion, thereby leading to increased heat denaturation (Ahn and Maurer, 1989b; Ahn and Maurer, 1989c; King and Whyte, 2006; Trout, 1989). In the present study, the percentage myoglobin denaturation (PMD) in cooked chicken breasts was increased (p<0.05) as salt level was increased to 2% NaCl. However, the PMD values of cooked chicken breasts with 3% NaCl were lower (p<0.05) than those of cooked chicken breasts with 2% NaCl. Presalting period did not significantly (p>0.05) influence the PMD values of cooked chicken breasts (Table 2). Although samples in this study were cooked to 75°C, approximately 15-20% of undenatured myoglobin remained in the products on both day 0 and day 3 (Table 2). Similarly, Jeong and Claus (2010) reported that approximately 15% of myoglobin was not denatured when presalted and stored ground turkey breasts were cooked to 79.4°C.

Table 2. Effects of presalting period and salt level on CIE L*, a*, and b* values, total myoglobin (Mb), and percentage myoglobin denaturation (PMD) in cooked ground chicken breasts[1)]

Traits	Presalting	NaCl level			
		0%	1%	2%	3%
CIE L*	Day 0	81.08±0.13^{A}	78.32±0.19^{B}	76.19±0.21^{C}	75.26±0.38^{D}
	Day 3	80.75±0.28^{A}	77.83±0.23^{B}	75.90±0.16^{C}	74.96±0.34^{D}
CIE a*	Day 0	3.59±0.12^{B}	3.74±0.05^{AB}	3.95±0.08^{Ax}	3.67±0.06^{Bx}
	Day 3	3.60±0.08^{AB}	3.90±0.12^{A}	3.02±0.24^{BCy}	2.44±0.38^{Cy}
CIE b*	Day 0	9.35±0.16^{A}	9.07±0.13^{Ax}	8.44±0.10^{B}	8.62±0.12^{B}
	Day 3	9.14±0.09^{A}	8.27±0.13^{Cy}	8.55±0.20^{BC}	8.84±0.24^{AB}
Mb (mg/g)	Day 0	0.24±0.01^{Ax}	0.22±0.01^{ABx}	0.21±0.01^{Bx}	0.23±0.01^{AB}
	Day 3	0.20±0.01^{Ay}	0.19±0.01^{ABy}	0.18±0.01^{By}	0.21±0.01^{A}
PMD (%)	Day 0	80.22±1.16^{B}	82.74±0.49^{AB}	84.93±0.30^{A}	81.91±1.48^{B}
	Day 3	80.49±0.87^{B}	81.94±0.76^{AB}	83.50±0.74^{A}	79.40±1.39^{B}

All values are presented as means ± standard error of triplicates.
[1)]Raw ground meat was salted (0, 1, 2, and 3% NaCl, respectively), stored for 0 or 3 d, and then cooked to 75°C in a 90°C water bath.
[A-D]Means within a row with different superscript letters are significantly different (p<0.05).
[x,y]Means within a column with different superscript letters are significantly different (p<0.05).

Table 3. Effects of presalting period and salt level on nitrite, nitrosyl hemochrome, and total pigment contents in cooked ground chicken breasts[1)]

Traits	Presalting	NaCl level			
		0%	1%	2%	3%
Nitrite (ppm)	Day 0	0.25±0.02^{Cx}	0.32±0.03^{Bx}	0.48±0.02^{A}	0.54±0.03^{A}
	Day 3	0.19±0.01^{Cy}	0.24±0.01^{Cy}	0.48±0.03^{B}	0.56±0.02^{A}
Nitrosyl hemochrome (ppm)	Day 0	2.54±0.53^{A}	1.35±0.38^{B}	1.55±0.37^{AB}	0.68±0.14^{B}
	Day 3	1.76±0.17^{A}	1.45±0.15^{A}	0.89±0.13^{B}	1.26±0.27^{AB}
Total pigment (ppm)	Day 0	14.79±0.37	15.02±0.21	15.19±0.27^{x}	14.45±0.21
	Day 3	15.30±0.31^{A}	15.19±0.34^{AB}	14.39±0.16^{By}	14.62±0.31^{AB}

All values are presented as means ± standard error of triplicates.
[1)]Raw ground meat was salted (0, 1, 2, and 3% NaCl, respectively), stored for 0 or 3 d, and then cooked to 75°C in a 90°C water bath.
[A-C]Means within a row with different superscript letters are significantly different (p<0.05).
[x,y]Means within a column with different superscript letters are significantly different (p<0.05).

Residual nitrite, nitrosyl hemochrome and total pigments contents

Residual nitrite contents were significantly ($p<0.05$) increased as NaCl level was increased from 0 to 3% on day 0 (Table 3). Products with 2% and 3% NaCl had the highest ($p<0.05$) nitrite contents. On day 3, nitrite contents of 0% and 1% NaCl samples were not significantly different ($p>0.05$) from each other. However, nitrite contents were increased with increasing salt level. The highest ($p<0.05$) nitrite contents were obtained for products containing 3% NaCl on day 3. These results on the effect of NaCl on residual nitrite are similar to those of Ahn and Maurer (1989a), which demonstrated that the addition of salt increased residual nitrite contents in oven roasted turkey breasts. These results suggest that adding salt might have inhibited the conversion from nitrite to nitric oxide. Depending on presalting period, nitrite contents of products with 0% and 1% NaCl on day 3 were lower ($p<0.05$) than those on day 0. Nevertheless, residual nitrite contents observed in this study were less than 1 ppm, the amount known to cause pink color in poultry products (Ahn and Maurer, 1987). Nitrosyl hemochrome, the heat stable pink pigment of cured meat products, can be formed from myoglobin reacting with nitric oxide reduced from nitrite upon cooking (Ahn and Maurer, 1987; Fox, 1987; Froning *et al*., 1969; Hornsey, 1956). In the current study, nitrite was not intentionally incorporated to determine pink color characteristics that developed naturally in cooked ground chicken. Even in uncured meat, when a small but sufficient amount of nitrite is present, it may form pink nitrosopigment (Holownia *et al*., 2003). In this study, nitrosyl hemochrome contents of cooked products ranged from 0.68 ppm to 2.54 ppm on day 0 and from 0.89 ppm to 1.76 ppm on day 3 (Table 3). Products without salt (0% NaCl) had higher trends in nitrosyl hemochrome contents than those with salt (1%, 2%, 3% NaCl) on both day 0 and day 3 (Table 3). According to Ahn and Maurer (1989a), salt addition to turkey breast resulted in less nitrosoheme pigment formation caused by limited conversion of nitrite to nitric oxide. However, no significant ($p>0.05$) differences in nitrosyl hemochrome content between day 0 and day 3 were found among all treatments.

Presalting period and salt level had very limited effect on total pigment contents of cooked ground chicken breasts (Table 3). Total pigment contents were similar ($p>0.05$) to each other across all treatments on day 0. On day 3, no significant ($p>0.05$) differences in total pigment content were found among treatments except that 2% NaCl products had lower ($p<0.05$) total pigment contents compared to 0% NaCl products.

Conclusion

Short-term presalting and salt level had limited effects on the development of pink color in cooked ground chicken breasts. When chicken meat with 3% salt was stored for 3 d, redness was reduced after cooking probably due to more denaturation of myoglobin. To prevent undesirable pink color defects in commercial production practices, processors should avoid storage of ground chicken meat when mixed with less than 1% NaCl. Further studies to better understand mechanisms to develop a natural pink color should focus on ground chicken breast with salt level lower than 2% when meat stored for longer than 3 d.

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