

**ARTICLE**

Limiting Pink Discoloration in Cooked Ground Turkey in the Absence or Presence of Sodium Tripolyphosphate Produced from Presalted and Stored Raw Ground Breasts

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Abstract The effects of pink inhibiting ingredients (PII) to eliminate the pink color defect in cooked turkey breast produced from presalted and stored raw ground turkey in the absence or presence of sodium tripolyphosphate (STP) were examined. Ground turkey breast was mixed with 2% sodium chloride and vacuum packaged. After storage for 6 d, ten PII were individually incorporated without or with added STP (0.5%) as follows: none (control), citric acid (CA; 0.1%, 0.2%, 0.3%), calcium chloride (CC; 0.025%, 0.05%), ethylenediaminetetraacetic acid disodium salt (EDTA; 0.005%, 0.01%), and sodium citrate (SC; 0.5%, 1.0%). Treatments were cooked at a fast or slow cooking rate, cooled, and stored before analysis. All PII tested were capable of lowering inherent pink color compared to the control (No STP: CIE a* pooled day reduction of 23.0%, 5.2%, 12.6%, and 12.6% for CA, CC, EDTA, and SC, respectively; STP: reduction of 21.5%, 17.4%, 6.0%, and 18.2% for CA, CC, EDTA, and SC, respectively). For samples without STP, fast cooking rate resulted in higher CIE a*. However, slow cooking resulted in more red products than fast cooking when samples included STP. Presalting and storage of ground turkey caused the pink discoloration in uncured, cooked turkey (CIE a* 6.24 and 5.12 for without and with STP). This pink discoloration can be decreased by inclusion of CA, CC, EDTA, or SC, but incorporation of CA decreased cooking yield. In particular, the addition of SC may provide some control without negatively impacting the cooking yield.

Keywords pink color defect, ground turkey breast, pink inhibiting ingredients, sodium tripolyphosphate

Introduction

The pink color defect in poultry products is a color quality problem, but not a food safety issue, which occurs sporadically in well-cooked, uncured products (Suman and Joseph, 2014). This defect can cause serious economic losses associated with consumer

complaints and buyer discounting because the pink colored products are perceived to be under-cooked (Cornforth et al., 1991; Holownia et al., 2003; Holownia et al., 2004). There are numerous mechanisms associated with the generation of the pink color defect that include nitrate and nitrite contamination (Ahn and Maurer, 1987; Froning et al., 1969a; Mugler et al., 1970), ammonia exposure (Shaw et al., 1992), nicotinamide (Cornforth et al., 1986; Schwarz et al., 1998), exhaust fumes (Froning et al., 1969b), oxidation-reduction potential (Cornforth et al., 1986; Cornforth et al., 1991), irradiation (Nam and Ahn, 2002), carbon monoxide and nitric oxide in oven gases (Ahn and Maurer, 1989; Cornforth et al., 1998; Nam and Ahn, 2002; Pool, 1956), undenatured pigment and hemochromes (Ghorpade and Cornforth, 1993; Trout, 1989), and cytochrome c (Ahn and Maurer, 1989; Ahn and Maurer, 1990a; Ahn and Maurer, 1990b; Girard et al., 1990). Because of the diversity of factors involved that can cause this defect, its commercial occurrence is unpredictable and difficult to prevent. Many studies have investigated the effects of various ingredients on eliminating or reducing the pink color defect in products in which the pink color was induced by incorporation of pink-generating ligands (nicotinamide, nitrite). Ingredients evaluated included dairy proteins (Sammel and Claus, 2003b; Slesinski et al., 2000a; Slesinski et al., 2000b), citric acid (CA; Kieffer et al., 2000; Sammel and Claus, 2003a; Sammel and Claus, 2006), metal chelators (Schwarz et al., 1999), calcium chloride (CC; Claus et al., 2010; Sammel and Claus, 2007) and sodium citrate (SC; Sammel and Claus, 2003a; Sammel et al., 2006). Sammel and Claus (2003a) reported that CA (0.2% and 0.3%) and SC reduced the pink color in ground turkey rolls but not in intact turkey breasts. However, in their study, CA decreased the pH and cooking yield, whereas SC did not. Schwarz et al. (1999) also found that ethylenedinitrilo-tetraacetic acid disodium salt (EDTA) was effective on reducing the pink color in cooked, uncured ground turkey. With respect to CC effect, Sammel and Claus (2007) examined its ability to reduce the pink color defect induced by sodium nitrite and nicotinamide in cooked ground turkey in the absence and presence of sodium tripolyphosphate (STP) and SC. They concluded that a combination of CC and SC in the presence of STP was the best means for inhibiting the pink discoloration in uncured ground turkey. Unlike these previous efforts on cooked pink products induced by pink-generating ligands, based on an industrial practice, Claus and Jeong (2018) evaluated the processing conditions associated with the formation of pink discoloration. They were the first to report that a pink defect can be reproduced without adding a pink-generating ligand to a fully cooked ground turkey if the meat had been presalted and stored before cooking. They found that when ground turkey that was salted, stored for 6 d, and cooked, it produced a cooked product with the most reducing condition and was one of the most red. In a similar processing condition for chicken breast, Bae et al. (2018) found that ground chicken with less than 2% salt added and stored 7 d can produce a pink color in cooked chicken breast among the samples with different salt levels (0% to 3%) without any addition of a pink-generating ligand. Similarly, Jeong (2017) suggested that presalting and storage for more than 3 d may result in a pink color in cooked chicken breast if less than 1% sodium chloride is added. In addition, myoglobin and cytochrome c denaturation which is affected by thermal input, not just endpoint temperature but time and temperature. A study of Ryan et al. (2006) showed that a difference in cooking rate affected the degree of meat pigments denaturation of cooked meat products. They found that fast cooking to the same endpoint temperature of ground beef patties caused a pinker appearance rather than slow cooking. Further, cytochrome c is more heat stable than hemoglobin or myoglobin and has great stability up to 80°C. However, when phosphate is added, the thermostability of myoglobin increases, whereas that of cytochrome c decreases (Ahn and Maurer, 1989; Trout, 1989).

Although recent research has documented the ability to produce a natural pink color defect associated with presalting and storage without adding pink-generating ligands, the efficacy of inhibiting this natural pink defect has not been evaluated with known pink inhibiting ligands (PII; Claus and Jeong, 2018). The main objective of this study was to determine the ability to remove the pink discoloration, associated with the storage of presalted ground turkey, in cooked ground turkey using PII

(CA, CC, EDTA, SC) in the absence or presence of STP. An additional objective included determining the impact of cooking rate on the presence of the pink color associated with the incorporation of the PII and absence or presence of STP.

Materials and Methods

Raw material preparation

The processing procedures (salting, grinding, storage of the raw turkey) reported by Claus and Jeong (2018) that produced the most intense and consistent pink color defect were used. Fresh, skinless, boneless, ground turkey breasts (1 d post-mortem; 0.64-cm plate; *pectoralis major*) were obtained from Jennie-O Turkey Store (Willmar, MN, USA). A total of 40 kg of ground turkey was used for each replication and mixed (Model A120T, Hobart Corporation, Troy, OH, USA) with 2% sodium chloride (meat weight basis, MWB) for 5 min, and then separated into 20 individual bags (~1.9 kg) before being vacuum-packaged (Item # 75001875, Prime Source Vacuum Pouches, Koch Supplies, Kansas City, MO, USA; Model EASY-PACK, Koch Supplies). The starting salted meat was stored for 6 days at 2°C to 3°C. Each batch (1,530 g salted meat) from 20 packaged samples was randomly selected. Each batch received 10% distilled, deionized water based on the meat weight (absence of STP) or 0.5% STP (MWB) in distilled, deionized water (10% MWB). In addition, each batch received a PII at a given level in 10% (MWB) distilled, deionized water such that each batch received a total of 20% added solution. One independent experiment was conducted without added STP and another independent experiment included added STP. In each experiment, ten PII treatments (T) that were made included a: Control (distilled water, no added PII), CA (0.1%, 0.2%, or 0.3%), CC (0.025% or 0.05%), EDTA (0.005% or 0.01%), and SC (0.5% or 1.0%). Each batch was mixed (Model Max Watts 300, KitchenAid, St. Joseph, MI, USA) for 5 min and then placed in a vacuum chamber (9 dial setting; Model EASY-PACK, Koch Supplies) to facilitate removal of air pockets. For CC treatments, the presalted ground turkey was first mixed with CC solution for 2 min after which the STP solution was added and then mixed for 3 min. This was done to minimize the formation of a calcium-phosphate complex prior to their incorporation (Sammel and Claus, 2007). Meat mixtures were stuffed into conical centrifuge tubes (approximately 50 g each) and centrifuged at 2,000×g for 10 min (Model J-6M, Beckman Instruments, Palo Alto, CA, USA) to remove air pockets (Claus and Jeong, 2018). The tubes from each batch were further separated into two groups depending on cooking rate. After all samples were stored overnight at 2°C to 3°C, cooking step was conducted using the procedure described by Bae et al. (2018). The fast cooking (5.43°C/min) was performed by placing the tubes in a preheated 90°C water bath (Isotemp 228, Fisher Scientific, Pittsburgh, PA, USA) and cooked to an internal temperature of 76.7°C. The slow cooking (2.59°C/min) was achieved by setting the water bath to 90°C immediately after loading the tubes into a 50°C water bath. The temperature was monitored using extra samples with thermocouples attached to a thermocouple scanner. Upon completion of the cooking process, the tubes were immediately cooled on ice for 20 min and stored at 2°C to 3°C overnight in the dark before being analyzed within two days. Depending on the absence or presence of STP and cooking rate, two randomly selected tubes were stored in the dark (2°C) for 14 d during which instrumental color and reflectance measurements were obtained.

Cooking yield and pH determination

Stuffed ground turkey meat samples were weighed prior to cooking and weighed again after cooking and cooling to determine cooking yield. Cooking yield was calculated as: $[\text{cooked sample weight} / \text{raw sample weight}] \times 100$. The pH value was measured on 10 g samples homogenized in 50 mL distilled, deionized water using a pH meter (Accumet AR50, Fisher Scientific).

Instrumental color and pigments determination

CIE $L^*a^*b^*$ was taken on cut surfaces of each cooked sample using a colorimeter (CR-300, 8 mm aperture, illuminant C; Minolta, Osaka, Japan) after calibration on a standard plate (CIE L^* 97.06, CIE a^* -0.14, CIE b^* 1.93). Each sample was cut parallel to the longitudinal axis and three measurements per slice were taken immediately after cutting. The reflectance of each sample was read using a multipurpose sample compartment (Model MPC-2200, Shimadzu, Kyoto, Japan) attached to an ultraviolet/visible scanning spectrophotometer (Model UV-2401PC, Shimadzu). Four readings were measured on cooked samples immediately following cutting. Nicotinamide hemochrome (rNIC) was estimated by the percent reflectance ratio of %R537 nm/%R553 nm (Schwarz et al., 1998). CIE color and reflectance ratios were taken on 1 and 14 days after cooking.

Myoglobin (Mb) contents and percentage myoglobin denaturation (PMD) determination

Mb was extracted from both uncooked or cooked turkey breast products using a procedure by Trout (1989) and Warriss (1979). Briefly, meat samples were homogenized with 4 volumes of 0.4 M phosphate buffer (pH 6.8), centrifuged, filtered, and then the absorbance was determined at 525, 572, and 700 nm (Krzywicki, 1979) using the UV/VIS spectrophotometer.

$$\text{Mb (mg/g)} = (A_{525} - A_{700}) \times 2.303 \times \text{dilution factor}$$

$$\text{PMD (\%)} = [1 - (\text{Mb concentration after cooking} / \text{Mb concentration before cooking})] \times 100$$

Nitrosyl hemochrome and total pigment analysis

Nitrosyl hemochrome and total pigments were measured on cooked turkey samples after extraction in 80% acetone and acidified acetone, respectively (Hornsey, 1956). After extraction, the absorbance at 540 nm (A_{540}) and 640 nm (A_{640}) were determined on the filtrate using the spectrophotometer. Nitrosyl hemochrome concentration (ppm) was determined by A_{540} times 290. Total pigment concentration (ppm) was determined by A_{640} times 680.

Statistical analysis

Data from the experiments were analyzed separately by the absence or presence of STP. Data for all dependent variables except for CIE color and reflectance data were analyzed as a split plot design with the PII, pink inhibiting ingredient treatment, represented the whole plot, and cooking rate (fast or slow) was the split plot. Data from CIE color and reflectance ratio were analyzed as a split-split plot design with pink inhibiting ingredient treatment as the whole plot, cooking rate (fast or slow) was the split plot, and storage days (1 and 14 d) represented the split-split plot. All data were statistically analyzed with the Proc Mixed procedure of the SAS program (SAS, 2000) to determine main and interaction effects. When significance ($p < 0.05$) was determined in the models, the significance of the means were further separated by pairwise comparisons using the pdiff option. Each experiment was replicated four times.

Results and Discussion

Effect of pink inhibiting ingredients without added sodium tripolyphosphate

Two or three levels of each PII were investigated in a ground turkey system without added STP in this experiment. The samples containing PII were compared to the control to evaluate their specific effectiveness on the pink color reduction.

Cooking yield

Cooking yield of cooked ground turkey breast was affected by a two-way interaction between treatment and cooking rate ($T \times C$, $p < 0.0001$; Table 1). At both cooking rate, the addition of CA (all levels) to samples resulted in a detrimental effect (an average reduction of 8.3%) on cooking yield and the greatest decrease (approximately 12.6%) occurring with the addition of 0.3% CA to samples was observed ($p < 0.05$). Conversely, cooking yield was increased ($p < 0.05$) by SC at both levels, resulting in the highest cooking yield for 1.0% SC (approximately 2.6%). This increase or decrease of cooking yield due to CA or SC might be related to differences in pH (Sammel and Claus, 2003a). However, at both cooking rates, cooking yield in either CC or EDTA samples did not differ ($p > 0.05$) from the control. Faster cooking caused higher ($p < 0.05$) cooking yield than slower cooking across all cooked samples tested (Table 1).

pH

The pH value of cooked ground turkey breast was affected by only treatment (T , $p < 0.0001$, Table 1). The pH value was

Table 1. Effects of pink inhibiting ingredients and cooking rate on cooking yield and pH values in cooked ground turkey breast

Main effects	Without added STP			With added STP		
	Cooking yield (%)		pH	Cooking yield (%)		pH
	Slow	Fast		Slow	Fast	
Treatment (T) ¹⁾						
Control	80.1 ^{cy}	87.9 ^{cx}	6.13 ^d	94.6 ^{ax}	93.9 ^{ax}	6.43 ^{de}
CA 0.1%	75.4 ^{dy}	81.5 ^{dx}	5.89 ^e	82.7 ^{by}	87.6 ^{bx}	6.24 ^g
CA 0.2%	71.3 ^{ey}	77.1 ^{ex}	5.61 ^f	81.7 ^{by}	87.9 ^{bx}	6.02 ^h
CA 0.3%	67.2 ^{fy}	73.1 ^{fx}	5.36 ^g	76.6 ^{cy}	83.2 ^{cx}	5.80 ⁱ
CC 0.025%	79.2 ^{cy}	88.0 ^{cx}	6.13 ^d	94.1 ^{ax}	94.0 ^{ax}	6.42 ^e
CC 0.05%	79.8 ^{cy}	87.8 ^{cx}	6.12 ^d	94.1 ^{ax}	93.1 ^{ax}	6.38 ^f
EDTA 0.005%	79.7 ^{cy}	87.4 ^{cx}	6.15 ^c	93.5 ^{ax}	93.2 ^{ax}	6.44 ^{cd}
EDTA 0.01%	79.9 ^{cy}	87.6 ^{cx}	6.16 ^c	92.1 ^{ax}	92.7 ^{ax}	6.45 ^c
SC 0.5%	84.2 ^{by}	90.4 ^{bx}	6.24 ^b	94.1 ^{ax}	94.4 ^{ax}	6.47 ^b
SC 1.0 %	88.6 ^{ay}	91.9 ^{ax}	6.29 ^a	94.2 ^{ax}	94.8 ^{ax}	6.50 ^a
(SEM)	(1.27)		(0.03)	(1.36)		(0.02)
Cooking rate (C) ²⁾						
Slow	78.5		6.01 ^a	89.78		6.32 ^a
Fast	85.3		6.01 ^a	91.50		6.31 ^b
(SEM)	(1.19)		(0.03)	(0.88)		(0.02)

¹⁾ Treatment: Ground meat was salted (2% NaCl) and stored for 6 days before pink inhibiting ingredients (PII: Control=no added PII; CA, CC, EDTA, SC) were incorporated, and then stored overnight prior to cooking.

²⁾ Cooking rate: The samples were cooked by loading the tubes into a 50°C water bath and then immediately setting the water bath to 90°C (slow cooking) or cooked to 76.7°C in a 90°C water bath (fast cooking).

^{a-i} Means within a column with unlike superscript letters are different ($p < 0.05$). Significant treatment by cooking rate interaction for cooking yield in the absence of STP ($p < 0.0001$) and the presence of STP ($p < 0.05$).

^{x-y} Means for cooking yield within an individual STP group (without or with) and row with unlike superscript letters are different ($p < 0.05$).

STP, sodium tripolyphosphate; CA, citric acid; CC, calcium chloride; EDTA, ethylenedinitrilotetraacetic acid disodium salt; SC, sodium citrate; PII, pink inhibiting ligands.

decreased ($p < 0.05$) with increasing levels of CA from 0.1% to 0.3% (range of pH 5.89 to 5.36). Conversely, EDTA increased ($p < 0.05$) pH value (range of pH 6.15 to 6.16) compared to the control (pH 6.13). Especially, pH value was increased ($p < 0.05$) as the SC level increased (range of pH 6.24–6.29) and higher ($p < 0.05$) than the control. However, the samples treated with CC showed a similar ($p > 0.05$) pH value to the control. Cooking rate effects on pH value were not observed ($p > 0.05$) and no significant ($p > 0.05$) interaction with treatment (T) on pH value was found.

CIE L*

CIE L* of cooked ground turkey breast was influenced by treatment (T, $p < 0.0001$), cooking rate (C, $p < 0.0001$), and storage day (D, $p < 0.0001$; Table 2). However, for CIE L*, interactions between main effects were not found ($p > 0.05$). All levels of CA increased CIE L* ($p < 0.05$) compared to the control, whereas incorporation of SC at 0.5% or 1.0% reduced ($p < 0.05$) CIE L* in cooked ground turkey (Table 2). However, EDTA or CC samples did not differ ($p > 0.05$) in CIE L* from the control. These results support the findings of Sammel and Claus (2007), who found that CC treatment did not affect the

Table 2. Effects of pink inhibiting ingredients without added STP, cooking rate¹⁾, and storage day²⁾ on CIE L*, CIE a*, CIE b* in cooked ground turkey breast

Main effects	CIE L*	CIE a*		CIE b*		CIE b*	
		Day 1	Day 14	Slow	Fast	Day 1	Day 14
Treatment (T) ³⁾							
Control	78.65 ^e	6.14 ^{ax}	6.33 ^{ax}	9.48 ^{ex}	9.22 ^{efy}	9.38 ^{dx}	9.32 ^{ghx}
CA 0.1%	79.39 ^{ab}	5.45 ^{cx}	5.03 ^{dey}	10.27 ^{cx}	10.20 ^{cx}	10.02 ^{cy}	10.45 ^{cx}
CA 0.2%	79.65 ^a	4.94 ^{dx}	4.65 ^{fx}	10.57 ^{bx}	10.57 ^{bx}	10.34 ^{by}	10.80 ^{bx}
CA 0.3%	79.23 ^b	4.47 ^{ex}	4.27 ^{gx}	10.89 ^{ax}	10.79 ^{ax}	10.64 ^{ay}	11.04 ^{ax}
CC 0.025%	78.61 ^e	5.84 ^{abx}	5.84 ^{bx}	9.55 ^{ex}	9.25 ^{efy}	9.31 ^{dx}	9.48 ^{fgx}
CC 0.05%	78.63 ^e	5.91 ^{abx}	6.05 ^{abx}	9.59 ^{ex}	9.34 ^{efy}	9.48 ^{dx}	9.45 ^{fgx}
EDTA 0.005%	78.65 ^e	5.96 ^{abx}	5.47 ^{cy}	9.85 ^{dx}	9.41 ^{ey}	9.39 ^{dy}	9.87 ^{ex}
EDTA 0.01%	78.50 ^e	5.51 ^{bex}	4.87 ^{efy}	10.26 ^{cx}	9.82 ^{dy}	9.83 ^{cy}	10.24 ^{dx}
SC 0.5%	78.12 ^d	5.83 ^{abx}	5.25 ^{edy}	9.50 ^{ex}	9.15 ^{fy}	9.05 ^{ey}	9.60 ^{fx}
SC 1.0 %	77.14 ^e	5.72 ^{bex}	5.00 ^{dey}	8.97 ^{fx}	8.82 ^{gx}	8.57 ^{fy}	9.22 ^{hx}
(SEM)	(0.25)	(0.29)		(0.19)		(0.19)	
Cooking rate (C)							
Slow	78.47 ^b	5.29 ^b		9.89			
Fast	78.85 ^a	5.57 ^a		9.66			
(SEM)	(0.23)	(0.26)		(0.18)			

¹⁾ Cooking rate: The samples were cooked by loading the tubes into a 50°C water bath and then immediately setting the water bath to 90°C (slow cooking) or cooked to 76.7°C in a 90°C water bath (fast cooking).

²⁾ Storage day: Samples were stored (2°C to 3°C) in the dark before color was determined.

³⁾ Treatment: Ground meat was salted (2% NaCl) and stored for 6 days before pink inhibiting ingredients (PII: Control=no added PII; CA, CC, EDTA, SC) were incorporated, and then stored overnight prior to cooking.

^{a-g} Means within a column with unlike superscript letters are different ($p < 0.05$). Significant treatment by storage day interaction for CIE a* ($p < 0.05$) and CIE b* ($p < 0.0001$). Significant treatment by cooking rate interaction for CIE b* ($p < 0.05$).

^{x-y} Means under CIE a* and CIE b* interaction effects within a row with unlike superscript letters are different ($p < 0.05$).

STP, sodium tripolyphosphate; CA, citric acid; CC, calcium chloride; EDTA, ethylenedinitrilotetraacetic acid disodium salt; SC, sodium citrate; PII, pink inhibiting ligands.

CIE L* of cooked ground turkey (0% STP). Faster cooking resulted in a higher ($p<0.05$) CIE L* than slower cooking (Table 2). Samples stored for 14 days after production had a slightly lower ($p<0.05$) CIE L* (78.8) than day 1 samples (78.5; SE, 0.23). Such a minor difference would not be expected to be perceptible by consumers.

CIE a*

Presalted, stored, cooked ground turkey without added PII showed the pink cooked color (CIE a* 6.24, control). Only one two-way interaction ($p<0.05$) between treatment (T) and storage day (D) was found for CIE a* (Table 2) and a three-way interaction was not observed ($p>0.05$). On day 1, samples at all levels of CA had a lower ($p<0.05$) CIE a* than the control, whereas the CC samples at both levels were not effective ($p>0.05$) at lowering in CIE a* (Table 2). In the case of EDTA and SC, a minimum of 0.01% EDTA or 1.0% SC was needed to reduce the CIE a* on day 1. All PII tested at all levels effectively lowered ($p<0.05$) the inherent pink color of cooked ground turkey breast on day 14 (except for 0.05% CC) compared to the control by an average reduction of 26.5%, 6.1%, 18.3%, and 19.0% for CA, CC, EDTA, and SC, respectively (Table 2). Increasing the level of an individual PII (CA, EDTA, SC) generally decreased CIE a* in the samples at 14 d. Inclusion of 0.3% CA was the most effective on reducing CIE a* (32.5% reduction, day 14) in cooked ground turkey. The addition of 0.1% CA and both levels of EDTA or SC to samples produced a lower ($p<0.05$) CIE a* on days 14 than on day 1, but other treatments including the control did not change ($p>0.05$) CIE a* through day 14.

CIE b*

Incorporation of PII had different effect on CIE b* in cooked ground turkey (Table 2). Two interactions ($T\times C$, $p<0.05$; $T\times D$, $p<0.0001$) were found for CIE b* (Table 2). In both cooking rates, samples with CA had significantly increased CIE b* as the amount of CA increased, whereas CC incorporation to samples did not change ($p>0.05$) the CIE b* compared to the control regardless of addition levels. This is similar to work done by Sammel and Claus (2007), who found that CC without STP addition had no impact on CIE b* in cooked ground turkey when samples were formulated without a pink inducing ligand or with sodium nitrite. When the samples cooked at slow rate, CIE b* was increased ($p<0.05$) by addition of EDTA at both levels or decreased ($p<0.05$) by 1.0% SC addition compared to the control. In the fast cooking rate, 0.01% EDTA or 1.0% SC was required to increase ($p<0.05$) or decrease ($p<0.05$) the CIE b* in comparison to the control (Table 2). With increasing cooking rate, lower CIE b* for the control, PII samples with both levels of either CC or EDTA, and SC at 0.5% were observed ($p<0.05$), but CA at all levels and SC at 1.0% were not affected ($p>0.05$) by cooking rate. On day 1, samples with either CA or EDTA were generally more yellow (higher CIE b*), whereas SC addition had less ($p<0.05$) yellow than the control (Table 2). However, the addition of CC to samples did not differ ($p>0.05$) in CIE b* than the control on day 1. The same trend was noted on CIE b* in samples stored for 14 days after production. As storage time increased from 1 to 14 days, samples containing CA, EDTA, or SC at all levels had a higher ($p<0.05$) CIE b*, whereas the control and samples with both levels of CC did not change the CIE b* (Table 2).

Myoglobin (Mb) content

Presalted ground turkey breasts treated with PII were stored overnight before Mb contents were measured prior to cooking. Mb contents of uncooked ground turkey were influenced by treatment (T, $p<0.05$; Table 3). Of the PII used, CA at 0.3%, EDTA at 0.005%, or SC at 1.0% resulted in lower ($p<0.05$) Mb contents than the control but no significant differences in Mb contents were found for any other of the ingredients at any level. The chemical state of myoglobin can be influenced by

Table 3. Effects of pink inhibiting ingredients without added STP, cooking rate¹⁾, and storage day²⁾ on Mb content, total pigments, PMD, rNIC, and nitrosyl hemochrome in cooked ground turkey breast

Main effects	Mb (uncooked, mg/g)	Total pigments (ppm)	PMD (%)		rNIC		Nitrosyl hemochrome (ppm)
			Slow	Fast	Day 1	Day 14	
Treatment (T) ³⁾							
Control	0.762 ^a	18.24 ^d	84.3 ^{bex}	85.9 ^{bex}	1.026 ^{bx}	1.004 ^{aby}	0.38 ^{cd}
CA 0.1%	0.753 ^{ab}	22.12 ^c	86.2 ^{abx}	86.6 ^{abex}	1.012 ^{dx}	1.001 ^{by}	0.40 ^{bcd}
CA 0.2%	0.708 ^{ab}	25.22 ^b	84.9 ^{bey}	87.3 ^{abx}	1.003 ^{ex}	0.998 ^{cy}	0.46 ^{abcd}
CA 0.3%	0.598 ^c	27.97 ^a	81.7 ^{dy}	86.2 ^{bex}	0.993 ^{fx}	0.991 ^{dx}	0.52 ^{ab}
CC 0.025%	0.738 ^{ab}	18.20 ^d	83.9 ^{ex}	85.3 ^{cx}	1.021 ^{ex}	1.003 ^{by}	0.35 ^d
CC 0.05%	0.759 ^a	18.26 ^d	83.6 ^{cy}	86.0 ^{bex}	1.020 ^{ex}	1.004 ^{aby}	0.38 ^{cd}
EDTA 0.005%	0.696 ^b	22.66 ^c	87.2 ^{ax}	86.0 ^{bex}	1.027 ^{abx}	1.006 ^{aby}	0.49 ^{abc}
EDTA 0.01%	0.762 ^a	23.09 ^c	87.2 ^{ax}	87.9 ^{ax}	1.020 ^{ex}	1.005 ^{aby}	0.53 ^a
SC 0.5%	0.744 ^{ab}	22.28 ^c	84.9 ^{bey}	86.9 ^{abex}	1.027 ^{abx}	1.006 ^{aby}	0.42 ^{abcd}
SC 1.0 %	0.695 ^b	22.32 ^c	84.2 ^{cy}	86.8 ^{abex}	1.031 ^{ax}	1.008 ^{ay}	0.36 ^d
(SEM)	(0.057)	(0.83)	(1.57)		(0.002)		(0.05)

¹⁾ Cooking rate: The samples were cooked by loading the tubes into a 50°C water bath and then immediately setting the water bath to 90°C (slow cooking) or cooked to 76.7°C in a 90°C water bath (fast cooking).

²⁾ Storage day: Samples were stored (2°C to 3°C) in the dark before color was determined.

³⁾ Treatment: Ground meat was salted (2% NaCl) and stored for 6 days before pink inhibiting ingredients (PII: Control=no added PII; CA, CC, EDTA, SC) were incorporated, and then stored overnight prior to cooking.

^{a-f} Means within a column with unlike superscript letters are different ($p < 0.05$). Significant treatment by cooking rate interaction for PMD ($p < 0.05$). Significant treatment by storage day interaction for rNIC ($p < 0.0001$).

^{x,y} Means under PMD and rNIC interaction effects within a row with unlike superscript letters are different ($p < 0.05$).

STP, sodium tripolyphosphate; Mb, amount of undenatured myoglobin; PMD, percentage myoglobin denaturation; rNIC, reflectance estimator of nicotinamide hemochrome (%R537 nm/%R553 nm); CA, citric acid; CC, calcium chloride; EDTA, ethylenedinitrotetraacetic acid disodium salt; SC, sodium citrate; PII, pink inhibiting ligands.

intrinsic and extrinsic factors such as pH and added ligands (Hunt et al., 1999). Perhaps EDTA and SC altered the chemical state of myoglobin which affected the absorbance at the wavelengths used to determine the content of undenatured myoglobin. In the case of 0.3% CA, it may have decreased the undenatured Mb content due to the pH lowering during storage overnight resulting in greater protein degradation (Gu et al., 2021). Therefore, the differences in Mb content due to the types and concentration of PII used in this study could not be sufficiently explained.

Total pigments

Total pigments were affected only by treatment (T, $p < 0.0001$) in cooked ground turkey breast (Table 3). No treatment by cooking rate interaction was found (T×C, $p > 0.05$; Table 3). Total pigments were increased ($p < 0.05$) as the addition level increased from 0.1% to 0.3% for CA and were higher ($p < 0.05$) for all levels of EDTA and SC than the CC samples and the control. However, no differences were found ($p > 0.05$) for total pigments between the CC and the control samples. Cooking rate did not affect total pigments in cooked ground turkey breast (C, $p > 0.05$).

Percentage myoglobin denaturation (PMD)

A two-way interaction (T×C, $p < 0.05$) was found for PMD (Table 2). When samples were cooked at slow rate, PMD decreased ($p < 0.05$) by 0.3% CA or increased ($p < 0.05$) at both levels of EDTA in comparison to the control. There were no

differences ($p>0.05$) in PMD for other ingredient treatments at any level. At fast cooking rate, PMD was not different ($p>0.05$) from the control for any of the ingredients used except 0.01% EDTA increased ($p<0.05$) PMD. Increasing cooking rate resulted in a greater ($p<0.05$) PMD in samples containing either 0.2% or 0.3% CA, 0.05% CC, or both levels of SC.

Reflectance ratio for nicotinamide hemochrome (rNIC)

The reflectance ratio of rNIC was used as an estimator of nicotinamide hemochrome with a greater value indicating higher concentrations (Table 3). An interaction between treatment and storage day ($T \times D$, $p<0.001$) for rNIC ratio was determined in the cooked ground turkey (Table 3). In addition cooking rate (C) affected the rNIC ratio (C, $p<0.05$; Table 3). The addition of all levels of CA, CC, or EDTA (except at 0.005%) were effective ($p<0.05$) on lowering the rNIC ratio on day 1 but 1.0% SC increased ($p<0.05$) the ratio compared to the control. On day 14, however, only either 0.2% or 0.3% CA was effective ($p<0.05$) to lower rNIC ratio than the control. With exception of CA 0.3% samples, the rNIC ratio of the control and all PII samples decreased ($p<0.05$) as storage time increased from day 1 to 14.

Nitrosyl hemochrome

Only treatment effects on cooked ground turkey breasts were observed for nitrosyl hemochrome (T, $p<0.05$) among the samples tested (Table 3). Nitrosyl hemochrome was similar ($p>0.05$) to the control with the exception of 0.3% CA and 0.01% EDTA across all ingredients tested. Cooking rate had no impact on nitrosyl hemochrome in cooked ground turkey breast (C, $p>0.05$). No two-way interaction ($T \times C$) was found ($p>0.05$) for nitrosyl hemochrome.

Effect of pink inhibiting ingredients with added sodium tripolyphosphate

This experiment has applicability to industrial use because STP is a commonly used non-meat ingredient in the manufacture of processed meats because it facilitates extraction of myosin to improve binding of meat pieces and it increases water-holding capacity. This experiment was conducted to investigate the combined effects of PII and STP on reducing a pink color defect in ground turkey breast products.

Cooking yield

A two-way interaction between treatment (T) and cooking rate (C) was found on cooking yield in cooked ground turkey breast ($T \times C$, $p<0.05$; Table 1). Cooking yield at both slow or fast cooking rate was reduced (11.6% reduction, $p<0.05$) by the incorporation of CA to samples (83.3%) with STP compared to the control (94.3%), and generally decreased with increasing the level of CA added in ground turkey breasts. This agrees with work by Sammel and Claus (2003a), who found that cooking yield was decreased as a result of CA addition to intact turkey breasts or ground turkey rolls in the presence of 0.5% STP. A slow cooking rate rather than a fast cooking resulted in less ($p<0.05$) cooking yield in the samples containing the same level of CA with STP and the least cooking yield was found ($p<0.05$) for 0.3% CA samples (19.0% reduction) cooked at slow cooking. However, other PII treatments, regardless of addition level at both cooking rate, were not different ($p>0.05$) from the control.

pH

The pH value of cooked ground turkey breast was affected by treatment (T, $p<0.0001$) and cooking rate (C, $p<0.05$; Table 1). No interaction ($p>0.05$) between treatment (T) and cooking rate (C) for pH value was found. Generally, the pH value was decreased by CA or CC, but increased by EDTA or SC addition compared to the control in the presence of STP (Table 1).

The same trend was previously observed in samples without STP in this study. The pH value was dramatically decreased ($p < 0.05$) as the level of CA increased and was the lowest for 0.3% CA samples with STP as was noted from works by Kieffer et al. (2000) and Sammel and Claus (2003a). Cooking at faster rate than at slower rate resulted in lower ($p < 0.05$) pH values in the cooked ground turkey products (Table 1).

CIE L*

Treatment (T) affected CIE L* of cooked ground turkey (T, $p < 0.0001$; Table 4). All levels of CA in samples containing

Table 4. Effects of pink inhibiting ingredients with added STP, cooking rate, and storage day on color and pigment properties in uncooked and cooked ground turkey breast

Main effects	CIE L*	CIE a*	CIE b*		Mb (uncooked, mg/g)	PMD (%)	rNIC	Nitrosyl hemochrome (ppm)	Total pigment (ppm)	
			Slow	Fast					Slow	Fast
Treatment (T) ¹⁾										
Control	75.07 ^{cd}	5.12 ^a	8.28 ^{dy}	8.69 ^{dx}	0.691 ^a	77.4 ^d	1.011 ^a	0.43 ^{bcd}	22.37 ^{ey}	24.54 ^{ex}
CA 0.1%	76.33 ^a	4.51 ^{cd}	9.14 ^{aby}	9.77 ^{ax}	0.787 ^a	85.4 ^{bc}	1.002 ^c	0.52 ^{ab}	27.66 ^{bex}	26.59 ^{ax}
CA 0.2%	75.97 ^{ab}	3.91 ^{fg}	9.36 ^{ax}	9.53 ^{abx}	0.728 ^a	87.3 ^{ab}	0.995 ^d	0.49 ^{abc}	28.60 ^{bx}	26.21 ^{aby}
CA 0.3%	76.11 ^a	3.64 ^g	9.48 ^{ax}	9.76 ^{ax}	0.736 ^a	89.2 ^a	0.990 ^d	0.55 ^a	30.34 ^{ax}	26.25 ^{aby}
CC 0.025%	75.46 ^{bc}	4.44 ^{cd}	8.44 ^{cdy}	9.44 ^{abx}	0.749 ^a	83.1 ^c	1.006 ^{abc}	0.49 ^{abc}	25.34 ^{dx}	26.00 ^{abex}
CC 0.05%	75.98 ^{ab}	4.02 ^{ef}	8.78 ^{bey}	9.61 ^{ax}	0.797 ^a	85.5 ^{bc}	1.003 ^{bc}	0.49 ^{abc}	26.48 ^{cdx}	25.36 ^{abex}
EDTA 0.005%	74.89 ^{de}	4.75 ^{bc}	8.08 ^{dy}	8.92 ^{cdx}	0.736 ^a	82.9 ^c	1.010 ^a	0.47 ^{abc}	25.70 ^{dx}	25.26 ^{abex}
EDTA 0.01%	74.78 ^{de}	4.88 ^{ab}	8.09 ^{dy}	8.86 ^{cdx}	0.712 ^a	83.1 ^c	1.009 ^a	0.54 ^a	26.12 ^{cdx}	26.09 ^{abex}
SC 0.5%	74.94 ^{cd}	4.36 ^{de}	8.27 ^{dy}	9.07 ^{bex}	0.748 ^a	84.1 ^c	1.009 ^a	0.42 ^{cd}	25.60 ^{dx}	25.50 ^{abex}
SC 1.0 %	74.40 ^e	4.02 ^{ef}	8.46 ^{cdy}	9.20 ^{bex}	0.734 ^a	85.3 ^{bc}	1.008 ^{ab}	0.36 ^d	25.45 ^{dx}	24.83 ^{bex}
(SEM)	(0.80)	(0.28)	(0.44)		(0.068)	(1.05)	(0.006)	(0.04)		(1.76)
Cooking rate (C) ²⁾										
Slow	74.76 ^b	4.67 ^a		8.64		82.6 ^b	1.008 ^a	0.48 ^a		26.37
Fast	76.03 ^a	4.06 ^b		9.28		86.1 ^a	1.001 ^b	0.47 ^a		25.66
(SEM)	(0.78)	(0.26)		(0.43)		(0.58)	(0.006)	(0.03)		(1.67)
Storage day (D) ³⁾										
Day 1	75.51 ^a	4.34 ^a		8.88 ^b			1.007 ^a			
Day 14	75.27 ^a	4.39 ^a		9.04 ^a			1.001 ^b			
(SEM)	(0.78)	(0.26)		(0.43)			(0.006)			

¹⁾ Treatment: Ground meat was salted (2% NaCl) and stored for 6 days before pink inhibiting ingredients (PII: Control=no added PII; CA, CC, EDTA, SC) were incorporated, and then stored overnight prior to cooking.

²⁾ Cooking rate: The samples were cooked by loading the tubes into a 50°C water bath and then immediately setting the water bath to 90°C (slow cooking) or cooked to 76.7°C in a 90°C water bath (fast cooking).

³⁾ Storage day: Samples were stored (2°C to 3°C) in the dark before color was determined.

^{a-g} Means within a column and main effect with unlike superscript letters are different ($p < 0.05$). Significant treatment by cooking rate interaction for CIE b* ($p < 0.05$) and total pigment ($p < 0.05$).

^{x,y} Means under CIE b* and total pigment interaction effects within a row with unlike superscript letters are different ($p < 0.05$).

STP, sodium tripolyphosphate; Mb, amount of undenatured myoglobin; PMD, percentage myoglobin denaturation; rNIC, reflectance estimator of nicotinamide hemochrome (%R537 nm/%R553 nm); CA, citric acid; CC, calcium chloride; EDTA, ethylenedinitrilotetraacetic acid disodium salt; SC, sodium citrate; PII, pink inhibiting ligands.

0.5% STP increased CIE L* ($p < 0.05$) compared to the control. Also, these results are similar to those of CA samples in the absence of STP in this study. Similarly, Kieffer et al. (2000) reported that adding 0.3% CA or 0.3% CA plus 1.0% nicotinamide to ground turkey with 0.5% STP were lighter than the non-treated samples. However, other PII treatments in the presence of 0.5% STP showed a similar CIE L* ($p > 0.05$) to the control except for 0.05% CC and 1.0% SC (Table 4). As cooking rate increased, CIE L* decreased ($p < 0.05$) in cooked ground turkey breasts. However, storage day (D) did not affect ($p > 0.05$) CIE L* and no interactions among the main effects were found ($p > 0.05$).

CIE a*

Significant effects on CIE a* of cooked ground turkey were found ($p < 0.0001$) in treatment (T) and cooking rate (C; Table 4). With the exception of EDTA at 0.01%, PII tested at all levels in the presence of STP were capable of lowering pink color in cooked ground turkey, thereby decreasing ($p < 0.05$) CIE a* compared to the control (average reduction of 21.5%, 17.4%, 6.0%, and 18.2% for CA, CC, EDTA, and SC, respectively). The similar trend was observed in the CIE a* of samples without STP, although 0.01% EDTA in the samples with STP was not effective at reducing inherent pink color. Schwarz et al. (1999) found that EDTA at 50 to 200 ppm with 2.0% NaCl and 0.5% STP were not effective at reducing CIE a* in cooked turkey breasts without pink generating ligands, which is partially similar to our result, although the addition of EDTA at 0.005% in our study affected the reduction of CIE a*. Mahoney and Graf (1986) reported that citrate and EDTA promote oxidative damage by increasing solubility and the oxidative-reduction of iron. Previously, Sammel and Claus (2006) found that CA and SC were effective on inhibiting CIE a* in ground turkey samples cooked prior to irradiation when samples were produced with 2% NaCl and 0.5% STP. They suggested that in the oxidized state, meat pigments are less likely to bind to the ligands that generate the pink color. Sammel and Claus (2007) also found that in the presence of phosphate, both CC and SC reduced pink cooked color induced by nicotinamide, and were the most effective in combination. As a general comparison, CA in STP containing cooked ground turkey may reduce the pink color (CIE a*) more effectively than in the absence of STP. Interestingly, EDTA appeared to be less effective on reducing the pink color in cooked ground turkey containing STP. CIE a* was lower ($p < 0.05$) with a fast cooking rate in the presence of STP compared to a slow cooking rate. This result was an opposite trend to our findings found in the absence of STP. However, storage day (D) did not affect ($p > 0.05$) CIE a* and interactions among the main effects were not found ($p > 0.05$). These results disagree with those of Claus et al. (1994), who found that increasing storage time generally increased CIE a* in turkey rolls treated with 2% nicotinamide. This disagreement could be due to the presence of pink generating agents in their samples during production prior to being stored.

CIE b*

A two-way interaction (T×C, $p < 0.05$) and storage day main effect (D, $p < 0.0001$) were observed for CIE b* in cooked ground turkey (Table 4). At slow cooking rate, incorporation of CA at all levels with 0.5% STP increased ($p < 0.05$) CIE b* compared to the control, but other PIIs tested had no effect ($p > 0.05$) on CIE b* regardless of the levels added (Table 4). When ground turkey samples were cooked at faster rate, CIE b* was increased ($p < 0.05$) by all levels of CA, CC, SC addition in the presence of STP but not EDTA ($p > 0.05$) which was not different from the control. With the exception of CA at 0.2% and 3%, the control and samples containing PIIs (CA, CC, EDTA, SC) with STP had higher ($p < 0.05$) CIE b* in fast cooking rate than slow cooking rate in the same treatments (Table 4). In addition, increasing storage day resulted in higher ($p < 0.05$) CIE b* in cooked ground turkey breasts (Table 4).

Myoglobin (Mb) content and percentage myoglobin denaturation (PMD)

Before cooking, the treatments had no effects on Mb contents of ground turkey breast (Table 4). Treatment (T, $p < 0.0001$) and cooking rate (C, $p < 0.0001$) had effects on PMD in cooked ground turkey breast (Table 4), but no treatment by cooking rate interaction was found ($p > 0.05$). Addition of PII in the presence of STP increased ($p < 0.05$) PMD compared to the control (Table 4). With the exception of CA, however, PMD was not affected ($p > 0.05$) by an increased level of PII added with STP. In the CA treatments with STP, 0.3% CA caused greater ($p < 0.05$) PMD in cooked ground turkey products than 0.1% CA, but 0.2% CA treatment was in between. This result may be due to the effect of pH by CA addition, as denaturation of myoglobin occurs more readily at lower pH (Renner, 1990; Trout, 1989). PMD at fast cooking rate was greater ($p < 0.05$) than a slow cooking rate (Table 4).

Reflectance ratio for nicotinamide hemochrome (rNIC)

The reflectance estimator of nicotinamide hemochrome was affected by treatment (T, $p < 0.0001$), cooking rate (C, $p < 0.0001$), and storage day (D, $p < 0.0001$; Table 4) in cooked ground turkey samples. Two-way or three-way interactions were not observed ($p > 0.05$) for rNIC ratio. Only samples containing CA (0.1%, 0.2%, or 0.3%) or CC (0.05%) with STP had a smaller ($p < 0.05$) rNIC ratio than the control (Table 4). For CA addition to ground turkey rolls, Sammel and Claus (2003a) reported that the reflectance estimator of nicotinamide hemochrome were reduced by 0.2% or 0.3% CA addition when samples were treated with nicotinamide in the presence of 0.5% STP. In their study, both 0.025% and 0.05% CC addition with 0.5% STP reduced the reflectance estimator of nicotinamide hemochrome compare to the control with added nicotinamide. These results agree with our results. However, other PII treatments had no effects ($p > 0.05$) on the rNIC ratio (Table 4). Fast cooking for turkey samples was more effective ($p < 0.05$) at lowering rNIC ratio compared to slow cooking and the rNIC ratio was also reduced ($p < 0.05$) as storage day increased from day 1 to 14.

Nitrosyl hemochrome and total pigments

Only a treatment effect was observed (T, $p < 0.05$) on nitrosyl hemochrome in cooked ground turkey breast (Table 4). Cooking rate (C, $p > 0.05$) did not affect nitrosyl hemochrome. A significant interaction was not found between independent variables ($T \times C$, $p > 0.05$). Most PII treatments did not affect (T, $p > 0.05$) nitrosyl hemochrome in cooked ground turkey. It is unknown why CA (0.3%) and EDTA (0.01%) resulted in more (T, $p < 0.05$) nitrosyl hemochrome than the control.

Total pigments were affected by a two-way interaction ($T \times C$, $p < 0.05$; Table 4). When ground turkey breast samples were cooked at slow rate, greater total pigments were found as a result of PII addition compared to the control ($p < 0.05$; Table 4) and the 0.3% CA treatment had the greatest ($p < 0.05$) total pigments among the PII treatments. In addition, at fast cooking rate, CA samples at all levels had greater ($p < 0.05$) total pigment than the control, but no differences ($p > 0.05$) in total pigments between the other PII treatments and the control were observed. As cooking rate increased, incorporation of CA at 0.2% and 0.3% produced samples with less ($p < 0.05$) total pigments, whereas other PII samples had the same effect ($p > 0.05$) by cooking rate on total pigments (Table 4).

Conclusion

The pink discoloration associated with storage of presalted ground turkey can be decreased by the addition of CA or CC. However, adding CA to ground turkey resulted in lower cooking yield. CIE a^* was generally reduced with increasing PII

levels in the absence and presence of STP. In the absence of STP, the slow cooking rate resulted a lower CIE a^* compared to the fast cooking rate. However, the opposite was observed in the presence of STP. These results indicate that the addition of SC may provide some control in reducing the unwanted pink color without negatively impacting the cooking yield when raw ground turkey is presalted and stored.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Claus JR, Jeong JY. Data curation: Claus JR, Jeong JY. Formal analysis: Claus JR, Jeong JY. Methodology: Claus JR, Jeong JY. Software: Claus JR, Jeong JY. Validation: Claus JR, Jeong JY. Investigation: Claus JR, Jeong JY. Writing - original draft: Claus JR, Jeong JY. Writing - review & editing: Claus JR, Jeong JY.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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