Evaluation of Induced Color Changes in Chicken Breast Meat During Simulation of Pink Color Defect

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ABSTRACT The objective of the study was to establish a pink threshold and simulate the pink defect in cooked chicken breast meat with treatment combinations that would induce significant changes in the color of raw and cooked meat. The subjective pink threshold used in judging pink discoloration was established at a* = 3.8. Samples of three color groups (normal, lighter than normal, and darker than normal) of boneless, skinless chicken breast muscles were selected based on instrumental color values. The in situ changes were induced using sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite at two levels: present and not present. Fillets in all treatments were subjected to individual injections, followed by tumbling, cooking, and chilling. Samples were analyzed for color [lightness (L*), red/green axis (a*), yellow/blue axis (b*)] and reflectance spectra. Simulation of the pink defect was achieved in eight of the 16 treatment combinations when sodium nitrite was present and in an additional two treatment combinations when it was absent. Pinking in cooked samples was affected ($P < 0.05$) by L* of raw meat color. Results confirmed that it was possible to simulate the undesired pinking in cooked chicken white meat when in situ conditions were induced by sodium chloride, sodium tripolyphosphate, and sodium nitrite. The continuation of the simulation study can aid in developing alternative processing methods to eliminate potential pink defects.

(*Key words*: chicken breast meat, color, pink defect, pinking in poultry, simulation)

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INTRODUCTION

Pinking (pinkness or pink tinge) of white meat in cooked poultry products is one of the quality defects that poultry industry is faced with. With this defect, white poultry meat displays areas that retain a pink color even after the meat has been heated to an internal temperature exceeding 71.1°C, the temperature required by the Food Safety and Inspection Service—USDA (FSIS-USDA, 1999). Restaurants, particularly fast food establishments, commonly use meat color rather than end-point cooking temperature to indicate proper procedures and may overcook their product due to this defect. Consumers, expecting off-white and gray hues of an uncured cooked white meat, may consider pinkness an indication of an undercooked product that is actually unsafe to eat. Even though food safety is not an issue in pink defect (no reports of illness have been associated with pinking in poultry), the defect may be responsible for substantial losses to the poultry industry due to rejection, rework, and condemnation. Factors related to pinking include various classes and types of pigments (Brown and Tappel, 1957; Fox, 1966; Ledward, 1974; Livingston and Brown, 1981; Izumi et al., 1982; Cornforth et al., 1986; Girard et al., 1989; Ghorpade and Cornforth, 1993); preslaughter factors such as genetics, feed, hauling and handling, heat and cold stress, and gaseous environment (Froning and Hartung, 1967; Froning et al., 1968a, 1969a, 1978; Babji et al., 1982; Ngoka et al., 1982; Sackett et al., 1986); stunning techniques (Ngoka and Froning, 1982; Froning, 1995; Young et al., 1996; Craig et al., 1999); incidental nitrate/nitrite contamination through diet, water supply, freezing, and processing equipment, and processing ingredients (Froning et al., 1969b; Mugler et al., 1970; Ahn and Maurer, 1987; Fleming et al., 1991); current industry procedures including the use of nonmeat ingredients and cooking methods and endpoint cooking temperatures (Froning et al., 1968b; Helmke and Froning, 1971; Janky and Froning, 1972, 1973; Ahn and Maurer, 1989a,b, 1990a,b; Trout, 1989; Claus et al., 1994; Cornforth et al., 1998); and, recently, irradiation

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Abbreviation Key: a* = red/green axis; b* = yellow/blue axis; L* = lightness.

of precooked products (Ahn and Maurer, 2002; Nam and Ahn, 2002). This well-documented pink discoloration also may be attributed to specific in situ conditions such as pH, reducing conditions, the chemical state and reactivity of pigments, degree of denaturation, and reactivity of endogenous meat compounds.

Good manufacturing practices concentrate on efforts to reduce or eliminate external contamination by nitrate and nitrite such that nitrosopigments would not significantly contribute to the pink discoloration. When studying the pinking phenomenon in white poultry meat, researchers attempt to use pink-generating ligands such as nicotinamide and nitrite to induce pinking (Schwarz et al., 1997, 1999; Slesinski et al., 2000a,b). Schwarz et al. (1997, 1999) hypothesized that because hemochromes are formed when a specific ligand binds to the pigment, it may be possible, without generating a pink color, to bind such a ligand to the sixth coordinate position of heme iron. In addition, there is an opportunity for some dairy proteins or some components of dairy proteins to reduce or eliminate the pink defect (Slesinski et al., 2000a,b). However, the mechanism responsible for this reduction is not yet established.

Interactions of factors and mechanisms associated with the pink defect are complex. We believe that the simulation of the pink defect is an essential tool to investigate procedures for its prevention and elimination. For that reason, a study was initiated to simulate the defect in the intact breast muscles. This defect was simulated by modifying the in situ conditions using the ingredients that are commonly used by the meat industry. Therefore, our specific objectives were establishing a subjective pink threshold for judging the existence of pinking and examining the effects of treatments that induce significant changes in the color of raw and cooked meat.

MATERIALS AND METHODS

Sample Collection and Preparation

Boneless, skinless, chicken fillets (pectoralis major) were obtained from two commercial processing plants. Breast fillets were selected based on three color groups: lighter than normal (light), normal (normal), and darker than normal (dark) (Fletcher, 1999; Fletcher et al., 2000). Previous research has shown (Fletcher, 1999; Fletcher et al., 2000) that muscle pH is highly correlated with meat color. Due to higher pH values observed in dark breast muscles and low pH values observed in light colored breast muscles (Fletcher, 1999) the selection into three color groups was important for this experiment. The initial differences in the endogenous conditions may indicate different responses to applied experimental treatments. Fillets were first sorted based on visual appearance at the deboning line or at the beginning of a further-processing line. Sorting was verified using Hunter Lab reflectance colorimeter;[2] fillets were selected based on the medial surface (bone side) lightness (L*) values. L* values for the three color groups were $L^* < 47$ for dark, $47 < L^* < 50$ for normal, and $L^* > 50$ for the light. The samples were then bagged according to color group (light, normal, and dark), packed with ice, and transported to the laboratory.

The experiment was repeated twice for each of the two processing plants. To introduce seasonal variation in the material, each sampling for replication was separated by a period of at least 4 mo. Two hundred forty fillets (eighty for each of the three color groups) were collected for each replication per plant resulting in 960 product samples. All 240 samples associated with each replication and plant could not be collected during a single visit due to unavailability of the desired number of breast fillets (80) in each color group at a given time. Thus two or three visits were made as needed.

To ensure collection of proper number of samples, meat was frozen before the experiment as a temporary storage method. To minimize effect of freezing and subsequent storage on meat protein and pigment degradations, all breast fillets (24 h postmortem) were vacuum-packed[3] into sampling polyethylene bags, labeled and coded individually, and then frozen and stored at –18°C for up to 3 wk before the experiment. After adequate thawing at 4 to 5°C (approximately 10 to 12 h) samples were ready for study. There were four treatment factors, namely sodium chloride[4] (1 g/100 g meat), sodium tripolyphosphate[5] (0.5 g/100 g meat), sodium erythorbate[6] (0.0546 g/100 g meat), and sodium nitrite[7] (1 ppm). Two levels of factors were selected: present and not present. Therefore, 16 treatment combinations were used. The level of each factor was chosen according to standard regulations and common industry applications. The injection method (12% of meat weight basis) was used to incorporate ingredients into the samples. The ingredients were prepared in stock solutions: 0.1% sodium nitrite, 10% sodium tripolyphosphate, and 1% sodium erythorbate. Next, the stock solutions were combined to obtain the final volume required for each sample. Finally, according to the weight of a given sample, sodium chloride was added as dry ingredient to the injection solution. Separate volumes of injection solutions were prepared and injected. Sixty-four injection solutions were required (4 muscles × 16 treatment combinations). Uniformly spaced multiple injections were carried out with a 35-mL syringe[8] equipped with a stainless steel 16-hole spray needle.[9] Once injected, samples were packed individually in moisture-impermeable polyethylene bags and closed, tumbled for 15 min

[2]MiniScan/XE 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA.
[3]Ultravac 250 Koch Packaging, Division of Koch Supplies, Inc., Kansas City, MO.
[4]Catalog No. S271-3, Fisher Scientific, www.fishersci.com.
[5]Catalog No. T-5883, Sigma Chemical Co., St. Louis, MO.
[6]Catalog No. 49,633-2, Aldrich Chemical Company, Inc., Milwaukee, WI.
[7]Catalog No. S-2252, Sigma Chemical Co.
[8]Monoject 35-cc syringe, Sherwood Medical Co., St. Louis, MO.
[9]Koch No. 30410306, Koch Supplies, N. Kansas City, MO.

with ice, and refrigerated for equilibration overnight (12 h) at 4 to 5°C before further experimentation.

The subjective pink threshold used in judging the pink discoloration was produced by injecting samples from the normal group with a solution containing sodium chloride and sodium nitrite to obtain a final concentration of 1, 2, 3, 4, and 5 ppm in the sample. The stock and injection solutions were prepared as explained above.

The tumbling process was done in a laboratory made small tabletop tumbler (outer diameter 40 cm, two equally spaced webs). Each time, four individually packed muscles (~ 25% load) from the same treatment were tumbled for 15 min at 45 rpm with 1.0 kg of ice in order to keep the product temperature at approximately 0°C.

The following morning, two fillets of four from the experimental units were designated for raw analysis, and the other two were subjected to cooking. The two fillets were placed in aluminum mini broiler pans[10] and covered with aluminum foil. Samples were cooked in a 167°C convection oven[11] to an internal temperature of 74°C and then cooled to 48°C within first 45 min in a temperature-controlled laboratory where the temperature was maintained constant at 21°C. Next, the samples were cooled to 4.4°C by placing them in a walk-in environmental cooler maintained at 4°C. The final targeted temperature of the samples (~ 4°C) was achieved within 4 h. Internal temperature of the product was monitored throughout the cooking and cooling process by using K-type chromel-alumel thermocouples inserted in the center of a cranial end of a muscle before cooking. One thermocouple was used per cooking pan and eight pans full (eight treatments with duplicate samples) were cooked at a time. HP BenchLink Data Logger 1.1 and data acquisition unit[12] was used for data collection.

Color Evaluation

Color was objectively evaluated, using reflectance spectrophotometry[2] (large area view, 25-mm aperture with illuminant D65-daylight, 10° standard observer, wavelength of 400 to 700 nm with 1.0 nm accuracy), for reflectance spectra and colorimetric CIE (1978) L*, red/green axis (a*), and yellow/blue axis (b*). In addition, subjective color evaluation was done, which included visual appraisal by the same experienced individual throughout the simulation study. Cooked samples were viewed using a light booth[13] (D65 as the illuminant source). The raw and cooked samples were sliced into halves, lengthwise horizontally. Immediately, color was visually appraised, and color measurements taken. Four color measurements were taken on each half of the fillet; two readings on the anterior and two on the posterior portion of the muscle, rotating the samples 90° between measurements. The average value of the eight measurements (four per half) was subjected to statistical analysis. Chroma and hue angle were calculated based on average a* and b* values to further explain color change between samples:

$$\text{chroma, } C = [(a^*)^2 + (b^*)^2]^{1/2}$$

$$\text{hue angle, } h^\circ = \tan^{-1}(b^*/a^*).$$

The reflectance data at each wavelength were averaged by treatment and converted into a graph with Microsoft Excel.[14]

Statistical Analyses

The experiment was a partially confounded 2^4 factorial. Only eight of the 16 treatments could be evaluated per day; therefore, the experiment was arranged into two blocks, thus introducing a confounding effect. The plant, replication, and treatments effects for L*, a*, b* color values were analyzed using the ANOVA option of the general linear models (GLM) procedures of SAS software (SAS Institute, 1989). All possible 15 orthogonal contrasts (treatment combinations) were analyzed and estimated. Planned comparisons between treatments were done using the LSD method only if ANOVA F-test and corresponding contrasts were significant at $P < 0.05$ (Fisher protected LSD). Otherwise, planned comparisons with the Bonferroni method ("data snooping") was performed (LS means option with PROG GLM procedure of SAS Institute, 1989). The three tested color groups in the experiment were subjected to individual statistical analyses.

RESULTS AND DISCUSSION

Subjective Pink Threshold

Establishment of a subjective pink threshold was an important step toward setting a cutoff for judging the existence of pinking during the experiment. Table 1 presents instrumental color values of the cooked samples examined to establish the pink threshold. Visual appraisal of the cooked samples in the light booth showed all the samples injected with nitrite to have pink discoloration. Samples injected with only 1 ppm of sodium nitrite had a light pink discoloration that was distinguishable from a control sample injected with sodium chloride only. Samples injected with 1 ppm sodium nitrite and 1% sodium chloride were compared to the controls injected with 1% sodium chloride; this comparison resulted in establishing the least discoverable visible pinking in cooked samples. During the six replications, a* values ranged from 3.79 to 3.85 for the 1 ppm level. Statistical analysis showed no significance for L* and b* between samples injected with different sodium nitrite levels (with the exception of the

[10]Mini Broiler Pans Size: 20 cm × 16.2 cm × 3.3 cm, Handi-Foil Corp., Wheeling, IL.
[11]Stabil-Therm Lindberg/Blue M Electric Oven, GS Lindberg/Blue M, Blue Island, IL.
[12]HP 34970A Data Acquisition/Switch Unit with 20 Channel HP 34901A Multiplexer, Hewlett-Packard Company, Loveland, CO.
[13]Byko-Spectra 1 CG-6050, BYK-Gardner, Columbia, MD.
[14]Microsoft Excel. 2000. Microsoft Corp., Redmond, WA.

TABLE 1. Mean (± SEM) CIE lightness (L*), red/green axis (a*), and yellow/blue axis (b*) color parameters of cooked breast fillets in pink threshold evaluation[1]

Sodium nitrite concentration (ppm)	L*	a*	b*
0 (Control)	81.17 ± 0.28	2.62 ± 0.37[d]	15.92 ± 0.53[a]
1	81.91 ± 0.27	3.81 ± 0.02[c]	12.36 ± 0.34[b]
2	82.26 ± 0.27	4.01 ± 0.15[bc]	12.50 ± 0.71[b]
3	80.98 ± 0.74	4.38 ± 0.16[b]	11.74 ± 0.59[b]
4	80.01 ± 0.04	4.71 ± 0.19[a]	11.32 ± 0.28[b]
5	80.07 ± 0.06	4.94 ± 0.34[a]	11.67 ± 0.71[b]

[a–d]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]n = 6. Normal color group muscles.

control sample). The a* value of the sample injected with 1 ppm of nitrite differed from those injected with 3, 4, or 5 ppm but not from that injected with 2 ppm. In samples injected with 4 or 5 ppm, a* values were significantly different from the rest of the samples. The change in a* value was accompanied by change in hue angle, which decreased from 72.86° in 1 ppm to 67.05° in 5 ppm sample. Chroma values were similar for all the pink samples and ranged from 12.26 to 13.12. The highest chroma value (16.13) and hue angle (80.65°) were found in the control sample. The results indicate that among pink samples, the a* value, but not L* or b* values, were affected by nitrite. Thus, when chroma values were similar, a* value seemed to cause the most changes in hue angle. Therefore, a* is the best choice to use as the threshold value within the range 79 to 84 for L* value and 10 to 16 for b* value. Previous research (Heaton et al., 2000) had also confirmed that the a* value correlates well with sensory (visual) scores of meat color. A comparison of results from a visual examination and colorimetric data established the least visible pinking at a* = 3.8. This value represents an average value from six replications. The pink threshold presented here is even lower than the a* values > 4.0 for white meat reported by other authors (Ahn and Maurer, 1989b; Heaton et al., 2000).

Colorimetric Analysis

Significance of Plant and Replication. The results of the combined ANOVA of raw and cooked meat for replication, plant, and contrast effects in color measurement are presented in Tables 2, 3, and 4 for light, normal, and dark groups, respectively. The replication effect within each plant was originally tested using a block-within-plant effect as a divisor in a confounding design. The most variation within plant was found in the light group, in which replication was significant for both plants with regard to L* in raw samples. In addition, the second plant showed significance of replication for a* and b* in raw samples and L* in cooked samples. There was only a significant replication effect in the second plant when L* of cooked samples was analyzed for the normal group. The dark group showed no significant effect of replication in either plant. The results indicated that some variability existed, especially in light muscles, even within a single plant. Testing the plant effect by using residual error as a divisor showed no significance ($P > 0.05$) for CIE L*a*b* colors of raw or cooked samples. Thus, in subsequent analyses the data were pooled across plants and replications.

Color of Raw Samples. The L* values of raw samples for the light, normal, and dark groups were significantly affected by 1% of sodium chloride, with decreases from 63.33 to 54.57, from 56.03 to 50.52, and from 50.94 to 38.02, respectively. The presence of sodium tripolyphosphate in injection solutions produced a negative effect ($P < 0.05$) on L* value for light and normal groups but not for the dark group. The a* value of the light and dark groups also was affected by sodium chloride. In the light and normal groups, a significant positive effect on a* value occurred for sodium nitrite when it was injected alone or in combination with other ingredients. The a* values ranged from 7.82 to 10.24 vs. 5.45 for the light control group, and from 8.71 to 11.88 vs. 7.81 for the normal control group. Thus, the presence of nitrite resulted in direct formation (without oxidation to metmyoglobin first) of red appearing nitric oxide myoglobin. The use of intact muscle for the simulation probably allowed the reducing activity of the breast muscles to be conserved. Values of b* of the raw samples in the dark group were influenced by salt and phosphate in the injection solutions. Whereas sodium chloride decreased the b* value from 17.63 (control) to 15.47 (salt treated), it increased the b* value to 20.04 for phosphate-treated samples.

The three preselected muscle color groups responded differently to the injection treatments. The light group showed a decrease only in the L* value. In contrast, the normal and dark groups showed both increased and decreased L* and a* values depending on treatment combinations. An increased a* value of raw muscles was observed in all color groups, especially when sodium tripolyphosphate was present in the injection solution. A similar tendency toward decreased L* and increased a* was reported by Yang and Chen (1993). In contrast, Allen et al. (1998) observed higher L* and lower a* in the marinated breast muscles from light and dark groups.

Color of Cooked Samples. Based on the pink threshold value of a* = 3.8, simulation of the pink defect was achieved in eight of the 16 treatment combinations in the light group, nine in the normal group, and 10 in the dark group (Table 5). Pinking in those samples was subjectively confirmed through visual evaluation in the light booth.

TABLE 2. Analysis of variance table[1] of CIE color parameters[2] for replication (within each plant), plant, and treatment contrast effect in the light group of raw and cooked samples

		Raw			Cooked		
Source	Statistic	L*	a*	b*	L*	a*	b*
Replication in							
Plant 1	F	116.70	0.03	4.20	3.85	0.10	11.35
	P	0.0085	0.8736	0.1769	0.1889	0.7835	0.0780
Plant 2	F	19.12	295.91	45.72	29.27	1.86	5.81
	P	0.0485	0.0034	0.0212	0.0325	0.3062	0.1375
Plant	F	0.13	0.75	1.13	0.62	0.19	0.01
	P	0.7575	0.4776	0.3996	0.5134	0.7085	0.9404
Treatments[3]							
S	F	58.40	8.56	3.71	9.56	0.01	8.10
	P	0.0001	0.0053	0.0602	0.0033	0.9301	0.0065
P	F	8.83	0.00	1.80	0.74	0.18	2.25
	P	0.0047	0.9936	0.1859	0.3932	0.6712	0.1403
SP	F	3.75	0.71	0.01	13.24	1.14	1.22
	P	0.0588	0.4030	0.9210	0.0007	0.2906	0.2746
E	F	0.09	0.00	0.00	0.41	0.02	0.11
	P	0.7671	0.9554	0.9657	0.5275	0.8838	0.7426
SE	F	0.00	0.01	1.31	0.04	0.07	0.05
	P	0.9770	0.9291	0.2583	0.5444	0.7961	0.8314
PE	F	1.08	0.10	0.05	0.22	0.05	0.11
	P	0.3034	0.7591	0.8271	0.6386	0.8239	0.7453
SPE	F	0.00	0.29	1.90	0.12	0.21	1.24
	P	0.9651	0.5956	0.1750	0.7335	0.6485	0.2708
N	F	0.49	7.03	0.02	4.01	209.91	73.71
	P	0.4876	0.0109	0.8983	0.0510	0.0001	0.0001
SN	F	0.43	0.60	0.62	0.00	0.59	1.48
	P	0.5152	0.4408	0.4346	0.9734	0.4448	0.2299
PN	F	0.19	0.28	0.02	0.02	2.42	0.61
	P	0.6617	0.5980	0.8865	0.8764	0.1262	0.4375
SPN	F	0.12	0.28	0.39	1.58	0.00	0.49
	P	0.7287	0.6019	0.5377	0.2145	0.9803	0.4894
EN	F	1.97	1.37	0.49	0.37	0.02	0.80
	P	0.1673	0.2476	0.4893	0.5432	0.8945	0.3759
SEN	F	1.39	0.03	0.09	1.37	0.55	0.28
	P	0.2448	0.8714	0.7679	0.2476	0.4609	0.6015
PEN	F	0.12	0.00	2.51	0.48	0.10	0.01
	P	0.7353	0.9718	0.1200	0.4932	0.7480	0.9434
SPEN	F	0.29	0.01	0.01	0.69	0.24	0.18
	P	0.5926	0.9219	0.9356	0.4096	0.6293	0.6725

[1]F = statistic; P = probability.

[2]L* = lightness; red/green axis; b* = yellow/blue axis.

[3]S = sodium chloride; P = sodium tripolyphosphate; E = sodium erythorbate; N = sodium nitrite.

There were no significant differences found in L* and b* values between tested treatments in the three color groups. L* values of 80.91 to 84.30, 79.07 to 83.27, and 78.88 to 82.26 were found in the light, normal, and dark groups, respectively (data not shown). The b* value varied between 9.48 and 16.33 in the light, normal, and dark groups (data not shown). Pinking in the eight cooked samples from the light group was directly related to the treatments containing sodium nitrite. The presence of sodium nitrite alone or in combination with other ingredients significantly increased a* value of the cooked fillets in the light group (Table 5). Samples containing nitrite also showed lower hue angle values (66.0° to 70.8°) when compared with other treatment combinations (79.2° to 81.7°). Lower hue angles were associated with redder samples. The effect was probably caused by both increased a* and decreased b* values. Pink samples in the light group did not show significant change in lightness compared to the rest of the treatments. Chroma values for these samples ranged from 10.5 to 13.2. These data are in agreement with previous published reports on the significant role of sodium nitrite manifesting pinking for concentrations as low as 1 ppm (Ahn and Maurer, 1989a; Heaton et al., 2000).

The pinking in the light group was not affected by a combination of salt, phosphate, and erythorbate; these ingredients, in combination influence pH, reducing conditions, and the solubility of proteins. However, because the light group had a well-established, low initial pH (Fletcher, 1999), it is unlikely that salt, phosphate, and erythorbate would cause enough changes under in situ conditions to promote pinking. A significant effect for sodium nitrite was found for the remaining color groups as well (Tables 3 and 4). The positive effect (increased a* value) was indicated by higher positive contrast estimates for the light, normal, and dark groups, respectively (data not shown). Estimated chroma and hue angle in the normal and dark groups showed the same trends as in the light group. Chroma values in pink samples containing sodium nitrite in the normal and dark groups ranged

TABLE 3. Analysis of variance table[1] of CIE color parameters[2] for replication (within each plant), plant, and treatment contrast effect in the normal group of raw and cooked samples

		Raw			Cooked		
Source	Statistic	L*	a*	b*	L*	a*	b*
Replication in							
Plant 1	F	0.39	0.48	0.13	2.72	0.02	7.44
	P	0.5946	0.5597	0.7562	0.2409	0.8910	0.1122
Plant 2	F	45.67	3.36	4.58	28.88	2.25	20.12
	P	0.0212	0.2083	0.1656	0.0329	0.2721	0.0463
Plant	F	0.96	10.52	5.37	14.47	0.00	0.93
	P	0.4304	0.0833	0.1463	0.0627	0.9589	0.4362
Treatment[3]							
S	F	68.21	0.54	0.05	13.41	5.49	1.58
	P	0.0001	0.4653	0.8323	0.0006	0.0234	0.2154
P	F	7.24	3.62	1.16	1.76	3.30	0.04
	P	0.0098	0.0632	0.2863	0.1916	0.0758	0.8446
SP	F	6.69	3.09	3.11	2.77	2.39	5.13
	P	0.0128	0.0853	0.0841	0.1027	0.1291	0.0282
E	F	0.33	0.14	0.05	0.87	5.15	0.15
	P	0.5685	0.7116	0.8162	0.3567	0.0278	0.6986
SE	F	0.44	0.04	0.41	0.30	0.31	1.65
	P	0.5096	0.8458	0.5256	0.5857	0.5792	0.2057
PE	F	0.38	0.00	0.06	2.00	0.00	0.01
	P	0.5386	0.9995	0.8046	0.1635	0.9751	0.9310
SPE	F	0.86	1.78	0.10	0.04	3.57	0.69
	P	0.3594	0.1890	0.7477	0.8376	0.0652	0.4110
N	F	0.45	12.35	0.00	2.21	144.68	85.13
	P	0.5043	0.0010	0.9490	0.1439	0.0001	0.0001
SN	F	0.63	1.09	0.21	0.00	6.05	0.09
	P	0.4299	0.3027	0.6511	0.9503	0.0177	0.7706
PN	F	1.15	0.07	0.45	0.08	3.16	0.11
	P	0.2894	0.7935	0.5038	0.7784	0.0820	0.7463
SPN	F	0.35	0.16	0.00	0.06	7.80	0.01
	P	0.5582	0.6893	0.9707	0.8108	0.0075	0.9237
EN	F	0.08	0.00	1.45	0.23	0.57	0.36
	P	0.7725	0.9477	0.2345	0.6339	0.4559	0.5511
SEN	F	3.22	0.36	0.36	0.44	0.01	0.07
	P	0.0794	0.5501	0.5540	0.5083	0.0906	0.7950
PEN	F	0.21	0.61	0.29	0.20	0.01	0.13
	P	0.6482	0.4403	0.5929	0.6597	0.0933	0.7155
SPEN	F	1.43	0.00	0.65	0.00	0.98	1.04
	P	0.2381	0.9977	0.4232	0.0985	0.3278	0.3120

[1]F = statistic; P = probability.

[2]L* = lightness; a* = red/green axis; b* = yellow/blue axis.

[3]S = sodium chloride; P = sodium tripolyphosphate; E = sodium erythorbate; N = sodium nitrite.

from 12.2 to 13.6 and from 11.9 to 14.7, respectively. Hue angles were affected by significantly higher a* values and significantly lower b* values (68.76° to 71.5° in the normal group and 62.1° to 69.5° in the dark group). It was also found that in all three color groups the presence of sodium nitrite significantly decreased b* values of the cooked fillets (Tables 2, 3, and 4). A similar significant decrease in b* values caused by sodium nitrite was reported by Ahn and Maurer (1989a). In addition to the eight treatments that induced pinking, a combination of sodium chloride, tripolyphosphate, and erythorbate also modified in situ conditions to an extent that produced pinking of cooked samples from the normal and dark groups. Those a* values exceeded the subjective pink threshold. The values were significantly different from a* values of the fillets with no pink defect (Table 5). Through a significant effect on the a* value, only a combination of sodium chloride with tripolyphosphate produced pinking in cooked samples from the dark group (Table 4).

Estimated chroma values for pink samples from the normal and dark groups ranged from 15.9 to 16.7. These chroma values for the normal and dark groups were similar to those of the respective control samples (15.2 to 16.1). Pink samples from the normal and dark groups lacking sodium nitrite had b* and chroma values similar to those of the control samples, showing that a* value was the main cause for lower hue angle and pinking in those samples. The hue angles of pink samples injected with sodium chloride and tripolyphosphate or the combination of sodium chloride, tripolyphosphate, and erythorbate ranged from 74.4° to 75.2°, whereas the control showed a range of 78.6° to 81.7°. Janky and Froning (1972) have previously shown that sodium erythorbate with polyphosphate increases redness of metmyoglobin in a meat system. The significant role of added phosphates, alone or in combination with sodium chloride, in increasing a* values has also been confirmed previously (Ahn and Maurer, 1989a,b).

Reflectance Spectra. The spectra for raw samples (Figure 1A) in all color groups were typical of myoglobin or mixtures of myoglobin (reflectance minimum ~560 nm) and metmyoglobin (reflectance minimum ~630 nm).

TABLE 4. Analysis of variance table[1] of CIE color parameters[2] for replication (within each plant), plant, and treatment contrast effect in the dark group of raw and cooked samples

		Raw			Cooked		
Source	Statistic	L*	a*	b*	L*	a*	b*
Replication in							
Plant 1	F	0.98	0.01	0.01	0.05	0.04	0.02
	P	0.4260	0.9998	0.9999	0.9994	0.8560	0.9997
Plant 2	F	0.24	1.17	0.02	0.41	15.46	0.32
	P	0.6747	0.3921	0.9954	0.5878	0.0509	0.6266
Plant	F	4.24	0.28	17.40	16.10	0.32	2.55
	P	0.1757	0.6509	0.0599	0.0565	0.6309	0.2514
Treatment[3]							
S	F	9.94	10.05	10.26	16.33	17.25	0.31
	P	0.0028	0.0027	0.0024	0.0002	0.0001	0.5815
P	F	0.12	0.91	8.26	1.19	1.32	20.82
	P	0.7298	0.3441	0.0061	0.2810	0.2567	0.0001
SP	F	10.37	1.60	1.69	0.01	2.17	6.03
	P	0.0023	0.2119	0.2001	0.9318	0.1473	0.0178
E	F	6.53	3.23	0.26	1.60	0.06	0.00
	P	0.0139	0.0785	0.6110	0.2117	0.8038	0.9965
SE	F	2.60	0.16	2.19	2.76	8.90	0.09
	P	0.1135	0.6903	0.1452	0.1032	0.0045	0.7614
PE	F	1.09	0.34	1.19	1.65	0.25	6.56
	P	0.3027	0.5603	0.2817	0.2059	0.6184	0.0033
SPE	F	0.00	1.85	0.86	0.00	0.01	0.81
	P	0.9593	0.1799	0.3588	0.9790	0.9309	0.3724
N	F	0.36	0.15	2.72	1.43	102.75	126.63
	P	0.5519	0.7032	0.1059	0.2375	0.0001	0.0001
SN	F	0.09	0.96	0.06	0.39	1.35	9.31
	P	0.7689	0.3331	0.8124	0.5346	0.2504	0.0037
PN	F	5.61	0.49	2.93	0.03	0.30	0.28
	P	0.0221	0.4862	0.0936	0.8680	0.5890	0.5967
SPN	F	1.35	0.32	6.11	0.00	11.21	0.82
	P	0.2504	0.5740	0.0171	0.9895	0.0016	0.3701
EN	F	2.87	0.28	2.34	3.64	0.68	3.64
	P	0.0967	0.1011	0.1325	0.0625	0.4125	0.0625
SEN	F	0.97	0.73	1.51	0.18	0.48	4.92
	P	0.3293	0.3965	0.2252	0.6713	0.4904	0.0314
PEN	F	0.03	1.56	17.25	0.01	1.65	0.28
	P	0.8638	0.2182	0.0001	0.9096	0.2055	0.5998
SPEN	F	0.05	0.21	0.01	2.47	0.00	4.73
	P	0.8328	0.6517	0.9280	0.1226	0.9892	0.0346

[1]F = statistic; P = probability.

[2]L* = lightness; a* = red/green axis; b* = yellow/blue axis.

[3]S = sodium chloride; P = sodium tripolyphosphate; E = sodium erythorbate; N = sodium nitrite.

TABLE 5. Means (± SEM) for the CIE red/green axis (a*) color parameter of cooked breast fillets

Treatment combination[1]	Light group	Normal group	Dark group
Control	2.60 ± 0.40^{b}	2.21 ± 0.45^{c}	3.24 ± 0.68^{e}
S	2.42 ± 0.34^{b}	2.59 ± 0.18^{bc}	2.96 ± 0.23^{ef}
P	2.38 ± 0.29^{b}	2.49 ± 0.18^{c}	3.03 ± 0.32^{ef}
SP	2.24 ± 0.52^{b}	3.25 ± 0.36^{b}	4.29 ± 0.10^{bcd}
E	2.73 ± 0.25^{b}	2.92 ± 0.32^{bc}	3.08 ± 0.31^{ef}
SE	2.43 ± 0.34^{b}	2.67 ± 0.19^{bc}	3.51 ± 0.12^{de}
PE	2.11 ± 0.45^{b}	2.45 ± 0.37^{c}	2.27 ± 0.40^{f}
SPE	2.36 ± 0.10^{b}	4.06 ± 0.09^{a}	4.29 ± 0.17^{bcd}
N	4.31 ± 0.21^{a}	4.20 ± 0.14^{a}	4.79 ± 0.16^{ab}
SN	4.41 ± 0.07^{a}	4.38 ± 0.12^{a}	5.00 ± 0.36^{ab}
PN	4.30 ± 0.16^{a}	4.47 ± 0.27^{a}	5.08 ± 0.39^{ab}
SPN	4.71 ± 0.23^{a}	4.09 ± 0.25^{a}	4.65 ± 0.50^{c}
EN	4.39 ± 0.15^{a}	4.41 ± 0.32^{a}	4.26 ± 0.22^{bcd}
SEN	4.22 ± 0.11^{a}	4.53 ± 0.20^{a}	5.63 ± 0.15^{a}
PEN	4.41 ± 0.11^{a}	4.48 ± 0.25^{a}	4.77 ± 0.31^{ab}
SPEN	4.54 ± 0.22^{a}	4.51 ± 0.23^{a}	5.56 ± 0.25^{a}

[a–f]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]n = 4. S = sodium chloride; P = sodium tripolyphosphate; E = sodium erythorbate; N = sodium nitrite.

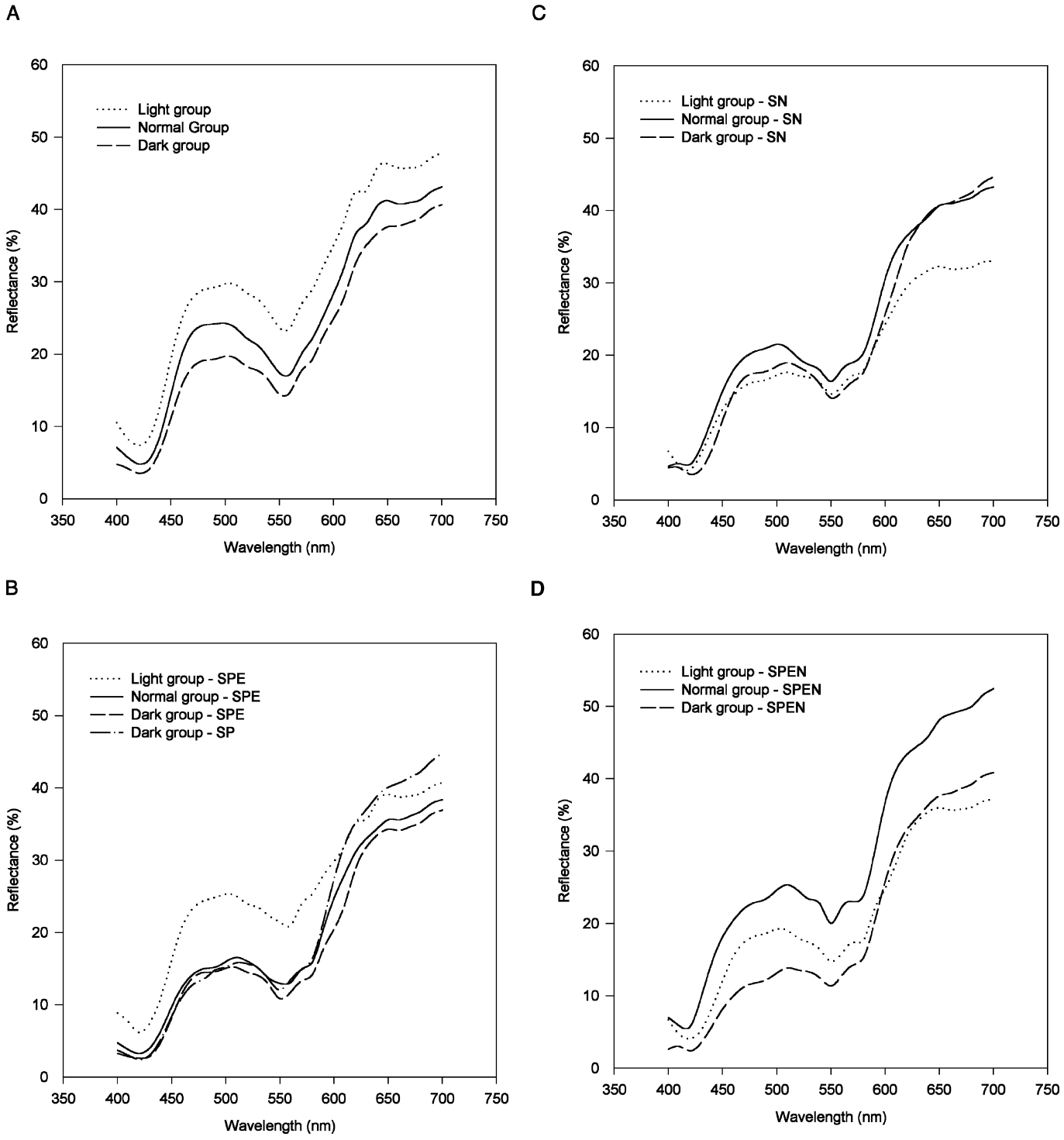

FIGURE 1. Reflectance spectra of raw chicken breast fillets from light, normal, and dark groups. A. Control samples; B. samples injected with a combination of sodium chloride, sodium tripolyphosphate, and sodium erythorbate (SPE) or injected with sodium chloride and sodium tripolyphosphate (SP); C. samples injected with sodium chloride and sodium nitrite (SN); D. samples injected with SPE and sodium nitrite (SPEN).

However, in samples injected with a combination of salt, tripolyphosphate with erythorbate, there was some evidence of oxymyoglobin formation as indicated by the reflectance minima at 540 and 580 nm (Figure 1B). Millar et al. (1996) also reported some evidence of oxymyoglobin in poultry meat. For the light, normal, and dark groups (Figure 1C,D), characteristic spectral curves of nitrosomyoglobin were found in raw samples injected with sodium nitrite or its combinations. These spectra had reflectance minima at about 421, 548, and 579 nm. Similar spectral curves of nitrosomyoglobin were found by Millar et al. (1996), even at low concentrations. The nitric oxide myoglobin that appears red in raw meat may be formed in the raw meat without the intermediate step of nitric oxide metmyoglobin formation (Livingston and Brown, 1981). When sodium nitrite was combined with sodium chlo-

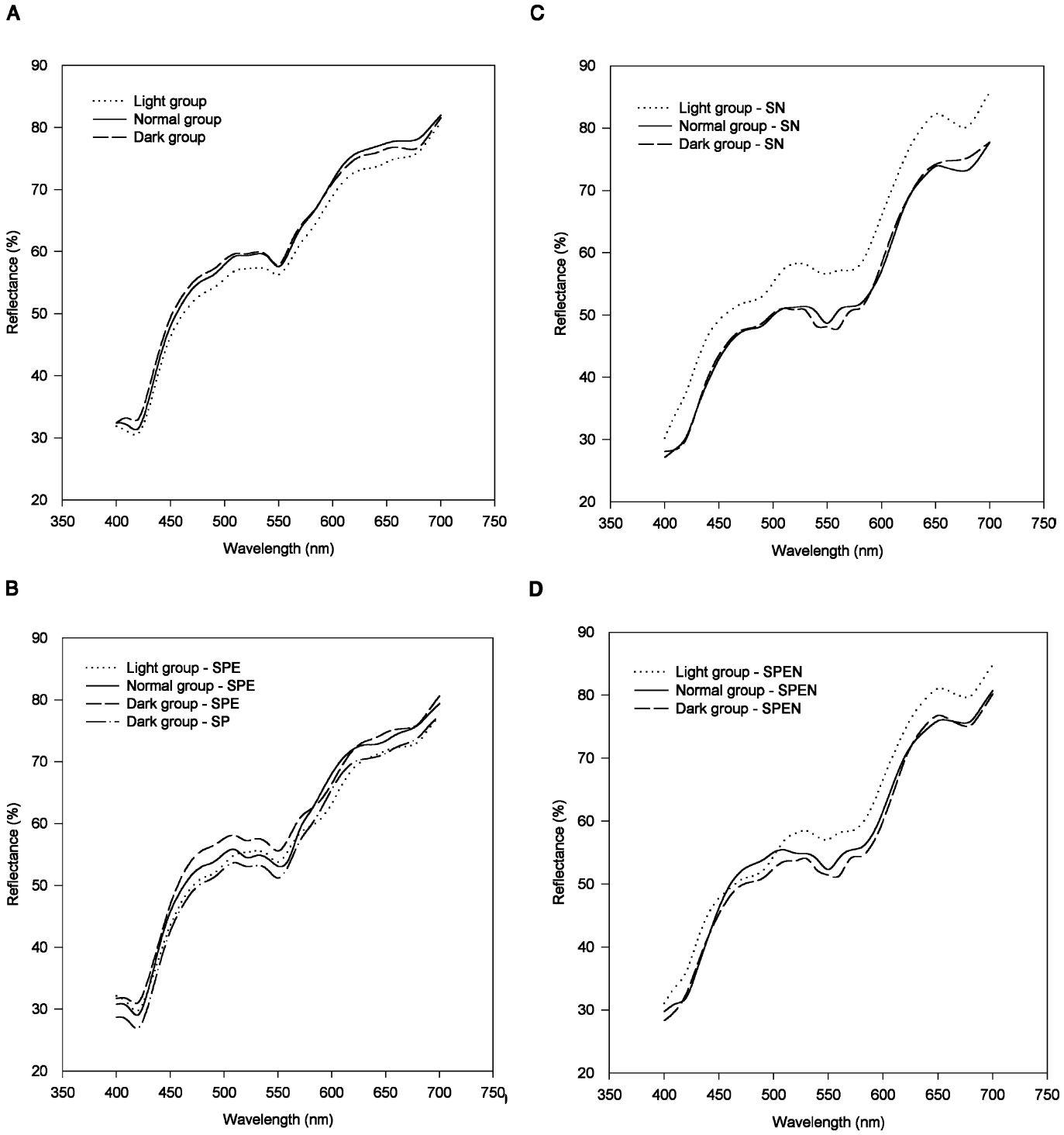

FIGURE 2. Reflectance spectra of cooked breast fillets from light, normal, and dark groups. A. Control samples; B. samples injected with a combination of sodium chloride, sodium tripolyphosphate, and sodium erythorbate (SPE) or injected with sodium chloride and sodium tripolyphosphate (SP); C. samples injected with sodium chloride and sodium nitrite (SN); D. samples injected with SPE and sodium nitrite (SPEN).

ride, tripolyphosphate, and erythorbate, higher reflectance values occurred for all three groups than when one of the three compounds was missing (Figure 1C,D).

No reflectance minima were identified at approximately 542 and 580 nm in the cooked control samples in all groups, which indicated that denaturation of myoglobin had occurred (Figure 2A). The very pronounced minimum at 550 nm was found for normal and dark control cooked fillets, which may indicate the presence of pink ferrocytochrom c (Cornish and Froning, 1974; Ahn and Maurer, 1989b). However, those samples exhibited no pinking after cooking. The reflectance spectra from normal and dark groups injected with a combination of salt, phosphate, and erythorbate (pink samples) showed that pinking was mainly due to reduced hemochromes (Figure 2B). These spectral curves, with reflectance minima at

about 555, 520 to 530, and 415 to 420 nm, were exactly those to be expected for reduced hemochromes (Brown and Tappel, 1957; Tappel, 1957; Akoyunoglou et al., 1963; Ledward 1974; Cornforth et al., 1986; Ahn and Maurer, 1990a; Girard et al., 1990; Ghorpade and Cornforth, 1993).

Pink ferrohemochromes usually have reflectance minima very similar to brown ferrihemochromes. Ferrohemochromes that are formed by reduction of ferrihemochromes cause a shift in Soret, α-, and β-minima toward the longer wavelength (Akoyunoglou et al., 1963). There is a problem in identifying the nitrogenous compound as a sixth ligand coordinating to the pigment molecule. It is difficult to distinguish between different hemochromes by reflectance measurement. They may be characteristic of many different compounds (Cornforth et al., 1986; Ghorpade and Cornforth, 1993). In addition, absorption maxima (reflectance minima) at 550, 520, and 415 nm, characteristic of ferrocytochrome c, have been reported by Ahn and Maurer (1990b) and Girard et al. (1990). Diffused reflectance minima at 540 and 565 nm, characteristic of nitrosohemochromes (Brown and Tappel, 1957), were found in all spectral curves of cooked samples containing sodium nitrite. Examples of these spectra are presented in Figure 2C and D. The intensities of these minima were more pronounced in cooked fillets from the normal and dark groups than from the light group. Even for samples from the normal and dark groups, which were injected with sodium nitrite in combination with other ingredients, minima between 520 and 530 nm were present (Figure 2C,D). This minima range is typical of nonnitrosyl hemochromes (Ahn and Maurer, 1990; Ghorpade and Cornforth, 1993).

Reflectance spectra measurement of meat surfaces cannot provide accurate information on pigment concentration because of variation in the light-scattering properties of meat; spectra can provide information on the state of pigment oxygenation or oxidation and on the presence of compounds that are not extractable (hemi- and hemochromes). Reflectance measurements were obtained to seek information whether the presence of the simulated pink defect in cooked samples is related to the presence of certain pigment classes.

CONCLUSIONS

It is possible to simulate a pink defect in cooked chicken breast fillets using the in situ conditions of raw meat that were induced by sodium chloride, sodium tripolyphosphate, and sodium erythorbate. The presence of sodium nitrite had the most significant effect on pinking, even at very low levels such as 1 ppm. The results proved the reproducibility of the pink defect because no significant plant effect was found. Simulation was most effective in the dark group, followed by the normal and light groups. The initial lightness (CIE L*) of the raw muscles was found to be the most critical condition for the occurrence of pinking.

The selection of samples into three color groups (light, normal, and dark) was important because the lightness of raw muscles may indicate different initial endogenous conditions of meat and as shown in this study different response to the experimental treatments. It is also recommended that poultry processors ensure that there is not significant variation in time postmortem for the meat that is processed in their plant. Such variation may cause changing muscle pH and associated lightness of muscles. The relationship of the induced in situ conditions and simulated pinking is the subject of a subsequent study.

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