Relating Induced In Situ Conditions of Raw Chicken Breast Meat to Pinking

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ABSTRACT Our objective was to simulate the pink color defect in cooked chicken breast meat with treatment combinations that would induce measurable changes in the conditions of raw meat. In addition, the feasibility of using induced raw meat conditions to develop a logistic regression model for prediction of pinking was studied. Approximately 960 breast fillets from 2 plants with 2 replications were used for inducing in situ conditions with 16 combinations of sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite (present and not present). Muscles in all treatments were subjected to individual injections, followed by tumbling, cooking, and chilling. Raw samples were analyzed for pH, oxidation-reduction potential, and pigment evaluation. Results indicated a significant role of induced in situ conditions of raw meat in the occurrence of pinking. Presence of 1 ppm or more of sodium nitrite in raw meat produced significant pinking of cooked meat. The light muscle color group was least affected and the dark group was most affected by induced pH, oxidation-reduction potential conditions, and metmyoglobin and nitrosopigment content. The predictive ability of the logistic model was more than 90% with nitrosopigment, pH, and reducing conditions being the most important factors. Moreover, validation of the model was confirmed by close association between observed pink samples and those predicted as pink.

(Key words: logistic model, pinking in poultry, prediction, raw chicken meat)

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INTRODUCTION

The pink color defect in white poultry meat has been a subject of research for over 40 yr. When and why the pinking phenomenon occurs is unclear. Therefore, it is challenging to study the pinking problem as it happens. Currently, major attention focuses on processing factors such as marinating ingredients, processing water, endpoint cooking temperature, and cooking methods. Froning et al. (1968) stated that pinking occurs in cooked turkey meat after refrigeration; Girard et al. (1989) found that pinking remained in turkey muscle after cooking to 85°C. Since then a number of research papers have been presented that report the investigation of cooked meat conditions related to pinking. The presence of nitrates in a concentration of 1 ppm or more causes pinking in cooked white poultry meat (Froning et al., 1969; Nash et al., 1985; Ahn and Maurer, 1987, 1989a; Heaton et al., 2000). Several studies have shown that different processing and marinating ingredients change pH and oxidation-reduction potential (ORP) of cooked meat significantly enough to induce pinking (Cornforth et al., 1986; Ahn and Maurer, 1989a,b; Trout 1989). A high pH in cooked meat (>6.4) is favorable for the heme-complex-forming reactions of pigments with most ligands (Ahn and Maurer, 1990). High pH also decreases the susceptibility of myoglobin to heat denaturation, allowing greater reactivity of pigments (Trout, 1989; Girard et al., 1990; Young et al., 1996b).

Several researchers have attempted to reduce pinking using different ingredients, such as ligands binding to heme iron, without formation of pink color in cooked products (Schwarz et al., 1997, 1999; Slesinski et al., 2000a,b). However, the mechanism of pink color reduction is not yet established. Moreover, adding the nonpink-generating ligands may or may not be effective depending on how the raw meat endogenous conditions were altered by processing procedures. There is not much information available relating raw meat conditions to pinking. There is a need for development of control methods and processing procedures to eliminate the development of the pink quality defect. In this study we made an attempt to use a simulated pink defect as a research tool to investigate how the occurrence of pinking is affected by certain endogenous conditions of raw meat.

Abbreviation Key: ORP = oxidation-reduction potential.
Logistic regression is a predictive statistical analysis, like linear regression, that can simultaneously relate both continuous and discrete independent variables to a binary (dichotomous) response (Afifi and Clark, 1990; Miller and Tierney, 1991; Hand, 1992). The logistic regression model uses the explanatory variables to predict the probability of a certain event’s occurrence. Logistic regression does not assume linearity of relationship between the input variables and the dependent (occurrence or nonoccurrence of an event) and does not require normally distributed variables (Hand, 1992). The logistic model is written as

\[
    \text{Prob} \text{(event)} = \frac{1}{1 + e^{-Z}}
\]

where \( Z = \alpha + \beta x_1 \). Logistic regression applies a maximum likelihood estimation after transforming the dependent variable into a logit variable (the natural log of the odds of the event occurring or not).

\[
    \text{logit}[\text{Prob(event)}] = \log \left[ \frac{\text{Prob(event)}}{1 - \text{Prob(event)}} \right] = \alpha + \beta x_1
\]

where \( 1 - \text{Prob(event)} = \text{Prob(not event)} \), \( \alpha = \) the intercept parameter, \( \beta = \) the vector of slope parameters, and \( x_i = \) the vector of explanatory variables.

The primary objective was to measure how changes in the in situ conditions (raw meat) caused by sodium chloride, sodium tripolyphosphate, sodium erythorbate, and sodium nitrite are related to pinking. The secondary objective involved relating the induced in situ conditions of raw meat to the occurrence of pinking in cooked meat using a binary logistic regression model approach with external validation of the predictive model.

**MATERIALS AND METHODS**

**Sample Collection and Preparation**

The experiment used boneless, skinless, chicken breast fillets (pectoralis major) obtained from processing plants and preselected based on 3 color groups: lighter than normal (light), normal (normal), and darker than normal (dark) (Fletcher, 1999; Fletcher et al., 2000). 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 a Hunter Lab reflectance colorimeter; fillets were selected based on the medial surface (bone side) CIE lightness (\( L^* \)) values (CIE, 1978). \( L^* \) values for the 3 color groups were \( L^* < 47 \) for the dark group, \( 47 < L^* < 50 \) for the normal group, and \( L^* > 50 \) for the light group. The samples were segregated according to color group (light, normal, or dark), packed with ice, and transported to the laboratory within 2 h.

Approximately 960 breast fillets were collected from 2 meat processing plants. Two hundred forty fillets (80 per each of the 3 color groups) were collected per replication during 2 or 3 visits to each of the 2 processing plants.

At 24 h postmortem, all breast fillets were vacuum packed\(^3\) into polyethylene sampling bags. Samples were labeled and coded individually and then frozen and stored at \(-18^\circ\text{C}\) for up to 3 wk. Experimentation commenced after samples were thawed at 4 to 5\(^\circ\text{C}\) (approximately 10 to 12 h). There were 4 treatment factors: 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 used: present and not present. Therefore, 16 treatment combinations were used. The level of each factor was chosen according to FSIS regulations (FSIS, USDA, 2002). 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 so that a 12% injection provided 0.5% sodium tripolyphosphate, 0.0546% sodium erythorbate, and 1 ppm sodium nitrite. For a 200-g fillet, the injection was prepared in a 50-mL beaker as follows: (1) 10 mL of 10% tripolyphosphate was added to the beaker; (2) 2 g of sodium chloride was added while stirring; (3) 0.2 mL of 0.1% sodium nitrite stock solution was stirred in; and (4) 10.92 mL of 1% sodium erythorbate was stirred in as the last ingredient. Finally, distilled water was added to bring the final volume to 24 mL, that is, 12% of the weight of the fillet. Sixty-four injection solutions were required (4 muscles \( \times \) 16 treatment combinations). Separate volumes of injection solutions were prepared and injected. The uniformly spaced multiple injections (35-mL syringes\(^8\) equipped with a stainless steel 16-hole spray needle) followed by overnight equilibration, tumbling (15 min at 0\(^\circ\text{C}\)), cooking to 74\(^\circ\text{C}\), and chilling to 4.4\(^\circ\text{C}\) during 4 h were performed in the same manner as described previously by Holownia et al. (2003).

**Evaluation of Pinking**

The subjective pink threshold established previously at CIE \( a^* = 3.8 \) (with \( L^* \) ranging from 79 to 84, and \( b^* \) from 10 to 16) was used in judging the existence of pinking in the cooked samples (Holownia et al., 2003). The threshold had been produced by injecting samples from the normal group with 1% sodium chloride and sodium nitrite solutions (1, 2, 3, 4, and 5 ppm). The experimental cooked samples were sliced horizontally into lengthwise halves. Immediately, color was objectively evaluated in

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\( ^3\) MiniScan/XE 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA.

\( ^4\) Ultravac 250 KOCH Packaging, Division of KOCH Supplies, Inc., Kansas City, MO.

\( ^5\) Sigma Chemical Co., St. Louis, MO.

\( ^6\) Aldrich Chemical Company, Inc., Milwaukee, WI.

\( ^7\) Sherwood Medical Company, St. Louis, MO.

\( ^8\) Koch No. 30410306, KOCH Supplies, N. Kansas City, MO.
the light booth (utilizing CIE standard Illuminant D65), and an $a^*$ value was measured with a Hunter Lab colorimeter. Four color measurements were taken on each half of the fillet, 2 readings in the anterior and 2 in the posterior portion of the muscle, rotating the samples 90° between measurements. The average value of 8 measurements (4 per half) was calculated. Both visual and instrumental results were used to judge the presence or absence of pinking.

In the following sections, the raw meat variables were analyzed and correlated with the pinking that was judged after additional samples from the same treatment combinations were cooked. This way, results of raw meat conditions are always discussed in association with the pink defect or pinking that by assumption means the defect in meat after cooking.

Analyses

pH Measurement. The muscle pH of raw meat was determined using the iodoacetate method described by Jeacocke (1977). For this analysis, approximately 25 g of meat tissue was removed from anterior portion of the fillet and ground. Duplicate pH measurements were determined for each sample by dispersing 2 g of each sample in 25 mL of 5 mM iodoacetate containing 150 mM KCl. After homogenizing for 30 s at 3,000 rpm, the pH of the slurry was measured by means of a pH meter (Model 525A+) equipped with a combination pH electrode (Catalog No. 8172BN). Mean values of the duplicate observations were recorded.

ORP. A platinum combination redox electrode (Catalog No. 9778BN) was firmly placed in the center of the cranial end of a raw breast fillet (3 to 5°C). ORP readings were recorded after 5 min of stabilization (Moiseev and Cornforth, 1999).

Percentage of Metmyoglobin. Myoglobin was extracted from the raw meat using an ice-cold (0°C) 0.04 $M$ phosphate buffer (Na$_2$HPO$_4$ and NaH$_2$PO$_4$cdotH$_2$O), pH = 6.8 (Warris, 1979). Twenty-five grams of the sample was removed from the anterior portion of the fillet and ground. Next, 5 g of the ground sample was homogenized with 50 mL of buffer at 10,000 rpm for 20 s. The homogenized samples were centrifuged for 30 min at 15,000 g at 4°C. The supernatant was then filtered through Whatman No.1 filter paper and filtered again through a 0.45-μm Puradisc filter device fitted on a syringe to remove any remaining suspended particles. Absorbance at 540 nm for nitrosopigment and 640 nm for total pigment was measured by an HP Spectrophotometer using a 1-cm cell against 80% acetone or acetone/HCl solution as a blank.

Statistical Analyses

The experimental design was a partially confounded 2$^4$ factorial design. Only 8 of the 16 treatments could be evaluated per day; therefore, the experiment was arranged into 2 blocks, thus introducing a confounding effect. The plant, replication, and treatments effects for response variables (pH, ORP, metmyoglobin, nitrosopigment, and total pigment) were analyzed using the MANOVA option of the general linear model procedures of SAS software (SAS Institute, 1989). The main effects and all contrasts (treatment combinations) were tested using a Wilks’ Lambda test. If the Wilks’ Lambda test was significant, univariate analyses were performed using an ANOVA F-test for each variable at $\alpha = 0.05$. If the ANOVA F-test was significant for a given variable, planned comparisons between treatments were made with the LSD method (Fisher protected LSD). Otherwise, planned comparisons with the Bonferroni method were performed using the LSMEANS option with the PROC GLM procedure of SAS software. The 3 tested color groups in the experiment were subjected to individual statistical analyses.

Pink defect data were coded by assigning 1 to a positive response when $a^* \geq 3.8$ (pink), and 0 when $a^* < 3.8$ (not pink). The stepwise forward selection option of the PROC LOGISTIC procedure was used with a DESCENDING
option for model event to relate the occurrence of pinking to raw meat conditions in the following logistic function:

\[
P(1/x) = \frac{\exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n)}{1 + \exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n)}
\]

where \(P(1/x)\) = the probability of the occurrence of pinking; \(x_1, x_2, \ldots, x_n\) = raw meat endogenous conditions; and \(\alpha, \beta_1, \beta_2, \ldots, \beta_n\) = the parameter estimates associated with the model terms. A significance level of 0.15 was used for the inclusion criterion.

The predictive value of the developed logistic model was evaluated by analyzing an independent set of samples from 1 of the processing plants for 3 color groups. Samples were subjected to the same treatment and analyses as in the main experiment.

RESULTS

Significance of Plant and Replication

Multivariate analysis of variance showed significant \((P < 0.05)\) effects of all tested treatment combinations based on the Wilks’ Lambda test. Therefore, further analyses with ANOVA and F statistics were performed for each independent variable separately. There was no effect \((P > 0.05)\) of replication per plant when all independent variables were tested using a replication-within-plant effect as a divisor (data not shown). Also, there was no plant effect \((P > 0.05)\) when tested using a residual error. Therefore, in subsequent analyses, data were pooled across plants and replications.

pH Conditions

The 3 color groups responded differently to the treatment combinations. There was an effect \((P < 0.05)\) of tripolyphosphate and sodium chloride on pH of raw samples from the normal group (Figure 1A). Samples injected with tripolyphosphate alone or in combination with other ingredients had the highest pH values, ranging from 6.230 to 6.296. These values were significantly different from those of other treatment combinations. High initial pH in raw meat, however, did not always correspond exactly to pinking after cooking. Among all the samples in the normal group with high pH, with one exception, only those containing sodium nitrite exhibited pinking. Fillets injected with a combination of sodium chloride, tripolyphosphate, and erythorbate and having a high raw meat pH of 6.240 also exhibited pinking after cooking. Among the normal group samples with the pink defect, those injected with tripolyphosphate had significantly higher pH values than those samples with no tripolyphosphate. A similar response to tripolyphosphate was observed in the light group. Light group samples containing tripolyphosphate showed pH values ranging from 6.153 to 6.248 (Figure 1B). In the light group, pinking was not observed in samples with a high raw meat pH except when sodium nitrite was also present in the injection solution. Among pink cooked fillets, those containing tripolyphosphate showed significantly higher pH values. The dark group showed marked variation in raw meat pH in response to injections (5.982 to 6.356) (Figure 1C). Pinking occurred in all samples containing sodium nitrite; thus, high pH caused by tripolyphosphate was not always the determining factor. A significantly positive effect of tripolyphosphate on pH was accompanied by a significantly negative effect of sodium erythorbate. Not all the samples with the pink defect had a significantly higher raw meat pH than samples with no pinking, and not all of the high pH samples exhibited pinking after cooking. In 2 instances, pinking occurred in the absence of sodium nitrite: the combination of sodium chloride and tripolyphosphate and the combination of sodium chloride, tripolyphosphate, and erythorbate. Interestingly, in these 2 instances,
pH values (6.305 and 6.244) of raw samples were not significantly different from that of the control (6.290).

**ORP**

The ORP was expressed as a change in ORP relative to the control (a “zero” value). The magnitude of change was obtained by subtracting the ORP of the control from that of the sample; when the ORP value was more negative, the reducing condition was greater. There was an effect ($P < 0.05$) of sodium erythorbate on ORP changes in the normal, light, and dark groups (Figure 2A,B,C). Only the treatment combination of sodium chloride, tripolyphosphate, and erythorbate in the normal group exhibited both pinking and a significant negative change in ORP (Figure 2A). Other treatment combinations in the normal group resulting in pink defect included (1) samples with sodium nitrite and positive ORP change, and (2) samples with sodium nitrite and negative ORP change. The light group was most prone to ORP changes caused by the addition of sodium erythorbate (Figure 2B). However, the light group did not show more pinking except when samples were injected with nitrite or with nitrite and erythorbate. As presented in Figure 2C, not all the samples from the dark group with pinking exhibited low ORP; substantial variation in ORP, not necessarily related to pinking, occurred in this group before cooking. The combination of sodium chloride, tripolyphosphate, and erythorbate caused pinking and lower ORP. On the other hand, a combination of sodium chloride with tripolyphosphate effected pinking while demonstrating a positive ORP change (3.49); this result indicated that the sample had an ORP value even higher than that of the control.

**Percentage of Metmyoglobin**

Widely varying percentages of metmyoglobin were present in raw fillets from the normal group with pinking, thus indicating that the percentage of metmyoglobin in raw meat was not a good indicator of pinking (Figure 3A). A similar trend was observed in the light group (Figure 3B). Among normal group samples with pinking defect, only the combination of sodium chloride, tripolyphosphate, and erythorbate and the combination of tripolyphosphate, erythorbate and nitrite showed a significantly lower content of metmyoglobin (Figure 3A). The other samples that exhibited pinking had a percentage of metmyoglobin that was not significantly different than that of the control sample. All of the samples in the light group, which were pink after cooking, had a significantly lower percentage of metmyoglobin except in 2 instances: the combination of tripolyphosphate and nitrite and tripolyphosphate with erythorbate and nitrite (Figure 3B). There was even more variation in the percentage of metmyoglobin in the dark group (Figure 3C). Samples with pink defect from the dark group injected with the combination of sodium chloride, tripolyphosphate, and erythorbate had a significantly lower percentage of metmyoglobin than the rest of the pink fillets. However, there was one exception—samples with pink defect injected with sodium chloride and tripolyphosphate.

**Nitrosopigment Content**

Nitrosopigment content showed a significant and positive effect on pinking in all 3 color groups. When the nitrosopigment content of raw meat was estimated to be greater than 1 ppm, all of the cooked fillets exhibited pinking (Figure 4). In addition, a combination of sodium chloride, tripolyphosphate, and erythorbate caused pinking of the normal and dark groups (Figure 4A and C). Only the dark group exhibited pink defect when injected with the combination of sodium chloride with tripolyphosphate. Pinking in those treatment combinations lacking

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**FIGURE 2.** Effect of treatment combinations on oxidation-reduction potential (ORP) in raw chicken breast fillets. $S =$ sodium chloride; $P =$ sodium tripolyphosphate; $E =$ sodium erythorbate; $N =$ sodium nitrite. Samples exhibited pinking after cooking. Bars with no common letters differ significantly ($P < 0.05$).
sodium nitrite was probably affected by other induced conditions such as pH and reducing conditions. Surprisingly, sodium nitrite in combinations with other ingredients caused a 2- to 3-fold increase in the nitrosopigment in fillets when compared with samples injected with sodium nitrite only. It was concluded that sodium chloride, tripolyphosphate, and erythorbate formed favorable conditions for nitrosopigment formation through metmyoglobin or myoglobin combination with naturally present and added nitrite.

### Total Pigment

The pigment content, expressed as parts per million of hematin, did not show specific trends toward pinking (data not shown). Hematin concentration ranged from 12.43 to 31.2 ppm in the normal group, from 13.34 to 26.84 ppm in the light group, and from 10.62 to 15.61 ppm in the dark group. Substantial variation in ppm hematin in the results of different treatment combinations could not be explained, and no firm conclusions were drawn with regard to the pink defect.

### Logistic Regression Analysis

The parameter estimates, the Wald chi-squared statistic, and the odds ratio estimates for all 3 color groups are presented in Table 1. As expected, the logistic model for the light group resulted in a complete separation of data points. This is attributed to the presence of sodium nitrite as one of the ingredients in the injection solution leading to pinking defect. All of the pink samples had more than 1 ppm of nitrite. Therefore, the only raw meat variable that met the 0.15 significance level of entry into the model...
TABLE 1. Results of logistic regression conducted to identify raw meat variables critical for pink defect in cooked meat expressed as probability of the pink defect

<table>
<thead>
<tr>
<th>Muscle color group</th>
<th>Source</th>
<th>df</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>Wald χ²</th>
<th>P &gt; χ²</th>
<th>Odds ratio estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Intercept</td>
<td>1</td>
<td>−16.5839</td>
<td>17.5338</td>
<td>0.8946</td>
<td>0.3442</td>
<td>. . . 4</td>
</tr>
<tr>
<td></td>
<td>Nitrosopigment²</td>
<td>1</td>
<td>15.9295</td>
<td>16.3764</td>
<td>0.9489</td>
<td>0.3300</td>
<td>&gt;999.999</td>
</tr>
<tr>
<td>Normal</td>
<td>Intercept</td>
<td>1</td>
<td>−9.0613</td>
<td>4.0392</td>
<td>5.0327</td>
<td>0.0249</td>
<td>. . .</td>
</tr>
<tr>
<td></td>
<td>Nitrosopigment</td>
<td>1</td>
<td>11.1729</td>
<td>5.6026</td>
<td>3.9770</td>
<td>0.0610</td>
<td>&gt;999.999</td>
</tr>
<tr>
<td></td>
<td>ORP³</td>
<td>1</td>
<td>−0.0418</td>
<td>0.0318</td>
<td>1.7269</td>
<td>0.1888</td>
<td>0.959</td>
</tr>
<tr>
<td>Dark</td>
<td>Intercept</td>
<td>1</td>
<td>−53.6647</td>
<td>27.9156</td>
<td>3.6956</td>
<td>0.0546</td>
<td>. . .</td>
</tr>
<tr>
<td></td>
<td>Nitrosopigment</td>
<td>1</td>
<td>8.7565</td>
<td>3.5405</td>
<td>6.1170</td>
<td>0.0134</td>
<td>&gt;999.999</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>1</td>
<td>7.5334</td>
<td>4.3809</td>
<td>2.9570</td>
<td>0.0855</td>
<td>&gt;999.999</td>
</tr>
</tbody>
</table>

1Significance, α = 0.15.
2Nitrosopigment, expressed as ppm of hematin.
3Oxidation-reduction potential change relative to the control.
4Not applicable.

was nitrosopigment content. Based on the likelihood ratio chi-squared test, at least one of the independent variables in the model was significant (P < 0.0001). The Wald statistic showed no significance with regard to the intercept and the nitrosopigment content. However, we decided to keep it in the model. Based on the nitrosopigment parameter estimate, the odds that the dependent variable would cause pinking in the light group reached infinity. This situation was expected because the nitrosopigment concentration in the raw meat was the only variable determining the presence of a pink defect in the light group. There were 2 variables, nitrosopigment content and ORP change, that entered the model at a 0.15 significance level for the normal group. Analysis of model parameters estimated that when the nitrosopigment concentration increased by 1 unit, with the ORP variable remaining unchanged, the odds would increase by a factor of e¹¹.¹⁷, that is, indefinitely. Considering the odds ratios corresponding to the ORP parameter in the model for the normal group, low and negative values of the estimate indicated that there was actually less chance of having a pink defect, given a one-unit increase in ORP change. Even though the ORP parameter was not significant (P > 0.15) in the model based on the Wald statistic, it was decided to keep it in the model as valuable information. When the logistic function was fitted to the data from the dark group, significance was found for the intercept, pH, and nitrosopigment content. Both the pH and nitrosopigment variables showed that the probability of pinking increases with higher pH and higher nitrosopigment content. The odds ratios estimated at e⁷.⁵³ = 1,863 for pH and e⁸.⁷⁵ = 6,310 for nitrosopigment indicated that the odds of occurrence of pink defect per unit increase increased by a factor of 1,863 for pH content and by 6,310 for nitrosopigment content, respectively. The c statistic is a rank correlation index computed from the pink/nonpink classification table (SAS Institute, 1995). The closer the value of c approaches 1, the better the predictability of the logistic regression equation. The light group logistic equation with its sole predictor variable and with complete data separation had a c value equal to 1, as expected (data not shown). The normal and dark groups also showed a high level of differentiation between pink and not pink events with group values of c = 0.987 and c = 0.968, respectively.

A classification table was used as a measure of model accuracy for each color group. The observed values for the pink defect and the predicted pinking (at a cutoff value of P = 0.5) were cross-classified (Table 2). The logistic function for the light group correctly classified 63 of the 64 observations (98.4%). Because 31 of the 32 pink events were correctly predicted in the light group, the sensitivity for the model was 96.9%. Furthermore, the model also correctly classified all of the nonpink samples yielding a specificity of 100%. None of the nonpink samples from the light group were falsely classified as pink samples (false positive). One pink sample was incor-

TABLE 2. Levels of correct classification from the logistic model (at a cutoff value of P = 0.50) for light, normal, and dark groups

<table>
<thead>
<tr>
<th>Muscle color group</th>
<th>Correct</th>
<th>Incorrect</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pink</td>
<td>Not pink</td>
<td>Pink</td>
</tr>
<tr>
<td>Light</td>
<td>31</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>32</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>Dark</td>
<td>36</td>
<td>22</td>
<td>2</td>
</tr>
</tbody>
</table>

¹Ratio consisting of the number of correctly classified pink over the total number of pink.
²Ratio consisting of the number of correctly classified nonpink over the total number of nonpink.
³Proportion of predicted pink responses that were observed as nonpink.
⁴Proportion of predicted nonpink responses that were observed as pink.
rectly classified as not pink, yielding a 3% false negative rate. The logistic function correctly classified samples from the normal group at 90.6%. There were 2 nonpink samples that were falsely predicted to be pink and 4 samples with pink defect that falsely predicted to be non-pink. Therefore, this model predicted the occurrence of pinking with 88.9% accuracy. More pink samples from the normal group were incorrectly predicted to be non-pink (false positive error) than nonpink samples were predicted to be pink (false negative error). The logistic model showed the same percentage for the dark group as for the normal group in predicting pinking. However, the proportion of pink samples that were predicted to be pink (sensitivity) was higher in the dark group compared with the normal group. On the other hand, the specificity of the model for the dark group was lower than that of the normal group. The model for the dark group produced more false negative errors than did the normal group model.

The logistic models for the 3 color groups correctly predicted more than 90% of the pinking in cooked samples. After transforming logit (p) into probability of pinking in the light, normal, and dark group, logistic regression equations were as follows:

Pink defect probability for the light group

\[
\frac{\exp(-16.58 + 15.95x_1)}{1 + \exp(-16.58 + 15.95x_1)}
\]

Pink defect probability for the normal group

\[
\frac{\exp(-9.06 + 11.17x_1 - 0.041x_3)}{1 + \exp(-9.06 + 11.17x_1 - 0.041x_3)}
\]

Pink defect probability for the dark group

\[
\frac{\exp(-53.66 + 8.75x_1 + 7.53x_2)}{1 + \exp(-53.66 + 8.75x_1 + 7.53x_2)}
\]

Where \(x_1\) = nitrosopigment (ppm); \(x_2\) = pH; \(x_3\) = ORP change relative to control (mV).

A new independent data set, from 1 plant with 1 replication, was used for model validation. The models (Equations 1, 2, and 3) generated with the SAS LOGISTIC procedure were used for verification and to generate the predicted values for observations from the independent data set. These predicted values were then compared with observed pinking in samples from the independent data set and cross tabulated in the 2-way frequency table using a FREQ procedure from the SAS software. In the TABLES statement, observed – pink x pink – predicted specified a table in which the columns are the observed pink events, and the rows are the predicted pink observations. Table 3 presents the cross-tabulation of observed pink and predicted pink. In the first cell, 100% of those samples with observed pink were predicted as pink in the light and normal groups, and 90% of pink samples were predicted as pink in the dark group. There was significant evidence of an association between observed pink samples from an independent data set and those predicted as pink. Fisher’s exact test yielded 2-sided \(P < 0.001\) for the light, normal, and dark groups. The results with an independent set of data confirmed the validation of the 3 logistic models built for the light, normal, and dark groups.

**DISCUSSION**

There is not much research available to date that attempts to relate raw meat conditions to pinking after cooking. The high pH (6.25) and abnormally red chicken breast muscle because of preslaughter factors (free struggle and excitation of birds) has been reported by Ngoka et al. (1982). They concluded also that when excitation and free struggle occur prior to slaughter it may cause some of the pinking problems encountered by the industry. Janky and Froning (1973) reported results indicating that the increased pH from 5.5 to 6.9 is associated with lower percentage of myoglobin denatured and may be related to pinking. In addition, Young et al. (1996a) related higher pH of raw turkey breast meat caused by a short postmortem time to the significantly increased CIE \(a^*\) values in raw meat. Also, according to Allen et al. (1998) there is a correlation between raw meat pH and raw meat color. Most of the information in regard to ORP in meat presents results that were obtained after cooking as an explanation of what conditions were present at the time the defect occurred. However, no information is available about observed ORP changes in raw meat in connection with pinking. Much greater amounts of nitrite introduced into the meat before cooking and causing pinking were reported compared to our findings. More than 200 ppm of nitrite-nitrogen present in chilling water was related to increased redness of meat after cooking (Mugler et al., 1970). Also, significant color development occurred when chicken carcasses were held in water or ice containing 3 ppm of nitrite (Nash et al., 1985). According to Froning et al. (1969), when birds were fed with a diet containing 25 or 50 ppm of sodium nitrite, the Gardner \(a_1\) values were reported to be more than 4.0. However, the authors did not present nitrite levels in raw meat. Ahn and Maurer (1985) found in their study that 1 ppm of added nitrite caused pinking in cooked turkey breast. Results from this study clearly indicate that 1 ppm of sodium nitrite in the raw meat is related to pinking.

**CONCLUSIONS**

The induced in situ conditions used in this study played a significant role in the occurrence of pinking. As expected, none of the ingredients alone, except sodium nitrite, significantly induced pinking. As reported in the literature, a small, almost residual, amount of nitrite may be present in meat depending on the preslaughter factors and processing conditions. Therefore, the probability of pinking is high whenever sodium nitrite (>1 ppm) is present in meat before cooking. In the absence of nitrites, the
other commonly used ingredients come into play: sodium chloride, sodium tripolyphosphate, and sodium erythorbate. These ingredients are well known to affect (1) solubility of meat proteins and their resistance to denaturation, (2) the increase in meat pH, and (3) the increase in reducing conditions. In the present study, we showed that it is unlikely that a change in only one of the above conditions would produce pinking. However, the combination of sodium chloride, tripolyphosphate, and erythorbate induced significant changes in pH, ORP, and percentage of metmyoglobin present. The natural variation in raw color of breast muscles also showed an effect on the occurrence of pinking, with the light group being least affected and the dark group most affected. The logistic regression and its validation as presented in this study demonstrated its use as a predictor of pinking based on the raw meat conditions. In addition, nitrosopigment content in raw meat, pH, and reducing conditions were shown to be the most important factors in pinking. These results are important in assisting the poultry industry in (1) assessing the potential for pinking and (2) investigating new procedures to prevent and eliminate the pink defect.

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