PROCESSING AND PRODUCTS

Poultry Meat Color: Heme-Complex-Forming Ligands and Color of Cooked Turkey Breast Meat

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ABSTRACT Heme-complex-forming ligands and their effects on color were studied in cooked turkey breast meat. Twenty-four ground meat samples were prepared with combinations of two levels of pigments (0 or .4 mg of myoglobin (Mb) and .4 mg of hemoglobin (Hb) per gram meat weight), two levels of NaCl (0 or 2.5%), and six ligands. Each combination of ingredients was dissolved in distilled water (15%, meat weight) prior to mixing with the meat samples. Each meat sample was hand mixed in a 250-mL beaker, covered with aluminum foil, and cooked to an internal temperature of 75 C in a water bath. Among the ligands, pyridine, nicotinamide, and histidine formed heme complexes with added pigments. Pyridine produced the highest a values (redness), followed by nicotinamide, histidine, and the control, respectively. Although the added BSA was expected to produce a pink color, it did not have any effect on the redness of the meat. The effect of the added salt appeared as great as that of the added pigments by apparently providing solubilized proteins to form heme complexes in the meat. The b and L values of the meat were usually decreased because of added nicotinamide or histidine. The added pigments and salt also generally decreased the b and L values of the turkey breast meat. Total pigment and extractable pigment increased when heme pigments were added. No relationships were observed between the redness and the total pigment or between the a value and the extractable pigment.

(Key words: color, heme-complex, pyridine, histidine, salt)

INTRODUCTION

The color-causing factors and the characterization of cooked meat pigments have been further investigated by several groups of researchers since the study by Tappel (1957) on the pink pigment in cooked meat. Most of these studies on the pink color problems in poultry meat focused on improper raising, handling, or processing conditions (Froning et al., 1968, 1969, 1978; Mugler et al., 1970; Helmke and Froning, 1971; Ngoka et al., 1982). Howe et al. (1982) reported that the pink color of cooked meat develops with refrigerated storage conditions but fades very rapidly upon exposure to light and oxygen.

The gray or brown pigment of cooked meats has been characterized as a mixed denatured globin nicotinamide hemochrome (Tappel, 1957) by sodium hydrosulfide reduction and comparison with known hemochromogens. Comforth et al. (1986) reported that the cooked meat pigment was unextractable with many solvents, such as acetone-HCl solution. Tarladgis (1962) interpreted the cooked meat pigment with quantum mechanical theories and explained that the compound responsible for the color of the cooked meats is a high spin, ferric-porphyrin coordination complex whose fifth and sixth coordination positions of the ferric iron of the compound are occupied by a carboxylate ion of the denatured globin molecule and water, respectively. However, Ledward (1971, 1974) suggested on the basis of optical and electron paramagnetic resonance (EPR) spectroscopy results that the pigments of the cooked meat are mainly di-imidazole complexes of ferric hematin with the imidazole residues being supplied by the histidine groups of the denatured proteins present in cooked meat.

In an effort to find possible nitrite substitutes for pigment formation in cured meat products, Akoyunoglou et al. (1963), Howard et al. (1973), and Dymicky et al. (1975) examined various kinds of chemicals and found that some of the pyridine derivatives were quite effective in producing a pink pigment under denatured conditions. However, those studies were limited to model systems or to subjective descriptions in a meat system. The results of Ahn and Maurer (1990a) have shown that histidine, proteins, pyridine, and

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nicotinamide are very effective heme-complex-forming ligands with myoglobin (Mb), hemoglobin (Hb), and cytochrome c. Methionine is also a good heme-complex-forming ligand with cytochrome c. The concentrations of the ligand (Ahn and Maurer, 1990a) and the pH of the system are very critical in the heme-complex-forming reactions of the pigments (Ahn and Maurer, 1990b). Although the previous studies with model systems suggest that histidine, proteins, and nicotinamide, which are naturally present in meat, are the main causes of the pink color problems in cooked meats, it is not certain that any or all of these would be the real cause of the pink color in a meat system. The conditions in the meat system are much more complicated than those of the model system.

The objectives of this study were 1) to examine the effects of the heme-complex-forming ligands on the color of the meat, and 2) to determine the relationships between the a value (redness) and the total pigment and between the a value and denatured pigment in cooked turkey breast meat containing different pigment and salt combinations.

MATERIALS AND METHODS

Materials

The Mb was prepared from turkey heart using the method described by Rothgeb and Gurd (1982), and Hb was purified from turkey blood using the method described by Riggs (1981). Chemicals were obtained from the following sources: L-glycine and L-histidine were purchased from US Chemical Co.; 2 BSA and nicotinamide from Sigma; 3 and pyridine from Fisher Scientific. 4

Preparation of Meat Samples

Fresh turkey breast meat was ground through a 3.2-mm (1/8") meat grinder plate, and 24 meat samples (100 g per sample) were prepared with the combinations of two levels of pigments (0 or .4 mg Mb plus .4 mg Hb/g meat), two levels of NaCl (0, 2.5%), and six ligands. Each combination of ingredients was dissolved in distilled water (15%, meat weight) prior to mixing with the meat samples. Each sample was hand mixed in a 250-mL beaker, covered with aluminum foil, and cooked to an internal temperature of 75°C in a water bath. After cooking, samples were cooled to room temperature (ca. 21°C) and stored in a 3°C cooler. The experiment was conducted four times.

Color Measurement

The color of cooked meat was measured at room temperature (21°C) by using a Hunter Lab color difference meter5 with a D25-9 reference. The colorimeter was standardized with a white ceramic tile having values: L, 92.14; a, -12.0; and b, 10.0. Before measuring the color of meat, each sample was cut into half and brushed with 1% sodium hydrosulfite solution (reducing agent) in 0.1 M phosphate buffer, pH 6.3 solution. The two pieces of meat were put together and wrapped with aluminum foil to minimize pigment oxidation. After 20 min, the cut surface of the meat was placed on a clean glass plate for measuring L (lightness), a (redness), and b (yellowness) color values. Two readings per slice were taken with ca. a 90° rotation between readings.

Total Pigment Measurement

Hornsey’s method (Hornsey, 1956) was used for total pigment extraction and calculation. After extraction of the pigment, the solution was first filtered through Whatman No. 42 filter paper, 6 and then filtered again through a 0.45 µ Prep-Disc membrane filter 7 just before absorbance measurement.

Statistical Analysis

A 2² × 6 factorial design was used. After analyzing the three-way ANOVA, no significant interaction effects between pigment and salt were found except some additive effect on L values by added salt and pigments. Twenty-four combinations of the turkey breast meat samples were sorted by pigment and NaCl combinations and then reanalyzed to determine the significant differences in mean values due to ligands under the given pigment and salt conditions. The statistical analysis system (SAS Institute, 1985)
TABLE 1. The effects of ligands on color values, total pigment, and extractable pigment of cooked turkey breast meat with no pigments or salt added

<table>
<thead>
<tr>
<th>Ligands</th>
<th>a value (redness)</th>
<th>b value (yellowness)</th>
<th>L value (lightness)</th>
<th>Total pigment (ppm)</th>
<th>Extractable pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.11 ± .74d</td>
<td>8.04 ± .12a</td>
<td>69.02 ± .8aabc</td>
<td>23.29 ± 3.44a</td>
<td>.253 ± .052ab</td>
</tr>
<tr>
<td>Pyridine</td>
<td>7.83 ± .79a</td>
<td>7.98 ± .38a</td>
<td>69.69 ± .4ab</td>
<td>21.08 ± 2.89a</td>
<td>.245 ± .050b</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>6.52 ± .39b</td>
<td>6.08 ± .15c</td>
<td>68.14 ± .50cd</td>
<td>24.82 ± 1.62a</td>
<td>.263 ± .036ab</td>
</tr>
<tr>
<td>Histidine</td>
<td>5.26 ± .49c</td>
<td>7.36 ± .18b</td>
<td>67.51 ± .69d</td>
<td>23.66 ± 1.71a</td>
<td>.337 ± .096a</td>
</tr>
<tr>
<td>BSA</td>
<td>2.74 ± .14d</td>
<td>7.84 ± .26a</td>
<td>70.23 ± .37a</td>
<td>23.97 ± 1.40a</td>
<td>.279 ± .041ab</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.84 ± .47d</td>
<td>7.88 ± .17a</td>
<td>68.67 ± .91ab</td>
<td>23.29 ± 3.26a</td>
<td>.270 ± .029ab</td>
</tr>
</tbody>
</table>

*a-d Mean scores (n = 4; ±SD) in the same column with no common superscripts are significantly different (P<.05).

1The amounts of the ligands used in this study were as follows: control: no ligand; pyridine: 10 mM; nicotinamide: 10 mM; histidine: 20 mg/g meat; BSA: 10 mg/g meat; glycine: 20 mg/g meat.

2The extractable pigment was expressed as the absorbance of the pigment solution at 415 nm.

was used for the ANOVA. Means shown in the tables are averages of four values; standard deviations are also listed. The error term used to determine significance was total sum of squares minus treatment sum of squares. Duncan's multiple range test was used to determine significant differences between mean values (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Ligands with No Pigment and No Salt Added

The data in Table 1 show the effects of the ligands on the color values, total pigment, and extractable pigment of cooked turkey breast meat with no pigment and no salt added. The a values (redness) of the turkey breast meat were significantly higher in samples with added pyridine, nicotinamide, and histidine when compared with samples with BSA, glycine, and the control. Among the ligands, pyridine produced the highest a values, followed by nicotinamide and histidine, respectively; all were significantly different (P<.05) from each other. Although the added BSA was expected to produce pink-colored meat, it did not have any effect on meat redness. The high a values caused by pyridine, nicotinamide, and histidine were due to heme complex formation, as in the model system that showed high absorbances at absorption maxima for these ligands (Ahn and Maurer, 1990a,b). The heme-complex-forming reactions of the pigments with pyridine, nicotinamide, and histidine were very strong in the model system, as well as in the meat system. Although the heme-complex-forming reaction of BSA was quite strong in a model system, that reaction was not observed in the meat system.

The b value (yellowness) of meat with nicotinamide was the lowest of all, followed by meat with histidine (Table 1). The b values of meat with nicotinamide and histidine were significantly different from each other; the b values of both were significantly lower than those of the other ligands examined. The BSA produced the highest L value (lightness), and histidine generated the lowest L value.

Extractable pigment was highest in meat with histidine and lowest with pyridine, but differences among the meats with different ligands were very small and generally not significant (Table 1). No relationships were observed between redness and total pigment, or a value and extractable pigment.

Ligands with No Pigment and 2.5% Added Salt

Although the relative effects of the ligands on the a values of meat with no pigment and 2.5% salt added were the same as those with no pigment and no salt added, the a values with salt-added meats were higher than those of the meats with no pigment and no salt (Tables 1 and 2). The increase in a values was caused by the added salt. With 2.5% added salt, myofibrillar proteins were solubilized, and the solubilized proteins likely provided more reactive amino acid side chains to the meat system. Histidine, methionine, cysteine, and added proteins were able to form heme complexes with some or all kinds of meat pigments under denatured conditions, and the higher the concentration of the reactive ligands, the more heme complexes formed (Ahn and Maurer, 1990a,b).
TABLE 2. The effects of ligands on color values, total pigment, and extractable pigment of cooked turkey breast meat with no pigments and 2.5% salt added

<table>
<thead>
<tr>
<th>Ligands 1</th>
<th>a value (redness)</th>
<th>b value (yellowness)</th>
<th>L value (lightness)</th>
<th>Total pigment (ppm)</th>
<th>Extractable 2 pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.92 ± 0.45d</td>
<td>8.56 ± 1.5bc</td>
<td>64.05 ± 0.4ab</td>
<td>22.44 ± 2.89a</td>
<td>.363 ± .03d</td>
</tr>
<tr>
<td>Pyridine</td>
<td>10.77 ± 0.93a</td>
<td>9.26 ± 1.5a</td>
<td>64.37 ± 0.27a</td>
<td>20.74 ± 2.80a</td>
<td>.281 ± .04b</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>9.14 ± 0.16b</td>
<td>6.34 ± 2.9e</td>
<td>62.89 ± 0.45c</td>
<td>22.95 ± 1.51a</td>
<td>.292 ± .055b</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.72 ± 0.21c</td>
<td>8.21 ± 2.8d</td>
<td>62.45 ± 0.80d</td>
<td>23.12 ± 3.09a</td>
<td>.338 ± .087b</td>
</tr>
<tr>
<td>BSA</td>
<td>4.94 ± 0.80d</td>
<td>8.74 ± 2.3b</td>
<td>64.02 ± 0.74ab</td>
<td>21.93 ± 2.85a</td>
<td>.299 ± .063b</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.16 ± 0.22d</td>
<td>8.38 ± 2.1cd</td>
<td>63.39 ± 0.51bc</td>
<td>22.78 ± 3.07a</td>
<td>.256 ± .033</td>
</tr>
</tbody>
</table>

*Mean scores (n = 4; ±SD) in the same column with no common superscripts are significantly different (P<0.05).

1The amounts of the ligands used in this study were as follows: control: no ligand; pyridine: 10 mM; nicotinamide: 10 mM; histidine: 20 mg/g meat; BSA: 10 mg/g meat; glycine: 20 mg/g meat.

2The extractable pigment was expressed as the absorbance of the pigment solution at 415 nm.

The b values of the meat were increased by added salt, but the effects of the ligands on the b values of the meat with 2.5% salt added were similar to meats with no pigment and no salt added (Tables 1 and 2). The b value was the lowest with the nicotinamide and the highest with the pyridine-added meat. Histidine and nicotinamide produced the lowest L values, and pyridine generated the highest L value. Extractable pigment values were the highest in the control and the lowest with glycine, but no relationships between extractable pigment and a value or total pigment and a value were found.

Ligands with Pigment and No Salt Added

The effect of the added (.4 mg myoglobin plus .4 mg hemoglobin per gram of meat) pigments on a values of the meats was nearly the same as that of the added salt, although the a values of the meats with BSA and glycine were more than 1 unit lower than those of the salt-added meats (Tables 2 and 3). With more heme available in the meat, more heme-complex-forming reactions resulted, as in the model system (Ahn and Maurer, 1990a). The a value of the control was higher than that of the meat with BSA added, but no difference in a value was found between meats with added pyridine or nicotinamide (Table 3).

The b value of the meat with nicotinamide was the lowest of all, followed by histidine (Table 3). The meats with pyridine and BSA produced the highest b values. The L value of histidine-added meat was significantly lower than that of the others. Although total pigment was not changed by ligands, added pigments doubled the amount of total pigment.

By comparing the general magnitude of the values in Tables 1 through 3 (significance not

TABLE 3. The effects of ligands on color values, total pigment, and extractable pigment of cooked turkey breast meat with pigments (.4 mg myoglobin plus .4 mg hemoglobin per gram meat) and no salt added

<table>
<thead>
<tr>
<th>Ligands 1</th>
<th>a value (redness)</th>
<th>b value (yellowness)</th>
<th>L value (lightness)</th>
<th>Total pigment (ppm)</th>
<th>Extractable 2 pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.93 ± 0.44c</td>
<td>7.11 ± 0.14ab</td>
<td>63.96 ± 0.40bc</td>
<td>45.22 ± 3.35a</td>
<td>.355 ± .028</td>
</tr>
<tr>
<td>Pyridine</td>
<td>10.17 ± 0.86a</td>
<td>7.34 ± 0.43a</td>
<td>63.71 ± 0.17bc</td>
<td>42.51 ± 5.57a</td>
<td>.360 ± .071</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>9.41 ± 0.80a</td>
<td>4.79 ± 0.50c</td>
<td>62.81 ± 0.00c</td>
<td>44.88 ± 1.11a</td>
<td>.404 ± .074</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.10 ± 0.76b</td>
<td>6.51 ± 0.21d</td>
<td>58.55 ± 0.28d</td>
<td>42.50 ± 1.96c</td>
<td>.403 ± .028</td>
</tr>
<tr>
<td>BSA</td>
<td>3.65 ± 0.30d</td>
<td>7.39 ± 0.57a</td>
<td>65.52 ± 0.79a</td>
<td>42.50 ± 4.39a</td>
<td>.362 ± .019</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.10 ± 0.10cd</td>
<td>7.10 ± 0.30ab</td>
<td>64.52 ± 0.85ab</td>
<td>42.83 ± 4.25a</td>
<td>.374 ± .061</td>
</tr>
</tbody>
</table>

*Mean scores (n = 4; ±SD) in the same column with no common superscripts are significantly different (P<0.05).

1The amounts of the ligands used in this study were as follows: control: no ligand; pyridine: 10 mM; nicotinamide: 10 mM; histidine: 20 mg/g meat; BSA: 10 mg/g meat; glycine: 20 mg/g meat.

2The extractable pigment was expressed as the absorbance of the pigment solution at 415 nm.
TABLE 4. The effects of ligands on color values, total pigment, and extractable pigment of cooked turkey breast meat with pigments (4 mg myoglobin plus 4 mg hemoglobin per gram meat) and 2.5% salt added

<table>
<thead>
<tr>
<th>Ligands</th>
<th>a value (redness)</th>
<th>b value (yellowness)</th>
<th>L value (lightness)</th>
<th>Total pigment (ppm)</th>
<th>Extractable pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.36 ± 33d</td>
<td>7.16 ± .02c</td>
<td>57.00 ± .87ab</td>
<td>45.56 ± 2.29a</td>
<td>.418 ± .044a</td>
</tr>
<tr>
<td>Pyridine</td>
<td>12.96 ± 87a</td>
<td>8.33 ± .29a</td>
<td>57.48 ± 1.56ab</td>
<td>41.14 ± 3.58a</td>
<td>.387 ± .033a</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>11.97 ± 76b</td>
<td>5.12 ± .72c</td>
<td>56.11 ± 1.76b</td>
<td>43.69 ± 3.35a</td>
<td>.394 ± .022a</td>
</tr>
<tr>
<td>Histidine</td>
<td>9.70 ± 33c</td>
<td>6.26 ± .70d</td>
<td>50.85 ± 1.42c</td>
<td>40.63 ± 5.04a</td>
<td>.400 ± .055a</td>
</tr>
<tr>
<td>BSA</td>
<td>5.67 ± .35e</td>
<td>8.00 ± .27ab</td>
<td>58.72 ± .41a</td>
<td>40.63 ± 2.63a</td>
<td>.394 ± .045a</td>
</tr>
<tr>
<td>Glicynne</td>
<td>6.16 ± .35e</td>
<td>7.46 ± .68bc</td>
<td>57.63 ± 1.62ab</td>
<td>41.48 ± 1.47a</td>
<td>.384 ± .056a</td>
</tr>
</tbody>
</table>

a,b Mean scores (n = 4; ±±SD) in the same column with no common superscripts are significantly different (P<.05).

The amounts of the ligands used in this study were as follows: control: no ligand; pyridine: 10 mM; nicotinamide: 10 mM; histidine: 20 mg/g meat; BSA: 10 mg/g meat; glycine: 20 mg/g meat.

The extractable pigment was expressed as the absorbance of the pigment solution at 415 nm.

reported), the powerful effect of heme complex formation on a values of the meat can be observed. When no heme-complex-forming ligand was present, the a value of the meat with added pigment was smaller than that of the histidine in Table 1. Even the values for heme-complex-forming ligands provided by solubilized proteins in salt-added samples were sometimes higher than those provided by added pigments. About 95% of the added pigments were denatured by the cooking procedure and were not extractable.

Ligands with Pigments and Salt Added

When both pigments and salt were added, the a values of the meat were the highest, and the b and the L values were the lowest among pigment and salt combinations. The a value with pyridine was the highest, followed by nicotinamide, histidine, and the control, respectively (Table 4). The a value of the control was significantly higher than in those treatments with BSA and glycine. The b value was lowest with nicotinamide and highest with pyridine; b values for pyridine and BSA were not significantly different. The effects of the ligands in meats with pigments and salt added on L-values were similar to the other pigment and salt combinations; meat with histidine had the lowest L value.

Overall, the a, b, and L values were generally affected by the added ligands. The increase in a values with added salt (Table 2) appeared as large as that with the added pigments (Table 3) by possibly providing solubilized proteins and reactable amino acid chains to form heme complexes in the meat. The b and L values of the meat were generally decreased by added nicotinamide or histidine. The added pigments and salt also decreased b and L values of turkey breast meat. The total pigment and the extractable pigment were affected only by added pigment.

The color of the meat with heme-complex-forming ligands faded very rapidly upon exposure to air, even after sodium hydrosulfite brushing, and returned to the pink color under air-tight conditions with a reducing agent. Although the heme complex itself, which was formed in the meat, was not extractable with acetone-HCl, and the total pigment cannot be used as an indicator of the redness of the meat, the hemes in the denatured pigments seemed to be extractable with the acetone-HCl solution. The pigment in the cooked meat may be ferri- or ferro-hemochromes in which the fifth ligand is a histidine and the sixth ligand is an N atom from the histidine residues of the solubilized proteins or pyridine derivatives, including the naturally present vitamin B6 group (Tappel, 1957; Ledward, 1971, 1974; Cornforth et al., 1986), rather than metmyochromogen in which the fifth ligand is occupied by a carboxyl group of heme protein and the sixth ligand by H2O (Tarladgis, 1962). The most important factors in the color of cooked meat are the amounts of heme-complex-forming ligands such as histidine, nicotinamide, and solubilized proteins (Ahn and Maurer, 1990a), meat pH (Ahn and Maurer, 1990b), and the oxidation-reduction potential (Ahn and Maurer, 1989).

REFERENCES

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