Effects of Added Pigments, Salt, and Phosphate on Color, Extractable Pigment, Total Pigment, and Oxidation-Reduction Potential in Turkey Breast Meat

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ABSTRACT  Ground turkey breast meat treatments were prepared with 16 combinations of myoglobin (0.4 mg/g meat), cytochrome c (0.1 mg/g meat), salt (NaCl 1.25%), and sodium tripolyphosphate (0.5%) before cooking to determine their effect on meat color, water extractable pigments, total pigment, and oxidation-reduction potential in cooked turkey breast meat. Addition of myoglobin did not increase extractable pigment but significantly (P<0.01) increased the redness (a-value) and total pigment of cooked meat. Cytochrome c greatly increased both extractable pigment and a-values (P<0.01). Most of the added myoglobin was denatured or became unextractable, but almost all of the added cytochrome c was extractable after cooking. A large portion of extractable pigment and drip was due to cytochrome c; added cytochrome c had a much stronger effect than myoglobin on pinkness of cooked turkey breast meat. Addition of pigments always decreased L-values (lightness) of breast meat. Salt significantly (P<0.01) increased a-values and decreased L- and b-values (yellowness). Extractable pigment was increased by salt in all pigment treatments except myoglobin-added meat; total pigment was hardly affected by salt. Phosphate significantly (P<0.01) increased a-values in no pigment and myoglobin-added meat, but was less effective than salt. Oxidation-reduction potentials were decreased by adding salt and phosphate. Those potential changes could have a strong effect on pinkness of cooked turkey breast meat if the oxidation-reduction potentials of the meat are around +90 mV or −50 mV.
(Key words: meat pigment, color values, sodium chloride, phosphate, oxidation-reduction potential)

INTRODUCTION

Although the concentration of myoglobin (Mb) varies between species, age groups, and muscle types within an animal carcass (Rickansrud and Henrikson, 1967; Froning et al., 1968; Nocito et al., 1973), Mb is the only pigment present in large enough quantity to color fresh meat. Hemoglobin (Hb) constitutes 5 to 30% of the total muscle pigments (Fleming et al., 1960; Rickansrud and Henrikson, 1967; Huffman, 1980; Niewiarowicz et al., 1986) depending on holding time and bleeding condition or methods, but in most cases Mb alone is used as an index of fresh meat color. The color of fresh meat varies not only with the number and amount of pigments present but also the state of the heme group of the pigments. The state of the heme group can be affected by various factors such as pH (Ashmore et al., 1972; Cornforth and Egbert, 1985), oxygen pressure (Shikama and Sugawara, 1978; Cornforth et al., 1985; Egbert and Cornforth, 1986), enzyme activity (Stewart et al., 1965), temperature (Rickert et al., 1957; Lanier et al., 1977; Cornforth et al., 1985), additives (Seman et al., 1986), electrical stimulation (Sleeper et al., 1983; Tang and Henrikson, 1980) and packaging method of the meat (Clause et al., 1984).

The concentration of cytochrome c in rat skeletal muscle is about 1/50 to 1/100 that of Mb (Akeson et al., 1960). However, cytochrome c could play a very important role in turkey breast meat, as the concentration of Mb in turkey breast muscle is very low (.58 mg/g meat) compared to that in other red muscles (Niewiarowicz et al., 1986), and the concentration of cytochrome c is comparatively high (.013 mg/g meat). Schollemeyer and Klingenberg (1962) reported that breast muscle of the pigeon contained as much as .72 mg cytochrome c/g meat, whereas white muscle of rabbit and smooth muscle contained .025 mg/g meat. Williams and Thorp (1969) reported that rat muscle contains .1 mg cytochrome c/g meat.

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The color-imparting materials in cooked meat are reported to be different from those of fresh meat. Ledward (1974) explained that the heme proteins responsible for the color of cooked meat were mainly di-imidazole complexes, the imidazole residues being supplied by the histidine groups of the bound protein. Tarladgis (1962) interpreted the compound that gives cooked meat its color as a high-spin ferric-porphyrin co-ordination complex, whose fifth and sixth co-ordination positions were occupied by a carboxylate ion of the denatured globin molecule and by water, respectively. Tappel (1957) and Cornforth et al. (1986) believed that mixed denatured globin nicotinamide hemochromes were responsible for a pink color defect in cooked meats, and the oxidation-reduction potential was the most influential factor affecting hemochrome formation. In his review paper, Giddings (1977) concluded that the possibility of a di-imidazole complex proposed by Ledward (1974) was unlikely, and the complex must be a reducible ferric complex, the sixth ligand of which could be replaced by such strong field ligands as -NO, -CO, and -CN to yield adducts with optical spectra and appearance similar to those of the native heme protein complexes. Falk (1964) explained that the donor ligands such as Cl⁻, F⁻, OH⁻, and OH²⁻ may be replaced by strong p-bonding ligands such as -CO, -CN, pyridine, nicotine, imidazole, etc. Among Hb derivatives, replacement of a donor by a p-bonding ligand causes a change from high to low-spin just as in the protein-free heme-complex (octahedral complex). Once one such ligand has been added to an iron-porphyrin, the affinity for a second is greatly increased, and low-spin octahedral complexes result (Castro, 1971). The prerequisite condition for heme-protein complex formation in cooked meat is an unfolding of polypeptides and exposure of heme to the outer environment. The conformational change of heme pigments by heat will allow heme to be attacked by the denatured globin or other proteins in meat.

Ahn and Maurer (1989) found that the denaturation rate of Mb and cytochrome c could be affected greatly by additives such as salt and phosphate in a model system. However, the situation in meat and a model system may be quite different. In order to solve this poultry industry meat color problem, it is very important to find the kinds of pigments that cause pinkness in cooked meat, and to determine the effects of additives on heat denaturation of meat pigments. Therefore, the objectives of this study were 1) to determine the effects of added pigments on color of cooked turkey breast meat, 2) to compare the heat stability of Mb and cytochrome c in a meat system, 3) to assess the effects of oxidation-reduction potential on pigment expression, and 4) to study the effects of added formula ingredients to turkey breast meat on the oxidation-reduction potential and cooked meat pigment formation.

MATERIALS AND METHODS

Purification of Myoglobin and Cytochrome c. The Mb and cytochrome c were prepared from turkey heart with the method described by Rothgeb and Gurd (1982) for Mb, and Brautigan et al. (1978) for cytochrome c. The purity for each purified Mb, Hb, and cytochrome c preparation was ascertained by a single band in SDS-PAGE.

Meat Sample Preparation. Fresh turkey breast meat 24 h postmortem was ground through a 3.2-mm (1/8") meat grinder plate and 16 meat samples (80 g meat/sample) were prepared with combinations of Mb (0, .4 mg/g meat), cytochrome c (0, .1 mg/g meat), salt (NaCl; 0, 2.5%) and sodium tripolyphosphate (0, .5%) before cooking to determine their effect on meat color, water extractable pigments, total pigment, and oxidation-reduction potential in cooked turkey breast meat. Distilled water was used to dissolve these ingredients prior to mixing with meat samples (15%, meat weight basis). Each sample was hand mixed in a 250-mL beaker, covered with aluminum foil, and cooked to an internal temperature of 77 C in a water bath. After cooking, samples were cooled to room temperature (ca 21 C) and stored in a 3 C cooler. The entire experiment was replicated four times.

Color Measurement. Color of the samples was measured at room temperature (21 C) by using a Hunter Lab color difference meter (Hunter Associates Lab., Reston, VA) with a D25-9 reference. The colorimeter was standardized with a white ceramic tile having values for L, 92.14; a, -1.20; and b, .10. Each sample was cut into half and placed on a clean glass plate for measuring L (lightness), a (redness), and b (yellowness) color values. Two readings per slice were taken with a ca 90° rotation between readings.
Absorption Spectrophotometry of Pigments. Drip from each cooked meat sample was filtered through Whatman No. 42 filter paper (Whatman, Clifton, NJ). Each filtrate was adjusted to pH 5.0 with .5 N HCl to precipitate extra proteins in the drip, and the filtrate was centrifuged at 1, 140 × g for 30 min. The final solution was transferred to a cuvette, and a few granules of sodium hydrosulfite were added to reduce the pigments. Absorbances were scanned by a spectrophotometer (Model No. 34, Beckman Instruments, Fullerton, CA) in the wavelength range of 600 nm to 380 nm.

To compare the heat stability of myoglobin and cytochrome c at 77°C in a meat system, pigments were extracted from cooked turkey breast meat, and their absorbances were measured at 415 nm. To extract cooked meat pigments, each 15 to 20 g of sample was minced and blended with the same amount of distilled water for 1 min and centrifuged at 6,000 × g for 30 min. The supernatants were collected, pH was adjusted to 5.0 with .5 N HCl, and their absorbances were recorded using the same method as described for the drip.

For the absorption spectra of purified Mb and cytochrome c mixtures, 1 mg/mL Mb and cytochrome c solutions in distilled water were made and then were mixed to give the desired ratios. The final concentration of each pigment mixture was adjusted to .4 mg/mL pigment solution. The spectra of purified Mb and cytochrome c mixtures were used as references for the composition of cooked meat extracts and drip.

To study the effect of oxidation-reduction potential on pigment expression, purified Mb and cytochrome c were used. Fifty mg of purified Mb or 50 mg of cytochrome c were dissolved in 100 mL of .1 M phosphate buffer, pH 6.3. The oxidation-reduction potentials of 5-mL portions were adjusted with 1% sodium dithionite solution or sodium hydrosulfite granules to desired values, and then the absorbances of each solution were scanned by using a spectrophotometer at wavelengths of 600 nm to 500 nm.

Total Pigment Measurement. Hornsey’s (1956) method was used for total pigment extraction and calculation. After extraction of pigment with acetone-HCl solvent, the solution was first filtered through Whatman No. 42 filter paper and then filtered again through a .45-μm Prep-Disc membrane filter (Bio-Rad, Richmond, CA) just before absorbance measurement to remove suspended particles.

Oxidation-Reduction (Redox) Potential Measurement. Fifteen to 20 g of cooked meat and two volumes of .1 M phosphate buffer, pH 6.3 were added to a blender; the air inside the blender was evacuated by vacuum action to minimize oxygen incorporation. Each sample was blended for 1 min. The redox potential was measured after 2 min of equilibration by using a combination platinum electrode attached to a pH meter (Model 7, Corning Glass Works, Corning, NY).

Statistical Analysis. Analysis of variance using the statistical analysis system (SAS, 1986) was applied. Sixteen combinations of turkey breast meat samples were sorted by pigments and then analyzed to determine significant differences in mean values due to the main effect of pigments, salt, and phosphate and their interactions. Values (+ or −) for main effects in the tables were expressed as the increase or decrease in each parameter caused by the added ingredient (e.g., for a salt main effect, an a-value of +1.1 (X = 3.12 in Table 1, no pigment added) means the actual a-value with added salt was 3.67, and 2.57 without salt. Their difference was 1.10. This difference was caused by added salt. For the interaction effect, the actual a-value of [(0% salt × 0% phosphate) + (2% salt × .5% phosphate)]/2 was 3.30, and that of [(0% salt × .5% phosphate) + (2% salt × 0% phosphate)]/2 was 2.95. Duncan’s multiple range test was used to determine significant differences in mean values (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Effect of Pigments. The Mb addition significantly increased mean a-values and total pigment but decreased L-values (Tables 1 and 2). Although a-values were significantly increased by added Mb, the amount of extractable pigment did not change.

The absorption spectra of extractable pigments are shown in Figure 1. The shapes of all four spectra are nearly the same. All of them have absorption maxima at 550 nm, 520 nm, and 415 nm, which are the characteristic absorption maxima of ferrocytochrome c in the 600 nm to 400 nm range.

Figure 2 shows absorption spectra of purified myoglobin, cytochrome c, and their combinations in various ratios. When the ratio
<table>
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<th>Source of variation</th>
<th>d.f.</th>
<th>No pigment</th>
<th>.4 mg Mb</th>
<th>.1 mg cyt. c</th>
<th>.4 mg Mb + .1 mg cyt. c</th>
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</tr>
<tr>
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<td>- .47</td>
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a-d Values within a row with no common superscripts are significantly different (P<.05).

1 Values (+ or –) for main effects in the tables were expressed as the increase or decrease in each parameter associated with the addition of an ingredient (e.g., for a salt main effect, an a-value of +1.1 (x = 3.12 in Table 1, no pigment added) means the actual a-value with added salt was 3.67, and 2.57 without salt. Their difference was 1.10. This difference was caused by added salt.

*Significant at P<.05.

**Significant at P<.01.
<table>
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<th>Parameter</th>
<th>Source of variation</th>
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<th>.1 mg cyt. c</th>
<th>.4 mg Mb + .1 mg cyt. c</th>
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<td>.238&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.718&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>20.87&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>− 1.28</td>
<td>− .34</td>
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<td>− .60</td>
<td>− .68</td>
<td>2.38</td>
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<td>− 43.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>− 51.69&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>+ .88</td>
<td>− 6.63</td>
<td>3.50</td>
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<sup>a,b</sup>Values within a row with no common superscripts are significantly different (P<.05).

<sup>1</sup>Values (+ or −) for main effects in the tables were expressed as the increase or decrease in each parameter associated with the addition of an ingredient (e.g., for a salt main effect, an a-value of +1.1 (x = 3.12 in Table 1, no pigment added) means the actual a-value with added salt was 3.67, and 2.57 without salt. Their difference was 1.10. This difference was caused by added salt.

*Significant at P<.05.

**Significant at P<.01.
was 9 parts Mb to 1 part cytochrome c, absorption maxima at 550 nm and 434 nm were observed, and the peak at 520 nm moved to 521 nm and was rather weak. With the ratio of myoglobin 4:cytochrome c 1, the absorption maxima at 550 nm, 521 nm, 434 nm and 416 nm were observed. When the ratio of myoglobin and cytochrome c became 2:1, the absorption maximum at 434 nm disappeared and absorption maxima at 550 nm, 520 nm, and 415 nm, which are the characteristic absorption peaks of cytochrome c, were observed. The absorption spectra of Hb were nearly the same as those of Mb except the maximum at 434 nm moved down to 428 nm in Hb (data not shown). From data shown in Figures 1 and 2 it could be concluded that almost all of the pigments in the cooked meat extracts were cytochrome c or the pigments with six ligands such as a heme complex with pyridine derivatives.

Howard et al. (1973) have shown that the heme complexes with pyridine derivatives have absorption spectra very similar to those of cytochrome c. Among the heme proteins, only Mb and Hb would be the major heme-protein complex-forming pigments, as the protein conformation of cytochrome c is different from that of Mb or Hb. Castro (1971) reported that there is considerable peripheral exposure in cytochrome c, but axial ligands are not easily available for substitution. Falk (1964) explained that donor ligands such as Cl\(^-\), F\(^-\), OH\(^-\), and OH\(_2\) may be replaced by strong p-bonding ligands such as -CO, -CN, pyridine, nicotine, imidazole, etc. Castro (1971), however, believed that strong p-ligands of biochemical import are O\(_2\), -CO, and CN\(^-\).
Imidazole and sulphydryl or thio-ester linkages are intermediate in this capacity, and carboxy, water, and amino are s-bonding. Thus, an imidazole and a thio-ester or two of each are required for generating a low spin complex, whereas a single carbon monoxide will suffice. Falk (1964) and Castro (1971) both agree that replacement of a donor by a p-bonding ligand causes a change from high to low spin just as in the protein-free heme complex (octahedral). Furthermore, once one such ligand has been added to an iron-porphyrin, the affinity for a second is greatly increased and a slow spin octahedral complex results. Therefore, the increase in heat denaturation rate of Mb in a meat system could be caused by the complex formation with Mb and p-bonding ligands such as nicotine, imidazole, and sulphydryl side chains of proteins present in meat systems (Falk, 1964).

Most of the added Mb became denatured or unextractable after cooking. The ratio of pigments in drip was also the same as that of extractable pigments (Figures 1 and 3). Although the heat stability of Mb in the model system was similar to or higher than that of cytochrome c (Ahn and Maurer, 1989), the behavior in the meat system was quite different from that in a buffer system. As was shown in Draudt’s (1969) study, the heat stability of Mb greatly decreased due to heme-protein complex formation with many proteins available in the meat system.

The a-value increase in Mb-added meat was not caused by native Mb; it could be caused by denatured Mb or an Mb-complex with ligands such as proteins, as the amount of extractable pigments in Mb-added samples was not increased by added Mb (Table 2). The effect of added cytochrome c on a-values is much greater than that of Mb, even though the amount of added cytochrome c is only 1/4 that of Mb. Unlike Mb, most of the added cytochrome c could be extracted after cooking.
Furthermore, the extinction percentage coefficient of native cytochrome c at 550 nm is about 3.5-fold higher than that of Mb at a similar wavelength (556 nm). Therefore, the a-value increase might be caused mainly by the undenatured cytochrome c in the cytochrome c-added samples. With Mb + cytochrome c, a-values were even higher than those of Mb or cytochrome c-added samples. Added cytochrome c had no effect on total pigment, but Mb significantly increased total pigment. Therefore, it can be assumed that undenatured cytochrome c and denatured Mb or Mb-complex both are very important in a-values of turkey breast meat. With addition of cytochrome c, the lightness of meat was decreased, but Mb had a larger effect than cytochrome c on lightness (Table 1) of turkey breast meat.

Effect of Salt. Salt significantly (P<.01) increased a-values (redness) but decreased b- (yellowness) and L-values (lightness) in all pigment treatments (Table 1). Salt significantly increased the amount of extractable pigment (P<.05) in no pigment added, cytochrome c added, and Mb + cytochrome c-added treatments. Although the amounts of pigments in drip (data not shown) were significantly lower in the salt-added samples than in others, the amounts of total pigment were not increased by added salt (Table 2). The highly significant increase in a-values but minor increase in the extractable pigment and no change in total pigment indicate that the amounts of undenatured pigments and acetone-HCl-extractable pigments are not the only color-generating factors; others such as heme protein complex, redox potential, pH, and texture of the meat also can be very important factors influencing color of turkey breast meat. With added salt (2.5%), most of the myofibrillar proteins become solubilized, and along with the sarcoplasmic proteins, they can take part in complex
formation with the heme proteins during the heating procedure.

Cornforth et al. (1986) indicated that the redox potential was the most important factor in a pink color defect of turkey rolls. Although the redox potential change by salt was not large, it was significantly (P < .05) lower in the Mb-added sample (Table 2). Figures 4 and 5
FIGURE 5. Absorption spectra and $A_{544}$ of myoglobin (Mb) at different oxidation-reduction (redox) potentials; redox potentials were adjusted with sodium hydrosulfite. A. Absorption spectra: $\square = -550$ mV; $\bullet = -50$ mV; $\circ = -15$ mV; $\circ = +115$ mV. B. $A_{544}$ of Mb: $Y = .3012 - 2.04 \times 10^{-4} X^2; r = .90$.

show the effect of redox potential on absorption spectra of Mb and cytochrome c. A redox potential change from $+130$ mV to $+30$ mV could double the absorbance of purified cytochrome c at 550 nm, but a further decrease in the potential did not change the $A_{550}$ of cytochrome c (Figure 4A). The $A_{550}$ increase of cytochrome c was followed by the quadratic
equation \( Y = 1.16 - 1.4 \times 10^{-3} X - 2.39 \times 10^{-5} X^2 \) (\( r = .97 \)), where \( Y \) is absorbance and \( X \) is redox potential change. The decreasing redox potentials were within a range from +130 mV to 0 mV (Figure 4B).

With purified Mb, the redox potential change from +115 mV to -15 mV hardly changed the absorbance of myoglobin in the red to pink color range (540 nm to 580 nm). With the redox potential ranging from -15 mV to -100 mV, two distinct absorption maxima were observed. The first one was at 542 nm and the second was at 580 nm. These two absorption maxima are the characteristic peaks of oxymyoglobin (MbO\(_2\)) in the 500 to 600 nm wavelength range. A further decrease in its potential to -550 mV reduced the Mb fully to the ferrous Mb state; only one peak was observed at 556 nm (Figure 5A). The \( A_{544} \) increase of Mb was followed by the equation of \( Y = .3 - 2.04 \times 10^{-4} X \) (\( r = .90 \)), where \( Y \) is absorbance and \( X \) is redox potential change. The decreasing redox potentials were within a range from +115 mV to -90 mV. The redox potential of raw meat varied from +100 mV to -100 mV, whereas cooked meat usually had lower redox potentials. During the refrigerated storage of cooked turkey meat for a few days, salt or phosphate or both could influence the redox potential and cause a significant change in a-value of the meat if the redox potential of the meat is around -50 mV (Figure 5B).

The heme pigment complexes in cooked meat were described by several researchers (Tappel, 1957; Tarladgis, 1962; Ledward, 1974; Cornforth et al., 1986). The characteristics of those complexes are 1) insoluble in water or other common solvents, 2) brown color but color can be changed to pink under reduced conditions, and 3) can be formed after heme pigment denaturation. Dymick et al. (1975) and Akoyunoglou et al. (1963) showed that amino acids, pyridine derivatives, and related compounds can react with a heme and can form pink color generating materials, or ferric- or ferrohemochromes. Falk (1964) also explained that the donor ligands such as \( \text{Cl}^- \), \( F^- \), \( \text{OH}^- \), and \( \text{OH}_2^- \) could be replaced by p-bonding ligands such as -CO, -CN, pyridine, nicotine, imidazole, etc.

Although the amount of unladenatured cytochrome c and the amount of denatured Mb appeared to be the most important factors in pink color in this study, the contribution from other factors such as heme complex formation also seemed to be very important in salt-added samples (Tables 1 and 2). The solubilization of myofibrillar proteins by added salt would provide more heme-complex-forming amino acid side chains in the meat system. Furthermore, the decrease in redox potential by salt might play quite a large role in the state of the complex and unladenatured pigments.

**Effect of Phosphate.** Phosphate significantly (\( P<.01 \)) increased the a-value of turkey breast meat with no pigment added and .4 mg Mb/g meat-added samples (Table 1). This increase in a-value was likely caused by the pH increase in phosphate-added samples. Shikama and Sugawara (1978) reported that the rate of autoxidation of MbO\(_2\) was increased with an increasing hydrogen ion concentration. The a-value increase in no pigment added and Mb-added meat was also enhanced by a redox potential decrease due to added phosphate (Tables 1 and 2). The L-values of the meat were significantly (\( P<.01 \)) decreased by added phosphate in all pigment-added samples (Table 1). The darkening of color was also observed in uncooked, phosphate-treated meat (Froning, 1965).

**Interactions.** A salt x phosphate interaction significantly (\( P<.05 \)) increased the a-value of no pigment-added samples (Table 1). Although added salt increased a-values, the increase in a-value was greater in the .5% phosphate treatment.

Extractable pigments were significantly increased by salt x phosphate interaction in Mb + cytochrome c-added samples (Table 2). In the 0% phosphate treatment, added salt did not change the amount of extractable pigment. With .5% phosphate, added salt greatly increased extractable pigments.

Overall, factors that could most affect color of cooked turkey breast meat would be the amount of unladenatured cytochrome c, the amount of added Mb, salt, some unknown heme-protein complex, and the changes in redox potential and pH of the meat caused by additives. The interaction of factors may have a minor influence on color of cooked turkey breast meat.

**REFERENCES**


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