Methods for Identification and Prevention of Pink Color in Cooked Meat

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Introduction

Consumers expect fresh meats to be red, and cooked meats to be brown. Consumers often interpret red or pink color in cooked meats as an indication of undercooking. Consumers are especially sensitive to pink color in pork, due to concern with trichinosis in undercooked pork. Processors are sometimes perplexed with pink color in cooked meats due to concern with standards. Total nitrate plus nitrite was determined by the AOAC (1984) procedure in which nitrite in an aliquot of extracted sample reacts with sulphanilamide to form an azo compound, followed by coupling with N-1-naphthylene-ethylenediamine to form a pink-colored complex that absorbs at 540 nm. Nitrate may also be determined by an AOAC (1984) method. Nitrates are again extracted from the sample in hot water. Any nitrite in the sample is oxidized to nitrate by addition of potassium permanganate. After precipitation of proteins and excess chloride, m-xylenol is added and the colored complex is distilled into dilute alkali. The color is measured by spectrophotometer at 450 nm. Nitrate is determined by comparison with standards, and corrected for nitrite content.

Froning et al. (1968) found that spray-dried egg albumin caused pink color when added as a binder to turkey rolls, but pan-dried albums did not affect color. They observed that spray-dried albumin raised meat pH to 6.2-6.3, while pan-dried albumin (acidified with lactic and citric acids) lowered meat pH to 5.9-6.0. They attributed the pink color to an egg conalbumin-iron complex stabilized at the higher pH, rather than an interaction with meat pigments. Another possibility not yet reported in the literature is that protein additives added as meat binders might be nitrosylated during drying by an open-flame procedure. Nitrogen oxides are produced in combustion, and may directly react with pigments in meat cooked in a gas oven (Pool, 1956), or perhaps react with egg, soy or milk proteins during drying. Ito et al. (1983) demonstrated that pink nitrosyl hemochromes were formed when myoglobin was incubated with nitrosated lysozyme or...
albumin and ascorbic acid. Thus, protein nitrosated during drying could conceivably release NO under reducing conditions in cooked meat, leading to pink color development.

If such a possibility is suspected, protein bound nitrite (possibly as tryptophyl-NO or cystyl-NO) can be determined (Olsmen and Leeuwen, 1977). Diluted samples are heated with mercuric chloride, cooled, and mixed with 30 percent zinc sulfate, resulting in dissociation and conversion of protein bound nitrite to free nitrite. Nitrite levels may then be determined by the AOAC (1984) method previously described.

Detection of precursors (nitrate, nitrite, gaseous nitric oxide) is not required to confirm presence of nitrosyl hemochrome. Nitrosylhemochrome can be directly and rapidly measured in cooked meats (Hornsey, 1956). Maximum pigment extraction is obtained at an acetone/water ratio of 1:0, allowing for the moisture present in meat. Nitrosopigment concentration is calculated from absorbance of the acetone extract at 540 nm. Extraction and filtration are done in subdued light to lessen pigment fading. The addition of cysteine during extraction is recommended to reduce fading (Hornsey, 1956). This is especially effective in non-meat model systems, where endogenous reductants from meat are absent.

**Carboxymyoglobin and Carbon Monoxide Hemochrome**

Fresh meat exposed to low levels of carbon monoxide will turn red with formation of carboxymyoglobin, but will turn brown upon cooking (Watts et al., 1978; Vahabzadeh et al., 1983). Tappel (1957) reported that cooked beef turned pink upon exposure to carbon monoxide. The reflectance spectra were similar to that of denatured globin hemochromes. The pink pigment was accordingly labelled as denatured globin carbon monoxide hemochrome. Pool (1956) reported that the surface of turkey breast meat turned pink when cooked in a gas oven. Pink color was observed when either CO or NO gas was circulated around birds cooked in an electric roaster. Interestingly, birds roasted in some electric tabletop roasters became pink when the element was set on high, and air in contact with hot elements also circulated past the bird. Apparently, temperatures near the heating element were sufficiently high to generate gaseous nitric oxides, which could generate pink color if allowed to come in contact with the meat. In birds or large roasts or rolls exposed to NO or CO gas, a pink ring of 1/2" thick or less will develop. This ring is common and even desired in Texas BBQ beef, where meat is slow cooked in a heavy smoke (Cornforth and Carpenter, 1988).

**Undenatured Myoglobin and Oxymyoglobin**

Undenatured myoglobin and oxymyoglobin may be present in sufficient concentration to cause red color in meats cooked to 71°C, if pH is greater than 6.0 (Trout, 1989; Schmidt and Trout, 1984). Hard-to-cook hamburger patties, characterized by persistent internal red color during cooking, are associated with high pH raw meat, such as bull meat (Mendenhall, 1989). The red color is apparent during and after cooking, and does not rapidly fade. Myoglobin has also been identified as the pigment responsible for surface discoloration with storage of vacuum-packaged bratwurst (Ghorpade and Cornforth, 1991). Product was brown after cooking, and red discoloration was associated with microbial growth. Microbial growth likely promoted reduction of undenatured metmyoglobin to myoglobin. Faustman et al. (1990) showed that filtered supernatant from a Pseudomonas culture caused a brown solution of metmyoglobin to turn red. Kacchayanand et al. (1989) reported pink discoloration in vacuum-packaged beef and proposed that a Clostridium spp. was the causative agent.

Undenatured myoglobin and oxymyoglobin can be extracted from cooked meat (Warriss, 1979). Meat is blended 1:10 with ice cold 0.04 M phosphate buffer, then kept on ice for 1 hour. After centrifugation, an absorption spectrum is recorded over the visible range from 400-700 nm. All forms of myoglobin exhibit a large absorption peak at about 420 nm. Myoglobin exhibits a single large absorption peak at 555 nm. Oxymyoglobin is usually the predominant pigment observed after blending in air. It exhibits two large absorption peaks, a and B, at 577 and 541 nm, respectively. Metmyoglobin has a broad absorption peak at 505 nm and a small peak at 630 nm. Often a mixed spectrum is observed in extracts of red-colored cooked meats. To confirm presence of fresh meat pigments, add 1 drop of 2% potassium ferriyanide and 1 drop of 2% potassium cyanide to the cuvette, converting all myoglobin forms to cyanometmyoglobin. It exhibits a single peak at 540 nm. Total myoglobin concentration, percent metmyoglobin and percent myoglobin denatured by cooking may be calculated from absorbance values at 525 and 572 nm after subtraction of absorbance at 700 nm to correct for turbidity (Trout, 1989; Krzywicki, 1979).

**Denatured Globin Hemochromes**

Denatured globin hemochromes are pink complexes between histidine side chains of heat-denatured proteins and reduced or ferrous iron of heme (Tappel, 1957). Free nitrilotriamide or arginine may also form pink-colored complexes with heme and denatured globin (Cornforth et al., 1986). Globin hemachromes have been identified as the pink pigments of canned tuna (Brown and Tappel, 1957) and turkey rolls (Cornforth et al., 1986). Since denatured globin hemochromes are poorly soluble, if at all, in most solvents, they are best identified in meat by their characteristic reflectance spectra. Oxy or carboxymyoglobin have reflectance minima (absorption maxima) at 541 and 577 nm, while various hemochromes exhibit a small reflectance minima at about 530 and a more pronounced minima at 555-560 nm (Tappel, 1957; Table 1).

Reflectance spectra are obtained with a diffuse reflectance attachment to a dual beam spectrophotometer. A white standard (powdered barium sulfate or meat sample bleached with hydrogen peroxide) is placed in the reference port, and the colored sample to be measured is placed in the sample port. The percent reflectance of the sample vs reference is automatically calculated by the spectrophotometer at each wavelength throughout the scanning range. Globin hemochromes require reducing conditions in order to maintain heme iron in the ferrous state. They rapidly oxidize and...
Table 1. Absorption Maxima (Reflectance Minima) for Various Pink or Red Meat Pigments.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>α</th>
<th>β</th>
<th>Y (soret)</th>
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<tbody>
<tr>
<td>Myoglobin⁴</td>
<td>540</td>
<td>577</td>
<td>420</td>
</tr>
<tr>
<td>Oxymyoglobin⁴</td>
<td>555</td>
<td>475</td>
<td>420</td>
</tr>
<tr>
<td>Cytochrome c²</td>
<td>550</td>
<td>521</td>
<td>415</td>
</tr>
<tr>
<td>Nitrosoyd Hemochrome³</td>
<td>558</td>
<td>530</td>
<td>422</td>
</tr>
<tr>
<td>Denatured Globin Carbon¹,⁴</td>
<td>558</td>
<td>529</td>
<td>420</td>
</tr>
<tr>
<td>Monoxide Hemochrome³</td>
<td>575</td>
<td>540</td>
<td>425</td>
</tr>
<tr>
<td>Globin Hemochrome⁵</td>
<td>558</td>
<td>530</td>
<td>422</td>
</tr>
<tr>
<td>Nicotinamide Hemochrome⁵</td>
<td>558</td>
<td>529</td>
<td>420</td>
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fades upon exposure to air. Brushing or soaking cooked meat surfaces with a solution of strong reductant, sodium dithionite, allows formation of pink globin hemochromes that resist fading long enough for reflectance spectra to be obtained. To obtain spectra of cooked meat surfaces without exposure to air or use of dithionite, Girard et al. (1990) sliced frozen meat to 2 mm thickness and placed meat between glass microscope slides separated by 2 mm spacers. Binder clips held the slides together. Samples were sealed in vacuum pouches and cooked at various temperatures before obtaining reflectance spectra. Girard et al. (1990) observed that pink complexes to occur in cooked meats between reduced heme and various nitrogenous ligands. For the various globin hemochromes of cooked meat, the ORP at which pink color appeared upon titration with a strong reductant ranged from -321 to -511 mV (Cornforth et al., 1986).

To measure ORP of cooked meat, the ORP electrode must be inserted into the meat, and attention given to exclusion of air while allowing the ORP reading to stabilize. For slurries or solutions, ORP measurement during titration also requires exclusion of air. This may be done by slowly bubbling nitrogen gas through the solution in a closed container with ports for 1) introduction of nitrogen, 2) the ORP electrode, and 3) venting of headspace gas.

PH measurements are often unstable in systems with little or no buffer capacity. Likewise, ORP measurements are more unstable in cooked meat systems with low reducing capacity (poising ability). Reducing capacity of a system may be determined by addition of 1 mM potassium ferricyanide to a buffered aliquot, and allowing 1 hr at 2°-4°C for reductants in the system to reduce ferricyanide. Reducing ability is expressed as absorbance of 1 mM potassium ferricyanide solution at 420 nm minus sample absorbance (Lee et al., 1981).

Microbial growth lowers ORP, and increases reducing capacity. Jacob (1970) reported that ORP of aerobic Bacillus subtilis cultures declined from +110 to -100 over a period of 12 hours, largely due to bacterial oxygen consumption. Deaeration of media lowered ORP from +110 to -250 mV. Inoculation of media with Clostridium paraputrificum resulted in further lowering of ORP to -350 mV in 9 hours. Clearly, anaerobic bacterial growth results in production of reductants capable of reducing heme iron in cooked meats. As mentioned earlier, Kalchayanand et al. (1989) reported pink discoloration in vacuum packaged beef and proposed that a Pseudomonas culture caused a brown solution of metmyoglobin to turn red.

Oxidation-Reduction Potential

Oxidation-reduction potential (ORP) is a measure of the tendency of a substance or system to gain or lose electrons, relative to an arbitrary standard, the hydrogen electrode. ORP is measured with an inert metal electrode such as platinum, a reference electrode (silver/silver chloride or hydrogen) and a pH meter. The potential between the solution to be measured and the reference is measured in millivolts. Temperature compensation is not used. Unlike pH electrodes, clean ORP electrodes are stable and normally do not need calibration before use. ORP electrode function may be checked by preparing the following solutions (Anon., 1983):

- Solution A (100 ml of 0.1 M potassium ferrocyanide, 0.05 M potassium ferricyanide), solution B (100 ml of 0.1 M potassium ferrocyanide, 0.05 M potassium ferricyanide, and 0.36 M potassium fluoride). The potential of solution A for a combination ORP electrode (platinum redox, silver/silver chloride reference) is about 234 millivolts. The potential in solution B should be about 66 mV greater than in solution A.
- Tables of standard oxidation-reduction potentials of organic compounds are available (Loach, 1970). Strong reductants (electron donors) have negative standard potential values, indicating electron flow from the substance in solution to the reference electrode when ORP measurements are taken.

Meat pigments may be thought of as oxidation/reduction indicators. Pink or red colors only occur under reducing conditions. In fresh meat, heme iron must be reduced (ferrous) in order for oxygen binding or bloom to occur. The standard ORP value (E°, pH 7.0) for MbMetMb is +46 mV (Loach, 1970). In cooked meats, heme is exposed due to globin denaturation, and the heme iron is more rapidly oxidized than in fresh meats upon exposure to air. Thus, relatively strong reducing conditions are needed for stable pink complexes to occur in cooked meats between reduced heme and various nitrogenous ligands. For the various globin hemochromes of cooked meat, the ORP at which pink color appeared upon titration with a strong reductant ranged from -321 to -511 mV (Cornforth et al., 1986).

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Prevention of Color Development

If nitrosoyl hemochrome is identified, the source of nitrate, nitrite or nitric oxide must be determined and eliminated. If NO₂⁻ contamination is identified, changes may be needed in oven temperature or type of oven used. High pH may lead to gel formation in hard-to-cook patties, so heat penetration is slowed during cooking. Rapid freezing after patties are formed would likely lead to more thorough cooking. Lowering pH of raw materials, or using a more acid phosphate blend (if used at all) should reduce incidence of hard-to-cook patties.
In vacuum-packaged products where microbial growth is associated with red discoloration, higher cooking temperature, lower pH and microbial inhibitors such as sodium lactate have been found to slow rate of discoloration. Red color from myoglobin or oxymyoglobin is eliminated at cooking temperature above 74°C.

Globin hemochromes are commonly present in canned or vacuum-packaged meats or large roasts cooked to temperatures above 74°C. Pink defect in turkey rolls is commonly observed in the extracted meat between muscle chunks, while the larger pieces may remain white. Processors report that rolls held for several hours before cooking are more likely to exhibit pink defect. Microbial growth and associated drop in ORP would promote pink color development in turkey rolls, but this has not been demonstrated to date. Meat emulsions containing 2% salt are more likely to develop pink color after cooking than emulsions without salt (Ahn and Maurer, 1990). Possibly an increased protein extraction in meat emulsions contributes to higher globin hemochrome formation and pink color after cooking. Further work is needed to understand factors causing the variable development of pink defect in poultry rolls. Milk proteins are sometimes promoted for their ability to lighten poultry rolls, and possible reduce pink color problems. This possibility also needs further investigation.

**Conclusions**

The three major pigments causing red or pink color in cooked meats are nitrosyl hemochrome, underdeveloped oxymyoglobin and globin hemochromes. Pigment identification by spectrophotometric procedures is the first step in eliminating color problems. Nitrosyl hemochrome formation is prevented by identifying and eliminating the source of nitrate, nitrite or gaseous nitric oxide. Higher cooking temperatures and lower meat pH will reduce oxymyoglobin levels in cooked products. Microbial inhibitors such as sodium lactate combined with higher cooking temperatures and lower pH will reduce the incidence of pink discoloration associated with microbial growth. Higher cooking temperatures (>74°C) and anaerobic or reducing conditions promote pink color from globin hemochromes. Practical procedures are needed for inhibiting development of these pink pigments.

**References**


Warriss, P.D. 1979. The extraction of haem pigments from fresh meat. J. Food Technol. 14, 75.

Discussion

D. Kropf: We have had some interest in adding vitamin E to rations—does this improve the reducing capacity of the muscle and if so, will this present a problem with persisting pink color? Perhaps you or Cameron (Faustman) would like to comment.

D. Cornforth: I am not aware of any increase in pink color problems in meat products with elevated antioxidant levels.

C. Faustman: Our experience with vitamin E in meat has been totally from a fresh meat color stability viewpoint. We have not investigated the potential for any cooked pink color defects.

N. Marriott: Two questions regarding your slides. One, what is your explanation of the pink color in the cooked fresh pork leg? Two, in your discussion of the Clostridium-induced color problems, did you indicate the species involved?

Cornforth: The color problem in the cooked pork did not appear until after the legs had first been refrigerated. Once the leg was sliced, the color defect could be observed, but faded quickly. We were able to rule out undenatured myoglobin and nitroso-pigments and thus concluded that we were working with undenatured hemochromes. The fact that the pigments did not become evident until refrigeration led me to infer that a reduction of heme iron from ferric to ferrous forms occurred during storage. The ferrous form would allow interactions with nitrogenous groups with ligands such as histidine to form pink pigments. The Clostridium which you referred to was not identified to the species level in our study. Kalchayanand and coworkers at the University of Wyoming have recently published work regarding involvement of Clostridium in beef color defects, but did not identify the species.

C. Bacon: I have observed a pink discoloration in the core of turkey franks which disappears during refrigerated storage. What is happening? Also, do you have any comments on the implication of gas-fired ovens for cooked meat color defects?

Cornforth: Were the turkey franks a cured product?

Bacon: Yes, but the pink discoloration in the core was much more intense than the typical cured color.

Cornforth: I cannot tell you what is happening. I would recommend some of the procedures outlined in my presentations for identifying pigments. Does anyone have any comments for Craig Bacon?

J. Claus: I have noticed that when working with processed meat batters where air is intentionally added, the outer surface will oxidize and turn brown while the center will have a cured color which does fade. In this case, we may be getting higher oxygen incorporation than normal coupled with a less-than-ideal cured. I would look at your comminution system and the efficiency of vacuum.

D. Epley: What methods can be used to lower meat pH below 6.0 on hard-to-cook ground beef?

Cornforth: A variety of acids work great in the laboratory. As far as methods likely to be USDA-approved for the commercial application, I do not know. Perhaps you can blend high pH ground beef with lower pH beef and through dilution obtain a product with pH less than 6.0.

Epley: Are any specific spices considered significant sources of nitrate?

Cornforth: Not that I know of. A processor in our area has experienced problems with certain spice blends causing brown discoloration in fresh pork sausage. We found that this was not due to the presence of nitrite or nitrate, but due to a lack of vitamin C activity in the implicated spice blends.

P. Lewis: The red color of the high pH patties cooked to the normal endpoint is not surprising given the temperature:pH relationship for myoglobin denaturation. A simple recommendation involves longer cooking time at the normal temperature.

Cornforth: I would agree with that.

C. Calkins: When discussing the red color in hard-to-cook ground beef, you also mentioned that rapid freezing could help to alleviate the problem. Why?

Cornforth: In the Utah State work by Von Mendenhall, it was noted that refrigerated storage of high pH beef patties caused these meat products to ‘gel up’ and cook slower. If you can rapidly freeze high pH patties following their manufacture (i.e., not allow refrigerated storage), then this problem should be avoided.

G. Schmidt: Has anyone performed a controled experiment looking at the interaction between redox potential, pH and color?

Cornforth: Not that I am aware of. That would be a good study.

Schmidt: We get lots of samples brought in with a variety of color problems and while I have no data to support it, I believe that the redox potential is a critical variable.

Cornforth: One point which is important to note is that measurement of redox potential in meat is difficult.

J. Acton: I have a comment. In the journal, Poultry Science during this past year, Art Maurer’s group at UW-Madison have looked at the effects of pH and redox potential on myoglobin in solution, but not in tissue.

Cornforth: We have data on myoglobin solutions as well-in work performed by Chuck Carpenter. We measured the redox point at which pink colors appear in solution. Also, Gerard and co-workers have published related data.

P. Sleper: We own several gas-fired ovens and I am wondering if your work with color and pH would help us because we suspect that we have a nitrosyl hemochrome problem in cooked chicken breast.

Cornforth: I would not expect that pH would be an issue with nitrosyl hemochrome pigments. To replace your ovens would be expensive. Poole (1956) demonstrated that nitric oxide or carbon monoxide exposed to cooking meats would form pink colors. Can you make the combustion process in your ovens more efficient? One suggestion would be to adjust the flame height and be sure the jets are clean.

Sleper: The pink discoloration in our products is present throughout and is not just found in a ring within the product. Are we attributing the problem to the wrong chemical species?

Cornforth: You may be. It is likely that the problem is not due to nitroso pigments.

[Elizundia]: We export to Japan and we have a pink color problem in chicken. Could drinking water which contains high levels of nitrate and which is used in the husbandry of the live birds result in pink discoloration problems postmortem?
They did not investigate the basis for the pink discoloration. You may want to see if the pink color you are observing is due to nitroso pigments.

[Elizundia]: Could nitrate/nitrite contamination of chill water be a problem?

Cornforth: Possibly. The important point would be the effectiveness of chill water penetration into the tissue.

Calkins: Froning also demonstrated that during transport, birds which were exposed to truck exhaust fumes (i.e. carbon monoxide) would actually show some pink color defect in the postmortem tissue.

Cornforth: Good point. Also, the authors of that study noted that the blood was extremely red-colored. I do not think that carbon monoxide derivatives of heme pigments are a common cause of pink discoloration in poultry. Because the birds must be nearly asphyxiated for the effect to be noticed.

C. Carpenter: Daren, would you comment on the effect of storage time prior to cooking on the occurrence of pink color defect? Also, can you elaborate further on the effect of pH on denatured globin hemochromes?

Cornforth: I will answer the last question first. Realize the relationship between pH and redox potential: High pH conditions generally result in higher redox potentials. The holding time prior to cooking may be an issue when considering microbial growth. The question is whether bacteria can cause pink discolorations. We have observed pink discolorations in cooked products which contained high microbial populations in the raw state. Faustman and others have recently reported that *Pseudomonas* can cause a color reversion in beef homogenates—Cameron, was this a reduction of ferric to ferrous myoglobin?

C. Faustman: This work was recently published in Fleischwirtschaft and was for a non-cooked system into which bacteria were intentionally inoculated for the purpose of effecting a brown-to-red color change. We were unable to definitively identify the pigment, but could rule out the process of metmyoglobin reduction.

Epley: Any recommendations on product handling if one is 'stuck' with gas-fired ovens?

Cornforth: Meat products can be placed in roasting bags to prevent contact between gases and the meat.

J. Acton: In your introduction, you mentioned working on a whitening aspect, trying to make meat appear lighter. Any comments?

Cornforth: We will be looking at the use of milk proteins for lightening the appearance of cooked meat products.

D. Bartholomew: How long can mitochondria actively consume oxygen in raw meat?

Cornforth: That is dependent upon pH. At higher pH, the mitochondria will remain active in postmortem muscle for a much longer time than at lower pH, on the order of weeks in high pH meat.